ABSTRACT

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Psyllium, a type of dietary fiber, has been shown to have various health benefits including cholesterol lowering, hypoglycemic, cancer prevention, and laxative effects. However, due to its extremely strong water-holding and gel-forming capacities, incorporation of psyllium into food products on the required amount per serving for health claim is difficult. This study evaluated the effect of acid treatment on water up-taking, swelling, gelling and bile acid binding capacities of psyllium samples. The acid treatments were conducted at different reaction temperatures (25, 37.5, and 50 °C) with different psyllium – solvent ratios (1:2.5, 1:5, 1:7.5, and 1:10 g/mL). The result showed that reaction temperature influenced the effectiveness of acid treatment on physical/chemical properties of psyllium samples significantly, while effects of different psyllium – solvent ratios were not significant. This implicated the acid modification at a high temperature might be a possible method to improve the physical/chemical properties of psyllium for incorporation in food.

ACID MODIFICATION OF PSYLLIUM

By

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1.1 Introduction

In 1953, Hipsley first applied the term dietary fiber for the non-digestible components which constitute the plant cell wall (Hipsley, 1953), since then, more concerns have been addressed by research in this area. Dietary fibers, which are usually a component of plant cell walls, show various beneficial effects on the human body associated with their intake. Due to their physical/chemical properties, dietary fibers may exert many functions such as bacterial fermentation (Yang, Manoharan, & Mickelsen, 1970; Prohaszka, Jayarao, Fabian, & Kovacs, 1990; Melcher, Leviit, & Slavin, 1991; Rao, Chou, Simi, Ku, & Reddy, 1998; Arimochi, Kataoka, Kuwahara, Nakayama, Misawa, & Ohnishi, 1999; Nakanishi, Kataoka, Kuwahara, & Ohnishi, 2003), water-holding capacity (Stephen & Cummings, 1979; Flourie, Vidon, Florent, & Bernier, 1984; Braaten, Wood, Scott, Riedel, Poste, & Clooins, 1991; Kim & Shin, 1996; Jekins, Vuksan, Kendall, Wursch, Jeffcoat, Waring, *et al.*, 1998; Muir, Yeow, Keogh, Pizzey, Bird, Sharpe, *et al.*, 2003), as well as absorption of some types of organic compounds (Kelsay, Behall, & Prather, 1979; Reinhold, Garcia, & Garzon, 1981; Fernandez & Philips, 1982; van der ARR, Fahey, Ricke, Allen, & Berger, 1983; Coudray, Demigne, & Rayssiguier, 2003). Positive correlations have been found between a higher intake of dietary fiber and promotion of health conditions. Base on this fact, the U. S. Department of Health and Human Services (HHS) as well as the U. S. Department of Agriculture (USDA) established a recommended consumption of

dietary fiber which is 14g/kcal (USDHHS & USDA, 2005). This recommendation revealed that American should significantly increase their current consumption of dietary fiber to achieve the recommended amount.

As a type of dietary fiber, psyllium, which mainly grows in Indian, is known as a mucilaginous material. Due to its strong water-holding capacity and gel-forming ability, psyllium has been used for treatment of constipation for a long time. However, recent studies on psyllium implicated that the beneficial effect associated with consumption of psyllium as a dietary fiber source may not only be limited to its laxative effect. Copious evidence showed that psyllium can also present functions such as lowering cholesterol, hypoglycemia, as well as preventing cancers (Yu, Lutterodt, & Cheng, 2009).

Much research has been conducted to investigate the effect of psyllium on health conditions, as well as the possible underlying mechanism, which will provide a more comprehensive view on this type of dietary fiber. Meanwhile, various efforts have been made to improve physical/chemical properties and functionalities as well as eliminate possible adverse effects of psyllium to achieve better applications of psyllium. In addition, a large number of studies have been done by researchers and food manufacturers on developing food products fortified with psyllium to offer consumers an easy way to get this dietary fiber.

The following review will first start with the discussion of dietary fiber. The definition, botanical functions, fiber chemistry, dietary fiber's physical/chemical properties, as well as analytical methods of measuring dietary fiber will be covered; in addition, source and consumption of dietary fiber will also be discussed. Then, as

an important type of dietary fiber due to its various health benefits, psyllium will be discussed in detail, with the focus on its beneficial health effects and possible underlying mechanisms. At last, effort on incorporating psyllium into food products will also be covered.

1.2 Dietary Fiber

1.2.1 Definition

Positive correlations between the consumption of dietary fiber and promoted health condition have been studied for many years. Meanwhile, the definition of dietary fiber has evolved by incorporating new scientific achievements of the time. In 1976, Trowell established a definition of dietary fiber which includes all indigestible polysaccharides (Trowell, Southgate, Wolever, Leeds, Gassull, & Jenkins, 1976). The 1976 definition rapidly gained wide acceptance and provided directions for research in the following 3 decades (Devries, 2003). Based on concerns on the 1976 definition, in December 1999, the American Association of Cereal Chemists (AACC) adopted a definition of dietary fiber: "dietary fibre is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fibre includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibres promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation" (Jones, 2000). This definition broadened the constituents of dietary fiber, including synthesized carbohydrate compounds as well as indigestible ('resistant') starch.

1.2.2 Botanical Functions of Fiber and Fiber Chemistry

Dietary fiber is mainly derived from plant foods. The major components of dietary fiber include cellulose, hemicelluloses, pectins, and lignin substances. Botanically, the primary functionality of fiber is to constitute the plant cell wall. For some types of fiber which are not cell wall components, the main function is to form plant secretions such as gums and mucilages.

Chemically, the major components of dietary fiber are polysaccharides and lignin. Cellulose, the most abundant organic compound in nature, is the most important structural polysaccharide in plants. It is a linear homopolymer chain made-up with β-1, 4-linked glucose units with a length from several hundred to over ten thousand. Cellulose can be hydrolyzed in concentrated acid solution but not in concentrated alkali. Hemicellulose, along with cellulose, presents in almost all plant cell walls. Comparing with cellulose, hemicellulose is a smaller size branched heteropolymer chain with backbones of β-1, 4-linked pyranoside sugars. Differing from cellulose, the random, amorphous structure of hemicellulose only has a little strength which means it can be easily hydrolyzed by dilute acid or alkali. Another component of dietary fiber is pectins, which serve as plant cell wall materials as well as intercellular cementing substances. Pectins backbone is formed by a linear chain α-1, 4-linked D-galacturonic acid. In this chain, some of the D-galacturonic acid units are replaced by 1, 2-linked L-rhamnose. This replacement leads to bends in the molecule. Many pectins have side chains formed by the linkages of neutral sugars. In general, pectins are water-soluble and gel-forming. Lignin is a non-polysaccharide component of dietary fiber. It is a polymeric material composed of phenylpropanoid

units such as coniferyl, sinapyl, and p-coumaryl alcohols. The primary functions of lignin in plants are imparting strength to cell walls, facilitating transportation of water, as well as impeding the degradation of wall polysaccharides.

1.2.3 Bacterial Degradation and Fermentation

Dietary fiber is resistant to human digestion in the small intestine. However, in the large bowel, dietary fiber is susceptible to bacterial digestion. In the human cecum and colon, the bacterial population is numerous and various. Scientists have found more than 50 genera and 400 species of bacteria in human feces, the majority of which are anaerobic bacteria (Topping & Clifton, 2001). Research also showed that these bacteria can constitute approximately 41% to 55% of dry weight feces (Stephen & Cummings, 1980).

The colonic bacteria fermentative reaction to dietary fiber is mainly due to the hydrolysis of polysaccharides. In an adult, the primary products of fermentation are short-chain fatty acids (SCFA), gases (including CO_2, H_2, and CH_4), and some heat. The major SCFA are acetate, propionate, and butyrate.

As the products of bacterial fermentation, SCFA have many metabolic effects in the large bowel. SCFA are weak acids with pK_a values about 4.8, thus, by fermentation of dietary fiber, colonic pH values will decrease. This was supported by a study using healthy human subjects (Melcher, *et al.*, 1991). The lowered pH may exert a function of inhibiting some pH-sensitive pathogens. Results from an animal study in pig conducted by Prohaszka *et al.* collaborates this finding. They reported that a high concentration of SCFA, which leads to relatively low pH of colon content, significantly lowered the number of *Salmonella* (Prohaszka, *et al.*, 1990). It was also

found that, in colon, SCFA were metabolized by colonocytes and served as main respiratory fuels (Yang, *et al.*, 1970).

In addition, a large body of evidence shows that short-chain fatty acids, especially butyrate, can inhibit carcinogensis in the colon. In 2003, Nakanishi and coworkers conducted an *in vivo* study using high amylase maize starch (HAS) in azoxymethan (AOM) -induced aberrant crypt foci (ACF) formation in rats (Nakanishi, *et al.*, 2003). HAS administered alone as well as together with *C. butyricum* strain MIYAIRI588 (CBM588) showed the effectiveness of decreasing the number of ACF. *C. butyricum* can produce SCFA and has been used as a probiotic. They also found that the β-glucuronidase activity in colonic content decreased significantly in two HAS-administered groups. Previous study demonstrated that there was a positive correlation between the number of AOM-induced ACF in the rat and fecal β-glucuronidase activity level (Arimochi, *et al.*, 1999). Results from another study provided support to this function of SCFA. Rao *et al.* (1998) administered rats with AOM-induced colonic ACF with dietary coffee fiber. Decreased numbers of ACF as well as fecal β-glucuronidase activity level was observed.

1.2.4 Water-holding Capacity

Many physiological effects of dietary fiber can be ascribed to the water-holding capacity. Initially, when dietary fiber is exposed to an aqueous medium, the surface absorbs water molecules. The sugar residues with polarity of polysaccharide provide a hydrophilic capacity to dietary fiber, whereas the intermolecular bonds such as ether cross-linkages present a hydrophobic effect. Meanwhile, the particle size of

dietary fiber may also have some effect for water-holding capacity by influencing the internal space in fiber matrix for water molecules to be entrapped (Kay, 1982).

By absorbing water molecules, dietary fiber becomes a viscous material. This property was demonstrated to have the effect of slowing the absorption of glucose in many studies. An *in vivo* animal study conducted by Kim and Shin (1996) used chicory water-soluble extract resulting in a reduced rate of glucose absorption in rats' jejunum. Studies in human subjects lent support to animal studies. In 1991, Braaten and coworkers first showed a β-glucan-rich oat gum from oat bran had significant effect of lowering the rate of glucose absorption in human (Braaten, *et al.*, 1991). Later, a stronger inhibition of absorption of glucose in human by higher viscosity dietary fibers than by lower viscosity ones was found by Leclere and colleagues in 1994. The mechanism of the effect of dietary fiber on glucose absorption was suggested to be due to viscous soluble fibers that increase the thickness of the "unstirred water layer" of the small intestine (Flourie, *et al.*, 1984).

The water-holding ability of dietary fibers can also affect the stool bulk and content. As early as 1979, Stephen and Cummings tested the *in vitro* water-holding capacities and fecal bulking abilities in 18 different dietary fibers (Stephen & Cummings, 1979). The water uptake was determined by centrifugation and using liquid capsules with dialysis tubing containing the materials. They found an inverse correlation between the water-holding property and fecal bulking ability in dietary fiber samples, which suggested that the fecal bulking effect was not only ascribed to the water uptaking capacity. In addition, they also demonstrated that the presence of charged group and the uronic acid content were related to the water-holding property,

while the particle size only had a little effect on it. Later *in vivo* studies showed the collaborative results. Furthermore, they suggested that the resistant starch also presented the ability of increasing fecal bulk. Jenkins *et al.* (1998) demonstrated the effect of resistant starch on increasing fecal bulk in human, while Muir and coworkers showed that the combination of resistant starch and wheat bran was more effective than wheat bran alone on fecal indexes (Muir, *et al.*, 2003).

1.2.5 Absorption of Organic Compounds

When passing the gastrointestinal tract, various organic compounds can bind to dietary fiber. The reversibly bound compounds include bile acids, toxic compounds, and some bacteria.

Several studies showed the ability of dietary fiber on binding different mineral elements. Binding mechanism might not be same for different elements. In 1979, Kelsay *et al.* conducted a study in human to investigate the effect of dietary fiber on calcium, magnesium, iron, and silicon balances (Kelsay, *et al.*, 1979). Subjects were administered with high fiber content diet or low fiber content diet. A significant decrease in calcium, magnesium, and silicon balance was observed in high fiber group compare with low fiber group.

