

Good research proposal on effect of dietary fibers source and particle size on ch...

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Introduction

Composition of Dietary Fiber

Dietary fiber is an essential component in nutrition and health (Mora et al., 2013; Klosterbuer, Roughead, & Slavin, 2011). Thus, the present study will explore the effect of particle size and source of fiber on the level of cholesterol in the human digestion system. Primarily, dietary fiber is a constituent of plant foods and includes materials that have diverse morphological and chemical structure. Such materials resist enzymatic action in the human digestion system (Dhingra et al. 2012). In the gastrointestinal tracts, the dietary fiber forms a matrix that has both amorphous and fibrous characteristics. The matrix' s physicochemical properties determine the therapeutic and homeostatic functions of fiber in

the human nutrition. Within the aqueous medium, which occur in the intestinal lumen, the fiber swells and takes up water, as well as small molecules. The pressure resulting from fiber swelling determines the diffusion rate and the responses of the intestinal smooth muscles; hence, it influences the bulk flow. Several linkages are formed between surrounding molecules and the fiber. The interactions, which include hydrogen bonds, ionic bonds, as well as the weaker dispersion and hydrophobic forces, may affect both steroid and mineral absorption. Moreover, there is a continuous modification in the matrix function and structure contingent on alterations in the surrounding osmolality, pH, and fiber matrix. The matrix experiences changes as the colonic bacterial enzymes degrade it selectively. Therefore, the effects of fiber in the lower and upper intestine may differ substantially. Principally, dietary fiber comprises of cellulose; a non-carbohydrate part called lignin and noncellulosic polysaccharides such as pectin substances and hemicelluloses. The structural components are found mainly in the cell wall of plants (Srivastava, Mudgal, & Jain, 2012). The cell wall development commences with the appearance of the middle lamella. Subsequently, the primary wall forms on the middle lamella's inner surface and comprises of cellulose fibrils embedded in the ground material of hemicellulose and pectin substances (Raven, Evert, & Eichhorn, 2012). The formation of the wall results from the layering on the middle lamella's interior. Later, a secondary cell wall, consisting of several layers of little pectin and abundant cellulose fibrils, is formed (Raven et al., 2012). The layers in the secondary cell wall are parallel and occur within the matrix of hemicellulose. As the maturation progresses, the lignin infiltration moves from the cell wall's exterior to its

interior part (Raven et al., 2012). Hence, the infiltration imparts extra rigidity and hydrophobicity to the plant structure. Lignification lowers the digestibility of various forms of fiber in the cell wall by the bacterial enzymes (Raven et al., 2012). Therefore, the maturation of plant cells is associated with a gradual change in fiber composition that favors increasing proportions of lignin and cellulose. However, some forms of plant fiber are not components of the cell wall but are formed in some specialized secretory plant cells. Such types include mucilage and plant gums.

The composition of dietary fiber depends on the anatomical source, species, and age of the plant material. The principal classes of dietary fiber are lignin and polysaccharides (Raven et al., 2012). Polysaccharides include cellulose, as well as diffuse category of substances called noncellulosic polysaccharides. Cellulose is the most plentiful of the naturally occurring molecules and is starch's beta isomer. In addition, it is a long linear polymer of 1, 4 beta-linked glucose units (Raven et al., 2012). The hydrogen bonding that occurs between the sugar residues in neighboring chains gives a crystalline microfibril structure. The noncellulosic polysaccharides (NCPs) include heteroglycans that comprise of a mixture of hexoses, uronic acids, and pentoses. However, the most significant of the NCPs include the pectin substances and hemicelluloses (Raven et al., 2012). Hemicelluloses refer to the cell wall polysaccharides that are solubilized by aqueous alkali following the removal of the pectin and water-soluble polysaccharides. Their backbones consist of beta-1, 4-linked pyranoside sugars (Raven et al., 2012). Nevertheless, hemicelluloses are different from cellulose in that they have a relatively smaller size and contain a variety of branched sugars. The

hemicelluloses are classified based on the primary monomeric sugar residue.

