THESIS

DIETARY FIBER CONTENT OF DRY PEA, CHICKPEA AND LENTIL DETERMINED USING THE CONSENSUS ANALYTICAL METHOD, AOAC 2011.25

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ABSTRACT

DIETARY FIBER CONTENT OF DRY PEA, CHICKPEA, AND LENTIL DETERMINED USING THE CONSENSUS ANALYTICAL METHOD, AOAC 2011.25

The health benefits of dietary fiber and its constituents have been well documented. However, the consumption of dietary fiber is lower than recommended levels worldwide, and the gap between actual consumption and recommended intake represents a widely unrecognized health risk. One approach to address the dietary fiber gap is to return to an ancient tradition that was abandoned in the last 50 years, i.e. the consumption of pulse crops as a dietary staple. To better advocate for an increase in pulse consumption, the determination of the fiber content of these crops using the consensus definition of dietary fiber published in 2009 and the method that conforms to that definition (AOAC 2011.25) is needed. The overall goal of this thesis was to analyze the dietary fiber content of three major pulse crops, dry pea (*Pisum sativum L.*), lentil (*Lens culinaris L.)* and chickpea (*Cicer arietinum L.*) using the consensus definition and method of analysis.

OBJECTIVE: The objectives of this project were: 1) to measure dietary fiber content of dry pea, lentil and chickpea using the AOAC 2011.25 method, and 2) to explore relationships among fiber content, pulse genotype (cultivar) and the environment in which these crops were grown.

REASERCH DESIGN AND METHOD: The pulse crops analyzed included 11 cultivars of dry pea, 13 cultivars of lentil and 24 cultivars of chickpea, each grown in two locations. Each cultivar was analyzed for insoluble dietary fiber, soluble dietary fiber, oligosaccharides and total dietary fiber content using the AOAC 2011.25 method of analysis.

RESULTS: Average total dietary fiber content was 24.6% for dry pea, 20.1% for lentil, and 21.8% for chickpea. The range in total dietary fiber was from 20.1 to 30.6% among dry pea cultivars, from 17.6 to

21.6% among lentil cultivars and from 15.8 to 25.8% among chickpea cultivars. Dietary fiber content in pulse crops varied significantly by cultivar while location of production had a limited effect.

CONCLUSION: The pulse crops assessed varied widely in dietary fiber content among cultivars within species indicating that breeding and selection can be used to improve pulse crops for this health beneficial trait, without concern that those improvements would be influenced by factors such as location of production. Overall, the pulse crops investigated had high dietary fiber content relative to other food crops, e.g. wheat *(T. aestivum L.)*, corn *(Zea mays L.*), or rice *(Oryza sativa L.)*, that are widely promoted in efforts to close the dietary fiber gap. Consumption of 2 to 3 servings per day of any of these pulse crops would eliminate the dietary fiber gap. The data presented also indicated that consumers can be guided to choose pulse crop cultivars that are enriched in fiber content as a way to further improve dietary fiber status without increasing caloric intake. Re-establishing pulse crops as a staple in Western diets illustrates the potential value of considering the pursuit of ancient solutions to solve 21st century challenges at the interface of agriculture and human health.

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CHAPTER 1

INTRODUCTION

1.1 Overview

An important contribution of edible components of plants to the human diet is the carbohydrate polymers that cannot be hydrolyzed via the endogenous enzymes of the human small intestine. These polymers are referred to as dietary fiber (1). Dietary fiber has many characteristics, some of which have been reported to affect physiological function. In addition, increasing levels of fiber in the diet are associated with a reduction in risk for a number of human disease conditions. A resurgence of interest in dietary fiber and human health has occurred because inadequate dietary fiber intake is widespread (2). The establishment of a consensus definition and the development and validation of an integrated method of dietary fiber analysis that complies with that definition (3) affords the opportunity to evaluate in a uniform manner the potential contribution of various food crops to human dietary fiber intake. The work reported in this thesis examines the dietary fiber content of three pulse crops, dry pea, lentil, and chickpea using an internationally accepted method for dietary fiber analysis.

1.2 The definition of dietary fiber and its constituents

Dietary fiber is an important non-nutritive component of food and has been reported to have various benefits to human health. In 1976, a definition of dietary fiber was published by Trowell (4). According to his definition, dietary fiber consists of chemical components which are resistant to digestion by the alimentary enzymes of humans. As research on dietary fiber progressed, it was realized that Trowell's definition of dietary fiber should be modified to include resistant starch (5;6). However, with the deeper understanding of carbohydrate chemistry and bioavailability, it was determined that oligosaccharides should also be included as a component of dietary fiber. The CODEX Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) published an internationally agreed upon consensus definition of dietary fiber in 2009. The current definition states that dietary fiber is comprised of carbohydrate polymers with ten or more monomeric units, which are not hydrolyzed by the endogenous enzymes in the small intestine of humans. Dietary fiber was classified into three categories: 1. consumed as naturally occurring in the food, 2. obtained from raw food by physical, enzymatic, or chemical means, and 3. synthetically produced (7). In the following sections, only naturally occurring dietary fiber will be discussed.

Naturally occurring dietary fiber has three main components: insoluble dietary fiber (IDF), soluble dietary fiber (SDF), and oligosaccharides. IDF is mainly comprised of cellulose and resistant starch. The constituents of SDF include β-glucan, arabinoxylan, pectin, inulin, arabinogalactan, polydextrose and galactomannan. Among them, arabinoxylan, pectin and inulin will be the focus of discussion since they are prominent components of pulse crop fiber. Oligosaccharides are carbohydrates containing 3 to 20 sugar units. Of the many oligosaccharides that exist, discussion will be limited to galactans since they are the major type of oligosaccharides in pulses. IDF is insoluble in both water and ethanol. SDF doesn't dissolve in ethanol but can dissolve in water, while oligosaccharides can dissolve in both solvents. The differential solubility of these three components in water and ethanol is used to isolate and quantify each component by the AOAC 2011.25 method.

1.2.1 Insoluble dietary fiber

Cellulose is one of the basic components of all plant materials and is composed of β-D-glucopyranose units linked by (1→4) glycosidic bonds (8). Cellulose is an important structural component of the primary cell wall of plants (9). It is a tough, water-insoluble material that is resistant to degradation in the human intestine due to the lack of cellulases, i.e., enzymes that cleave the β 1-4 glycosidic linkage. Cellulose accounts for a large proportion of dietary fiber.

Resistant starches are defined as the portion of starch which is not digested in the upper gastrointestinal tract (10). Resistant starch usually occurs as granules in plant tissues. Resistant starch can be classified as types 1, 2, 3 and 4. Type 1 is physically inaccessible to digestion by entrapment in a non-digestible matrix. Type 2 is ungelatinized resistant granules with type B crystallinity that are slowly hydrolyzed by α-amylase. Type 3 is retrograde starch formed when starch containing foods are cooked and cooled. Type 4 refers to chemically modified resistant starches and industrially processed food ingredients (11).

1.2.2 Soluble dietary fiber

β-glucan is a linear polysaccharide of glucose monomers with β-1,4 and β-1,3 linkages and was first identified in fungal cell walls by Van Wisselingh (12). β-glucan is water soluble and highly viscous at low concentration (13). β-glucans occur in the cell walls of cereal grains (14) and in pulses (10).

Arabinoxylan (AX) is a non-digestible carbohydrate and contains a large number of 1,4-linked xylose units (13). AX is a major hemicellulose component in primary cell wall and a minor component in secondary cell wall (14). Part of AX can be digested in colon by AX-degrading enzymes (15).

Pectin is a linear polymer of galacturonic acid connected with α-1,4 bonds (16). It is found in the cell walls of plants where it functions as a hydrating agent and cementing material for the cellulosic network (17). It is a very abundant class of macromolecule in the cell matrix. Pectin is a water-soluble polysaccharide that bypasses enzymatic digestion of the small intestine and can be degraded by the microflora of the colon (13).

Inulin is a storage polysaccharide in numerous plants and is comprised of fructose monomers linked via a β-(2,1) bond (18). Inulin is not digested in the human upper gastrointestinal tract because the β-(2,1) chemical linkage between fructose monomers is not digestible by human enzymes, e.g. either ptyalin or amylase (19)

1.2.3 Oligosaccharides

Three main kinds of oligosaccharides which were measured in our experiments are raffinose, stachyose and verbascose. These oligosaccharides consist of galactosyl residues linked with α-(1,6) glycosidic linkage. The difference among these three oligosaccharides is the number of galactose monomers they contain. Among them, raffinose has one galactose, stachyose has two and verbascose has three galactose

(20). Oligosaccharides are distinguished from soluble dietary fiber because of their solubility in water and ethanol. Oligosaccharides are naturally produced or derived from polysaccharides (21). These oligosaccharides are resistant to gastric acidity and are not hydrolyzed by human digestive enzymes.

1.2.4 Summary

Insoluble dietary fiber, soluble dietary fiber and oligosaccharides are three main components in dietary fiber of pulse crops and they are quantified in our experiments. Different constituents within dietary fiber have been introduced in this section. Studies have been done on the relationships among these chemical components of dietary fiber and their health benefits as discussed in the next section.

1.3 The health benefits of dietary fiber

Denis Burkitt (22;23) realized the importance of dietary fiber to human health in 1984. He hypothesized that dietary fiber has protective effects against the development of Western diseases, including diabetes, hypercholesterolemia, heart disease, diverticular disease and colon cancer. Based on his work, a large number of investigations have been carried out to explore the health benefits of dietary fiber.

1.3.1 Prebiotic effects and gut health

The microbial community in the small intestine and colon affect the function of the human digestive system. It has been reported that some components in dietary fiber can function as prebiotics, i.e., they provide growth promoting substrates to the gut microbial community (24). It has been reported that the fermentation potential of each fiber type is microbial species and strain dependent and that dietary patterns with different types and amounts of fibers will differentially modulate the composition of the intestinal microbiome (25). For example, galacto-oligosaccharides and inulin, which are two components of dietary fiber, have been reported to increase the abundance of bifidobacteria, a type of bacteria that is associated with gut health in reducing the metabolism of hydrogen-producing fermentative bacteria (26). Dietary fiber also induces microbial communities to produce increased amounts of short chain fatty acids such as butyrate which have been reported to promote cellular health (27).

1.3.2 Weight maintenance

Increasing dietary fiber intake has been shown to be associated with a reduction in body weight as reported in a recently published systematic review and meta-analysis of 21 randomized control studies (28,29). To illustrate the nature of the evidence reported, a significant weight loss (6.0 kg ± 4.2 kg) was observed in fifty-four postmenopausal women given a low-fat and high fiber diet for 8 months (30). A follow-up study from Liu et al based on 121,700 registered nurses for 12 years reported that the group with high dietary fiber intake gained an average of 1.52 kg less weight than individuals with low intake (31). In a survey on U.S. women, increased dietary fiber intake was also reported to be associated with lower body mass index (BMI) (32). Nonetheless, as noted in (29), additional studies are required to establish the actual magnitude of protection against weight gain. Mechanisms proposed to explain the weight maintaining effect of dietary fiber include: 1) dietary fiber can lead to bulking, gel formation, viscosity, a change in gastric content and a delay in gastric emptying, resulting in a suppression of appetite (33;34); 2) dietary fiber can stretch the walls of the gastrointestinal tract, stimulating the satiety reflex and slowing the absorption of nutrients like fatty acids and carbohydrates (34,35); 3) soluble dietary fiber can be fermented in the large intestine, producing glucagon-like peptide and peptide tyrosine tyrosine (PYY) which in turn can induce a sense of satiety (36,37); and 4) high fiber food has low caloric density that results in reduced total caloric intake over time (38).

1.3.3 Diabetes prevention

Diabetes mellitus is a common disease worldwide. The International Diabetes Foundation predicts that by 2030, 10% of the world's population will be diabetic (39). Many studies have reported that the increasing dietary fiber intake from whole grains can reduce the risk of diabetes. In a prospective study of 162,000 U.S. women without a history of diabetes, the relative risk of developing type 2 diabetes was found to be 0.62 (95% confidence interval 0.57-0.69, $p < 0.001$) when comparing the highest quintile of whole grain intake with the lowest (40). Similarly, Ye et al. reported a 26% lower risk of type 2 diabetes among individuals in the American Association of Retired Person (AARP) cohort who consumed 48-80 g/d whole grains (41).

Greater than 90% of diabetic patients have the type 2 form of the disease which is mainly attributed to insulin insensitivity. There are several reasons that dietary fiber may reduce the risk of diabetes. Dietary fiber can reduce extremely high blood glucose levels due to its ability to decrease the intestinal transport of glucose, thus slowing the appearance of glucose in blood (42). It is also suggested that soluble fiber causes the slow absorption and digestion of carbohydrates, which leads to slower absorption of glucose and reduced demand for insulin (43). B-glucan and arabinoxylan are two important components of dietary fiber that increase food viscosity. The formation of a viscous solution in the stomach can delay gastric emptying and significantly lower the postprandial glucose response (44). Thus, via glucose regulation and improved insulin sensitivity, dietary fiber can reduce the risk of diabetes.

1.3.4 Cardiovascular Diseases (CVD)

Over the past five decades, CVD has risen to become the single largest cause of death worldwide, accounting for 30% of total deaths according to WHO (45). Studies in the 1970s indicated that the dietary fiber consumption is inversely associated with the risk of CVD and this was confirmed in 2004 by the observation that a significant inverse relation exists between dietary fiber intake and the risk of CVD among both men and women (46). It was estimated that for every 10 g/day increase in dietary fiber intake, risk of all coronary events was reduced by 12% (47). There are many factors that can increase the risk for CVD, such as overweight, elevated blood pressure, high fasting total cholesterol and elevated blood glucose. A number of studies have indicated that the benefits of dietary fiber in reducing CVD are through affecting these processes (48-52).