Dietary fiber's ability of binding to iron is well studied. Fernandez and Phillips (1982) proposed that fiber components have a potential binding site with high affinity but low capacity. Later studies suggested the phytate content in dietary fiber might be mainly ascribed to its iron binding capacity as well as for other mineral elements (Coudray, *et al.*, 2003). Further study on iron-binding property was conducted by Reinhold and colleagues (Reinhold, *et al.*, 1981). The *in vitro* study

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showed that the binding of iron rose rapidly when pH changed from 5.0 to near 7.0. Meanwhile, binding to iron by dietary fiber could be interfered by certain inhibitors, such as ascorbic, citric and phytic acids, EDTA, as well as some amino acids such as cysteine.

The effect of dietary fiber on other mineral elements was tested in chick by van der Arr and coworkers (van der Arr, *et al.*, 1983). They asserted that for different elements, the mechanisms are various. For zinc, the absorption might be because of the phytate content, bulk-providing property, as well as altered microflora in the gut by fibers. The effect on copper might be due to fibers' phytate content and also the chelating properties.

Although dietary fiber showed negative effect on the absorption of many mineral elements, recent studies found that the magnesium absorption was enhanced by consuming dietary fiber (Coudray, *et al.*, 2003). Several mechanisms were postulated to explain this effect on magnesium. One mechanism suggested that the fermentation of dietary fiber resulted in the production of SCFA and acidification of luminal contents, which had effects on microflora in the large intestine and might lead to the presence of a large magnesium pool. Another mechanism relating the SCFA to the enhancement of magneisum absorption involves a cation exchange function. In addition, the rich content of magnesium in dietary fiber may affect the reduction in magnesium absorption.

1.2.6 Methods of Measuring Dietary Fiber

In order to establish a labeling regulation for consumers to better understand and receive beneficial health effects from dietary fiber, a defined analytical

methodology for dietary fiber measurement in foods is needed. To meet the requirements of the definition, the method should also be applicable in competent laboratories worldwide. Critical characteristics of the required method include the ability to accurately simulate the enzymes-involved human digestion behaviors as well as the capacity of being performed with acceptable precision (DeVries, 2003).

Several AOAC (Association of Official Analytical Chemists) official methods have been developed based on aforementioned Trowell's 1976 definition of dietary fiber. The most widely accepted and used enzymatic gravimetric methods are AOAC 985.29 and AOAC 991.43. Since the AOAC 985.29 does not allow the separation of soluble and insoluble fiber content, it can only be applied for the measurement of total fiber content of foods. The later one, AOAC 991.43, evolved from the former method and can be adapted for both soluble and insoluble fiber contents. This method uses MES-TRIS buffer and briefly includes steps as removing starch and protein by enzymatic treatment, precipitation of soluble fiber using ethanol, filtration and weighing of the dietary fiber residue, and determination of protein and ash in residue for correction (Association of Official Analytical Chemists, 2000; method 985.29; Association of Official Analytical Chemists, 2000; method 991.43). These methods are well applied, however, problems with filtration step was encountered when the sample has strong gel-forming ability (such as psyllium). A modification of AOAC 985.29 and AOAC 991.43 was made by Lee and coworkers by using sonication and high-speed centrifugation to separate the soluble dietary fiber fractions from insoluble fractions (Lee, Rodriguez, & Storey, 1995).

With the adaption of AACC's definition of dietary fiber, non-digestible oligosaccharides and resistant starch are now considered as a part of dietary fiber because of their physiological functionalities. It is becoming increasingly clear that modifications of previous analytical methods must be made to accommodate the newer definition.

The most commonly available non-digestible oligosaccharides include fructo-oligosaccharides, *trans*-galacto-oligosaccharides, polydextrose, and fibersol 2 (McCleary, 2003). Hoebregs developed a method using enzymatic hydrolysis combined with high performance anion-exchange chromatography (HPAEC) with pulse amperometric detection to analyze the fructo-oligosaccharide content in food products (Hoebregs, 1997). Another method of measuring the fructo-oligosaccharides, based on the hydrolysis by specific enzymes, was established by McCleary and coworkers (McCleary, Murphy, & Mugford, 2000). The use of enzymatic hydrolysis together with HPAEC was also applied in the analysis of *trans*-galacto-oligosaccharide (De Slegte, 2002) and polydextrose (Craig, 2001). Fibersol 2 was measured by using a modification of AOAC method 985.29, in which high performance liquid chromatography was used to analyze the combination of ethanol solution and washings (Gordon & Okuma, 2002). Although the analytical methods of determination for a particular non-digestible oligosaccharide were established, no universally adaptable method has been developed yet.

For the measurement of resistant starch, the method established by McCleary and Monaghan offered result in accordance with data obtained from previous *in vivo* study. Amyloglucosidase and α-amylase were used to digest the soluble starch

fraction in this enzymatic method. Afterward, the resistant starch fraction was separated. The digestion of this fraction was conducted in acetate buffer with the addition of potassium hydroxide (KOH) and amyloglucosidase. Then the digestion product, glucose, was incubated with glucose oxidase-peroxidase (GOPOD) and analyzed using spectrometric method (McCleary & Monaghan, 2002).

1.2.7 Source of Dietary Fiber

The main source of dietary fiber is plants. The content and chemical composition of dietary fiber can be various in different plant materials. In 1988, Anderson and Bridges investigated the dietary fiber content in selected foods (Anderson & Bridges, 1988). The tested foods included cereal products, vegetables, legumes, and fruits. It was seen that in cereal products, corn bran had the highest total dietary fiber (85.19%) while oat bran had the most soluble dietary fiber (7.84%). Frozen kale was the richest in total fiber (33.48%) and frozen broccoli was the richest in soluble fiber (13.63%) among the tested vegetables. In legumes, canned green beans contained most total dietary fiber (33.97%), dried raw pinto beans contained most soluble fiber (8.15%). Canned pear contained more total dietary fiber (32.18%) than all other tested fruits, while canned purple plum was the richest in soluble fiber (9.92%).

Studies have also been conducted to determine the dietary fiber content in diet. Anderson and colleagues investigated a simulated American diet for its total fiber content (Anderson, Bridges, Tietyen, & Gustafson, 1989). The simulated American diet was made on the basis of household Nationwide Food Consumption

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Survey data. The result that the stimulated diet provided 5.6 total dietary fiber/1000 kcal suggested a lower dietary fiber level than recommended.

1.2.8 Consumption of Dietary Fiber in Different Populations

The consumption of dietary fiber has been measured in different populations. A total dietary fiber intake of 19.9 ± 5.3 g/day in the British population was found by Bingham and coworkers (Bingham, Cummings, & McNeil, 1979). This result matched the calculated value of 19.7 g/day from the Nation Food Survey conducted in 1976 in Britain. Data from the Second National Health and Nutrition Examination Survey (NHANES II) was analyzed by Lanza *et al.* for dietary fiber intake in United States adult population (> 19 years age) (Lanza, Jones, Block, & Kessler, 1987). They reported a value of 13.3 g/day dietary fiber intake according to Southgate values. They also found that in every age groups, women consume more dietary fiber than men, on a per 1000 kcal basis. Another study was conducted to investigate the trends from 1965 to 1996 of US adolescent (11-18 years of age) on food intake (Cavadini, Siega-Riz, & Popkin, 2000). The studied data was collected from four nationally representative United States Department of Agriculture surveys (the Nationwide Food Consumption Survey 1965 and 1977, and the Continuing Survey of Food Intake by Individuals 1989-91 and 1994-96). The dietary fiber intake per day slightly increased from 13.5 ± 0.3 g in 1965 to 15.0 ± 0.4 g in 1994-96 in the tested age group.

In the Dietary Guideline for Americans, 2005 published by U.S. Department of Health and Human Services and U.S. Department of Agriculture (USDHH & USDA, 2005), the currently recommended dietary intake is 14 g per 1000 calorie

consumed. It is realized that this objective might be hard for some Americans to achieve, but more consumption of fiber-rich foods can be helpful.

1.3 Psyllium

1.3.1 History and Natural Source of Psyllium

There are over 200 species in genus *Plantago,* a number of plants in this genus are commonly known as psyllium. The seeds of psyllium, which are commercially used for production of mucilage, are mainly derived from *Plantago afra* L. and *Plantago indica* L.. The husks are derived from the seeds of *Plantago ovata Forsskaol*. The common name of *Plantago ovata* in India, ispaghula, comes from the words "isap" and "ghol", with the meaning of "horse ear" in Persian, which describes the shape of the *Plantago ovata*'s seeds.

Plantago ovata is an annual herb mainly cultured in North Gujarat in India. The plant is normally 12 to 18 inch in height, with numerous small white flowers. The seeds are enclosed in capsules. Generally, psyllium is cultured for its mucilage content, which is a white fibrous material with hydrophilic property. The mucilage can be obtained by mechanical milling/grinding, and is usually referred to as husk.

Interest in psyllium arose due to a variety of health benefits. In world market, India is the primary country that produces and exports psyllium, while the United States is the largest importer of psyllium, and over 60% of imported psyllium husk is used in pharmaceutical products.

1.3.2 Chemical Composition and Structures

Many studies have been done to investigate the chemical composition of psyllium's polysaccharide chain. As early as 1950s, Laidlaw and Percival extracted the polysaccharide obtained from *Plantago ovata* with first cold water, then hot water and reported two components as a polyuronide and a neutral arabinoxylan (Laidlaw & Percival, 1949; 1950). Later, Kennedy and coworkers reported a single polysaccharide chain in psyllium, which was a highly branched arabinoxylan (Kennedy, Sandhu, Southgate, 1979). More recently, Fischer and colleagues conducted a study on the chemical composition and structure of the gel-forming fraction of psyllium (Fischer, Yu, Gray, Ralph, Anderson, & Marlett, 2004). They applied compositional and methylation analysis, associated with NMR spectroscopy, on the gel-forming fraction, which is so-called fraction B. The result provided a figure of a highly branched arabinoxylan main chain, with densely substituted 1, 4 – linked xylopyranose residues, some carrying single xylose units at position 2, as well as some carrying trisaccharide branches at position 3, with the structure as L-Araf-α-1,3-D-Xylp-β-1, 3-L-Araf.

1.3.3 Cholesterol Lowering Effect

It is a widely accepted statement that elevated plasma cholesterol level is a major factor leading to development of several lethal diseases, such as cardiovascular disease (CVD) and coronary heart disease (CHD). Although medical technologies improved rapidly in past many years, CHD is still the leading cause of death in the United States and other western countries. According to the guidelines published in 1994 by the National Cholesterol Education Program (NCEP), an undesirable high

serum cholesterol level was found in approximate 30% of Americans. Statistical data from the American Heart Association showed that mortality from CVD accounted for 36.3% of all deaths in 2004, or one of every 2.8 deaths in USA (American Heart Association, 2008).

A copious body of evidence elicited the strong linkage between intake of psyllium and lowering cholesterol level. An *in vivo* animal study conducted by Anderson and coworkers used 10 different types of dietary fiber including psyllium to feed male Sprague-Dawley rats for 3 weeks (Anderson, Jones, & Riddell-Mason, 1994). The result showed significantly decreased serum and liver cholesterol concentrations in rats fed soluble fiber-rich diets, especially for psyllium, which reduced the most cholesterol levels. A conclusion that intake of soluble fibers has more effect of lowering cholesterol level than intake of insoluble fibers was drawn. Another animal study with rats showed similar function of psyllium in terms of reducing cholesterol level (Matheson, Colon, & Story, 1995). Different fibers were fed to 10 male rats for 4 weeks and activity of cholesterol 7α-hydroxylase, which is the rate-limiting step in bile acid synthesis, was measured. Elevated enzyme activity was found in the psyllium hydrocolloid-feeding group in contrast with the cellulose-feeding group. This result lent support to the hypothesis that the cholesterol lowering effect of soluble fibers was affected by increasing synthesis of bile acids.

In 1997, Arjmandi *et al.* determined that psyllium which was partially hydrolyzed by storage had comparable ability of lowering cholesterol level with native psyllium (Arjmandi, Sohn, Juma, Murthy, & Daggy, 1997). The study used male Sprague-Dawley rats as animal subjects. After feeding cellulose, native

psyllium, as well as psyllium with various storage temperatures as 5 °C and 40 °C for 21 days, liver total cholesterol and total lipid concentrations was tested. No significant differences of reducing cholesterol level abilities among native and stored psyllium were observed. Meanwhile, serum triglyceride and HDL cholesterol levels showed no significant change among groups.