The neutral or acidic forms differ in the content of galacturonic and glucuronic acids. The formation of uronic acid involves oxidizing $-CH_2OH$ to $-COOH$, and has biological significance because the sugar residues are available for the formation of cation complexes, as well as the amidation and methylation processes. Hemicelluloses, particularly the uronic acid and the hexose components, are more accessible to bacterial enzymes than cellulose.

Pectin substances are complex polysaccharides with the D-galacturonic acid as their principal constituent (Raven et al., 2012). Moreover, they are structural components in cell walls of plants and function as intercellular cementing substances. Pectin's backbone structure is an unbranched chain consisting of units of the axial-axial- α -(1 \rightarrow 4)-linked D-galacturonic acid (Raven et al., 2012). Blocks of L-rhamnose-rich units interrupt the long chains of galacturonan, hence, causing bends in the molecule. Neutral sugars are covalently linked as side chains to most pectins. The sugars are primarily galactose and arabinose. Sometimes, however, small quantities of glucuronic acid are joined to pectin in the side chain (Raven et al., 2012). The galacturonic acids' carboxyl groups are partially methylated while the secondary hydroxyls are, sometimes, acetylated. Pectin has high water-solubility and is almost metabolized completely by colonic bacteria (Raven et al., 2012).

In contrast, lignin is a non-polysaccharide (Srivastava et al., 2012). It is also complex random polymer with almost forty oxygenated phenylpropane units

(Raven et al., 2012). The units include sinapyl, coniferyl, and p-coumaryl alcohols. Lignins vary in methoxyl content and molecular weight (Raven et al., 2012). In addition, lignin is highly inert due to the strong intramolecular bonding, which includes the carbon-carbon linkages. Lignin is chemically measured as Klason lignin, which is a cell wall residue that is insoluble in 72 percent sulfuric acid. Lignin shows greater resistance to the digestion enzymes than other polymers, which occur naturally (Raven et al., 2012). In addition to lignin and polysaccharides, the human diet has several plant-derived materials that resist digestion in the upper intestine. The substances include suberin and cutin, which are fatty acids' polymeric esters. Cutin is a water-impermeable material deposited onto the surface of plants, whereas suberin is secreted during the later developmental stages of the cell wall (Raven et al., 2012). Suberin and cutin are acid- and enzyme-resistant substances that are recoverable in the fraction of lignin. Other dietary components associated with the lignin residue include the products that result from the Maillard reaction (Tessier & Birlouez-Aragon, 2012). The components occur due to the enzyme resistant linkages between the carbonyl groups of reducing sugars and the amino groups of proteins during heat treatment. Some fiber-rich diets have significant amounts of plant sterols (Winkler-Moser, 2011). Sterols are crucial in human health and include hormones, cholesterol, and vitamin D. The most significant sterol in the human diet is cholesterol (Shazamawati, Alina, Mashitoh, & Juhana, 2013). However, cholesterol occurs only in animal products such as egg yolks. Thus, plants lack cholesterol but may contain phytosterols (Shazamawati et al., 2013).

Importance of Dietary Fiber

It is widely known that an increased intake of dietary fiber promotes wellness and health (Calorie Control Council, 2014). The health gains of consuming dietary fiber are so numerous that researchers are still discovering them (Calorie Control Council, 2014). Fibers reduce constipation, enhance the colon's health (Havenaar, 2011; Kaczmarczyk, Miller, & Freund, 2012), and control diabetes (American Diabetes Association, 2013; Jiang et al., 2012; Post et al., 2012). In addition, they lower the level of cholesterol and blood pressure. Consequently, they reduce the risk of illnesses such as cardiovascular diseases and ameliorate the control of blood sugar after meals. Moreover, dietary fiber helps in weight loss (Ebbeling et al., 2012; Kraschnewski et al., 2010; Sumithran et al., 2011) and reduces diarrhea (Calorie Control Council, 2014).