1.3.5 Cancer prevention

Cancer is a disease that accounts for nearly 15% of human deaths globally (53). One possible benefit of dietary fiber is that it can reduce cancer risk. There are reports that dietary fiber prevents a number of cancer types including colon cancer (54), esophageal cancer (55), and breast cancer (56). Aune et al. (57)

reported an inverse correlation between fiber intake and the risk of colorectal cancer. According to their analysis, a 16% (95% Confident Interval 0.77-0.92 Relative Risk = 0.84) increase in cancer risk was observed among people with low intake of dietary fiber. Among different types of dietary fiber, fiber from whole grains was associated with a 20% reduction of risk (58) while vegetable and fruit fiber did not lead to an apparent reduction (59) possibly because the intake of these foods is relatively low. Murphy et al. (60) also suggested that dietary change can lead to a reduction of cancer risk. They found that with a 10 g/day increase in cereal fiber intake, that colorectal cancer risk was reduced by 11%. It was found that the esophageal cancer risk has a strong inverse correlation with the dietary fiber intake (55).

Breast cancer is the most common cancer in women and the second leading cause of death from cancer in United States. Dietary fiber has been associated with a significant inverse correlation with the risk of breast cancer. For an increase of 10 g/d of dietary fiber intake, there is a 7% decrease in breast cancer risk (95% Confident Interval 0.88-0.98, Relative Risk = 0.93) (56). One possible mechanism to explain this relationship is that dietary fiber decreases the circulating estrogen concentration by suppressing bacterial B-glucuronide activity in the gut. This process may inhibit the reabsorption of estrogens in the colon, increasing the excretion of estrogens in feces, and thereby reducing the risk of breast cancer by lowering circulating concentrations of this hormone.

1.4 The dietary fiber intake gap

While dietary fiber is not considered an essential nutrient in the human diet, the U.S. Department of Agriculture and Department of Health and Human Services (2010) recommend consumption of 14 g/1000 Kcal, which is about 25 g of fiber per day for women and 38 g for men. Nonetheless, the importance of dietary fiber has not been fully recognized by consumers and there is a dietary fiber intake gap all over the world. As reported by King et al. (2012) (61), the average daily intake of dietary fiber for U.S people during 1999-2000 is only 15.6 g/day, much lower than the recommended level. Inadequate dietary fiber intake occurs not only in United State, but also in the other parts of the world. According to the Chinese Dietary Fiber Intake White Book (2016 Fourth China dietary fiber industry forum by China Association of Pharmaceutical Biotechnology), the average dietary fiber intake for both city and rural populations in

China is about 13 g/day, far less than the daily requirement standard from WHO. According to investigations in Europe, the average daily dietary fiber intake is less than 20 g/day (62). Meanwhile, for South America, average daily dietary fiber consumption is only about 6g/1000 kcal (63). Among these four areas, China and South America have lower dietary fiber intake than in North America and Europe. One possible reason for such a difference is that in developing countries, the importance of the dietary fiber to people's health has not been widely realized. For East Asia countries, inadequate intake of dietary fiber may also be due to the dietary structure. The main source of carbohydrate in many East Asian countries is rice, which contains only about 3% w/w dietary fiber.

1.5 Pulse crops, potential candidates to the close the dietary intake gap

Pulse crops such as dry pea, lentil and chickpea as well as common bean (*Phaseolus vulgaris L.*) are widely consumed worldwide and have been the very important foods in most population centers for thousands years ago. Pulses have high nutritional quality and high content of fiber. However, while the Green Revolution (late 1960s) greatly increased agricultural production worldwide, saving over a billion people from starvation, it also changed diet structure with increased consumption of high-yielding crops like wheat, rice and corn with a concomitant decrease of pulse crop consumption (64), leading, in part, to the dietary fiber intake gap. Increasing pulse crop consumption consciously in our diet structure is, we believe, a practical way to fill the dietary fiber intake gap.

1.6 The objectives of the study

As discussed in the above sections, dietary fiber has been demonstrated to be important for human health; however, intake is inadequate for maximal benefit to be attained. It is reasonable to assume that this gap can be closed by increasing pulse consumption. In view of the fact that the fiber content of dry pea, chickpea and lentil, has not been determined using AOAC 2011.25, information that is essential for the promotion of increased consumption of these pulse crops, those analyses were performed in the experiments reported in this thesis. The objectives of this project were: 1) accurately measure dietary fiber content in dry pea, lentil and chickpea, and 2) to explore relationships between the fiber content, pulse genotype (variety) and the environment in which these crops were grown. Due to the importance of these pulse crops and especially due to their potential value in closing the dietary fiber gap worldwide, we believe the work reported herein has potential for significant clinical impact.

CHAPTER 2

MATERIALS AND METHODS

2.1 Experimental design

Chickpea, dry pea, and lentil seed was selected by plant breeders familiar with commercial cultivars of each pulse crop grown in the United States. Chickpea cultivars were selected by Dr. George Vandemark (USDA-ARS) and dry pea and lentil were selected by Rebecca McGee (USDA-ARS). Chickpea seed was harvested from Pullman and Dayton, WA. Lentil seed was harvested from Fairfield and Pullman, WA. Dry pea seed was harvested from Genesee and Dayton, WA. Within each location, two samples (replicates) of the same cultivar were obtained from different field plots. The seeds were obtained from 24 varieties of chickpea, 13 varieties of lentil and 11 varieties of dry pea. The annual average daily temperature of Pullman is 8.7°C, and the average precipitation is about 51.8 cm. The annual average daily temperature of Dayton is 10.7°C and the average precipitation is about 49 cm. The annual average daily temperature of Fairfield is 13.4°C and the average precipitation is about 33.8 cm. For Genesee, the annual average daily temperature is 12.2°C and the average precipitation is about 31.2 cm. The plants were grown from seed. After harvest, dry seed samples were collected for fiber analysis.

2.2 Sample preparation

Each of the seed samples were cooked and prepared for the AOAC 2011.25 Integrated Total Dietary Fiber Analysis. Dry seeds were ground into a fine powder using a mechanical grounder (KRUPS Tipo203). Two replicates of ground samples were weighed to approximately 1 g. The range of sample weighing was between 0.9950 to 1.0050 g. The powdered sample was then transferred into a 50 ml plastic Falcon tube and 8 ml nanopure water (18 mega ohm) was then added. The sample was vortexed (Baxter S/P vortex mixer) until the powder was well dispersed in water. Samples were then cooked at 115°C under a pressure of 76 kPa for 65 minutes (Market Forge Autoclave). After cooking, the pasty like sample was cooled down to room temperature and homogenized using a Polytron® PT10/35 with an S type probe, for approximately 15 seconds on speed 6. The probe was then washed with 6 ml nanopure water twice in a VWR Culture tube 17 x 100 mm to remove all seed particles. The liquids from the two washings were transferred to the original 50 mL conical tube containing the homogenized seed sample. Final sample volume was approximately 20 mL. The conical tubes with the homogenized seed sample were stored at -80˚C until analysis.

2.3 AOAC 2011.25 Integrated Total Dietary Fiber Assay

The total integrated dietary fiber method (AOAC 2011.25) was conducted using a commercial assay kit (K-INTDF) purchased from Megazyme International, Wicklow, Ireland). The method is based on the definition of dietary fiber, which was proposed in 2009 by CODEX Committee on Nutrition and Foods for Special Dietary Uses. The method was accepted and put into use in 2009 by Codex Alimentarius Commission. In the present study, the assay was carried out with modifications as published in detail by our laboratory (65). Briefly, the analysis of insoluble and soluble dietary fiber was according to AOAC 2011.25 without modification. Since oligosaccharides in pulses are limited to galactans, HPLC separation with electrochemical detection was employed to improve the sensitivity and specificity of the assay for raffinose, stachyose and verbascose.

2.4 Buffer Preparation

For buffer preparation, 23.2 g maleic acid was added into a 2 L flask filled with a suitable amount of nanopure water. The mixture was stirred with a stirring bar until maleic acid was fully dissolved. 4 M sodium hydroxide was used to adjust pH to 6.0. 1.2 g calcium chloride and 0.8 g sodium azide were added into the flask. This buffer was stored at -20 ℃ freezer until it was used.

2.5 Sample Digestion

Seed samples were taken out from -80 ℃ freezer and thawed to room temperature. Amyloglucidase and pancreatic α amylase were added to the buffer solution and stirred until well proportioned. The enzyme amount was calculated according to the volume of buffer. The concentration of amyloglucidase was 3.3 Units/mL and 50 Units/mL for pancreatic α-amylase. 250 ml blank square glass bottles were prepared and samples were transferred into the glass bottles. The Falcon tube was washed with 20 ml buffer and the buffer was added into the sample. The square bottle was placed in Grant OLS200 shaking incubation water bath and the temperature was kept at 37 °C for 16 hours, which simulates the digestion processes. The square bottle was taken out and 3.0 ml Trizma base was added to adjust pH to approximately 8.0 pH and pH was recorded for comparison. The precision water incubator was warmed up to 90°C. The square bottle was then placed into the incubator and the sample was kept at 90°C for about 20 minutes in order to inactivate amyloglucidase and pancreatic α amylase, and also for protein denaturation. The square bottle was taken out and cooled down to 60°C. After that, 0.1 ml protease solution was added into the sample. The square bottle was then placed in the Grant OLS 200 incubator stable at temperature of 60°C and shaken for 30 minutes, enabling protease to digest and denature protein from large pieces to small fragments. After 30 minutes, the square bottle was taken out from water bath and cooled down to room temperature. 4 ml of 2 M Acetic acid was added to the sample for pH adjustment to 4.3 in order to accelerate precipitation. The sample was stirred and stored at 60°C for 1 hour before gravimetric filtration.

2.6 Analysis of insoluble and soluble dietary fiber

Gravimetric Filtration was applied twice in the analysis. The first gravimetric filtration separated insoluble dietary fiber from sample and the second filtration isolated soluble dietary fiber from sample. The details of the first filtration are as follows:

Three hundred and twenty milliliter of 95% ethanol was added to a 500 ml glass Pyrex bottle. The bottle was placed on the Fisher Scientific Isotemp and the ethanol was warmed up to 60°C before use. A weighed 50 ml Pyrex crucible was used for filtration. 1.0 mg Celite was added onto the crucible and the crucible was placed in oven at 130°C overnight to dispel water. Before weighing, the crucible was placed in desiccator until it was cooled down to room temperature. To collect the precipitate, crucible was wetted by 15 ml of 78% ethanol under vacuum suction to help distribute the Celite at the bottom of the crucible. Sample from the square bottle was then vacuum-filtered into a 1L flask. The prepared 320 ml 95% ethanol was mixed with it in a 500ml glass bottle. The precipitated portion of the sample was washed with

30 ml 78% ethanol, 30 ml 95% ethanol and 30 ml acetone, subsequently. The crucibles were placed into an oven and dried it at 105℃ overnight. The crucible was moved into a desiccator and cooled down to room temperature. The crucible was accurately weighed and the weight was recorded as weight 1. The sample was separated into Rep 1 and Rep 2 for protein and ash correction. The dry weight of insoluble dietary fiber was labeled as $R_{IDF.}$

The soluble dietary fiber contained in the aqueous phase was precipitated when it was mixed with ethanol. The second filtration was then carried out by the following procedures:

Seventy eight percent of ethanol was added to the crucible under vacuum suction in order to distribute the celite in the base of crucible. The sample in the 500 ml glass bottle was vacuum-filtered into a 1 L flask. The soluble dietary fiber precipitated on the crucible was washed with 30 ml 78% ethanol, 30 ml 95% ethanol and 30 ml acetone, subsequently. The crucible was placed in the oven and was dried at 105 ℃ overnight. The crucible was moved into a desiccator and cooled down to room temperature. The crucible was accurately weighed and the weight was recorded as weight 2. Samples were separated into Rep 1 and Rep 2 for protein and ash correction. The soluble dietary fiber weight is labeled as R_{SDF}.

The volume of the aqueous phase which contains oligosaccharides was measured with a graduated cylinder and was recorded for calculation. 10 ml of the solution was transferred into a 10 ml Falcon tube for HPLC measurement.

2.7 Analysis of Oligosaccharides

The aqueous phase obtained after second gravimetric filtration was evaluated by high performance liquid chromatography. The samples were taken from freezer and thawed to room temperature. A 1 ml syringe was used to take 1 ml of samples. The 1 ml sample was then filtrated through a 25 mm diameter 0.22 µm pore size filter into a 2 ml HPLC test tube and analyzed by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). The experiment was performed with a Shimadzu instrument (Shimadzu Corp., Kyoto, Japan) equipped with SCL-10Avp system controller, LC10ATvp pump, DFU-20A5 online degasser, and SIL-10A autosampler with a 20 uL fixed loop. The oligosaccharides were separated by a Dionex CarboPac PA10 anion-exchange resin analytical column with a Dionex CarboPac PA10 guard column. The mobile phase consisted of 120 mM NaOH solution at a flow rate of 1.0 ml/min under isocratic conditions. The mobile phase was prepared by diluting a carbonate-free 50% (w/w) NaOH solution in Milli-Q water. The detection was accomplished by an ED40 electrochemical detector with a gold working electrode and an Ag/AgCl reference electrode. Standard was made by using pure sucrose, raffinose, stachyose and verbasecose powder dissolved in pure water and mixed evenly together. The concentration used to make a standard curve is from 0.005 mM to 1 mM.

2.8 Protein and Ash Correction

The dietary fiber sample residues from the first filtration and second filtration were separated into two parts. Replicate 1 was used for protein correction, while replicate 2 was used for ash correction. The precipitate from replicate 1 was moved from the crucibles into a vial for protein detection. Before detection, the sample was homogenized with a glass rod. About 0.2 g of replicate 1 was weighed and covered with tinfoil. LECO TruSpec equipment was applied to detect the nitrogen content in the sample with Dumas method. A conversion of 6.25 was used to convert nitrogen content to protein content. The protein content is labeled as P_{IDF} and P_{SDF}.

Crucibles with Rep 2 were placed in furnace. Temperature was adjusted to 495℃ and the samples were kept to burn for 5 hours. The furnace was turned off and cooled down to about 100℃. The crucibles were moved into desiccator and wait until the crucibles were cooled down to room temperature. The dry weight of crucible with ash was weighed to the nearest 0.1 mg. Ash weight is labeled as A_{IDF} and A_{SDF}.