The aforementioned studies were all conducted in male rats with experiment length from 3 weeks to 4 weeks. To investigate the hypocholesterolemic effect of psyllium for a longer period, Terpstra and coworker performed an 8 weeks psyllium-feeding experiment using female rats (Terpstra, Lapre, de Vries, & Beynen, 2000). Lowered plasma cholesterol and liver cholesterol concentrations were observed in the psyllium-fed group, and plasma cholesterol was determined to be mainly very low-density lipoprotein (VLDL). The psyllium in diet in this study was at a lower concentration as 3% than previous studies. A less striking hypocholesterolemic effect on liver cholesterol level in female rats than in male rats was also found. This was asserted to be an indication that male rats store excess cholesterol in liver rather than in plasma. In this study, the cholesterol lowering effect of psyllium was ascribed to its association with a higher excretion of bile acid, which is because of the viscosity of psyllium.

Besides rats, hamster was also used in many studies due to the similarities in cholesterol metabolism between it and human, as well as the response of plasma LDL levels to various dietary and pharmaceutical treatments (Turley, Daggy, & Dietchy, 1994). Furthermore, the biliary bile acid composition in hamster is in common with that in human, which is mainly glycine conjugated cholic acid and

choenodeoxycholic acid. Whereas in rat, it is cholic acid and several muricholate isomers conjugated to taurine (Daggy, O'Connell, Jerdack, Stinson, & Setchell, 1997). Studies in hamster showed results which lend support to the effect of psyllium on lowering cholesterol level. In addition to investigate this effect, in 1999, Trautwein and coworkers tested the changes in the circulating bile acid pool in hamster administered psyllium (Trautwein, Kunath-Rau, & Erbersdobler, 1999). Comparing to hamsters fed cholestyramine, psyllium-fed hamsters showed significantly higher glycine to taurine conjugation and cholate to chenodeoxycholate ratios in bile acid pool.

The hypocholesterolemic effect of psyllium was also studied in hamsters when combined with cholestyramine, which is a well-known bile acid binding resin. Daggy *et al.* and Turley *et al.* showed an augmentation of the cholesterol lowering effect of psyllium when used together with cholestyramine. Furthermore, they asserted that the effects of psyllium and cholestyramine on cholesterol lowering were conducted by different underlying mechanisms (Turley, *et al.,* 1994; Daggy, *et al.,* 1997).

Guinea pig was also used to study the cholesterol reducing effect of psyllium. This animal model was considered to be appropriate since not like other animals, guinea pig, similar to humans, has lower level of HDL than LDL, and the lowered plasma cholesterol level induced by dietary fibers mainly happens to the atherogenic LDL (Fernandez, Ruiz, Conde, Sun, Erickson, & McNamara, 1995). Studies in this species offered collaborative results with studies on other animal models, in aspect of the hypocholesterolemic effect of psyllium (Fernandez, 1995; Fernandez, Vergara-

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Jimenez, Conde, Behr, & Abdel-Fattah, 1997; Eomero, West, Zern, & Fernandez, 2002).

In addition to the cholesterol lowering effect of psyllium, Vergara-Jimenez and colleagues found an increased stability of LDL to oxidation in psyllium-fed guinea pigs (Vergara-Jimenez, Furr, & Fernandez, 1999). After administering a psyllium containing diet for 4 weeks, LDL oxidation susceptibility, as well as α-tocopherol (a lipid soluble antioxidant) concentration in LDL from individual samples were tested. The LDL showed higher α-tocopherol concentration and was more resistant to oxidation. The results showed that by feeding psyllium, the number and cholesterol concentration of LDL particles were reduced, and the remaining time of LDL in circulation was shortened.

A copious body of evidence from studies using human subjects lends supports to the hypocholesterolemic effect of psyllium. In 1988, Arbaham and Mehta conducted a study which involved 7 healthy males to investigate the cholesterol lowering effect of psyllium (Abarham & Mehta, 1988). Subjects were administered 21 g/day psyllium husk incorporated into their basal diet for 3 weeks after having control diet for the first 3 weeks. After 10 days and after 3 weeks of psyllium intake, reduced total cholesterol as well as LDL cholesterol and HDL cholesterol were observed, which demonstrated the effect of psyllium supplementation on cholesterol lowering. In 1993, another study involved 24 healthy men showed similar results (Stoy, LaRosa, Brewer, Mackey, & Meusing, 1993). In the same year, a study done by Sprecher and coworkers involved as many as 118 healthy men and women with primary hypercholesterolemia, while the treatment of psyllium along with high-fat or

low-fat diets was as long as 16 weeks (Sprecher, Harris, Goldberg, Anderson, Bayuk, Russell, *et al.,* 1993). In the high-fat group, the total cholesterol level and LDL cholesterol level decreased 5.8% and 7.2% respectively, while in the low-fat group, the reductions were 4.2% and 6.4%, respectively. The lowered total cholesterol level and LDL cholesterol level indicated that psyllium could provide a modest while still significant hypocholesterolemic effect in subjects on either high-fat or low-fat diets.

The evaluations of the reducing cholesterol effect of psyllium were also conducted in human subjects with hypercholesterolemia. Twenty six men with mild to moderate hypercholesterolemia were administered 3.4 g psyllium for 3 times per day for 8 weeks after 2 weeks of base line period. The serum cholesterol level, LDL cholesterol level, as well as LDL cholesterol to HDL cholesterol ratio were found to be reduced by the psyllium treatment comparing to the control which used cellulose (Anderson, Zettwoch, Feldman, Tietyen-Clark, Oeltgen, &Bishop, 1988). Later studies conducted within similar subjects with mild to moderate hypocholesterolemia with time lengths from 40 days to 24 weeks offered collaborative results in terms of the hypocholesterolemic effect of psyllium supplementation (Bell, Hectorn, Reynolds, & Hunninghake, 1990; Everson, Daggy, McKinley, & Story, 1992; Davidson, Maki, Kong, Dugan, Torri, Hall, *et al.,* 1998).

High serum cholesterol level is an important risk factor of cardiovascular disease (CVD). Studies have been done to explore the effect of psyllium on patients with CVD. In 2007, Sola and coworkers administered the soluble fraction from psyllium incorporated into a low-fat diet to 28 men with CVD, while using insoluble fiber for control. After a time period of 8 weeks, plasma lipid profile was measured.

Comparing with the control group, the results showed an enhancement of HDL concentration, as well as lowered total to HDL ratio and LDL to HDL ratio. Thus, a conclusion that soluble fraction of psyllium presented a more beneficial effect than insoluble fiber for CVD secondary prevention was drawn (Solar, Godas, Ribalta, Vallve, Girona, Anguera, *et al.,* 2007).

Whether psyllium, as a hypocholsterolemic supplementation, provides similar effect in children was also investigated by scientists. Davison and coworkers conducted a double-blind study involved 25 children with hypercholesterolemia aged from 6 to 18 years to test the efficiency of psyllium (Davidson, Dugan, Burns, Sugimoto, Story, & Drennam, 1996). After the diet stabilization for 8 weeks, subjects were administered either psyllium-rich cereal or control cereal for 6 weeks, followed with a 6-week wash out period, then a 6-week crossover treatment. Comparing the time period of consuming control cereal, significant drops in total and LDL-cholesterol concentration were noted when consuming psyllium-rich cereal. It was concluded that psyllium was an efficient adjunction to low-fat diet in the pediatric population due to its easy incorporation into a variety of food products. Results from another study in 1995 lent support to this conclusion. Williams and colleagues demonstrated that psyllium can enhance the hypocholesterolemic effect of the Step I Diet in 2 to 11 years old children in terms of treating hypercholesterolemia (Williams, Bollella, Spark, & Puder, 1995).

Another topic which should be paid attention to is whether psyllium must be administered with other foods to offer the hypocholesterolemic effect. Wolever *et al.* explored this question by administering hypercholesterolemic subjects with psyllium

mixed with food or between two meals (Volever, Jenkins, Mueller, Boctor, Ransom, Patten, *et al.,* 1994). Significant reductions in total, LDL, and HDL cholesterol were observed between the group administered psyllium mixed with food and the control group, which was administered wheat-bran cereal. However, no significant differences were found between the control group and the psyllium-administered groups when psyllium was given between two meals. It showed that to have a maximum effect of cholesterol lowering, psyllium must be mixed with other foods. Psyllium is thought to act as a hypocholesterolemic supplement due to its function of inhibiting fat absorption, as well as increasing bile acid excretion. Since fat absorption and bile acid excretion is meal-related, psyllium can perform the most effect when combined with meal.

Gender difference and hormonal status may also influence the psyllium effect on hypercholesterolemia. Scientists have postulated that plasma triacylglycerol, HDL and VLDL concentration are gender dependent (Cobb, Greenspan, Timmons, & Teitelbaum, 1993). In addition, in men, high plasma LDL-cholesterol concentration is closely related with cardiovascular disease risk, while in women, low HDL-cholesterol concentration and high triacylglycerol concentration are highly related (Castelli, 1988). Furthermore, Bonithon-Kopp and coworkers, based on their study, found that comparing with premenopausal women; postmenopausal women have a higher risk for coronary heart disease due to significantly higher plasma LDL cholesterol, triacylglycerol, as well as apo B concentration (Bonithon-Kopp, Scarabin, Darne, Malmejac, & Guize, 1990).

To investigate whether the response to a high cholesterol diet as well as the effect of lowering cholesterol by soluble dietary fiber including psyllium differ by gender, Fernandez *et al.* conducted a study in guinea pigs (Fernandez, Vergara-Jimenez, Romero, Erickson, & McNamara, 1995). They found that female guinea pigs were more responsive to the challenge of high dietary cholesterol than males, which lead to a reduction in the cholesterol lowering effect of soluble dietary fiber when taking high cholesterol diet. However, with low cholesterol diets, the hypocholesterolemic effects of dietary fiber were found to be similar. In 1999, Roy and coworkers published results from their study which was conducted to explore the gender effect on soluble dietary fiber intake (Roy, Vega-Lopez, & Fernandez, 1999). Male and female guinea pigs were involved, as well as menopause-mimicking females by ovariectomy. Higher HDL-cholesterol concentration and large LDL particle size were found in female guinea pigs. Furthermore, postmenopausal guinea pigs were observed to have higher plasma cholesterol, apo B and triacylglycerol concentration compared to males and premenopausal females, even in the group which consumed soluble dietary fibers. Meanwhile, the reduction of the susceptibility to oxidation of LDL in males and premenopausal females by administering soluble dietary fiber was not found in postmenopausal females. The discrepancy of the LDL oxidative susceptibility between postmenopausal female guinea pigs and others was interpreted to be lead by estrogen deprivation. Sack and colleagues showed that by infusing the estradiol into postmenopausal women to mimic the premenopausal women's serum estradiol concentration, the LDL oxidative susceptibility decreased significantly (Sack, Rader, & Cannon, 1994).

Human studies on the influence of gender and hormonal status on the hypocholesterolemic effect showed collaborative results (Vega-Lopez, Vidal-Quintanar, & Fernandez, 2001; Vega-Lopez, Conde-Knape, Vidal-Quintanar, Shachter, & Fernandez, 2002; Vega-Lopez, Freake, & Fernandez, 2003). After the intake of soluble dietary fiber such as psyllium, enhanced triglyceride level was found in postmenopausal women, while it decreased in men, and no significant change was observed in premenopausal women. In human, apo C III and apo E play an important role on influencing plasma triglyceride concentration (Vega-Lopez *et al.,* 2003). Apo C III, which is a major component of VLDL, has the function of inhibiting the hydrolysis of triglyceride by lipoprotein lipase, while apo E contributes to hypertriglyceridemia by both promoting VLDL synthesis and impairing VLDL hydrolysis. In postmenopausal women, it was found that the concentrations of apo C III and apo E in plasma elevated in response to psyllium, which might be due to the influence from gender and hormonal status. In addition, estrogen, which is lacking in postmenopausal women, was postulated to have the function of providing a better lipoprotein profile with enhanced HDL-cholesterol level and reduced LDL-cholesterol level (Vega-Lopez *et al.,* 2001).

From the aforementioned studies, it could be concluded that psyllium is a very useful dietary fiber in terms of lowering cholesterol with great application potential. The underlying mechanisms of the hypocholesterolemic effect of psyllium were explored for many years. It was found that psyllium does not function as a bile acid sequestrant which is the main function of cholestyramine. Indeed, it was proved that psyllium does not show a bile acid binding capacity *in vitro* (Anderson, Floore, Geil,

O'Neal, & Balm, 1991). However, the results from several studies did show that psyllium presented the function of altering hepatic cholesterol homeostasis and stimulating bile acid synthesis (Fernandez *et al.,* 1995; Daggy *et al.,* 1997; Everson *et al.,* 1992). It was postulated that this effect might be due to the interruption of the enterohepatic circulation of bile acid and decreased the absorption of cholesterol lead by psyllium's viscosity (Fernandez *et al.,* 1995). A study done by Satchithanandam and coworkers showed that intake of psyllium may lead to increased levels of gastric in lumen and colon (Satchithannadam, Klurfeld, Calvert, & Cassidy, 1996). Mucins are responsible for the gelling properties of mucus, which can provide internal organs with lubrication and protection, enhance the removal of microorganisms, as well as act as intestinal bacteria substrates.