Specific forms of viscous fibers, such as the beta-glucans, guar gum, and psyllium, thicken the food content in the intestinal tract (Calorie Control Council, 2014). In addition, they decrease the absorption of glucose and cholesterol (Calorie Control Council, 2014). Various studies have also linked a high fiber diet with melioration in serum lipids, low-density lipoprotein cholesterol, total cholesterol, triglycerides, and high-density lipoprotein cholesterol (Calorie Control Council, 2014). Moreover, epidemiological studies have observed a decrease in the rate of heart diseases among individuals who consume adequate amounts of fiber (Anderson, et al., 2009; Grooms et al., 2013; Threapleton, et al., 2013). Yikyung, Hollenbeck, & Schatzkin (2011) reported that the consumption of a high-fiber diet lowered the risk of early death, which results from causes such as heart disease,

cancer, and infectious diseases. In another study, Sherry et al. (2010) reported that the consumption of fiber improves the body's immune system. Roberfroid et al. (2010) observed an increased mineral absorption due to the consumption of dietary fiber.

Purpose of the Project

The study aims at determining the in vitro effect of subsamples, comprising of fibers of different particle size, on the cholesterol level. In addition, the research will explore the effect of various forms (sources) of fiber on the cholesterol level.

The study will test the hypothesis that if different forms of dietary fiber are passed through in vitro human digestion system, then the particle size and source of the dietary fiber will influence the cholesterol level.

The research is significant and justifiable because it will determine the significance of dietary fiber in regulating the cholesterol level. The in vitro study will also help to explain the role of fiber in human health. Since cholesterol levels in human blood are linked to cardiovascular disease, the study will explain the correlation between dietary fiber and cardiovascular illness.

Literature Review

Role of Fiber in Cholesterol Reduction

Functional fibers are similar to dietary fiber in their effects but are extracted either artificially or naturally (Coleman, 2009). For example, pectin, which is used as a gelling agent in jellies and jams, is isolated from citrus peel.

In late 2002, the Food and Nutrition Board showed that the Adequate Intake

(AI) is about fourteen grams of fiber per 1,000 kcal. Therefore, adult men aged fifty years old and below should consume at least thirty-eight grams of fiber every day while women should eat 25 gm/day. However, due to a reduced food intake, older men should eat 30 gm of fiber while older women should consume twenty-one grams of fiber per day. The AI levels were derived from studies demonstrating that dietary fiber protects against heart disease (Coleman, 2009). In 2001, guidelines by the National Cholesterol Education Program recognized the beneficial effect of fiber in decreasing LDL cholesterol (Coleman, 2009). As a result, the guidelines recommend including viscous fiber, of between ten to twenty-five grams per day in the Lifestyle Changes diet (Coleman, 2009). Dietary fiber protects against cardiovascular illness (Eshak, et al., 2010; Kokubo, et al., 2011) by ameliorating blood lipid profiles, reducing inflammation indicators, and lowering blood pressure (Anderson et al., 2009).

Several dietary fiber sources reduce LDL cholesterol levels (Coleman, 2009; Stadler & Thye, 2009). The sources include foods such as barley, apples, legumes, oatmeal, fruits and vegetables, rice hulls and oat bran (Coleman, 2009). Purified sources include guar gum, beet fiber, karaya gum, locust bean gum, knojac mannan, psyllium seed husk, pectin, xanthan gum and soy polysaccharide (Coleman, 2009).

Adequate research conducted on the beta-glucans in oats, psyllium husk, and barley has led the Food and Drug Administration (FDA) to permit a certain health claim (Bureau of Nutritional Sciences). One such claim is that foods consisting of between 0.75 grams to 1.7 grams of soluble fiber can lower the risk of heart disease (Coleman, 2009).