2.9 Calculations

Total dietary fiber content (TDF %) was calculated as TDF= IDF+SDF+Oligosaccharides content. Oligosaccharides were analyzed by HPLC and the calculation was mentioned in chapter 2.7. IDF and SDF were calculated by the following two equations:

$IDF\%=(R_{IDF}-A_{IDF}-P_{IDF}-B_{IDF})/S*100\%$

$SDF\% = (R_{SDF} - A_{SDF} - P_{SDF} - B_{SDF})/S * 100\%$

For insoluble dietary fiber sample residues, R_{IDF} is IDF sample residue dry weight, A_{IDF} is the ash dry weight in the residue, P_{IDF} is the protein dry weight in the residue, B_{IDF} is the residue blank. S is the seed sample dry weight.

For soluble dietary fiber sample residues, R_{SDF} is the sample residue dry weight, A_{SDF} is the ash dry weight in the residue, P_{SDF} is the protein dry weight in the residue, B_{SDF} is the residue blank. S is the seed sample dry weight.

2.10. Statistical Analysis

Analysis of variance was conducted to compare entry and location means for all variables using the Proc GLM procedure in SAS version 9.2 (SAS Institute Inc., Cary, NC). Two replicates per entry were used for all variables except as noted. Tukey's multiple-mean comparison method ($p < 0.05$) was used to determine significance among entry means for all variables. Correlation analysis was used to determine the relationship between dietary fiber components. SPSS version 20 (International Business Machines Corps., Armonk, NY, USA) and SAS version 9.2 (SAS Institute Inc., Cary, NC, USA) were used.

CHAPTER 3

DIETARY FIBER CONTENT IN DRY PEAS

3.1 Introduction

Dry pea is among the world's oldest crops. It was domesticated in the Middle East approximately 9000 years ago and has been grown worldwide for human and animal consumption (66;67). Dry pea is an inexpensive and nutritious food being an excellent source of carbohydrate, protein, B-vitamins, folate, and minerals such as calcium, iron and potassium(67). Dry pea has low fat and sodium content, which makes it healthier than protein containing foods from animal sources. Dry pea has also been reported to have disease prevention effects, such as lowering serum cholesterol, reducing heart disease, and decreasing risk of type 2 diabetes (67).

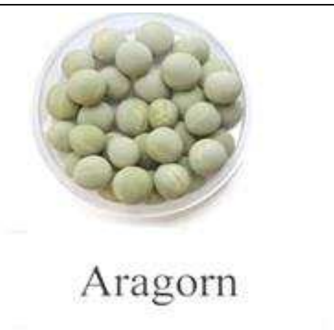
Total dietary fiber content in dry pea has been measured with different methods (68). Those methods included gas chromatographic (GC) measurement, enzymatic-gravimetric AOAC methods, and the methods used in United Kingdom (UK) for dietary fiber measurement. Compared with AOAC methods, the UK method does not include the measurement of lignin and has an extraction step for removing starch that is not hydrolyzed by amylase, resulting in the underestimation of total dietary fiber content. The GC method applied to measure dry pea fiber and the total fiber content was reported to be 16.7% w/w. On the other hand, Dodevska et al.(69) reported that the dietary fiber content in dry pea samples using AOAC Method 995.16 was 20.7%. Such inconsistencies caused by the differences in the definition of dietary fiber and measurement methods demonstrate the importance of carrying out a re-analysis of dry pea fiber content using the newly developed consensus definition and method.

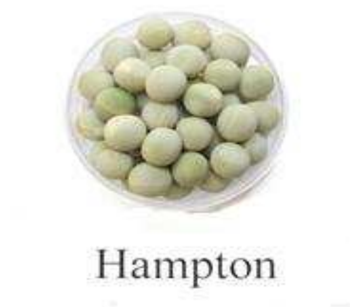
In the current work, the modified state-of-the-art method, AOAC 2011.25, which is based on the consensus definition of dietary fiber, was used for the first time to estimate the content of different components of dietary fiber of dry pea. Eleven dry pea cultivars grown in two different locations were studied. The main objectives of this experiment were: (1) to update the value of dietary fiber components of dry pea, and (2) to determine if variation exists for dietary fiber among different cultivars. The long term goal of this study was to obtain results about genetic variation in dietary fiber to develop dry pea cultivars with improved human health benefits.

3.2 Sample description and measurement

Eleven dry pea cultivars were obtained from two different locations, Pullman and Genesee, WA, except for the cultivar Melrose, WA. Melrose was only obtained from the Pullman, WA. Photographs of these samples are shown in Figure 3.1. Samples were divided into different economically important market classes based on recognized seed phenotypic characteristics. Among them, Columbian, Aragorn, PS07100471, PS03101445 and Hampton belong to the dry green pea market class. The primary use of this market class is as whole food. Columbian was a very old cultivar with a small dimple on its surface and is generally used for split pea soup. Aragorn is developed in New Zealand with good color quality. PS07100471 and PS03101445 are breeding lines and are similarly as Aragorn. Hampton is a high yielding cultivar. It has very good color quality and resistance to many plant diseases. Carousel, DS. Adminal, and PS08101022 belong to the dry yellow pea market class. They are used primarily as animal feed or for food ingredients after fractionation. The starch fractions are used to make noodles in Asia and the protein component is used to make foods like energy bars. Spector belongs to the winter market class, which has a clear seed coat with ‘ghost mottling’ and small seed size. Granger and Melrose, with dark pigmented seed coat and black hilum, belong to the Austrian winter pea market class. All of these dry pea varieties, i.e., Granger, Melrose and Spector, were planted in the autumn, overwinter as seedlings, and are mature about three weeks before spring-sown dry peas. They are cold tolerant and don’t require vernalization to flower. They are not very palatable and are usually used for animal feed or as a cover crop that improves soil nitrogen and organic matter content.

Dry Green





Dry Yellow

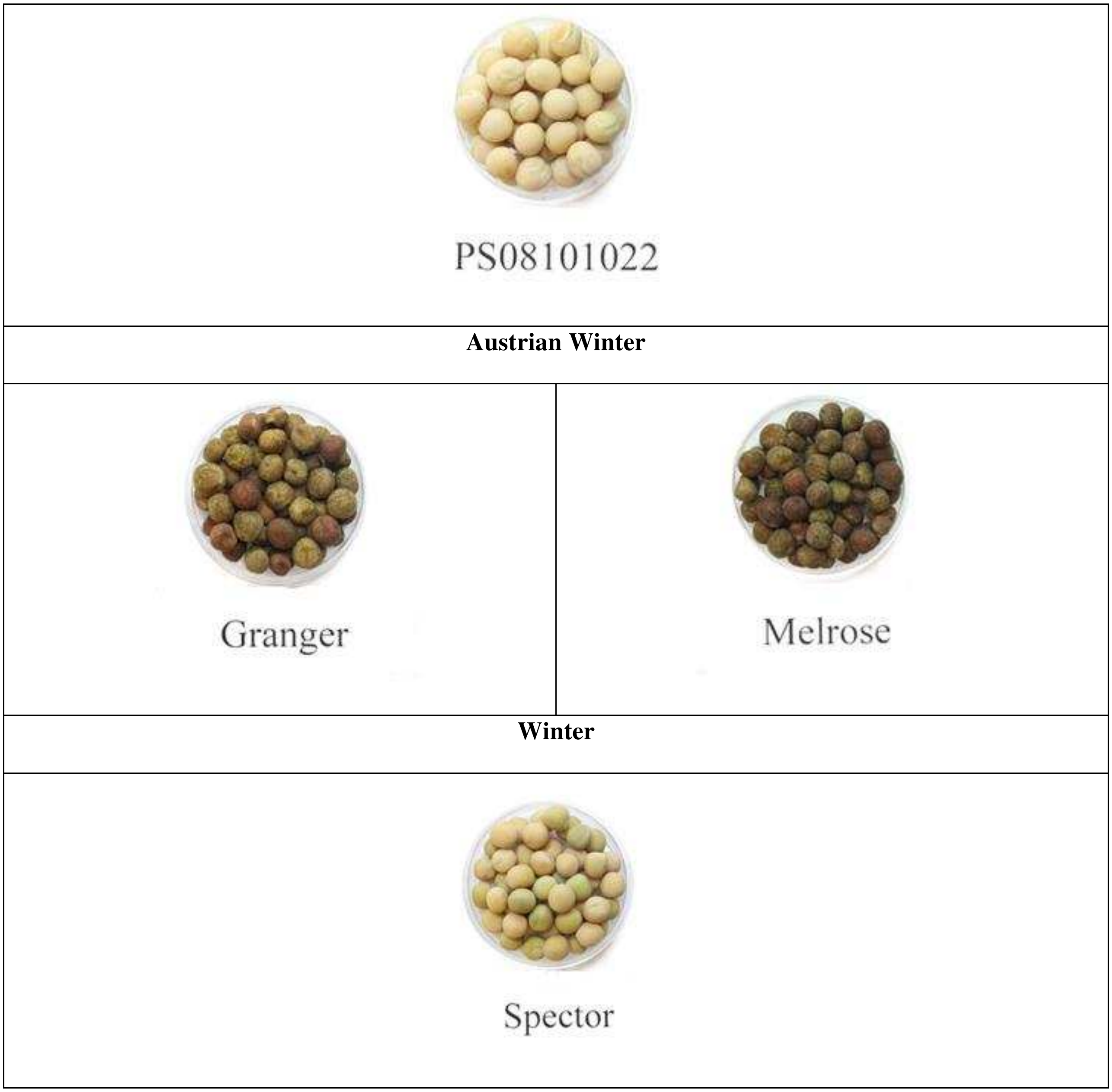


Figure 3.1 Seed types for dry pea cultivars evaluated

3.3 Results

3.3.1 Dietary fiber content and the cultivars

The content of dietary fiber in dry pea cultivars, including IDF, SDF, raffinose, stachyose, verbascose and the total oligosaccharides are shown graphically in Table 3.1, Figures 3.2 and 3.3.

IDF content accounted for about 65% of total TDF content. Based on F-test, IDF differed among cultivars ($P = 0.004$) and ranged from 14.2 to 19.8% with mean 16.0%. Among tested cultivars, Granger had the highest IDF content (19.1%) and PS03101445 had the lowest (14.2%). SDF content for dry pea did not differ among cultivars ($P = 0.079$) and ranged from 3.3 to 5.2% with a mean of 3.9%. Total oligosaccharide content differed among cultivars ($P = 0.026$) and ranged from 4.0 to 5.4% with mean 4.7%. The highest total oligosaccharide content cultivar was Columbian (5.4%) and Melrose (4.0%) was the lowest. When the individual components of oligosaccharides were considered, raffinose did not differ among cultivars ($P = 0.179$). The raffinose content for dry pea samples ranged from 0.6% to 1.1% with a mean of 0.8%. Stachyose content for dry pea samples did not differ among cultivars ($P = 0.214$). The mean stachyose content was 2.5% and ranged from 2.2 to 3.2%. Among dry pea cultivars, verbascose content was not significantly different ($P = 0.087$). The mean verbascose content was 1.4% and ranged from 1.0 to 1.9%. Stachyose content (2.5%) was markedly higher than raffinose (0.8%) and verbascose (1.4%) among dry pea cultivars. The TDF content for dry pea samples differed among cultivars ($P = 0.006$) and ranged from 22.3 to 28.0% with mean 24.9%. Granger had the highest TDF content (28%) while Hampton had the lowest (22.3%) among tested cultivars.

Table 3.1 Insoluble dietary fiber (IDF), soluble dietary fiber (SDF), raffinose, stachyose, verbascose, total oligosaccharides and total dietary fiber (TDF) among dry pea cultivars

Variety	IDF[a]	SDF[a]	Raffinose	Stachyose	Verbascose	OLIGO[a]	TDF[a]
ARAGORN	14.8 C	5.2	0.6	2.5	1.2	4.4 CDE	24.5 BC
CAROUSEL	15.0 C	4.4	1.1	2.5	1.3	4.8 ABCD	24.1 BC
COLUMBIAN	15.4 BC	3.3	1.1	3.2	1.1	5.4 A	24.1 BC
DS. ADMINAL	16.0 BC	3.5	0.8	2.2	1.3	4.3 CDE	23.8 C
GRANGER	19.1 A	3.8	0.7	2.4	1.9	5.0 ABC	28.0 A
HAMPTON	14.6 C	3.6	0.6	2.2	1.4	4.1 DE	22.3 C
MELROSE	19.8 A	3.9	0.6	2.2	1.2	4.0 E	27.6 A
PS03101445	14.2 C	3.5	0.9	2.6	1.7	5.2 AB	22.9 C
PS071019971	15.5 BC	3.8	0.9	2.5	1.0	4.4 BCDE	23.8 C
PS08101022	15.8 BC	3.7	0.7	2.3	1.6	4.6 ABCDE	24.2 BC
SPECTOR	17.6 AB	4.1	0.8	2.6	1.6	4.9 ABCD	26.7 AB
Overall Mean	16.0	3.9	0.8	2.5	1.4	4.7	24.6
P-value[b]	0.004	0.079	0.179	0.214	0.087	0.026	0.006

[a]Abbreviations: IDF: insoluble dietary fiber; SDF: soluble dietary fiber; OLIGO: total oligosaccharides (raffinose + stachyose + verbascose); TDF: total dietary fiber (IDF + SDF + OLIGO). Values are expressed as percent of dry weight; Means followed by same letters are not significantly different at P<0.05. Mean separations were performed using Tukey's honestly significant difference test. [b]For significant of the F-test in the analysis of variance.