Besides, another mechanism was considered to be of more importance. Bile acids is thought to be the primary form for cholesterol to be excreted from human body, thus, altered bile acid metabolism is considered to be one of the mechanisms for dietary fibers' hypocholesterolemic effect. In human, the rate-limiting enzyme of the synthesis of cholesterol is known to be 3-hydroxy-3 methylglutaryl CoA (HMG-CoA) reductase, while for the catabolism of cholesterol, it is cholesterol 7α-hydroxylase. The changes in activities of these two enzymes as well as the mRNA levels by feeding psyllium were tested by Buham and coworkers (Buham, Furumoto, Konkin, & Story, 2000). They observed that after administered psyllium, the activity of cholesterol 7α-hydroxylase was significantly higher than the control group which was fed cellulose. This change was correleated with the change in cholesterol 7α-hydroxylase mRNA level. Interestingly, an increased level of HMG-CoA reductase

had also been observed, which indicated an up-regulated HMG-CoA reductase activity. This phenomenon was reported in another study done by Fernandez *et al.* (Fernandez *et al.,* 1995). Intake of a diet rich in cholesterol will lead to a feedback inhibition of HMG-CoA reductase activity. However, they found that in guinea pigs, intake of psyllium together with a high-cholesterol diet enhanced the HMG-CoA reductase activity to the level which was comparative with the intake of a low-cholesterol diet. This implicated a more complex interaction of psyllium with HMG-CoA reductase as well as cholesterol 7α-hydroxylase. One possible explanation is that the increased cholesterol 7α-hydroxylase activity and bile acid excretion lowering the serum cholesterol level, which enhanced the HMG-CoA reductase activity to potentially synthesis more cholesterol as a complement. Similar increase of HMG-CoA reductase activity was observed in guinea pigs administered citrus pectin (Fernandez, Sun, Tosca, & McNamara, 1994). More studies are required on this topic to further reveal the mechanism.

Besides, scientists found that by intake of psyllium, the activity of hepatic acyl CoA: cholesterol acyltransferase (ACAT) was suppressed. The primary function of ACAT is catalyzing the intracellular cholesterol esterification and cholestryl esters formation. A positively correlation between ACAT activity and hepatic total cholesterol concentration was reported in previous studies (Fernandez, *et al.,* 1994). An increased hepatic cholesterol concentration could lead to an up-regulated ACAT activity, whereas a suppressed ACAT activity indicates a smaller hepatic cholesterol pool size. It implicated the interruption of the enterohepatic circulation of bile acid and decreased the cholesterol absorption due to psyllium intake.

A lowering level of LDL-cholesterol was found as an appreciable effect of the intake of psyllium in many studies. LDL level has been recognized to have a close relationship with the risk of cardiovascular disease (Goldstein & Brown, 1983). LDL is the main carrier of cholesterol, which comprises approximate 60% of total serum cholesterol. It has the function of delivering cholesterol to tissues. LDL can be recognized by the LDL receptor on cell surfaces via the apolipoprotein B (apo B) embedded in the LDL particle. Thus, the number of apo B of hepatic membrane may strongly influence the LDL level. An up-regulation of apo B receptors associated with intake of psyllium together with high cholesterol diet in guinea pigs was reported by Fernandez and coworkers (Fernandez, *et al.,* 1995).

It was also found that large particles of very low-density lipoprotein (VLDL) rich in cholestryl esters associated with high cholesterol intake are more readily converted to intermediate-density lipoprotein, and then LDL. By administering psyllium, secretion of smaller particles of VLDL was observed. This alternation of VLDL was postulated to lead to a better plasma cholesterol index (Fernandz *et al.,* 1995, 1997).

Taken together, psyllium produces the hypocholesterolemic effect by interrupting enterohepatic bile acid circulation and absorption of cholesterol, up-regulating the activity of HMG-CoA reductase as well as 7α-hydroxylase while suppressing ACAT activity, enhancing apo B receptor, and inducing the secretion of smaller VLDL particles.

1.3.4 Hypoglycemic Effect

Diabetes mellitus is considered as a major factor which causes disability and hospitalization all over the world lead to big financial loss (Foster, 1994). Type 2 diabetes, which is also referred as non-insulin-dependent diabetes, is known to significantly increase the risk of atherosclerosis as well as other macrovascular diseases (Laakso & Lehto, 1997). Studies show that psyllium may serve as a hypoglycemic agent in health human subjects as well as patients with diabetes.

In 2001, 10 healthy female volunteers were involved in a study which was conducted by Sierra and colleagues to investigate the effects of psyllium husk as well as guar gum on postprandial glucose and insulin concentrations in healthy subjects (Sierra, Carcia, Fernandez, Diez, Calle, Sahagun, *et al.,* 2001). After an overnight fasting, glucose was administered orally associated with or without fiber. Afterwards, serum concentrations of glucose and insulin were monitored. A significant reduction in mean serum insulin concentration was detected when both fibers were administered, while psyllium significantly decreased the area under the curve (AUC) of glucose concentration, compared to guar gum.

Studies conducted in human subjects with diabetes offered similar description of psyllium's postprandial hypoglycemic effects. In different assays, psyllium was administered via various ways. Pastors and coworkers gave psyllium immediately before breakfast and dinner (Pastors, Blaisdell, Balm, Asplin, & Pohl, 1991), while Clark *et al.* incorporated psyllium into meals (Clark, Gardiner, MuBurney, Anderson, Weatherspoon, Henry, *et al.,* 2006). However, intake of psyllium lead to reduced postprandial glucose concentration and insulin concentration independent of the

methods of taking. Furthermore, Pastors *et al.* found that the glucose and insulin lowering effect of psyllium in diabetes patients who were treated by oral hypoglycemic drugs or diet alone did not differ significantly. In addition to the postprandial hypoglycemic effects of psyllium for the first meal after intake, psyllium also reduced serum glucose and insulin concentrations after the intake of second meal in several studies (Pastors, *et al.,* 1991; Anderson, Allgood, Turner, Oeltgen, & Daggy, 1999).

The mechanism of the hypoglycemic effect of psyllium was postulated to be similar with other soluble dietary fibers. One major function of psyllium may due to its viscosity which leads to a more difficult access of glucose to small intestine and slower absorption. Meanwhile, this viscous dietary fiber may also slow gastric emptying as well as carbohydrate uptake (Pastors, *et al.,* 1991, Hannan, Ali, Khaleque, Akhter, Flatt, & Abdel-Wahab, 2006). Dikeman and coworkers tested the effects of different types of dietary fibers on the viscosity of solutions and simulated human gastric and small intestinal digesta (Dikeman, Murphy, & Fahey Jr., 2006) offered support to this demonstration. Among all tested fibers, psyllium showed the highest viscosity of fiber solutions, as well as viscous characteristics during small intestinal simulation which implicated the potential of psyllium to attenuate blood glucose. The second-meal effect of psyllium may involve many mechanisms. Pastors and coworkers postulated that by smoothing the significant change in postprandial glucose concentration, especially for diabetes patients, psyllium can provide an improved insulin response to later meals (Pastors, *et al.,* 1991).

1.3.5 Cancer Prevention

Many studies have shown that psyllium may also possess anticarcenogenic properties, especially for colon cancer and breast cancer. Although the underlying mechanisms of psyllium's cancer prevention capacity are still not thoroughly clear, a large body of research has been done to reveal the possible effects.

Short chain fatty acids (SCFA), the fermentation products of dietary fibers, have been found to benefit colon health (Cummings, 1981). Among SCFA, *n*-butyrate was reported to have the function of slowing the proliferation of cancer cells (Kim, Tsao, Siddiqui, Whitehead, Arnstein, Bennet, *et al.,* 1980). In addition, studies have shown that most tumors occur in the distal colon in both human and experimental animal models. However, the main fermentation sites of dietary fiber are cecum and proximal colon (Cummings & Englyst, 1987). This fact implicates the potential importance of developing a method to shift the fermentation site of dietary fibers to the distal colon thus enhancing the production of *n*-butyrate. Morita and coworkers conducted an assessment to test whether psyllium can present the function of shifting the fermentation site of high-amylose cornstarch in rats (Morita, Kasaoka, Hase, & Kiriyama, 1999). They reported that the concentration of butyrate in the cecum was significantly enhanced by feeding rats with high-amylose cornstarch with or without psyllium. However, the concentration of butyrate as well as SCFA decreased along the colon in the rats fed a diet without psyllium. The psyllium-fed rats exhibited concentrations of butyrate and SCFA, which elicited that psyllium was able to shift the fermentation site of high-amylose cornstarch to distal colon thus providing the cancer prevention effect.

Psyllium's cancer prevention effect may also be due to its function on enzymes related to cancer promotion. Sphingomyelin (SM), a type of sphingolipid, can be hydrolyzed sequentially by two enzymes, sphingomyelinase (SMase) and ceramidase. The hydrolysis products are ceramide and sphingosine, which are reported to have the anti-proliferation function towards tumor cells (Hannun & Bell, 1989). The effect of psyllium in diet on the activities of sphingomyelinases and ceramidase has been investigated (Cheng, Ohlsson, & Duan, 2004). After feeding mice with psyllium containing diet or high-fat diet for 4 weeks, the activities of acid, neutral and alkaline SMase as well as the activity of ceramidase were determined. Previous studies showed that primary enzymes responsible for sphingolipids in the gut are alkaline SMase and neutral ceramidase, and mice fed with psyllium-containing diet exhibited enhanced activities of alkaline SMase and neutral ceramidase, which indicated that psyllium can promote the activities of these anti-proliferation related enzymes. In contrast, a high-fat diet led to suppressions of alkaline SMase and neutral ceramiase activities.

Many studies have also been conducted to reveal the possible cancer prevention effects of psyllium towards breast cancer. Estrogen has been found to be closely related to the development and growth of breast cancer, while free estrogens entered circulation are mainly the products of bacterial hydrolysis of estrogen conjugates in colon. In addition, β-D-glucuronidase is also of great importance since it has been found to be a key enzyme of diet-responsive estrogen-deconjugating (Cohen, Zhao, Zang, Wynn, Simi, & Rivenson, 1996). Whether psyllium, alone or mixed with wheat bran in different percentages, has effects on circulating estrogen

and β-D-glucuronidase activity have been tested in rats induced n-methylnitrosourea (MNU) (Cohen, *et al.,* 1996). After 19 weeks of feeding period, the tumor number in rats as well as β-D-glucuronidase activity decreased in MNU induced rats. The result indicated that psyllium, with properties similar with other insoluble dietary fibers, can suppress the activity of β-D-glucuronidase which led to a reduction of circulating estrogen, and thus reduce the tumor cell number.

In addition, scientists hypothesized that the reversible inhibition of gap junctional intercellular communication (GJIC) as well as apoptosis play an important role in tumor promotion, thus the prevention of the down regulation of GJIC caused by tumor promoting chemicals as well as the restoration of GJIC in tumor cells with GJIC-deficient could be possible strategy of cancer prevention and therapy (Trosko & Ruch, 1998). Since many chemical compounds, such as carotenoids, lovastatin, and caffeic acid, have been demonstrated to prevent the inhibition of GJIC and restoring GJIC, the functionality of psyllium in this aspect has also been tested (Nakamura, Yoshikawa, Hiroki, Sato, Ohtsuki, Chang, *et al.,* 2005). In this study, the ethanol soluble part of psyllium seed husks was extracted, purified, and then tested for its functions. The purified compounds showed the abilities of restoring GJIC in GJIC-deficient cells. Furthermore, the compounds were analyzed by gas chromatography, and showed to primarily contain β-sitosterol, which has been reported to have the function of preventing the uncontrolled cancer cell proliferation. It can be concluded that the antitumor ability of psyllium may partially due to its β-sitosterol component.

In conclusion, although the mechanisms of psyllium's effect on cancer prevention still remain unclear, scientists have offered many possible explanations.

This function may due to psyllium's ability to shift the fermentation site to distal colon, its effects on certain enzymes closely related to cancer promotion, and certain chemical components present in psyllium.