In order to carry the claim, oat products should comprise of at least 0.75 gm of oat fiber (beta-glucan) for every serving (Coleman, 2009). The 0.75 gm amount represents about one-fourth of the three-gram amount needed to attain a mean of six mg/dL reduction in total cholesterol (Coleman, 2009). Practically, an intake of three grams of beta-glucan is equivalent to 1 cup of cooked oat bran (Coleman, 2009).

Nevertheless, the magnitude of the reduction is related to an individual's initial cholesterol level. Persons with higher cholesterol levels show an increased response to beta-glucan than individuals with lower cholesterol levels. Therefore, oat fibers are a valuable tool for lowering cholesterol. Most people tolerate the fiber well because it produces little bloating, gastrointestinal distention, and gas.

Study related to the Present Research

Brouns et al. (2012) conducted research on the cholesterol-lowering properties of various forms of pectin in mildly hypercholesterolemic individuals. They observed that viscous fibers reduce the total cholesterol (TC) in humans by 3–7% (Brouns et al., 2012). The pectin's cholesterol-lowering properties are influenced by physicochemical properties such as molecular weight (MW), viscosity, and the degree of esterification. In the study, various types and sources of well-characterized pectin in humans were examined (Brouns et al., 2012). Crossover studies were conducted in mildly hypercholesterolemic individuals who received either 15 g/day of cellulose or pectin with food for four weeks. The relative low-density lipoprotein cholesterol lowering was as follows: citrus pectin's degree of esterification (DE) -70 = apple pectin's DE-70 > apple pectin's DE-35 (Brouns

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et al., 2012). Moreover, apple pectin's DE-35 = citrus pectin's DE-35 > orange pulp fiber's DE-70 (Brouns et al., 2012). An accompanying 3-week trial involving citrus' DE-70, 6 g/day pectin, and a high MW pectin of DE-70 lowered the low-density lipoprotein cholesterol against the control (Brouns et al., 2012). In both studies, high MW and DE were essential for the lowering of cholesterol level. The pectin source was also significant because the citrus and apple's DE-70 pectin had more effectiveness than the orange pulp fiber's DE-70 pectin (Brouns et al., 2012). However, pectin had no effect on the inflammatory markers of the plasma homocysteine and high-sensitivity C-reactive protein (Brouns et al., 2012). The researchers concluded that the type and source of pectin (MW and DE) affect the lowering of cholesterol (Brouns et al., 2012).

Materials and Methods

The sample materials will be oat, beet, carrot, chokeberry, cranberry, cacao, apple, flax dietary fiber. For each of the samples, two subsamples of fine and coarse substances will be obtained. The subsamples will be bound to cholesterol and passed through in vitro human digestion system. Next, the subsamples will be analyzed, and the level of cholesterol determined using Gas Chromatography (GC). The GC method involves the direct saponification of samples, extraction using hexane, and injection of specimens into a capillary GC column (Madzlan, 2008).

The technique described by Madzlan (2008) will be followed when analyzing the cholesterol level. 5 α -cholestane (> 99% purity) and cholesterol reference standard will be used to determine the level of cholesterol. Methanolic-potassium hydroxide solution (0.5 M) will be prepared by dissolving 14 g of <https://assignbuster.com/good-research-proposal-on-effect-of-dietary-fibers-source-and-particle-size-on-cholesterol-level-measured/>

potassium hydroxide and stirring in methanol. Next, the solution will be diluted to 500 ml with methanol. Sample saponification and separation will be performed as described by Madzlan (2008). In particular, the direct saponification technique will be used to hydrolyze the specimens because it is time- and cost- effective (Dinh et al. 2011). Sources of cholesterol for the experiment will include egg yolk (Naviglio et al., 2011).

The fine and coarse dietary fiber will be bound in a food matrix containing cholesterol. Two grams of the food matrix will be weighed in 15 ml sample preparation tube containing 0.02 grams of 5 α -cholestane, which will function as an internal standard. Next, about 5 ml of methanolic KOH will be added. The tube will be capped tightly and then vortexed for 20 s.