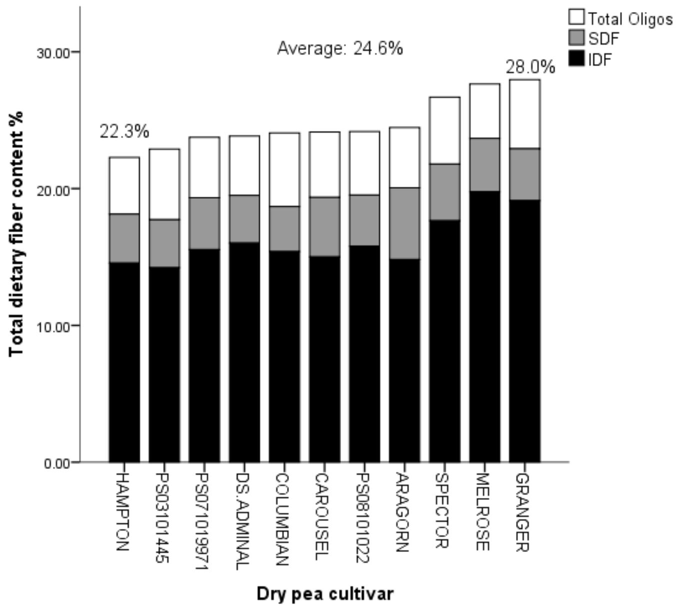


Figure represents dietary fiber content among dry pea cultivars. Values are expressed by percent of dry weight. Abbreviations for the legends: IDF = insoluble dietary fiber; SDF = soluble dietary fiber, Total Oligo = total oligosaccharides.

Figure 3.2 Mean percentage IDF, SDF, total oligosaccharides and TDF among dry pea cultivars.

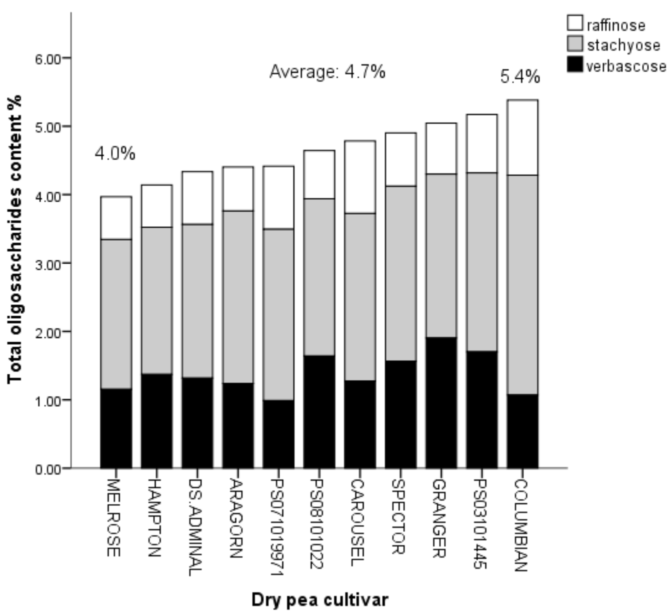


Figure represents oligosaccharides content among chickpea cultivars. Values are expressed by percent of dry weight.

Figure 3.3 Mean percentage of raffinose, stachyose, verbascose and total oligosaccharides among dry pea cultivars.

3.3.2 Correlation analysis among different components of dry pea samples

Pearson correlation analysis was performed to explore possible associations among dietary fiber components of the dry pea cultivars. The results are shown in Table 3.2. Raffinose had a strong positive correlation with stachyose. At the same time, verbascose had strong negative correlation with both raffinose and stachyose. The biosynthesis pathway for raffinose family of oligosaccharides (RFO's) is raffinose to stachyose to verbascose and it is reversible (70). From the correlation test, the higher raffinose content was associated with higher stachyose and lower verbascose. Enzymes in the biosynthesis pathway may have large impact on adjusting the balance of these three components.

Table 3.2 Pearson correlations for all dry pea samples

Correlations	IDF	SDF	Raffinose	Stachyose	Verbascose	Total Oligos
IDF	1					
SDF	-0.12					
Raffinose	-0.13	-0.08				
Stachyose	-0.09	-0.02	0.61**			
Verbascose	0.26	0.05	-0.48**	-.37*		
Total Oligos	0.04	-0.01	0.57**	0.82**	0.16	1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Abbreviations: IDF: insoluble dietary fiber, SDF: soluble dietary fiber, Total Oligos: total oligosaccharides

3.3.3 The influence of market class on dietary fiber content

Mean values of IDF, SDF, raffinose, stachyose, verbascose and total oligosaccharides among dry pea market classes (Table 3.3) are shown in Figure 3.4. IDF content differed among market classes (P=0.01). SDF, raffinose, stachyose, verbascose and total oligosaccharides were not different among market classes (P>0.05). TDF content also differed among market classes (P=0.01). Austrian winter and winter dry pea market classes had higher and dry green and dry yellow market class had lower TDF content.

Table 3.3 Dietary fiber content for dry pea samples by market classes

Class	IDF[a]	SDF	Raffinose	Stachyose	Verbascose	Total Oligos	TDF
Austrian Winter	19.3 [A]	3.8	0.7	2.3	1.7	4.7	27.9 [A]
Dry Green	14.9 [B]	3.9	0.8	2.6	1.3	4.7	23.5 [B]
Dry Yellow	15.6 [B]	3.9	0.9	2.3	1.4	4.6	24.1 [B]
Winter	17.7 [A]	4.1	0.8	2.6	1.6	4.9	26.7 [A]
P-value[b]	<0.001	0.97	0.66	0.48	0.18	0.87	<0.001

[a]Means with the same letters are not significantly different at P<0.05. Mean separations were performed using Tukey's honestly significant difference test. Abbreviations: IDF: insoluble dietary fiber, SDF: soluble dietary fiber, Total Oligos: total oligosaccharides, [b]P-value for significant of the F-test in the analysis of variance.

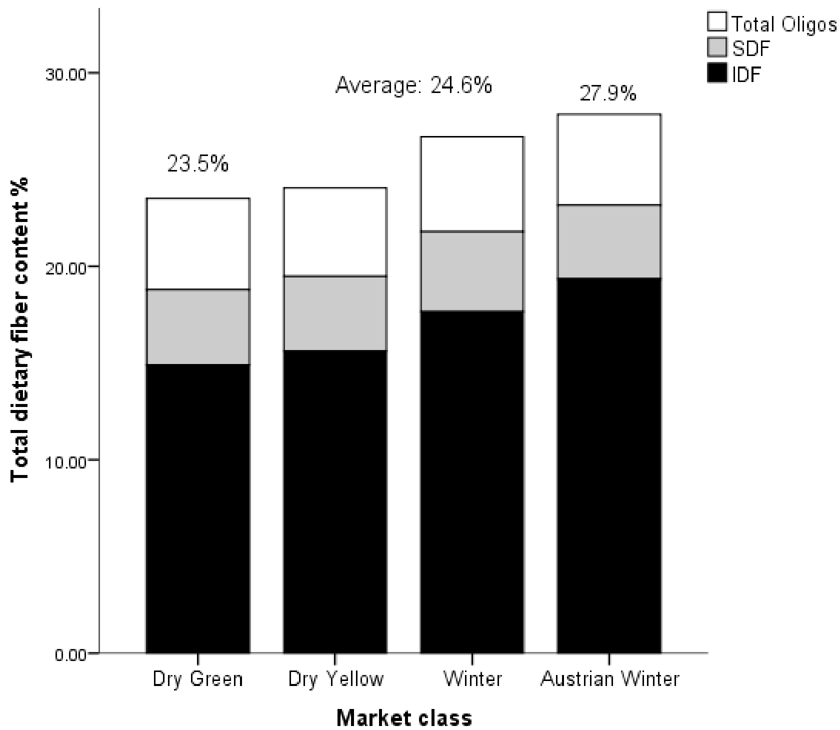


Figure represents dietary fiber content among dry pea market classes. Values expressed by percent dry weight. Abbreviations: IDF = insoluble dietary fiber, SDF = soluble dietary fiber, Total Oligo = total oligosaccharides

Figure 3.4 Mean dietary fiber contents for dry pea samples among different market classes

3.3.4 The influence of location

The effect of the location of production on dietary fiber content was also studied. Seed samples grown at two locations, namely Pullman and Genesee, WA, were compared (Table 3.4). There were no significant differences in mean IDF, SDF, total oligosaccharides or TDF content grown at the two locations.

Table 3.4 IDF, SDF, Total Oligosaccharides, and TDF content between two locations of samples

Cultivar/DF	IDF[a]		SDF[a]		Total Oligo[a]		TDF[a]	
Location	Pullman	Genesee	Pullman	Genesee	Pullman	Genesee	Pullman	Genesee
ARAGORN	16.3	13.3	3.6	6.9	4.5	4.3	24.4	24.6
CAROUSEL	15.6	14.4	4.8	3.9	5.3	4.2	25.8	22.5
COLUMBIAN	15.8	15.0	3.4	3.2	5.2	5.5	24.4	23.7
DS. ADMINAL	15.6	16.4	3.1	3.9	4.1	4.6	22.8	24.9
GRANGER	18.2	20.0	3.4	4.2	4.5	5.6	26.1	29.8
HAMPTON	15.5	13.6	3.6	3.5	4.5	3.8	23.6	21.0
MELROSE		19.8		3.9		4.0		27.6
PS03101445	13.0	15.5	3.6	4.0	4.8	4.1	24.0	23.5
PS071019971	15.6	15.5	4.3	3.2	4.7	4.6	24.4	24.0
PS08101022	15.3	16.3	3.1	3.9	5.5	4.9	21.6	24.2
SPECTOR	17.4	17.8	4.3	4.0	4.8	5.0	26.5	26.9
Mean	15.8	16.1	3.7	4.1	4.8	4.6	24.4	24.8
P-value[b]	0.65		0.30		0.36		0.55	

[a]Values are expressed as percent of dry weight; dietary fiber content between two locations (Pullman and Genesee) are measured with F test. Abbreviations: IDF: insoluble dietary fiber; SDF: soluble dietary fiber; OLIGO: total oligosaccharides (raffinose + stachyose + verbascose); TDF: total dietary fiber (IDF + SDF + OLIGO), [b]P-value for significant of the F-test in the analysis of variance.

3.3.5 Discussion

Despite the important health benefits of dietary fiber in the prevention of chronic diseases and efforts over many years to educate consumers about these health benefits, insufficient intake of dietary fiber is still widespread, with less than 10% of all Americans meeting recommended intake levels (71). In a comparison of 70 different food items, pulse crops were found to have the highest dietary fiber content (72). In addition to being high in dietary fiber, pulse crops are also high in protein, very low in fat and supply essential vitamins and minerals. Our laboratory has advanced the idea that the lack of pulse consumption in countries such as the United States is a significant contributor to the dietary fiber gap, since these crops provide two to three times more dietary fiber per 100 g edible portion than cereal grains, the type of foods frequently advertised as sources of dietary fiber (72). The high protein and low fat content of pulses further strengthens the importance of emphasizing their regular consumption to close the dietary fiber gap.

Dry pea is one of the four highest consumed pulse crops. Our results have shown that the averages IDF, SDF, total oligosaccharides and TDF among dry pea samples were 24.6%, 16.0%, 3.9% and 4.7%, respectively. For oligosaccharides, the averages values for raffinose, stachyose and verbascose were 0.8%, 2.5% and 1.4%, respectively. Comparing the AOAC 2011.25 method with the old method AOAC 995.16, by which TDF content was reported to be 20.6% for dry pea, the new method includes more components, e.g., fructo-oligosaccharides, galacto-oligosaccharides, polydextrose and maltodextrins. There is difference between method of 4 g/100g dry weight where TDF between these methods shows about an 18% in percentage difference. The comparison shows that dietary fiber content in dry pea has been underestimated by the old method. It is necessary to reevaluate dietary fiber content in dry pea with the new method. The recommended daily dietary fiber intake amount is 25 g for women and 38 g for men, while the average daily dietary fiber intake for U.S population is 15.6 g/day. Based on our results, one serving of dry pea contains an average 4.3 g dietary fiber.

Significant differences in the content of TDF, IDF and total oligosaccharides were observed among dry pea cultivars. There was a 22.7% difference in TDF between the highest content cultivar and the lowest. Such substantial differences in fiber content among available cultivars can be used to further improve gains in fiber intake without the need to change dietary habits. This also provides a rationale for cultivar-based food labeling. At the same time, the above results indicate that the genetic effects on dietary fiber content in dry pea samples are marked. Thus, it may be possible to use either traditional approach to breeding and selection as well as transgene and hybridization technology to develop new cultivars with higher fiber content.

Genotype-environment interactions are important factors of consideration in breeding for specific characteristics. Our results show that mean dietary fiber content among cultivars of dry pea was not different between two locations. This finding suggests that the environment impact on dietary fiber content for dry pea is small. Nonetheless, additional experiments are needed across more diverse growing conditions in order to more vigorous determine the magnitude of effect that environment imposes on the expression of genes that regulates fiber synthesis.

Oligosaccharides were shown to be important nutrient sources for some microbes that populate the human intestine. Raffinose, stachyose and verbascose are the prominent dry pea oligosaccharides, which had been reported to promote the growth of beneficial intestinal bacteria (26). Among three oligosaccharides for dry pea, raffinose was positively association with stachyose and negatively associated with verbascose. However, oligosaccharide content was not correlated with either IDF or SDF (Table 3.2). Thus, it is valuable to report oligosaccharide content separately rather than including it as a component of SDF. While more work is needed, it is possible that consumption of high oligosaccharide dry peas could have specific health benefits. However, for individuals who have poor tolerance of galatans, the consumption of low oligosaccharides content cultivars may improve tolerance.

Market classes are based on the phenotype of the seeds (color, size or shape) that determine the usage and economic value. Marked differences in dietary fiber content were observed among different market classes of dry pea. The dry green and dry yellow market classes had the lowest dietary fiber content (human food), while the winter and Austrian winter classes (animal feed) had the highest TDF. Unfortunately, dry pea cultivars with highest content of dietary fiber are not suitable for human consumption due to their unfavorable taste. This result suggests that it may be possible to introgress genes from high fiber content market classes into cultivars that are preferred by consumers. It is noteworthy that within the dry green market class, there is a significant difference in fiber content between Aragorn which had the highest SDF content and PS03101445 which had the second lowest. The fact that differences in fiber content exist within a market class supports the feasibility of efforts to further increase dietary fiber content through breeding and selection.