1.3.6 Laxation

For many years, psyllium has been used as an effective treatment towards irritable bowel syndrome due to its laxative effect. Many studies have been conducted to assess the effectiveness of psyllium, and the results were seen to be collaborative. In 1987, Prior and Whorwell conducted a double blind study involving 80 patients with irritable bowel syndrome (Prior & Whorwell, 1987). Besides the reported improved abdominal pain and bloating in both placebo and psyllium-administered group, the latter group also presented a significant improvement in constipation compared with the former one. In the same year, a study conducted by Kumar and associates showed similar effect of psyllium on ameliorating irritable bowel syndrome. In addition, they tested the optimum dosage of psyllium for this treatment using three different doses: 10g, 20g, and 30g daily. The test was divided into two parts. In part I, patients were administered 10g psyllium every day for 17 days, and then the dosage increased to 20g, for subsequent 17 days, finally, 30g psyllium were given to patients daily for the last 17 days. In part II, to avoid the possible "spill over" from one dose to the next one, three different dosages of psyllium were administered in a random order and a one week wash out period was included between two dosages. By clinical assessments of irritable bowel syndrome in patients, a dosage of 20g psyllium per day was postulated to be the optimum dosage (Kumar, Kumar, Vij, Sarin, & Anand, 1987). Furthermore, for the treatment of chronic constipation,

psyllium's effect was evaluated to be superior to other marketed stool softener laxatives, such as docusate sodium (McRorie, Daggy, Morel, Diersing, Miner, & Robinson, 1998).

The laxative effect of psyllium was considered to be mainly due to its water soluble and gel-forming capacity. Marlett *et al.* showed that the intake of psyllium may lead to increase viscosity and moisture of stool, as well as stool weight (Marlett, Kajs, & Fischer, 2000). This effect was ascribed to the unfermented part of psyllium which was caused by the inaccessibility of microbial enzymes due to the structure of psyllium-formed gel. Although many dietary fibers may decrease the gastrointestinal transit time thus alleviating irritable bowel syndrome (Bijkerk, Muris, Knottnerus, Hoes, & De Wit, 2004), whether psyllium may present this capacity remains controversial. In the studies conducted by Marlett *et al.* and Kumar *et al.,* no change in transit time was detected, while Prior and Whorwell reported a significantly decreased transit time in psyllium-treated group. However, by taking a close look at Prior and Whorwell's study, it can be seen that the decrease transit time most likely happened in patients possessing high initial transit time. In patients whose transit time was relative short, reduction in transit time was not significant compared with the placebo group.

1.3.7 Food Products Fortified with Psyllium

Due to psyllium's various health benefits, many efforts have been made by nutritionists and food manufacturers to develop methods for incorporating it into food products. According to the Dietary Guideline for Americans, 2005, the recommended consumption of dietary fiber is 14g/kcal, which means the current daily intake of

dietary fiber of Americans needs to be doubled. Park and cowokers developed a type of fortified bread incorporated with wheat fiber and psyllium husk fiber in a ratio of 7:3 (w/w), as well as three antioxidants, which were fat-coated ascorbic acid, protein-encased β-carotene, and cold-water-dispersible all-*rac*-α-tocopherol acetate (Park, Seib, & Chung, 1997). By calculation, one serving size of this kind of bread (28g) can provide 2.1g of dietary fiber, which approximately equals 8% of recommended daily value. Meanwhile, this type of product was found to have good retention time for β-carotene and α-tocopherol, but not ascorbic acid.

Psyllium has also been incorporated into ready-to-eat cereal bars. Ringe and Stoll (1991) developed a type of psyllium-fortified ready-to-eat cereal bar which contained about 2% to 37% psyllium with the minimum content of soluble fiber of 3g/oz. Compared to former ready-to-eat cereal products fortified with dietary fiber, this product contained a higher proportion of soluble fiber, which will provide more health benefits. Furthermore, they claimed that this type of product offered a more pleasing organoleptical effect.

Methods were established to add psyllium into aqueous food products. In 1984, Colliopoulos and colleagues filed a patent on developing a psyllium hydrophilic mucilloid with increased mixablility as well as dispersibility (Colliopoulos, Paul, & Young, 1984). This was achieved by coating psyllium particles with a film which can be made from various types of hydrolyzed carbohydrates.

1.4 Research Statement

The health condition of Americans may benefit from an increased consumption of psyllium as a source of dietary fiber. Incorporating psyllium into food products might be a good way via which a sufficient intake of psyllium will be accomplished. However, due to its strong water up-taking ability and gel-forming capacity, the addition of psyllium in food products, especially in aqueous products was is problematic. Many studies have been done to improve the physical/chemical properties of psyllium to achieve better applications in food industry. The following study was conducted to investigate the effects of different reaction temperatures (25 °C, 37.5 °C, and 50 °C) and psyllium – solvent ratios (1:2.5, 1:5, 1:7.5, and 1:10 g/mL) on swelling, water up-taking, gelling, as well as bile acid binding capacities of psyllium samples modified by using hydrochloric acid.

Chapter 2: Acid Modification of Psyllium, A Preliminary Study

2.1 Introduction

Dietary fiber is a ubiquitous plant-derived material which is resistant to human digestion in gastrointestinal tract. It mainly contains cellulose, non-cellulose polysaccharides including hemicelluloses and pectic substances. In the gastrointestinal tract, dietary fiber may function as a polymer matrix with properties such as susceptibility to bacterial fermentation, water-holding, cation-exchange, and absorptive functions (Kay, 1982).

Dietary fiber may have potential health benefits such as lowering serum cholesterol level, lowering risk of coronary heart disease (Kay, Sabry, & Csima, 1980; Morris, Marr, & Clayton, 1977), lowering risk of cholelithiasis (Pomare, Heaton, Low-Beer, & Espiner, 1976; Watts, Jablonski, & Toouli, 1978; Meyer, DenBesten, & Mason, 1979), reducing blood pressure, reducing risk of certain types of cancer (such as colon cancer) (Painter & Burkitt, 1975; Hill, 1974; Burkitt, 1975; Silverman & Andrew, 1977), better glycemic index control and body weight management (Jenkins, Leeds, & Gassull, 1976; Schwartz & Levine, 1980; Tasman-Jones, Jones, & Owen, 1978; Holt, Heading, Carter, Prescott & Tothill, 1979) and improving gastrointestinal function (Stephen & Cummings, 1980). These benefits can be ascribed to a variety of functions of dietary fiber, including increasing stool bulk, shortening transit time and enhancing fecal bile acid output. (Kay, 1982; Anderson,

Smith, & Gustafson, 1994). The recommended dietary fiber intake, according to the "Dietary Guidelines for Americans 2005", is 14 g per 1000 calories consumed (USDHHS & USDA, 2005).

Psyllium is known as a mucilaginous dietary fiber material. The primary source of psyllium is the seed husk from the *Plantago* genus including but not limited to *P. ovata, P. indica* and *P. psyllium*, which mainly grow in India and other sub-tropical regions. The main mono sugar compositions in psyllium are D-xylose, L-arabinose, L-rammonose and D-galacturonic acid (Chakrabortty & Patel, 1992).

Recent evidence shows that psyllium may have the health benefit of cholesterol lowering. Scientists have demonstrated that, in both *in vivo* animal studies and human subject studies, psyllium reduced blood cholesterol levels and the risk of coronary heart disease (CHD) (Anderson*, Zettwoch, Feldman, Tietyen-Clark, Oeltgen, & Bishop, 1988; Anderson, Allgood, Laurence, Altringer, Jerdack, Hengehold, & Morel, 2000; Anderson, Davidson, Blonde, Brown, Howard, Ginsberg, *et al.,* 2000; Everson, Daggy, McKinley, & Story, 1992; Ganji & Kies, 1996; Gupta, Agrawal, Singh, & Chatak, 1994; Olson, Anderson, Becker, Anderson, Hunninghake, Jenkins, *et al.,* 1997; Pastors, Dlaisdell, Balm, Asplin, & Pohl, 1991; Roe, Kalkwarf, & Stevens, 1988; Sola, Godas, Ribalta, Vallve, Girona, Anguera, *et al.,* 2007; Sprecher, Harris, Goldberg, Anderson, Bayuk, Russel, *et al.,* 1993; Turley*, Daggy, & Dietschy, 1994). The mechanism of this function has not been dearly established. One of the proposed underlying mechanisms is that psyllium has the ability of binding bile acid in the similar way of bile acids binding resins, which will inhibit the reabsorption of bile acids (Everson *et al.*, 1992; Turley *et al.,* 1994; Wolever, Jenkins, Mueller,

Boctor, Ransom, Patten, *et al.,* 1994). In addition, psyllium stimulates bile acids synthesis by promoting cholesterol 7α-hydroxylase activity, as well as suppressing acyl CoA cholesterol acyltransferase (ACAT) activities (Buhman, Furumoto, Donkin, & Story, 2000; Matheson, Colon, & Story, 1995; Fernandez, Ruiz, Conde, Sun, Erickson, & McNamara, 1995). Since bile acids are synthesized from cholesterol, reduced reabsorption and increased production of bile acids will lead to lower serum cholesterol levels.

Psyllium was also observed to have hypoglycemic effect. Research on animal and human subjects administered psyllium indicated the effectiveness of reducing hyperglycemia (Anderson, Allgood, Turner, Oeltgen, & Daggy, 1999; Hannan, Ali, Khaleque, Akhter, Flatt, & Abdel-Wahab, 2006; Song, Sawamura, Ikeda, Igawa, & Yamori, 2000). The possible mechanism has been investigated and it turns out that the capacity of suppressing gastrointestinal glucose absorption is the primary function that leads to this health benefit of psyllium.

Furthermore, psyllium has been associated with cancer prevention, especially for colon and breast cancers (Morita, Kasaoka, Hase, & Kiriyama, 1999; Nakamura, Trosko, Chang, & Upham, 2004; Nkamura, Yoshikawa, Hiroki, Sato, Ohtsuki, Chang, *et al.,* 2005). The underlying mechanism is still not clearly explicated. It was proposed that the fermentation of psyllium by intestinal microflora results in the formation of short chain fatty acids (SCFA) in the distal colon, where colon cancer usually occurs. SCFA was demonstrated to be associated with the inhibition of the cancer cell proliferation and promotion of mucosa cell differentiation (Morita *et al.,* 1999). In addition, the laxative effect of psyllium has been observed for a long time.

It has large swelling volume in water and can form a lubricating gel, which leads to a larger stool bulk and facilitated bowel movement.

Due to its various health benefits, many efforts have been made to promote psyllium utilization and safety in food industry. However, inherent in its physical/chemical properties are problems of adding psyllium in food products (specifically in water or aqueous media). Various approaches have been tested to improve the physical/chemical properties as well as reduce the allergenic effects of psyllium to widen its application in food. In general, efforts have been made in aspects of physical and mechanical approaches (Powell & Patel, 1982; Rudin, 1985; Wullschleger, 1993; Barbera, 1993, 1995; Barbera & Burns, 1993), chemical modification (Kumar & Verma, 2007; Mishra, Srinivasan, & Gupta, 2003), conventional enzymatic approaches (Nielsen, 1993; Yu, DeVay, Creek, Lai, Simmons, & Neisen, 2001), and solid-state enzymatic procedures (Yu, 2003a, b; Yu & Perret, 2003 a, b; Yu, Perret, Parker, & Allen, 2003). Physical and mechanical methods might include changing the particle size, coating, and changing pH (Powell & Patel, 1982; Rudin, 1985; Wullschleger, *et al.,* 1993; Barbera, 1993, 1995; Barbera & Burns, 1993). However, none of them had remarkable improvements on gelling and water up-taking properties of psyllium.

In terms of conventional enzymatic modification, in 2001, Yu *et al.* successfully improved functionalities of psyllium, including gelling property by carbohydrase treatment (Yu *et al.,* 2001). An earlier study conducted by Nielsen (1993) also showed that the allergic effects of psyllium can be reduced by enzymatic procedure, mainly hydrolytic reactions (Nielsen, 1993). The disadvantage of

conventional enzymatic modification is that it requires a further step of freeze-drying to remove the moister in psyllium induced by previous steps. It encumbered the wide commercial realization of this method.

In 2003, Yu and others developed a solid-state enzymatic procedure to improve the functionalities of psyllium as well as avoid previous problems of psyllium modification. This research was conducted with food-grade enzymes, which leads to no addition of chemicals and no generation of chemical waste. In addition, the further freeze-drying processing step is not required. The modified psyllium showed improved water absorbing capacity and gelling property, and retained the hypolipidemic effect (Yu, 2003a, b; Yu & Perret, 2003a, b; Yu *et al.,* 2003).

The enzymatic modification methods showed several applicable properties comparing to conventional methods, however, the application of enzymatic modifications in large industrial scale could be limited due to the high cost of enzymes. Research on the modification of psyllium with lower cost chemicals and reagents is still a possible approach. The present study was conducted to use hydrochloric acid (HCl) to modify psyllium samples and determine the effects of reaction temperature and psyllium-solvent ratio on water up-taking, swelling, gelling, and bile acid binding capacities of the acid-modified psyllium samples.