Subsequently, the lower half of the specimen tube will be immersed in a water bath at 80 °C for about 15 min. Next, the tube will be removed and agitated on a vortex mixer for five seconds at five-minute intervals. The tube will be cooled, and the cap removed. Five ml of hexane and one ml of water will be added into the tube and then vortexed vigorously for one minute. The tube will be centrifuged at 7,000 rpm for fifteen minutes. Then, one μ l of the upper phase will be injected into the GC for analysis.

The analysis of cholesterol will be performed using a Hewlett-Packard (HP) 5890 GC, which will be equipped with a FID detector and an on-column capillary injector (Madzlan, 2008). A capillary column of cross-linked methyl siloxane will be used. In addition, a splitless inlet will be used to inject specimens into the capillary column. A ramped oven temperature will be utilized in the analysis. The initial temperature will be 250 °C and held for two minutes (Madzlan, 2008). Next, the temperature will be increased to 300

°C at a rate of ten degrees Celsius/minute, and then held for 2.5 minutes. The inlet temperature will be kept at 280 °C while the detector temperature will be 300 °C (Madzlan, 2008). Helium will be used as the carrier gas and will constantly flow at a rate of 1.0 ml/min. The Chemstation software will be used to integrate the area of each peak, and the amount of cholesterol will be calculated using the 5 α -cholestane internal standard (Madzlan, 2008).

The calculation of cholesterol will be performed as follows:

Calculation of response factor, Rf:

Calculation of cholesterol level:

Results and Discussion

Cholesterol is a crucial eukaryotic structural component that occurs in lipoprotein and cell membranes. It functions as a precursor for bile acids and steroid hormones (Shazamawati et al., 2013). In humans, researchers have found a correlation between the blood levels of cholesterol and the risk of heart disease in humans, as well as the premature atherosclerosis' development (Stadler & Thyne, 2009). Consequently, various national and international groups have urged the reduction of cholesterol total intake in the developed countries. The goal of reducing the incidences of coronary heart malady has sparked the emergence of such campaigns.

There are some conflicting results and views in relation to the analytical techniques for cholesterol. The colorimetric determination of cholesterol concentration in food products has been questioned due to overestimation that may result from interfering compounds (Shazamawati et al., 2013).

Therefore, chromatographic methods such as gas chromatography (GC) and the High Performance Liquid Chromatography are favored because they

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quantify and separate the amounts of cholesterol specifically. However, the GC method that will be used in the study is preferred to HPLC because of its relatively high sensitivity (Shazamawati et al., 2013).

For each subsample, a chromatogram of 5 α -cholestane and cholesterol will be obtained. Figure 1 illustrates a chromatogram.

Figure 1. Cholesterol and 5 α -cholestane (Source: Madzlan, 2008). The figure illustrates a chromatogram of Cholesterol and 5 α -cholestane

The chromatograms will be used to determine the effect of particle size and fiber source on the cholesterol level. The cholesterol level will be read from each of the chromatograms. In each of the samples, the cholesterol levels of the fine and the coarse subsamples will be compared using statistical tests. In the comparison, a one-way ANOVA will be used to compare the means of the cholesterol levels of the subsamples.

Differences in the observed cholesterol levels will support the research hypothesis. However, the theory will be rejected if no difference in cholesterol level will be observed.

Conclusion

The present study is significant because it will explain the influence of dietary fiber on cholesterol level. In addition, it will determine whether the source and particle size of dietary fiber have any effect on the level of cholesterol. Various past studies have examined the role of fiber in human health. Minimal errors are expected in the current research, which will ensure that the study supports previous literature. Thus, the subsamples of the various specimens are expected to yield different cholesterol levels on

the chromatograms. Such observation will lead to the illation that the in vitro cholesterol level is influenced by the source and particle size of dietary fiber.

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