3.3.6 Summary

The average total dietary fiber content for dry pea cultivars is 24.6%. Among different dry pea cultivars and market classes, dietary fiber content was significantly different. Among four tested market classes, human food cultivars have lower dietary fiber than animal feeding cultivars. Location has limited effect on dietary fiber content. Correlations exist among raffinose, stachyose and verbascose. For dry pea, genetic difference has higher impact than environmental effects. The dietary fiber content tested by AOAC 2011.25 method is higher than those tested by old methods. It is valuable to reevaluate dry pea dietary fiber content with the new method.

CHAPTER 4

DIETARY FIBER CONTENT OF LENTIL SAMPLES

4.1 Introduction

Lentil is a pulse crop that was domesticated in the Middle-East and is now cultivated worldwide (73,74). The country that produces the most lentils is Canada. Lentil has high protein and carbohydrate, but low lipid content. They are also a good source of minerals such as calcium, potassium, manganese and zinc. Lentil has been reported to promote weight maintenance, reduce the risk of type 2 diabetes, cardiovascular disease and cancer (75).

Dietary fiber content of lentil samples has been measured by various methods. By using the method of Van Soest and Wine (76)and McQueen and Nicholson (77), content of neutral detergent fiber (NDF) and acid detergent fiber (ADF) in lentils were 21.2% and 7.2%, respectively (78). In a separate study reported by de Almeida Costa et al. (79), insoluble dietary fiber content of lentil was approximately 19.0% and SDF was approximately 1.4%.

In the current work, the newly developed method AOAC 2011.25 was employed for the first time to estimate the content of different components of dietary fiber of lentil. Thirteen cultivars representing six market classes were studied. The objectives of the study were: (1) accurately measure dietary fiber content in lentils. (2) To determine if variation exists for dietary fiber among different cultivars. The result of the current work, we believe, can be applied to guide breeding programs to develop lentil cultivars with improved health benefits.

4.2 Sample description and measurement

Thirteen cultivars of lentil were produced from two locations, Fairfield and Pullman, WA. Cedar, Crimson and Merrit were only produced from Pullman. For each location, two replicates of the same cultivar were collected from different field plots. The thirteen lentil cultivars belong to six different market classes (Table 4.1). Photographs of these thirteen lentil cultivars are shown in Figure 4.1

Table 4.1 Different market classes of lentil

Market class	Cultivar	Characteristic
Small green	Eston	Green unpigmented seed coat; Yellow cotyledons
Medium green	Brewer, Merrit, Richlea, Avondale, CDC-viceroy	Green unpigmented seed coat; Yellow cotyledons
Large green	Pennell, LC6601734L	Green unpigmented seed coat; Yellow cotyledons
Zero tannin	Cedar	Translucent seed coat; Red cotyledons
Spanish brown	Pardina, LC08600113P	Brown pigmented seed coat; Red cotyledons
Turkish red	Crimson, Morton	Brown pigmented seed coat; Red cotyledons

Small Green	
 Eston	
Medium Green	
 Merrit	 Brewer
 Avondale	 CDC Viceroy

Large Green

Turkish Red

Spanish Brown

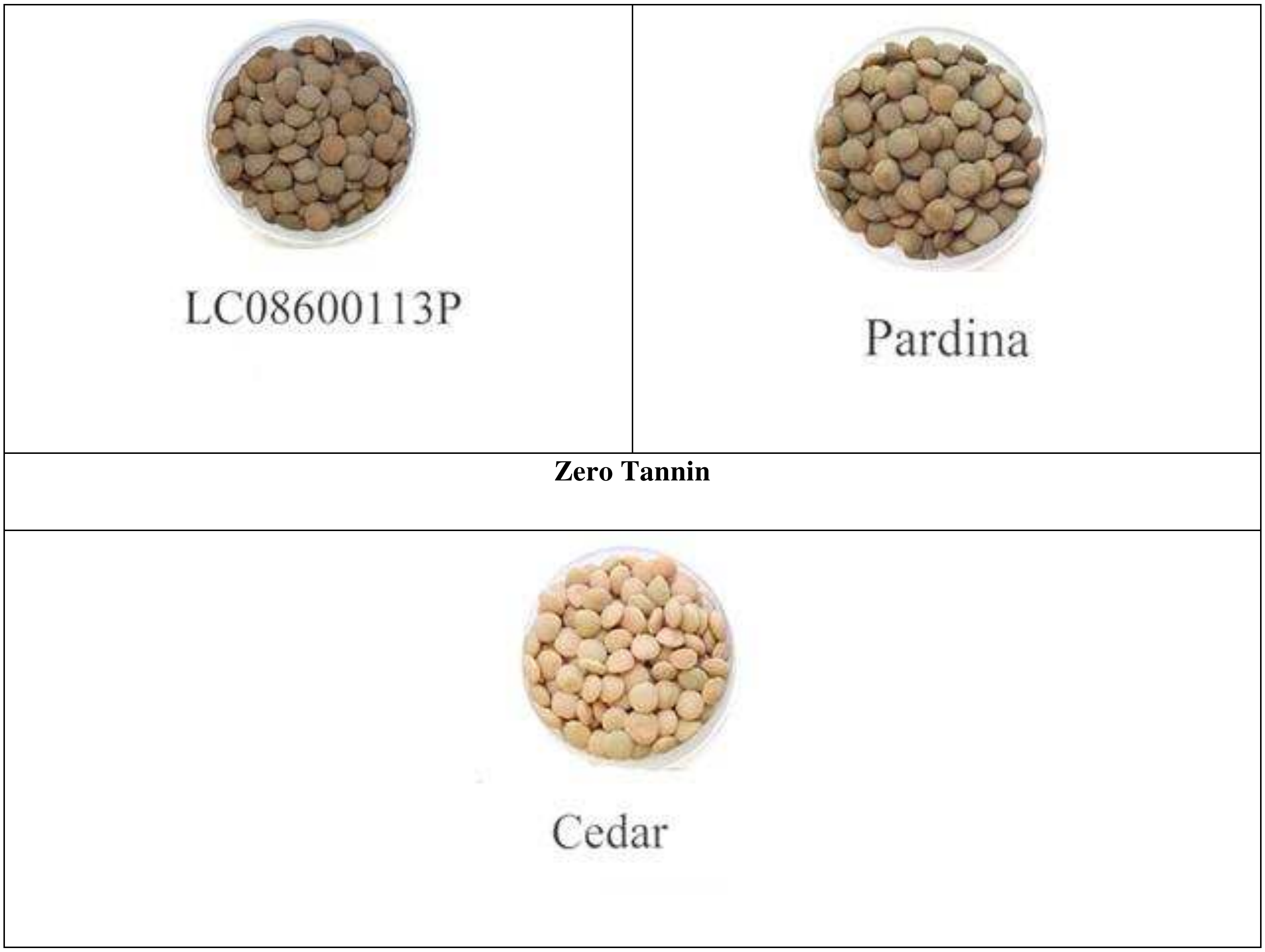


Figure 4.1 Seed types for lentil cultivars evaluated

4.3 Results

4.3.1 The dietary fiber content and the cultivars

The content of dietary fiber in lentil cultivars, including IDF, SDF, raffinose, stachyose, and verbascose and total oligosaccharides are shown in Table 4.2, Figures 4.2 and 4.3. Based on F-test IDF content among cultivars was different ($P = 0.016$) and ranged from 12.2 to 14.7% with mean 13.6%. Crimson had the highest IDF content (14.7%) and Cedar had the lowest (12.2%). SDF content differed among lentil cultivars ($P < 0.01$) and ranged from 2.7 to 3.9% with a mean of 3.2%. Pennell had the highest SDF content (3.9%) and Morton had the lowest (2.7%). Total oligosaccharide content did not differ among cultivars ($P = 0.115$) and ranged from 3.0 to 3.7% with a mean 3.3%. Brewer had the highest total oligosaccharide content (3.7%) and Crimson had the lowest (3.0%). When the individual component oligosaccharides were considered, raffinose content differed among cultivars ($P = 0.041$). The raffinose content for lentil samples ranged from 0.3 to 0.4% with a mean of 0.3%. Stachyose content for lentil samples did not differ by cultivar ($P = 0.521$). The average content of stachyose was 2.0% and ranged from 1.8 to 2.2%. Among lentil cultivars, verbascose content was not different ($P > 0.081$). The mean verbascose content was 1.0% and ranged from 0.8 to 1.2%. Stachyose content (2.0%) was markedly higher than raffinose (0.3%) and verbascose (1.0%) in all lentil cultivars. The TDF content for lentil samples differed among cultivars ($P < 0.001$) and ranged from 18.4 to 21.3% with mean 20.1%. Pennell had the highest TDF content (21.3%) while Cedar had the lowest (18.4%). The percent difference between the TDF content of these two cultivars was 14.6%.

Table 4.2 Insoluble Dietary Fiber (IDF), soluble dietary fiber (SDF), raffinose, stachyose, verbascose, total oligosaccharides and total dietary fiber (TDF) among lentil cultivars

Variety	IDF	SDF	Raffinose	Stachyose	Verbascose	OLIGOS	TDF
Avondale	13.6 ^{B}	3.1 ^{BCD}	0.3 ^{ABCDE}	2.1	1.1	3.3	20.0 ^{CD}
Brewer	13.8 ^{B}	3.3 ^{B}	0.3 ^{AB}	2.2	1.0	3.7	20.8 ^{AB}
CDC Viceroy	13.4 ^{B}	3.0 ^{BCDE}	0.3 ^{BCDEF}	2.2	1.2	3.4	19.8 ^{CDE}
Cedar	12.2 ^{C}	3.1 ^{BCD}	0.3 ^{F}	2.0	0.8	3.0	18.4 ^{F}
Crimson	14.7 ^{A}	2.9 ^{CDE}	0.3 ^{BCDEF}	1.9	0.8	3.0	20.6 ^{B}
Eston	13.5 ^{B}	3.0 ^{BCDE}	0.3 ^{ABCD}	2.2	1.1	3.3	19.9 ^{CDE}
LC06601734L	13.7 ^{B}	3.3 ^{B}	0.3 ^{ABC}	2.1	1.1	3.3	20.3 ^{BC}
LC08600113P	13.4 ^{B}	3.0 ^{BCDE}	0.3 ^{CDEF}	2.0	1.0	3.4	19.8 ^{CDE}
Merrit	13.5 ^{B}	3.3 ^{B}	0.3 ^{BCDEF}	2.0	0.9	3.5	20.3 ^{BC}
Morton	13.7 ^{B}	2.7 ^{E}	0.3 ^{F}	1.8	0.8	3.0	19.4 ^{E}
Pardina	13.5 ^{B}	2.8 ^{DE}	0.4 ^{DEF}	2.0	0.9	3.2	19.5 ^{DE}
Pennell	13.8 ^{B}	3.9 ^{A}	0.3 ^{A}	1.8	0.9	3.6	21.3 ^{A}
Richlea	13.7 ^{B}	3.2 ^{BC}	0.3 ^{CDEF}	1.9	0.9	3.1	20.0 ^{CD}
Overall Mean	13.6	3.2	0.3	2.0	1.0	3.3	20.1
P-value	0.016	<0.001	0.041	0.521	0.081	0.115	<0.001

[a]Abbreviations: IDF: insoluble dietary fiber; SDF: soluble dietary fiber; OLIGO: total oligosaccharides (raffinose + stachyose + verbascose); TDF: total dietary fiber (IDF + SDF + OLIGO). Values are expressed as percent of dry weight; Means followed by same letters are not significantly different at P<0.05. Mean separations were performed using Tukey's honestly significant difference test. [b]For significant of the F-test in the analysis of variance.

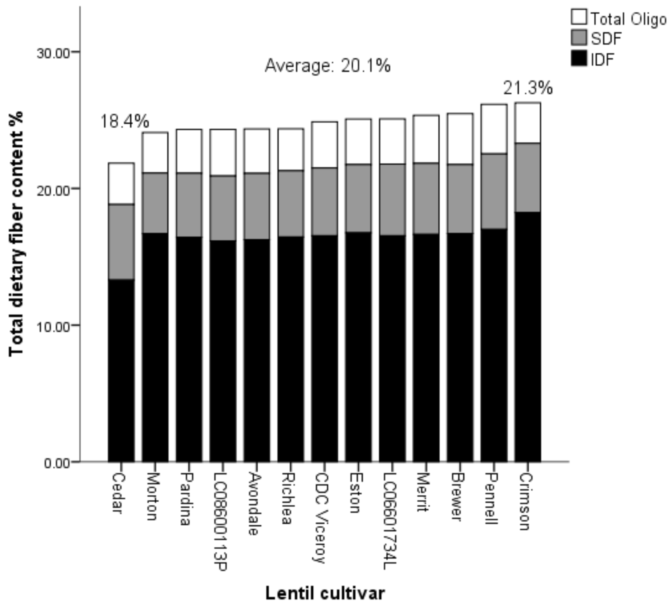


Figure represents dietary fiber content among lentil cultivars. Values are expressed by percent of dry weight. Abbreviations for the legends: IDF = insoluble dietary fiber; SDF = soluble dietary fiber, Total Oligo = total oligosaccharides.