2.2 Materials and Methods

2.2.1 Materials

Psyllium husks used in this study were kindly provided by the Bio-Products Co (Joliet, IL). 34% - 37% hydrochloric acid (HCl) was purchased from Fisher

Scientific (Pittsburgh, PA). All other chemicals and solvents were of the highest commercial grade and used without further purification.

2.2.2 Sample Preparation

The solvent used for psyllium husks treatment was ethanol with hydrochloric acid (HCl) at the concentration of 0.72% (v/v). The study was conducted to investigate the effect of psyllium – solvent ratio and reaction temperature on physical/chemical properties of the acid modified psyllium samples. Three different reaction temperatures (25, 37.5, and 50 °C) as well as four different psyllium – solvent ratios (1:2.5, 1:5, 1:7.5, and 1:10 w/v, g/mL) were tested. Thus, twelve 20 g psyllium samples were divided into 3 groups for treatments with different temperatures. Four samples in each group were designated for different psyllium – solvent ratios. After the addition of the solvent, samples were incubated for 48 hours. Afterward, samples were vacuum filtered, rinsed with 95% ethanol and 100% for 2 times each, then dried and stored. Control group was treated with 100% ethanol, and follow the steps of preparation above.

2.2.3 Swelling Volume

The swelling volume assessment was conducted following the procedure from Zhou *et al.* (2006) and U.S. Pharmacopoeia (2006) with slight modifications. Briefly, 35 mL of simulated intestinal fluid without enzyme was transferred into a 100 mL graduated cylinder with 0.5 g psyllium sample. Afterward, the graduated cylinder was sealed and incubated at 37 °C for 8 h while gently shaking for 1 min every 30 min and then kept at ambient temperature for 16 h for the sediment to settle. The volume

of the sediment was recorded. The swelling volume capacity was calculated as volume of the sediment/dried weight of sample (mL/g).

2.2.4 Water Up-Taking Rate

According to the method described by Yu and Perret (2003), 1.0 g psyllium sample was equilibrated in 10% relative humidity (RH) desiccator for 48 h. After accurately weighing, all the samples were transferred into a 90% RH desiccator and exposed to moisture for 30 min. The moisture-absorbed samples were then accurately weighed and the weights were recorded. The amount of the absorbed water was presented as weight change of the dry matter after exposure to high RH environment. The data were reported as the average amount of water taken up by each gram of the psyllium samples per minute (mg/(g×min)).

2.2.5 Gelling Property

Gelling property assessment was performed using the method described by Yu and Perret (2003). Psyllium samples were prepared by mixing 1.5 g psyllium into 20 mL of water and then settled for 24 h. All samples were analyzed by a TA-XT PLUS texture analyzer (Texture Technologies Corp., Scarsdale, NY) for double compression test. All the experiments were conducted in duplicate, and the data was expressed as the mean ± SD in gram force for hardness and adhesiveness.

2.2.6 Bile Acid Binding Capacity

The bile acid binding capacities of acid modified samples were investigated as described by Zhou, *et al.* (2006) and Camire and Dougherty (2003) with a slight modification. Briefly, 100 mg psyllium sample was incubated with 1 mL of 0.01 N

HCl at 37 °C for 1 h with continuous shaking. Then, the pH of samples was adjusted to 7.0 with 0.1 N sodium hydroxide (NaOH). The suspension was mixed with 5 mL of 0.01 M phosphate buffer (pH 7.0) and 5 mL of 400 μM individual bile acid standards (cholic acid and chenodeoxycholic acid) prepared in 0.01 M phosphate buffer (pH 7.0). The mixture was then vortexed and incubated at 37 °C for 1 h. Afterward, the mixture was centrifuged at 2,000 rpm for 10 min at ambient temperature. The supernatant was carefully collected for bile acid quantification.

The quantification of each bile acid (cholic acid and chenodeoxycholic acid) in the supernatant was conducted using a commercial kit from Sigma-Aldrich (St. Louis, MO). The final assay mixture contained 200 μL of supernatant or bile acid standards, 0.25 mL of 2.5 mM nicotinamide adenine dinucleotide (NAD), 0.25 mL of 0.61 mM nitro blue tetrazolium salt (NBT), 0.2 mL of 625 units/L 3-α hydroxysterol dehydrogenase (3-HSD) and 0.2 mL of 625 units/L diphorase. The mixture was incubated at ambient temperature for 1 h. To stop the reaction, 200 μL of 1.33 M phosphoric acid was added into the mixture. The absorbance at 503 nm was spectrometrically determined. The cholestyramine was used as a positive control and the 0.01 M phosphate buffer (pH 7.0) without bile acids was used for a reagent control. The levels of the unbound bile acids were determined by a standard curve prepared with each bile acid standard. The bile acid binding capacities of psyllium samples were expressed as mg bile acid/g psyllium sample.

2.2.7 Statistical Analysis

Data were reported in the form of mean±SD. One-way analysis of variance and Tukeys honestly significant difference tests were conducted (Minitab Statistics

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Software, Version 15.1.1.0., 2007, Minitab Inc., State College, PA) to determine differences among means. Statistical significance was declared at P < 0.05.

2.3 Result and Discussion

There is growing research and consumer attention to functional foods which may help people to reduce the risk of certain diseases as well as to improve health conditions (Salon, 2000; Drecher, 1997). It is a widely held conviction that a high dietary fiber intake may lead to several favorable effects on health. Psyllium is well known as a good source of both soluble and insoluble dietary fiber. Over many years, scientists and manufactures have been trying to incorporate psyllium into functional food products. However, due to its extremely strong water up-taking ability and gelling capacity, the addition of psyllium into foods is still a big challenge, because a required quantity of soluble fiber of psyllium must be incorporated in order to have a health claim on the label according to the regulation of U.S. Food and Drug Administration (U.S. FDA). These facts severely limit the type of food which can be enriched with psyllium and arise a high demand of improvements on psyllium modification on physical/chemical properties as well as safety issues. This study was conducted with the purpose of investigating the effects of reaction temperature and psyllium-solvent ratio on water up-taking, swelling, gelling, and bile acid binding capacities of acid-modified psyllium samples.

2.3.1 Swelling Volume

The psyllium samples were incubated with 0.72% hydrochloric acid in different volumes of ethanol with the psyllium – solvent ratio of 1:2.5, 1:5, 1:7.5, and

1:10 (g/mL). The control group was treated with pure ethanol, with the psyllium – solvent ratio as 1:2.5 (g/mL). The swelling volumes of psyllium samples (Fig 2.1) with same psyllium – solvent ratio increased significantly when the reaction temperature increased from 25 °C to 37.5 °C, and then decreased significantly when the temperature was increased from 37.5 °C to 50 °C. Swelling volume of the 25 °C control sample was significantly lower than the 37.5 °C control, but was not statistically different from the 50 °C control. A similar reslut was found for the samples treated with the psyllium – solvent ratio of 1:7.5 (w/v, g/mL). In groups with psyllium – solvent ratios of 1:2.5, 1:5, and 1:10 (w/v, g/mL), comparing with samples treated at 25 °C, changes in swelling volume at reaction temperature of 50 °C were significant, but not as big as changes at 37.5 °C.

The effect of different psyllium – solvent ratios was also investigated at 25 °C, 37.5 °C, and 50 °C. At the reaction temperature of 25 °C, the swelling volumes of samples treated with psyllum – solvent ratios of 1:2.5, 1:5, 1:7.5, and 1:10 (w/v, g/mL) were significantly reduced comparing with the control group. When the reaction temperature was 37.5 °C, comparing with the control group, no significant difference was detected among different psyllium – solvent ratio groups. At the reaction temperature of 50 °C, significant difference was found between control and all tested groups. In conclusion, the swelling volumes of acid modified psyllium samples were affected stronger by different temperatures than by different psyllium – solvent ratios.
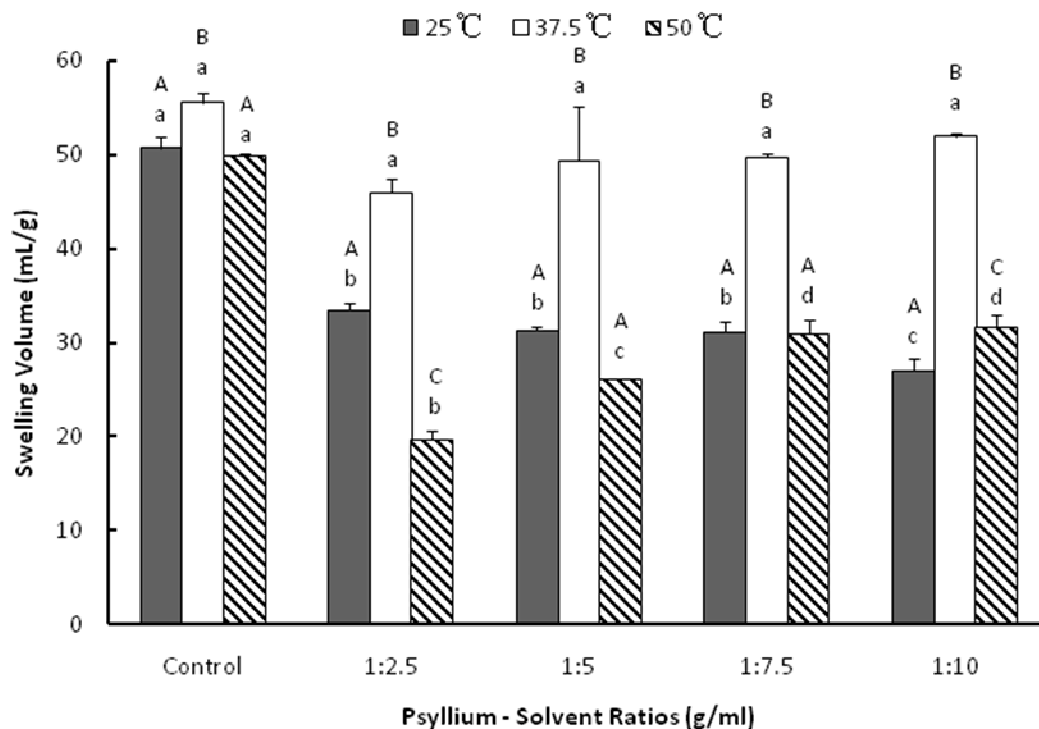
Figure 2.1 Swelling Volume of Acid Modified Psyllium Samples Columns represent different incubation temperatures (25, 37.5, and 50 °C) for psyllium samples, while the x axis represents different psyllium – solvent ratios (w/v, g/mL) as control, 1:2.5, 1:5, 1:7.5, and 1:10. Control contains no acid and with a psyllium – EtOH ratio of 1:2.5 (w/v, g/mL). Data are reported in form of mean ±SD (n = 2). Different capital letters represent statistical significance (P < 0.05) among same psyllium – solvent ratio groups, while different small letters represent statistical significance (P < 0.05) among samples with the same reaction temperature. In each different psyllium – solvent ratio groups, the swelling volume increased by increasing temperature from 25 °C to 37.5 °C, and then decreased from 37.5 °C to 50 °C.

2.3.2 Water Up-Taking Rate

The effect of reaction temperatures on water up-taking rate can be seen from Figure 2.2. In the control group, no significant decrease of water up-taking rate was seen by increasing reaction temperature from 25 °C to 50 °C. In the group with the psyllium – solvent ratio of 1:2.5 (g/mL), no significant difference was found between the samples treated at 25 °C and 37.5 °C, while a significant reduction in water up-taking rate was observed when samples were treated at 50 °C. When the psyllium – solvent ratio was 1:5 (w/v, g/mL), comparing with samples treated at 25 °C, water up-taking rate of sample treated at 37.5 °C decreased but not significantly, while samples treated at 50 °C showed a significant reduction. In the group with the psyllium – solvent ratio of 1:7.5 (w/v, g/mL), similar reductions of water up-taking rate were observed, samples treated at 37.5 °C and 50 °C, although showed no significant difference between each other, all presented significant differences with samples treated at 25 °C. A very similar result was seen when psyllium – solvent ratio was 1:10 (w/v, g/mL). No significant difference between samples treated at 37.5 °C and 50 °C, while they were all significantly reduced in water up-taking rate comparing to samples treated at 25 °C.