Figure 4.2 Mean percentage IDF, SDF, total oligosaccharides and TDF among lentil cultivars

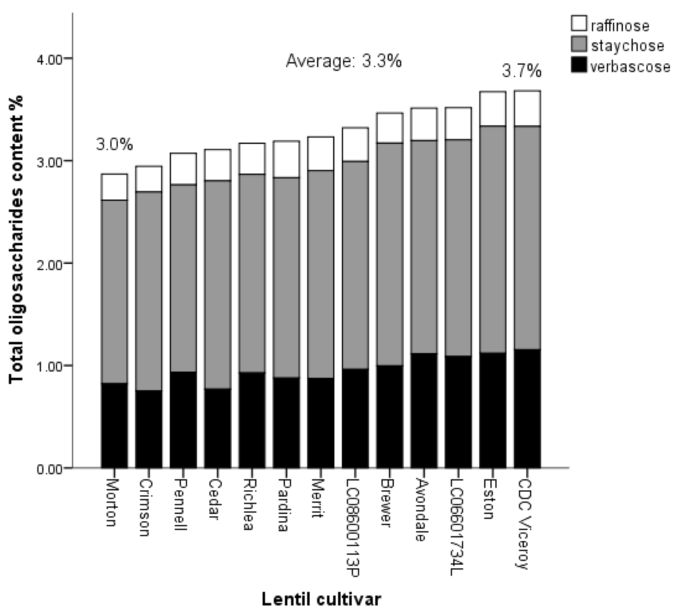


Figure represents oligosaccharides content among lentil cultivars. Values are expressed by percent of dry weight.

Figure 4.3 Mean percentage raffinose, stachyose, verbascose and total oligosaccharides and TDF among lentil cultivars

4.3.2 Correlation analysis among different fiber components of lentil samples

Pearson correlation coefficients were computed to explore associations among fiber components. The results were shown in Table 4.3. Raffinose had a strong positive association with SDF and verbascose, SDF also had positive association with both verbascose and total oligosaccharides. The biosynthesis pathway for raffinose family of oligosaccharides (RFO's) is raffinose to stachyose to verbascose and its reversible(70). From the correlation test, higher raffinose content is associated with both higher stachyose and verbascose.

Table 4.3 Pearson correlations for all lentil samples

Correlations	IDF	SDF	Raffinose	Stachyose	Verbascose	Total Oligo
IDF	1					
SDF	0.01					
Raffinose	.34*	.51**				
Stachyose	-.35*	0.14	.30*			
Verbascose	-0.06	.47**	.43**	0.27		
Total Oligo	-0.19	.43**	.55**	.75**	.83**	1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Abbreviations: IDF: insoluble dietary fiber, SDF: soluble dietary fiber, Total Oligos: total oligosaccharides

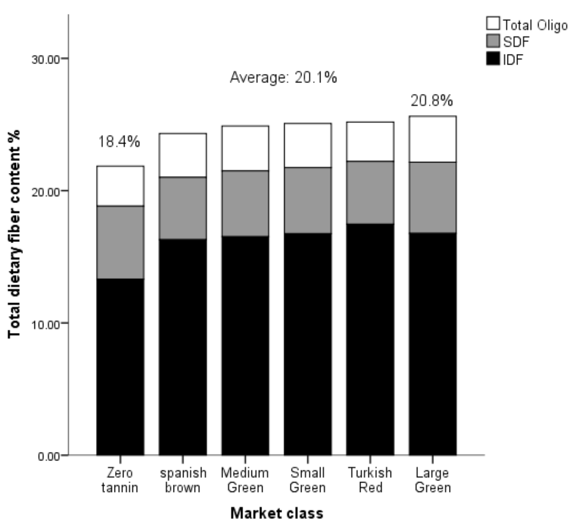
4.3.3 The influence of market classes on dietary fiber content

The mean values of IDF, SDF, raffinose, stachyose, verbascose and total oligosaccharides (Table 4.4) among different market classes were shown graphically in Figure 4.4. IDF, SDF, raffinose, stachyose, verbascose, total oligosaccharides and TDF content differed among market classes ($P < 0.05$). The Large green market class had the highest dietary fiber content and zero tannin class had the lowest.

Table 4.4 Dietary fiber content for lentil samples of different market classes

Class	IDF[a]	SDF[a]	Raffinose[a]	Stachyose[a]	Verbascose[a]	Total Oligo[a]	TDF[a]
Small Green	16.8 ^{AC}	5.0 ^{AD}	0.3 ^{AC}	2.2 ^{AB}	1.1 ^{A}	3.3 ^{AB}	19.9 ^{AB}
Large Green	16.8 ^{AC}	5.4 ^{B}	0.3 ^{C}	2.0 ^{AC}	1.0 ^{AC}	3.5 ^{A}	20.8 ^{C}
Spanish Brown	16.3 ^{A}	4.7 ^{CD}	0.3 ^{A}	2.0 ^{AC}	0.9 ^{ABC}	3.3 ^{AB}	19.7 ^{B}
Zero Tannin	13.3 ^{B}	5.5 ^{B}	0.3 ^{ABC}	2.0 ^{ABC}	0.8 ^{ABC}	3.0 ^{AB}	18.4 ^{D}
Turkish Red	17.5 ^{C}	4.8 $_{ACD}$	0.3 ^{B}	1.9 ^{C}	0.8 ^{BC}	3.0 ^{B}	20.0 ^{AB}
P-value[b]	0.02	<0.001	0.01	0.03	<0.001	0.01	<0.001

[a]Means with the same letters are not significantly different at P<0.05. Mean separations were performed using Tukey's honestly significant difference test. Abbreviations: IDF: insoluble dietary fiber, SDF: soluble dietary fiber, Total Oligos: total oligosaccharides, S: significant. [b]P-value for significant of the F-test in the analysis of variance.



*abbreviations: IDF = insoluble dietary fiber, SDF = soluble dietary fiber, Total Oligo = total oligosaccharide

Figure 4.4 Mean dietary fiber contents for lentil samples among different market classes

4.3.4 The influence of location

The effect of location of production of lentil on dietary fiber content was also studied. Samples from two locations, namely Pullman and Fairfield, were compared (Table 4.5). In general there was no significant location effect among lentil cultivars for IDF, SDF, total oligosaccharides or TDF content.

Table 4.5 IDF, SDF, Total Oligosaccharides, and TDF content between two locations of samples

Cultivar	IDF		SDF		Total Oligo		TDF	
	Pullman	Fairfield	Pullman	Fairfield	Pullman	Fairfield	Pullman	Fairfield
Avondale	13.4	13.8	3.1	3.2	3.3	3.2	19.8	20.2
Brewer	14.2	13.3	3.3	3.3	3.7	3.8	21.2	20.4
CDC Viceroy	13.7	13.1	3.0	3.1	3.3	3.4	20.0	19.6
Eston	13.7	13.4	3.0	3.1	3.4	3.3	20.1	19.7
LC06601734L	14.0	13.5	3.3	3.2	3.2	3.4	20.6	20.1
LC08600113P	13.3	13.5	3.1	2.9	3.4	3.4	19.8	19.9
Merrit	13.8	13.2	3.3	3.2	3.6	3.4	20.7	19.9
Pardina	13.4	13.6	2.8	2.8	3.2	3.2	19.4	19.6
Pennell	13.8	13.8	3.8	3.9	3.7	3.5	21.3	21.3
Richlea	13.7	13.6	3.3	3.1	3.1	3.1	20.1	19.8
Cedar	13.0	11.5	3.1	3.1	3.1	3.0	19.1	17.6
Crimson	15.0	14.5	2.9	2.9	3.2	2.8	21.1	20.1
Morton	14.3	13.1	2.6	2.8	3.0	3.0	19.9	18.9
Mean	13.8	13.4	3.1	3.1	3.3	3.3	20.2	19.8
P-value	0.07		0.93		0.79		0.12	

[a]Values are expressed as percent of dry weight; dietary fiber content between two locations (Pullman and Fairfield) are tested with F-test. Abbreviations: IDF: insoluble dietary fiber; SDF: soluble dietary fiber; OLIGO: total oligosaccharides (raffinose + stachyose + verbascose); TDF: total dietary fiber (IDF + SDF + OLIGO). [b]P-value for significant of the F-test in the analysis of variance.

4.3.5 Discussion

Our laboratory has proposed that increasing pulse consumption in countries such as the United States is a practical way to resolve the dietary fiber gap. Lentil has valuable nutritional characteristics. In addition to being high in dietary fiber, lentil is high in protein and contains very low fat (80). Mineral and vitamin content in lentil are also high. Moreover, lentils have the highest phenolic component content among pulse crops (80), which suggests that they may have specific health benefits in addition to their nutritive value. These characteristics of lentil strengthen its importance for regular consumption in addition to its potential role in resolving the dietary fiber gap.

While FAO data indicate that lentil is one of the widely consumed pulse crops (81), there are only limited reports of the dietary fiber content of this crop. Our results show the values of IDF, SDF, total oligosaccharides and TDF among lentil cultivars were 20.1%, 13.6%, 3.2% and 3.3%, respectively. For oligosaccharides, the average values of raffinose, stachyose and verbascose were 0.3%, 2.0% and 1.0%, respectively. The value for TDF is similar to that reported by de Almeida Costa et al. (79) using method AOAC 1975; however, when comparing the content of individual components, differences among results become evident. Whereas, 19% for IDF and 1.4% for SDF were reported by using AOAC 1975 method, our results were 13.6% for IDF and 3.6% for SDF. The differences were as high as 33% for IDF and 88% for SDF by percentage. The portion of each component in dietary fiber has large differences, which is mainly because of the different definitions and different components measured. When calculating TDF by serving size, our results indicate that one serving of lentil contains an average of 6.0 g dietary fiber. Significant differences in the content of TDF, SDF and IDF were observed among different lentil cultivars. The TDF difference between the highest content cultivar and the lowest was 2.9 g per 100 g dry weight which is a difference of 14.6%. Statistically, the differences in TDF, SDF and IDF content among different lentil cultivars are significant, which suggest that dietary fiber content may be altered by breeding and selection. It is notable that the content of total oligosaccharides did not show large variation among lentil cultivars. These results suggest that oligosaccharides maybe more difficult to increase in a breeding program. The most feasible way to increase oligosaccharides content in lentil is to affect stachyose content.

Gene-environmental interactions are an important factor of consideration in breeding for specific characteristics. Our results indicate that environment has limited impact on dietary fiber content. However, additional experiments are needed under more diverse growing conditions in order to test more vigorously whether or not environment imposes effects on the expression of genes that regulate fiber synthesis.

Marked difference in dietary fiber content were observed among different lentil market classes, although caution is warranted in interpreting this data since there were an unequal number of cultivars in each market class. Nonetheless, among the six market classes into which lentils were grouped, large green and zero tannin differed in TDF by 12.5%. From the perspective of consumers interested in increasing dietary fiber intake, the cultivar Pennell could be marketed for superior dietary fiber content.

4.3.6 Summary

The average total dietary fiber content for lentil cultivars is 20.1%. Among different lentil cultivars and market classes, dietary fiber content was different. Six market classes of lentil had been tested, among which the highest dietary fiber content market class is large green and the lowest is zero tannin. Location has limited effect on dietary fiber content. Correlations exist among raffinose, stachyose and verbascose. For lentil, genetic factors have a higher impact than environmental effects. The dietary fiber content determined by AOAC 2011.25 method is higher than those reported using older methods. It is important to advocate the use of the method for lentil dietary fiber content analysis as work in this field progresses.

CHAPTER 5

DIETARY FIBER CONTENT OF CHICKPEA SAMPLES

5.1 Introduction

Chickpea is a widely consumed pulse crop. World consumption of chickpea is estimated to be 14.2 million MT (81). There are two different types of chickpea, Desi and Kabuli. The Desi type of chickpea has pink flowers, anthocyanin pigmentation on stems, and a colored and thick seed coat. The Kabuli type has white flowers, lacks anthocyanin pigmentation on stems, and has white or beige-colored seeds with a ram's head shape (82). Compared to Kabuli chickpea, Desi seed color is darker and seeds are smaller. Desi types are mostly grown in Asia and Africa while Kabuli types are usually grown in West Asia, North Africa, North America and Europe. As a food, Desi chickpea is typically split to make stew and Kabuli chickpea is eaten whole or processed for making hummus. We focused on Kabuli chickpeas in the work reported herein.

Chickpea contains a high concentration of protein, fiber, carbohydrate and a low concentration of lipid. Chickpea is a good source for vitamins and minerals and contains phytochemicals like carotenoids and flavonoids which can provide many human health benefits (83). The impact of chickpea consumption has been reported to include the regulation of chronic vascular diseases such as cardiovascular disease, blood pressure and diabetes risk reduction, weight maintenance, and cancer prevention (84).

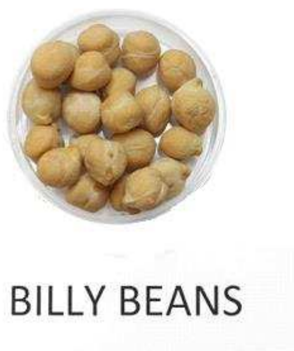
Dietary fiber content of chickpea has been studied using traditional methods. Giovana et.al. (79) employed AOAC 1975 to determine the total dietary fiber content and found that the TDF of chickpea is about 13.9% and the SDF is close to zero. Dalgetty and Baik (2003) (85) applied the AOAC 2001 method to estimate the fiber content of chickpea and found that the TDF ranged from 18.0 to 22.0% (86).

To the best of our knowledge, there is no application of the AOAC 2011.25 method for measuring dietary fiber content in chickpea, which was established based on the new consensus definition of dietary fiber. Due to the health benefits of dietary fiber and the economic value of chickpea, the accurate determination of dietary fiber contents of this pulse crop is of importance. The purpose of the current work, therefore, was to carry out a systematic measurement of dietary fiber content of different cultivars of chickpea by employing the AOAC 2011.25 method.

5.2 Sample description

Twenty-four cultivars of Kabuli chickpea, which were harvested from two locations, Pullman and Dayton, WA, were tested in this experiment. Within each location, two replicates of the same cultivar were collected from different field at each location. According to the USDA National Agricultural Statistics Service, Kabuli chickpeas can be divided into two categories based on the diameter of the seeds: Small, with a diameter <7.9 mm, and Large, with a diameter >7.9 mm. For example, Sierra is a "Large" Kabuli chickpea and it is the dominant cultivar in the U.S. CDC-Frontier and Billybean are the mostly used in making hummus in U.S, and they belong to the "Small" Kabuli category (87). The photographs of the twenty-four cultivars of chickpea evaluated are shown in Figure 5.1.