The effects of different psyllium – solvent ratios on the water up-taking rate of psyllium samples were also investigated at three different reaction temperatures: 25 °C, 37.5 °C, and 50 °C. When the reaction temperature was 25 °C, no significant reduction was observed between the control group and psyllium – solvent ration 1:2.5 (w/v, g/mL) group, while comparing with the control group, samples treated with the psyllium –solvent ratio 1:5 (w/v, g/mL) and lower ratios showed significant

reductions in water up-taking rate. At 37.5 °C, samples with psyllium – solvent ration

of 1:2.5 and 1:5 (w/v, g/mL) did not show any significant decrease comparing with

the control group, while water up-taking rates reduced significantly in groups with

psyllium – solvent ratio of 1:7.5 and 1:10 (w/v, g/mL). When the reaction temperature

increased to 50 °C, water up-taking rates of psyllium samples in different psyllium –

solvent ratios did not reduce significantly comparing with the control group. Taken

together, in a selected psyllium – solvent ratio, increasing reaction temperature from

25 °C to 50 °C reduced the water up-taking rate of acid modified psyllium samples.

However, at a selected reaction temperature, effect of different psyllium – solvent

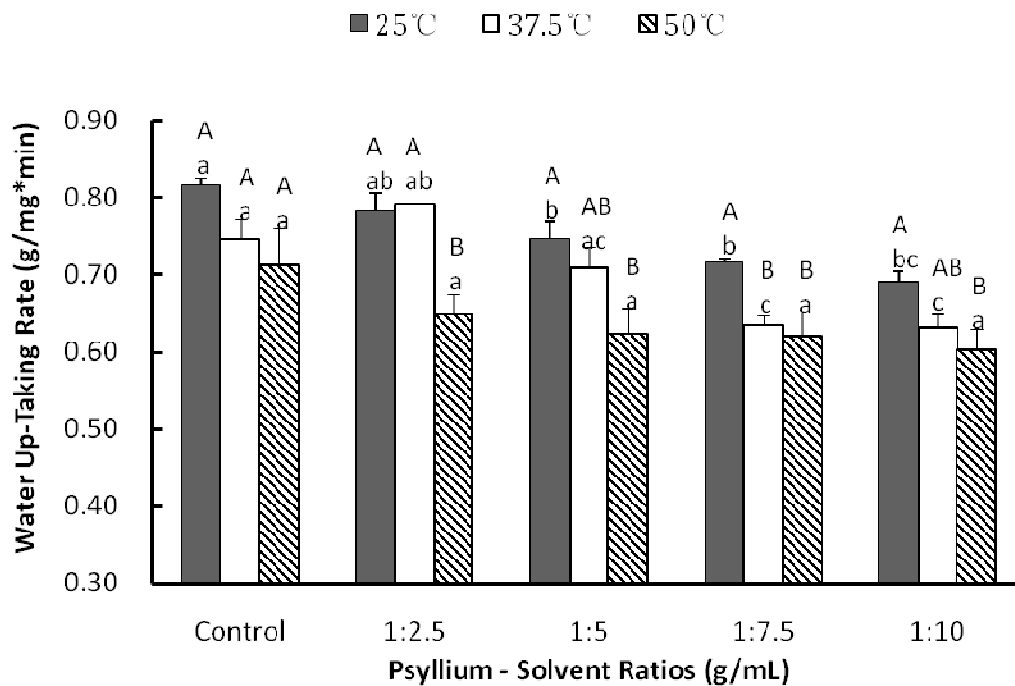ratio on reducing water up-taking capacity of psyllium was not significant.

Figure 2.2 Water Up-Taking Capacities of Acid Modified Psyllium Samples
Columns represent different incubation temperatures (25, 37.5, and 50 °C) for

psyllium samples, while the x axis represents different psyllium – solvent ratios (w/v, g/mL) as control, 1:2.5, 1:5, 1:7.5, and 1:10. Data are reported in form of mean $\pm$SD (n = 2). Different capital letters represent statistical significance (P < 0.05) among same psyllium – solvent ratio groups, while different small letters represent statistical significance (P < 0.05) among samples with the same reaction temperature. Water up-taking rate decreased by increasing temperature in each different psyllium – solvent ratio groups. Meanwhile, at each specific temperature, slightly decreased water up-taking rate was seen by lowering the psyllium – solvent ratio from 1:2.5 to 1:10 (w/v, g/mL).

2.3.3 Gelling Property

The gel hardness is shown in Figure 2.3. In the control group, the hardness for the gel decreased by increasing the reaction temperature from 25 °C to 50 °C. In the control group, comparing with samples treated at 25 °C, samples treated at both 37.5 °C and 50 °C showed significant reduction in gel hardness. When the psyllium – solvent ratio were 1:2.5 and 1:7.5 (w/v, g/mL), by increasing the reaction temperature from 25 °C to 37.5 °C, gel hardness decreased slightly but still significantly. Comparing with this, when the reaction temperature shifted from 37.5 °C to 50 °C, large and significant reductions were observed in gel hardness. When the psyllium – solvent ratio was 1:7.5 (w/v, g/mL), the gel hardness increased by increasing the reaction temperature from 25 °C to 50 °C, and similar to other psyllium – solvent ratios, a large and significant reduction was found at the reaction temperature 50 °C. No significant difference was found in gel hardness in samples with the psyllium – solvent ratio of 1:10 (w/v, g/mL) when reaction temperature increased from 25 °C to

37.5 °C, while a large and significant reduction was also found when the temperature increased to 50 °C.

The effects of different psyllium – solvent ratios on gel hardness of psyllium samples were also investigated at reaction temperature of 25 °C, 37.5 °C and 50 °C. When the reaction temperature was 25 °C, comparing with the control group, no significant reduction in gel hardness was found in samples with the psyllium – solvent ratio of 1:2.5 (w/v, g/mL), while gel hardness was decreased significantly in samples with the psyllium – solvent ratios of 1:5, 1:7.5, and 1:10 (w/v, g/mL). At the reaction temperature of 37.5 °C, comparing with the control group, no significant differences were found in samples treated with psyllium – solvent ratio of 1:2.5 and 1:5 (w/v, g/mL). Significant changes were found in samples with psyllium – solvent ratios of 1:7.5 and 1:10 (w/v, g/mL). When the reaction temperature was 50 °C, comparing with the control group, significant reductions in gel hardness were found in tested samples.

The adhesiveness of the psyllium-formed gels was tested (Figure 2.4). In the control group, no significant changes were found in samples treated at different temperatures of 25 °C, 37.5 °C and 50 °C. When the psyllium – solvent ratios were 1:2.5 and 1:5 (w/v, g/mL), no significant changes were observed when the reaction temperature increased from 25 °C to 50 °C while large and significant reduction were found at 50 °C. In samples treated with psyllium – solvent ratios of 1:7.5 and 1:10 (w/v, g/mL), by increasing the reaction temperature from 25 °C to 50 °C, the gel adhesiveness decreased significantly, and similar with other groups, the reductions were large at reaction temperature of 50 °C.

At the reaction temperature of 25 °C, comparing to the control group, no significant effect of different psyllium – solvent ratios on gel adhesiveness were found in samples with psyllium – solvent ratios as 1:2.5, 1:5, and 1:7.5 (w/v, g/mL), while a significant change was found in samples treated with the psyllium – solvent ratio of 1:10 (w/v, g/mL). When the reaction temperature was 37.5 °C, comparing with the control group, no significant differences were found in samples treated with psyllium – solvent ratio of 1:2.5 and 1:5 (g/mL). Significant changes were found in samples with psyllium – solvent ratios of 1:7.5 and 1:10 (w/v, g/mL). At the reaction temperature of 50 °C, significant changes were found in samples treated with psyllium – solvent ratios of 1:2.5, 1:5, 1:7.5, and 1:10 (w/v, g/mL), comparing with the control group. In conclusion, the effects of different reaction temperatures were significant, especially for a high temperature as 50 °C, while the effects of different psyllium – solvent ratios were not significant.

Previous studies showed that by enzymatic modification, the hardness and adhesiveness of psyllium formed gels can be reduced as high as 23% for both parameters in convention enzymatic treatment and 48% and 55% in solid-state enzymatic procedure, respectively (Yu, 2001; Yu & Perret, J., 2003a, 2003b). In this study, the gel hardness and adhesiveness reduction of samples under reaction temperatures 25 °C and 37.5 °C appeared to be similar to psyllium under solid-state enzymatic modification. This observation may be due to HCl, under the study conditions, having comparable ability with enzymes such as Viscozyme L of breaking polysaccharide molecule networks. The sharp decrease in both hardness and adhesiveness of samples in 50 °C group may be due to the stronger reaction between

HCl and psyllium under 50 °C which may alter the molecular structure of psyllium and inhibit the formation of junction zones.
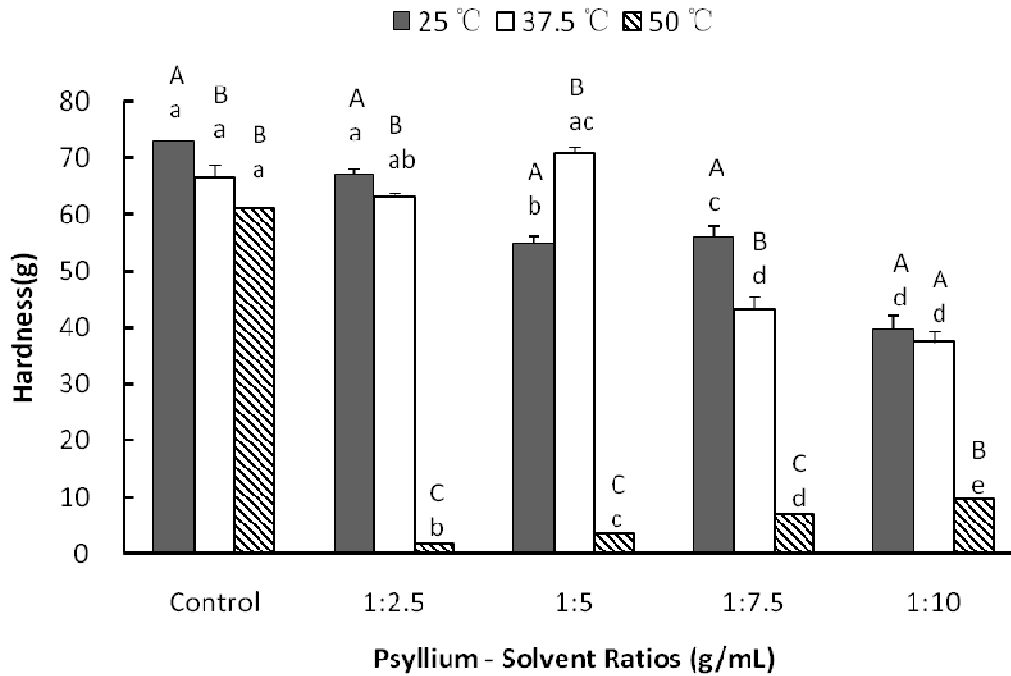

Figure 2.3 Gelling Properties of Acid Modified Psyllium Samples – Gel Hardness Columns represent different incubation temperatures (25, 37.5, and 50 °C) for psyllium samples, while the x axis represents different psyllium – solvent ratios (w/v, g/mL) as control, 1:2.5, 1:5, 1:7.5, and 1:10. Data are reported in form of mean $\pm$SD (n = 2). Different capital letters represent statistical significance (P < 0.05) among same psyllium – solvent ratio groups, while different small letters represent statistical significance (P < 0.05) among samples with the same reaction temperature. Gel hardness decreased by increasing temperature in each different psyllium – solvent ratio groups except in sample with the psyllium – solvent ratio of 1:5 (w/v, g/mL). Large and significant reductions were found at reaction temperature of 50 °C. Meanwhile, at each specific temperature, no appreciable trend of changes in gel

hardness was observed by lowering the psyllium – solvent ratio from 1:2.5 to 1:10 (w/v, g/mL).
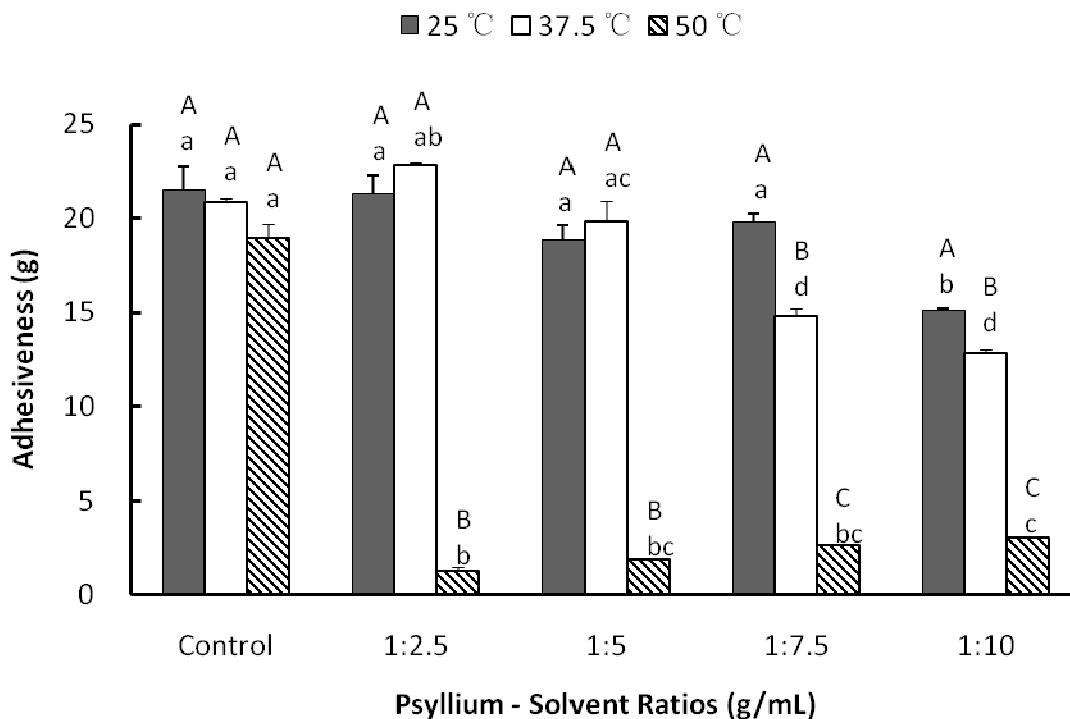