Kabuli


BILLY BEANS


CAO0690B0250C


CAO690B0427C


CAO790B0034C


CAO790B0042C


CAO790B0043C

CAO790B0053C

CAO790B0054C

CAO790B0547C

CAO790B0549C

CAO790B0642C

CAO790B0733C

CAO890B0085W

CAO890B0429C

CAO890B0434C

CAO890B0496C

CAO890B0531C

CAO890B0551C

CAO890B0628W

CAO890B0648W

CAO4900843C

CDC-FRONTIER

Figure 5.1 Seed types of chickpea cultivars evaluated

5.3. Results

5.3.1 Dietary fiber content and the cultivars

The content of dietary fiber in chickpea cultivars, including IDF, SDF, raffinose, stachyose, verbascose and total oligosaccharides are shown in Table 5.1, Figures 5.2 and 5.3. IDF content accounted for about 70% of TDF. Based on F-test IDF content among cultivars did not differ (P = 0.498) and ranged from 14.4 to 17.1% with mean 15.8%. CDC-Frontier had the highest IDF content (17.1%) and CAO890B0085W (14.4%) had the lowest. SDF content for chickpea differed among cultivars (P < 0.001) and ranged from 2.0 to 5.8% with a mean of 3.5%. CA0790B0042C had the highest SDF content (5.8%) and CAO790B0053C (2.0%) had the lowest. Total oligosaccharide content was different among cultivars (P < 0.001) and ranged from 1.0 to 3.5% with a mean of 2.5%. The cultivar with the highest oligosaccharide content was Sierra (3.5%) and CA0890B0551C had the lowest (1.0%). When individual components of total oligosaccharides were considered, raffinose content among cultivars was different (P < 0.001). The raffinose content for chickpea cultivars ranged from 0.1 to 0.9% with mean 0.6%. Stachyose content for chickpea samples differ by cultivar (P < 0.001). Mean stachyose content was 1.8% and ranged from 0.7 to 2.4%. Among chickpea cultivars, verbascose content differed (P < 0.001). The mean verbascose content was 0.2% and ranged from 0.1 to 0.3%. Stachyose content (1.8%) was markedly higher than raffinose (0.6%) and verbascose (0.2%) in all chickpea cultivars. The TDF content for chickpea samples differed among cultivars (P < 0.001) and ranged from 19.5 to 24.9% with mean 21.8%. CAO790B0034C had the highest TDF content (24.9%) while CAO890B0496C had the lowest (19.5%). The percentage difference between these two varieties was 24.3%.

Table 5.1 Insoluble dietary fiber (IDF), soluble dietary fiber (SDF), raffinose, stachyose, verbascose, total oligosaccharides and total dietary fiber (TDF) among chickpea cultivars

Entry	**IDF**[a]	**SDF**[a]	Raffinose	Stachyose	Verbascose	**OLIGO**[a]	**TDF**[a]
Billy beans	15.4	3.2 ^{EFG}	0.8 ^{ABC}	2.4 ^{A}	0.1 ^{HIJ}	3.2 ^{AB}	21.8 ^{BCDEFG}
CA04900843C	16.9	3.4 ^{DEF}	0.6 ^{CDE}	2.3 ^{AB}	0.2 ^{EFGHI}	3.1 ^{ABC}	23.4 ^{ABC}
CA0690B0427C	15.1	5.8 ^{A}	0.4 ^{FGH}	1.2 ^{HIJ}	0.2 ^{ABCD}	1.9 ^{GH}	22.8 ^{ABCDEF}
CA0690B250C	16.7	3.3 ^{DEFG}	0.7 ^{ABCD}	2.2 ^{ABC}	0.1 ^{FGHIJ}	3.1 ^{ABC}	23.2 ^{ABCD}
CA0790B0034C	16.5	5.7 ^{A}	0.6 ^{CDE}	2.0 ^{ABCDEFG}	0.1 ^{HIJ}	2.7 ^{ABCDEF}	24.9 ^{A}
CA0790B0042C	15.9	5.8 ^{A}	0.7 ^{BCDE}	2.0 ^{ABCDEFG}	0.2 ^{BCDEF}	2.8 ^{ABCDE}	24.5 ^{A}
CA0790B0043C	15.6	2.0 ^{I}	0.9 ^{AB}	2.3 ^{AB}	0.1 ^{GHIJ}	3.3 ^{AB}	21.0 ^{DEFG}
CA0790B0053C	15.0	2.0 ^{I}	0.9 ^{ABC}	2.0 ^{ABCDEF}	0.1 ^{J}	3.0 ^{ABC}	19.9 ^{G}
CA0790B0054C	15.6	2.4 ^{HI}	0.7 ^{ABCDE}	2.1 ^{ABCDE}	0.1 ^{IJ}	2.9 ^{ABCD}	20.9 ^{DEFG}
CA0790B0547C	15.6	3.2 ^{EFG}	0.4 ^{FGHI}	1.5 ^{EFGHI}	0.2 ^{ABCDEF}	2.1 ^{DEFGH}	20.9 ^{EFG}
CA0790B0549C	15.8	3.2 ^{EFG}	0.5 ^{EFG}	1.7 ^{BCDEFGH}	0.3 ^{A}	2.5 ^{BCDEFG}	21.4 ^{CDEFG}
CA0790B0642C	16.7	2.8 ^{GH}	0.4 ^{FGHI}	1.5 ^{EFGHI}	0.2 ^{CDEFG}	2.1 ^{EFGH}	21.5 ^{CDEFG}
CA0790B0733C	15.0	3.0 ^{EFG}	0.3 ^{FGHI}	1.3 ^{HIJ}	0.2 ^{ABCDE}	1.8 ^{GH}	19.8 ^{G}
CA0890B0085W	14.4	3.3 ^{DEF}	0.3 ^{GHI}	1.5 ^{FGHI}	0.2 ^{DEFGH}	1.9 ^{FGH}	19.6 ^{G}
CA0890B0429C	15.9	2.9 ^{FGH}	0.5 ^{EFG}	1.4 ^{GHI}	0.2 ^{CDEFGH}	2.1 ^{DEFGH}	20.9 ^{DEFG}
CA0890B0434C	16.0	3.0 ^{FG}	0.5 ^{DEF}	1.6 ^{CDEFGHI}	0.2 ^{ABCD}	2.4 ^{CDEFGH}	21.3 ^{CDEFG}
CA0890B0496C	14.7	3.0 ^{FG}	0.4 ^{FGHI}	1.3 ^{HIJ}	0.2 ^{ABCDE}	1.9 ^{FGH}	19.5 ^{G}
CA0890B0531C	16.2	2.9 ^{FG}	0.2 ^{HI}	1.1 ^{IJ}	0.2 ^{ABC}	1.6 ^{HI}	20.7 ^{FG}
CA0890B0551C	15.5	3.8 ^{CD}	0.1 ^{I}	0.7 ^{J}	0.1 ^{GHIJ}	1.0 ^{I}	20.3 ^{G}
CA0890B0628W	14.6	4.4 ^{B}	0.3 ^{FGHI}	1.5 ^{DEFGHI}	0.2 ^{ABC}	2.1 ^{DEFGH}	21.1 ^{DEFG}
CA0890B0648W	15.7	4.3 ^{BC}	0.5 ^{EFG}	2.1 ^{ABCD}	0.2 ^{CDEFGH}	2.8 ^{ABCDE}	22.8 ^{ABCDEF}
CDC-Frontier	17.1	3.6 ^{DE}	0.8 ^{ABC}	2.2 ^{ABC}	0.2 ^{CDEFG}	3.2 ^{AB}	23.8 ^{AB}
Sawyer	16.6	3.4 ^{DEF}	0.9 ^{A}	2.3 ^{AB}	0.2 ^{ABC}	3.4 ^{A}	23.4 ^{ABC}
Sierra	16.1	3.5 ^{DEF}	0.9 ^{A}	2.3 ^{AB}	0.3 ^{AB}	3.3 ^{A}	23.1 ^{ABCDE}
Overall Mean	15.8	3.5	0.6	1.8	0.2	2.5	21.8
P-value	0.498	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[a]Abbreviations: IDF: insoluble dietary fiber; SDF: soluble dietary fiber; OLIGO: total oligosaccharides (raffinose + stachyose + verbascose); TDF: total dietary fiber (IDF + SDF + OLIGO). Values are expressed as percent of dry weight; Means followed by same letters are not significantly different at P<0.05. Mean separations were performed using Tukey's honestly significant difference test. [b]For significant of the F-test in the analysis of variance.

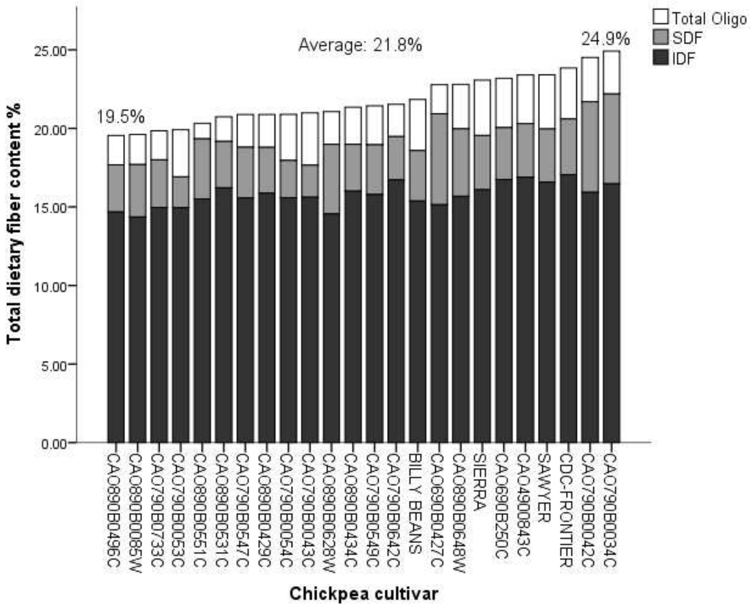


Figure represents dietary fiber content among chickpea cultivars. Values are expressed by percent of dry weight. Abbreviations for the legends: IDF = insoluble dietary fiber; SDF = soluble dietary fiber, Total Oligo = total oligosaccharides.

Figure 5.2 Mean percentage of IDF, SDF, total oligosaccharides and TDF among chickpea cultivars

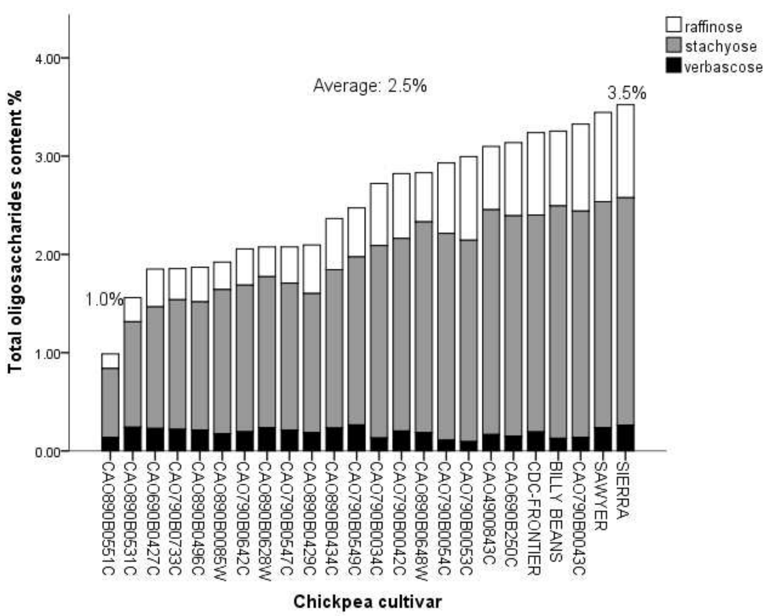


Figure represents oligosaccharides content among chickpea cultivars. Values are expressed by percent of dry weight.

Figure 5.3 Mean percentage of raffinose, stachyose, verbascose and total oligosaccharides among chickpea cultivars

5.3.2 Correlations among different components of dietary fiber

Pearson correlation analysis was conducted to identify the possible associations among dietary fiber components. The results are shown in Table 5.2. Raffinose had strong positive association with stachyose, while IDF had a positive association with raffinose, stachyose and total oligosaccharides. The biosynthesis pathway for raffinose family of oligosaccharides (RFO's) is raffinose to stachyose to verbascose and it's reversible (70). From the correlation test, similarly with dry pea, for chickpea cultivars, the higher raffinose content will lead higher stachyose and lower verbascose.

Table 5.2 Pearson correlations among all chickpea samples

Correlations	IDF	SDF	Raffinose	Stachyose	Verbascose	Total Oligo
IDF	1					
SDF	-0.01					
Raffinose	0.26*	-0.17				
Stachyose	0.25*	-0.11	0.88**			
Verbascose	-0.05	0.16	-0.16	-0.11		
Total Oligo	0.26*	-0.12	0.94**	0.99**	-0.05	1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Abbreviations: IDF: insoluble dietary fiber, SDF: soluble dietary fiber, Total Oligos: total oligosaccharides

5.3.3 The influence of location

The effect of the location of production on dietary fiber content was also studied. Samples from two locations, namely Pullman and Dayton, WA, were compared (Table 5.3). In general there was no difference in mean IDF, SDF, total oligosaccharides and TDF content among cultivars between two locations from which chickpea cultivars were harvested.

Table 5.3 IDF, SDF, total oligosaccharides, and TDF between two locations of samples

Cultivar	IDF[a]		SDF[a]		Total Oligo[a]		TDF[a]	
Location	Pullman	Dayton	Pullman	Dayton	Pullman	Dayton	Pullman	Dayton
SIERRA	15.7	16.5	3.5	3.3	3.5	3.5	22.7	23.4
SAWYER	15.9	17.3	3.4	3.3	3.5	3.4	22.8	24.0
CDC-FRONTIER	15.8	18.3	3.6	3.5	3.1	3.4	22.5	25.2
BILLY BEANS	14.5	16.3	3.4	3.0	3.3	3.3	21.1	22.6
CAO4900843C	17.4	16.4	3.6	3.2	3.2	3.0	24.2	22.6
CAO690B250C	16.7	16.8	3.3	3.3	3.1	3.2	23.1	23.4
CAO690B0427C	16.0	14.2	5.7	5.9	2.4	1.3	24.2	21.5
CAO790B0034C	16.2	16.8	6.0	5.4	2.4	3.1	24.6	25.2
CAO790B0042C	16.7	15.2	5.8	5.7	2.7	2.9	25.2	23.9
CAO790B0043C	14.8	16.5	2.1	2.0	3.2	3.5	20.1	21.9
CAO790B0053C	16.1	13.8	2.0	1.9	2.9	3.1	21.1	18.8
CAO790B0054C	16.2	15.0	2.5	2.3	3.0	2.9	21.6	20.3
CAO790B0547C	15.5	15.6	3.0	3.5	1.8	2.3	20.3	21.5
CAO790B0549C	14.2	17.4	2.9	3.5	2.0	3.0	19.1	23.8
CAO790B0642C	17.1	16.4	2.6	2.9	2.3	1.8	22.0	21.1
CAO790B0733C	14.5	15.4	3.0	3.0	2.0	1.8	19.5	20.2
CAO890B0429C	15.5	16.3	2.8	3.0	1.7	2.5	19.9	21.8
CAO890B0085W	15.3	13.4	3.2	3.5	2.1	1.8	20.5	18.7
CAO890B0434C	15.8	16.2	3.2	2.8	2.4	2.4	21.4	21.3
CAO890B0496C	15.7	13.7	3.0	3.0	2.8	0.9	21.5	17.6
CAO890B0531C	17.9	14.5	3.0	2.9	0.9	2.2	21.9	19.6
CAO890B0551C	16.1	14.9	3.8	3.9	1.6	0.4	21.4	19.2
CAO890B0628W	13.8	15.3	4.1	4.8	2.2	1.9	20.1	22.1
CAO890B0648W	15.8	15.6	4.6	4.0	2.9	2.8	23.3	22.4
Mean	15.9	15.5	3.5	3.5	2.4	2.3	21.8	21.3
P-value[b]	0.86		0.95		0.88		0.83	

[a]Values are expressed as percent of dry weight; dietary fiber content between two locations (Pullman and Fairfield) are compared with F-test. Abbreviations: IDF: insoluble dietary fiber; SDF: soluble dietary fiber; OLIGO: total oligosaccharides (raffinose + stachyose + verbascose); TDF: total dietary fiber (IDF + SDF + OLIGO. [b]P-value for significant of the F-test in the analysis of variance.

5.3.4 Discussion

Chickpea is one of the oldest domesticated legumes which originated in the Middle East. India produces the most chickpea, globally, which accounts for about 67% of the production (88). However, in India and in other developing countries, the development of food technology and its associated effect in changing the pattern of food consumption is resulting in a higher risk of chronic diseases such as type-2 diabetes.

Consumption trends reveal a decrease in pulse crop consumption in India since the 1990s (89). The same pattern has also occurred in United States and globally. This raises the possibility of a causal relationship between decreased pulse consumption and the global pandemic of obesity, and the related diseases, i.e., cardiovascular disease, type-2 diabetes, and cancer, whose prevalence has also increased over the same time interval. The identification of a causal link might relate to dietary fiber intake. Thus accurate data on the fiber content of chickpeas and other pulse crops is essential.

Our experiment measured the dietary fiber content of twenty-four chickpea cultivars, including IDF, SDF, raffinose, stachyose, verbascose and the total oligosaccharides by AOAC 2011.25. The average values of TDF, IDF, SDF and the total oligosaccharides among chickpea cultivars were 21.8%, 15.8%, 3.5% and 2.5%, respectively. For oligosaccharide content, the average values of raffinose, stachyose and verbascose were 0.6%, 1.8% and 0.2%, respectively. Compared with the older method AOAC 2001, in which chickpea dietary fiber was reported to be 18 to 22% (86), our results ranged from 19.5 to 24.9%. There is about 13% difference in dietary fiber content between these two different methods. Once again, it suggests that older methods underestimated dietary fiber content in comparison to AOAC 2011.25. Based on the data reported herein, one serving of chickpea contains an average of 7.3g of dietary fiber. Accordingly, consumption of two servings of chickpea/d could resolve the current dietary fiber gap. There is a 24.7% difference in TDF content between the highest chickpea cultivar and the lowest. This magnitude of difference supports the feasibility of breeding and selection to increase dietary fiber content. For individual components, SDF and total oligosaccharides content were different by cultivar, but IDF was not. This was surprising since there was considerable variation in IDF among cultivars of other pulse crops that we have evaluated. It is possible that such lack of variation in chickpea can be explained via comparative genomics since the complete genetic sequences of various pulse crops are now being reported.

A significant difference in oligosaccharide content among chickpea cultivars was also observed in this study. Comparing the highest oligosaccharide content cultivar with the lowest, there was almost a four-fold difference (Table 5.2). This result suggests that it would be feasible to alter the total oligosaccharide content of chickpea using either traditional breeding approaches or emerging gene editing technologies. Similar to dry pea and lentil, the dietary fiber content of chickpea was not statistically different between two locations, which indicate that environment has limited impact on dietary fiber content. Nonetheless, additional experiments are needed using more diverse growing conditions in order to establish the magnitude of effect that environment imposes on the expression of genes that regulate dietary fiber synthesis.

5.3.5 Summary

The average total dietary fiber content for chickpea is 21.8%. Among different chickpea cultivars, dietary fiber content was significantly different. Oligosaccharides content for chickpea has large variation which is up to 3.5 fold. Current work indicates that location has a limited effect on dietary fiber content. Genetic factors have a large impact on dietary fiber content. The dietary fiber content tested by AOAC 2011.25 method is higher than those tested by older methods. It is important to promote the use of AOAC 2011.25 method for chickpea dietary fiber content for encouraging work in the field.

CHAPTER 6

PULSE CROP SUMMARY: COMMON BEAN, PEA, LENTIL AND CHICKPEA

Dietary fiber is an important non-nutritive component of food with various health benefits. In many countries, such as the United States and Canada, the intake of dietary fiber is 50% to 70% below the recommended levels in greater than 95% of the population. Pulse crops, which have of high protein and low fat content can provide two to three times more dietary fiber per 100 g edible portion than cereal grains which are frequently advertised as a key source of dietary fiber. It has been advanced recently (90) that the lack of pulse consumption is a significant contributor to the dietary fiber gap and that increasing pulse crop consumption in the daily diet is a potential approach to resolve the dietary fiber intake gap.
Of the seventeen pulses recognized by FAO, chickpea, common bean, dry pea and lentil are the most widely consumed pulse crops globally and are both affordable and accessible to the majority of consumers. It is of specific importance and interest to compare the fiber content of these four commonly consumed pulse crops.

6.1 Total dietary fiber contents among different pulse crops

Table 6.1 shows the mean values among pulse crops for total dietary fiber content and its principal components, namely insoluble dietary fiber, soluble dietary fibers and total oligosaccharides (i.e. raffinose + stachyose + verbascose). Among them, the data for common bean samples were evaluated by Dimas Echeverria of our laboratory by analyzing 26 common bean entries which belong to the market classes commonly grown in North America. All four pulse crops have high content of total dietary fiber. Common bean and dry pea have the highest TDF value, which is higher than chickpea and lentil.
Besides using the unit g dietary fiber/100g dry weight, the units of g/100kcal and g/serving were also calculated and evaluated. The same ranking can be observed from the perspective of TDF per 100 kcal.

However, when comparing TDF per serving (half cup), the sequence becomes different. Usually, g/serving is used in daily life to calculate total dietary fiber intake (Table 6.1). Common bean and chickpea contain more dietary fiber than dry pea and lentil per serving.

Table 6.1 Dietary fiber content of four pulse crops

Crop	IDF[a]	SDF[a]	OLIGO[a]	TDF[a]	TDF[a] g/serving	TDF[a] g/ 100 kcal
Chickpea	15.8 [A]	3.5 [A,B]	2.5 [C]	21.8 [B]	7.1	5.3
Common bean	13.9 [B]	7.7 [A]	4.2 [A]	25.8 [A]	8.1	6.6
Dry pea	16.2 [A]	3.9 [A,B]	4.6 [A]	24.7 [A]	4.3	6.4
Lentil	13.6 [B]	3.1 [B]	3.3 [B]	20.0 [C]	6.0	5.2
p-value[b]	<0.001	<0.001	<0.001	<0.001		

[a]Abbreviations: IDF: insoluble dietary fiber; SDF: soluble dietary fiber; OLIGO: total oligosaccharides (raffinose + stachyose + verbascose); TDF: total dietary fiber (IDF+SDF+OLIGO). [b]Values are expressed as percent of dry weight; means with the same letters are not significantly different at P<0.05. Turkey's honestly significant difference test was applied for mean separation.

Table 6.2 Range of dietary fiber content (%) among pulse crop cultivars

Crop	IDF[a]	SDF[a]	OLIGO[a]	TDF[a]
Chickpea	14.4 to 17.1	2.0 to 5.8	1.0 to 3.5	19.5 to 24.9
Common bean	12.3 to 15.7	5.8 to 9.8	3.6 to 5.2	24.1 to 27.4
Dry pea	14.2 to 19.8	3.3 to 5.3	4.0 to 5.4	22.3 to 28.0
Lentil	12.3 to 14.7	2.7 to 3.9	3.0 to 3.7	18.4 to 21.3

[a]Abbreviations: IDF: insoluble dietary fiber; SDF: soluble dietary fiber; OLIGO: total oligosaccharides (raffinose + stachyose + verbascose); TDF: total dietary fiber (IDF+SDF+OLIGO). Values are expressed as percent of dry weight.

Table 6.2 summarized the variation in TDF among four most commonly consumed pulse crops. Dry pea, lentil, common bean (90) and chickpea samples differ in fiber content. The magnitude of variation among different cultivars suggests the value of adding food labels for cultivar identities, to provide consumers with more guidance on fiber content among cultivars. Based on data presented in Table 6.2, calculations were performed to reveal that high fiber cultivars can increase total fiber intake by as much as 30% when the same amount of the crop is consumed. This significantly increases the feasibility of reaching the recommended level of dietary fiber consumption without needing to increase caloric intake.

Compared with the data on fiber content determined by older methods, the results from the new method AOAC 2011.25 are higher in amount and in how dietary fiber is evaluated among its components. According to the previous studies (91), the dietary fiber content determined by method AOAC 991.43 for common bean, chickpea, lentil and dry pea are 5.4, 6.2, 5.9 and 10.6%, respectively. The enzymatic-gravimetric method used by Schakel et al., (92) gives the result of 3.5g/100g for pea and 11.4g/100g for lentil. However, according to our results by employing the new method AOAC 2011.25, the total fiber contents of common bean, chickpea, lentil and dry pea are 25.8, 24.7, 20.08 and 21.8%, respectively, demonstrating that the total dietary fiber content for these pulse crops are higher than suggested by the older methods of analysis. Such a difference clearly indicates the importance of using fiber contents of the pulse crops determined using AOAC 2011.25.

Oligosaccharides content in pulse crops have some variations among cultivars. Among the pulse crops tested, oligosaccharide content in chickpea is markedly different among cultivars. This suggests that for people who are intolerant to oligosaccharides, it is possible to choose cultivar with lower oligosaccharide content. The correlations among different oligosaccharides components varied among different pulse crops and cultivars. Oligosaccharides components in dry pea and chickpea present are similarly correlated and the correlations differ from those observed in lentil. The correlation of three components, raffinose, stachyose and verbascose in oligosaccharides provides valuable understanding of the enzymatic pattern in biosynthesis pathway operation in pulse crops. However, genetic sequence data is needed to better understand these relationships.

One common question asked by scientists and consumers is whether specific components of dietary fiber should be emphasized, e.g., insoluble versus soluble dietary fiber, or oligosaccharides. The answer to this question has varied. The current consensus is that total fiber intake rather than consumption of a specific component is the most important consideration in terms of human health benefit (93;94).

Oligosaccharides are known to be associated with flatulence and have been considered to be an unfavorable component of dietary fiber. However, recent studies have shown that oligosaccharides can exert prebiotic effects, and thus their consumption is thought to be also beneficial (25).

6.2 Summary

In summary, the newly developed method AOAC 2011.25 was first applied to determine the total dietary fiber content of four pulse crops and the amount of each component of dietary fiber in commonly consumed pulse crops, namely dry pea, lentil and chickpea, and in previous work, common bean. The impacts of cultivar, market class, and environment on dietary fiber content were studied and the following conclusions can be drawn:

1. All four pulse crops tested have high dietary fiber content relative to some other food crops, e.g. corn or rice. Consumption of 2 to 3 servings intake of these pulse crops per day could close the dietary fiber gap.
2. IDF composes 70% of total dietary fiber content, and stachyose accounts for over half of oligosaccharide content for all four pulse crops.
3. In comparison with older methods, the AOAC 2011.25 method gives markedly higher values for TDF. Based on our data, it is clear that other methods underestimate and misclassify the amount of various fiber components present in pulse crops. It is important to promote the consensus of definition and method for dietary fiber analysis in not only pulse crops but other food crops as work in the field progresses.
4. While the environment has limited influence on the fiber content of the pulse crops tested, both cultivar and market classes had impact on the total dietary fiber content and some dietary fiber components, indicating that the genetic effect is a dominant factor in regulating dietary fiber content of pulse crops. The information about market class and genetics is important for consumers who are interested in increasing their consumption of dietary fiber and for plant breeders who are interested in developing cultivars with enhanced dietary fiber content.

5. People who are not tolerant to oligosaccharides usually have concerns about oligosaccharides content in pulse crops. Our results suggest that oligosaccharide content vary widely by cultivars. For example, there is a 3.5 fold difference in oligosaccharide content of chickpea. Knowledge of these differences not only provides guidance to interested consumers but also inform efforts to alter oligosaccharide content of pulse crops by breeding and selection.

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