Figure 2.4 Gelling Properties of Acid Modified Psyllium Samples – Gel Adhesiveness Columns represent different incubation temperatures (25, 37.5, and 50 °C) for psyllium samples, while the x axis represents different psyllium – solvent ratios (w/v, g/mL) as control, 1:2.5, 1:5, 1:7.5, and 1:10. Data are reported in form of mean $\pm$ SD (n = 2). Different capital letters represent statistical significance (P < 0.05) among same psyllium – solvent ratio groups, while different small letters represent statistical significance (P < 0.05) among samples with the same reaction temperature. Gel adhesiveness decreased significantly when the reaction temperature increased to 50 °C. At each specific temperature, no appreciable trend of changes in gel hardness

was observed by lowering the psyllium – solvent ratio from 1:2.5 to 1:10 (w/v, g/mL).

2.3.4 Bile Acid Binding Capacity

Effects of different reaction temperatures (25 °C, 37.5 °C, and 50 °C) and different psyllium – solvent ratios (1:2.5, 1:5, 1:7.5, and 1:10 w/v, g/mL) on bile acid binding capacities towards chenodeoxycholic acid of psyllium samples were studied (Figure 2.5). In the control group, no significant change was found among samples treated with different reaction temperatures. In the groups with psyllium – solvent ratio of 1:2.5, 1:5, and 1:10 (w/v, g/mL), no significant differences were found between samples treated at 25 °C and 37.5 °C, while significant decreases were found in samples treated at 50 °C. In samples with psyllium – solvent ratio of 1:7.5 (w/v, g/mL), no significant change was observed. In terms of effects of different psyllium – solvent ratios, at 25 °C, no significant difference was found among samples in control group as well as samples treated with different psyllium – solvent ratios. When the reaction temperature was 37.5 °C, comparing with the control group, no significant change was found in samples with psyllium – solvent ratio of 1:2.5 (w/v, g/mL), however, significant increases were found in samples with psyllium – solvent ratios of 1:5, 1:7.5, and 1:10 (w/v, g/mL), while no significant difference was observed among them. At 50 °C, comparing with samples in the control group, significant changes were found in psyllium samples treated with different psyllium – solvent ratios. Among these samples, bile acid binding capacities did not change significantly between samples treated with psyllium – solvent ratios 1:2.5 and 1:5 (w/v, g/mL), as well as samples treated with psyllium – solvent ratios 1:7.5 and 1:10 (w/v, g/mL).

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However, enhanced bile acid binding capacities were found when psyllium – solvent ratio decreased from 1:5 to 1:7.5 (w/v, g/mL). Taking together, at a high reaction temperature as 50 °C, bile acid binding capacities of acid modified samples decreased significantly, while no appreciable trend of effects of different psyllium – solvent ratios was found.
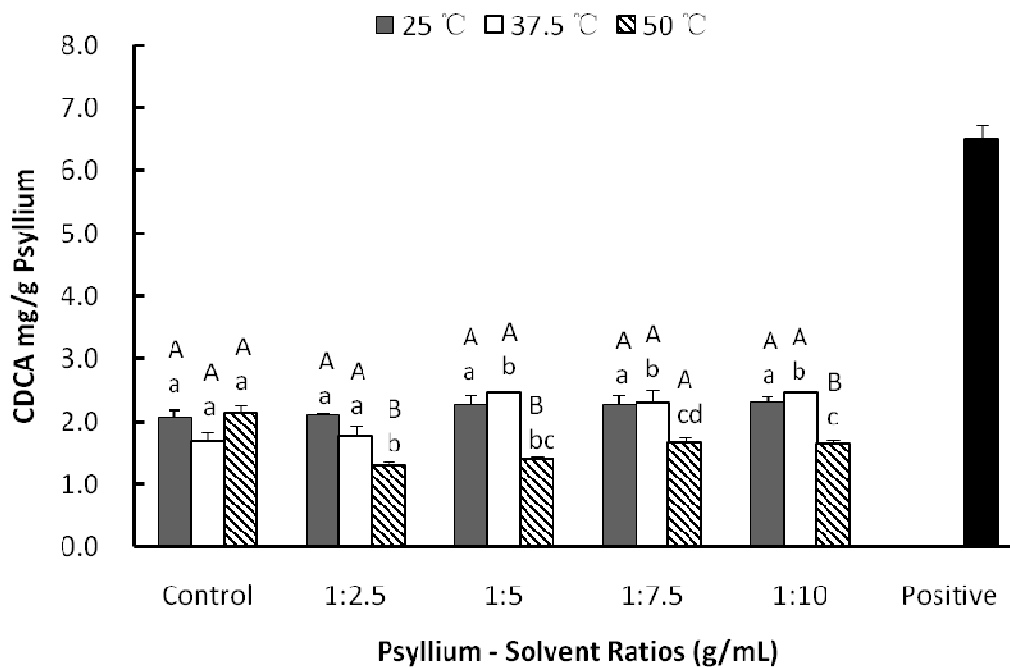

Figure 2.5 Bile Acid Binding Capacity for Chenodeoxycholic Acid (CDCA) of Acid Modified Psyllium Samples Columns represent different incubation temperatures (25, 37.5, and 50 °C) for psyllium samples, while the x axis represents different psyllium – solvent ratios (w/v, g/mL) as control, 1:2.5, 1:5, 1:7.5, and 1:10. Data are reported in form of mean $\pm$SD (n = 2). Different capital letters represent statistical significance (P < 0.05) among same psyllium – solvent ratio groups, while different small letters represent statistical significance (P < 0.05) among samples with the same reaction temperature. Bile acid binding capacities decreased significantly in

different psyllium – solvent ratio groups when reaction temperature was 50 °C, while no appreciable trend of effects of different psyllium – solvent ratios was found.

The effects of different reaction temperatures and psyllium – solvent ratios were also tested for the bile acid binding capacity toward cholic acid (Figure 2.6). In terms of effects on different reaction temperatures (25 °C, 37.5 °C, and 50 °C), in the control group, the bile acid binding capacities were enhanced significantly by increasing reaction temperature from 25 °C to 50 °C. However, in groups with psyllium – solvent ratios as 1:2.5, 1:5, 1:7.5, and 1:10 (w/v, g/mL), the bile acid binding capacities decreased significantly when reaction temperature changed from 25 °C to 50 °C. The effects of different psyllium – solvent ratios were studied. When the reaction temperature were 25 °C and 50 °C, significant difference was observed between the control group and acid modified psyllium sample groups, however, no significant change in bile acid binding capacities was found among psyllium samples treated with different psyllium – solvent ratios. At 37.5 °C, no significant difference was found among all psyllium samples. Meanwhile, the bile acid binding capacities of acid modified psyllium samples is stronger for chenodeoxycholic acid than for cholic acid. In conclusion, enhanced reaction temperature led to a decrease in bile acid binding capacities towards cholic acid of acid modified psyllium samples. While no significant effects of different psyllium – solvent ratios were observed.

The results showed that the acidic treatment of psyllium may lead to a loss of bile acid binding capacity toward chenodeoxycholic acid. However, in contrast, the ability of binding cholic acid might be improved. This fact indicates that the bile acid binding capacity assessment need to be done on more kinds of bile acid to gain a

comprehensive view of this method. Meanwhile, it can be deducted that a stronger binding ability towards one selected bile acid does not provide a certainty of stronger binding affinity to other bile acids. Furthermore, *in vivo* study might be necessary to investigate the effectiveness of modified psyllium sample on hypolipidemic functionality, which partially due to the bile acid binding capacity of psyllium.
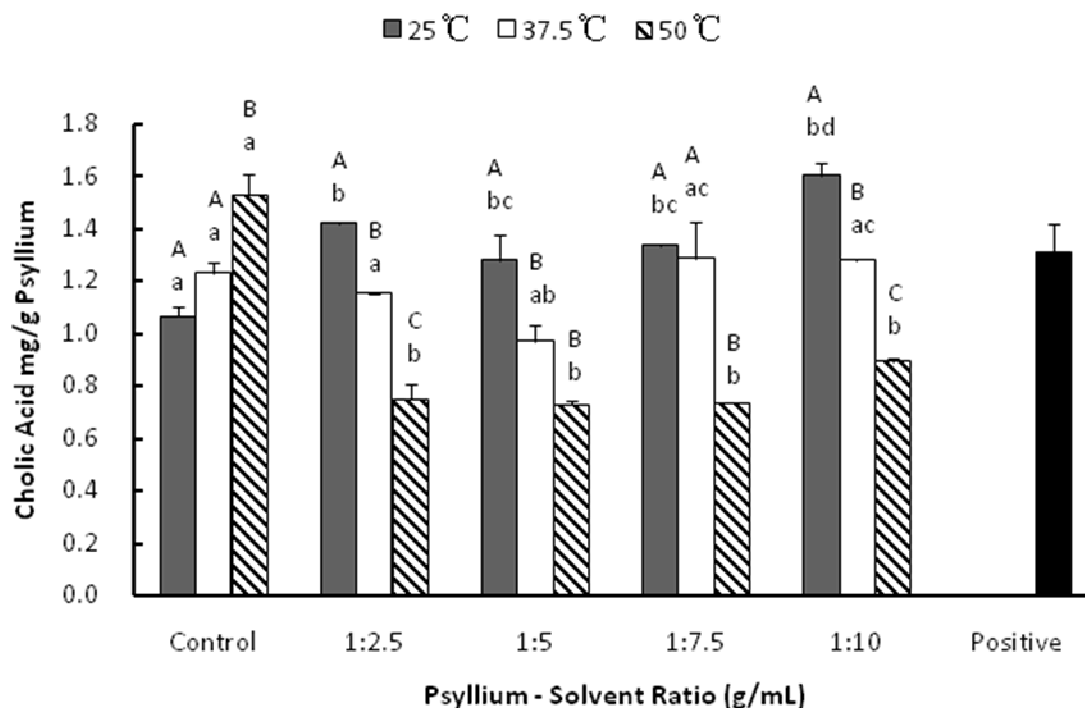

Figure 2.6 Bile Acid Binding Capacity for Cholic Acid Columns represent different incubation temperatures (25, 37.5, and 50 °C) for psyllium samples, while x axis represents different psyllium – solvent ratios (w/v, g/mL) as control, 1:2.5, 1:5, 1:7.5, and 1:10. Data are reported in form of mean ±SD (n = 2). Different capital letters represent statistical significance (P < 0.05) among same psyllium – solvent ratio groups, while different small letters represent statistical significance (P < 0.05) among samples with the same reaction temperature. Bile acid binding capacities decreased significantly in different psyllium – solvent ratio groups by increasing the

reaction temperature for 25 °C to 50 °C, while no significant effects of different psyllium – solvent ratios were found.

2.4 Conclusion

In conclusion, this study reported the swelling volume, water up-taking rate, bile acid binding capacity and gelling property of acidic modified psyllium samples under different reaction temperatures and psyllium – solvent ratios. Results showed although not as effective as enzymatic treatment, acidic modification still can improve the water absorbing property and gelling capacity, as well as bile acid binding capacity towards certain bile acids. Considering its lower cost than enzymatic methods, acidic modification presents a competitive potential of being applied in food industry. Further research is necessary to investigate more aspects of altered physical/chemical characteristics and reveal the underlying mechanisms.

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