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THE NUTRIENT AND PHYTONUTRIENT COMPOSITION OF
ONTARIO GROWN BEANS AND THEIR EFFECT ON
AZOXYMETHANE INDUCED COLONIC
PRENEOPLASIA IN RATS

by

Helen Anita Millie Samek

A thesis submitted in conformity with the requirements
for the degree of Masters of Science
Graduate Department of Nutritional Sciences
University of Toronto

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THE NUTRIENT AND PHYTONUTRIENT COMPOSITION OF
ONTARIO GROWN BEANS AND THEIR EFFECT ON
AZOXYMETHANE INDUCED COLONIC
PRENEOPLASIA IN RATS

Master of Science, 2001
Helen A. Samek
Graduate Department of Nutritional Sciences
University of Toronto

ABSTRACT

Beans have long been recognized for their nutritional quality. Epidemiological studies have shown that populations consuming substantial amounts of beans in their daily diets are at low risk of developing many chronic diseases including colon cancer. The first aim of this thesis was to investigate both the nutritional and phytonutrient composition of raw and processed Navy, Kidney and Fava beans. The nutrient and phytonutrient analysis of raw was in good agreement with published literature. The nutrient and phytonutrient content of canned beans, homogenized with the liquid content found in the can, appeared to be slightly higher than those beans that were not homogenized with the liquid contents, suggesting leaching. The second aim was to investigate the effects of canned navy bean consumption on the development of preneoplastic lesions in the colons of azoxymethane-treated F344 Fisher rats. The incidence of aberrant crypt foci (ACF) and aberrant crypts (AC), induced by azoxymethane in rats fed canned navy
beans showed significant reductions in the number and size compared to rats consuming a bean-free diet. This study demonstrates that Navy, Kidney and Fava beans contain anticarcinogenic phytonutrient compounds capable of inhibiting chemically-induced preneoplastic lesions in the rat colon.
ACKNOWLEDGEMENTS

I would like to extend my deepest appreciation and gratitude to my supervisor Dr. A. Venketeshwer Rao, whose endless support and supervision guided me through this thesis. Thank you for your continued patience, encouragement and for giving me the opportunity to see myself grow as a strong, capable individual.

I will forever be indebted to Dr. David Yeung for his expertise, his continued belief in my capabilities, his desire to see me succeed and above all for being such a good friend. Your have instilled the desire in me to reach for the stars.

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A big thank you to the H.J. Heinz Company of Canada and The Ontario Bean Growers Association for generously helping to fund this research project. I am also grateful to the University of Guelph for assisting me with some of my analysis.

I would like to thank my fellow graduate students Sujatha Chakravarthi and Linda Kim for their support when the hill seemed almost too big to climb, and to Debbie Gurfinkle whose insight always helped me see the brighter side of things. I would also like to thank all of the members of Dr. Rao’s Lab; Dr. Sanjiv Agarwal, Anita Agarwal, Nalini Shiwnarain, Judlyn Fernandes and Honglei Chen whose friendship was often at times a driving force.

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To Rose Ricupero, Nicole Dollo and Anna-lisa Masci. You have all been an endless pillar of strength. Your love and support continues to redefine the true meaning of friendship.

I want to thank my father whose zest for knowledge has always inspired me to be the best I can be. I have looked up to you from day one and you have continued to support all my endeavors with much support and understanding. You will always be a part of all my successes. I would also like to thank my mother and my brothers for their encouragement and support. Without your love, little would be possible.

To John Emantoilidis. You have been there through it ALL. Your belief that I can accomplish all that I desire has allowed me to continue through the toughest of times. I could not have done this without your everlasting encouragement, understanding and patience, your unwavering support, and above all, your love.
<table>
<thead>
<tr>
<th>TABLE OF CONTENTS</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>List of Tables</td>
<td>ix</td>
</tr>
<tr>
<td>List of Figures</td>
<td>xi</td>
</tr>
<tr>
<td>Publications Arising from this Thesis Research</td>
<td>xiii</td>
</tr>
<tr>
<td>CHAPTER 1: INTRODUCTION</td>
<td></td>
</tr>
<tr>
<td>1.1 Introduction</td>
<td>2</td>
</tr>
<tr>
<td>CHAPTER 2: HYPOTHESIS AND OBJECTIVE</td>
<td></td>
</tr>
<tr>
<td>2.1 Rationale</td>
<td>8</td>
</tr>
<tr>
<td>2.2 Hypothesis</td>
<td>9</td>
</tr>
<tr>
<td>2.3 Objectives</td>
<td>10</td>
</tr>
<tr>
<td>2.3.1 Overall Objective</td>
<td>10</td>
</tr>
<tr>
<td>2.3.2 Specific Objectives</td>
<td>10</td>
</tr>
<tr>
<td>CHAPTER 3: LITERATURE REVIEW</td>
<td></td>
</tr>
<tr>
<td>3.1 History</td>
<td>12</td>
</tr>
<tr>
<td>3.2 Patterns of Bean Consumption</td>
<td>13</td>
</tr>
<tr>
<td>3.3 Nutrient Profile</td>
<td>14</td>
</tr>
<tr>
<td>3.3.1 Macronutrient Composition</td>
<td>15</td>
</tr>
<tr>
<td>3.3.1.1 Protein</td>
<td>18</td>
</tr>
<tr>
<td>3.3.1.2 Carbohydrate</td>
<td>19</td>
</tr>
<tr>
<td>3.3.1.3 Fat</td>
<td>21</td>
</tr>
<tr>
<td>3.3.2 Micronutrient Composition</td>
<td>22</td>
</tr>
</tbody>
</table>
3.3.2.1 Vitamins
3.3.2.2 Minerals

3.4 Health Benefits of Beans
3.4.1 Coronary Heart Disease
3.4.2 Diabetes Mellitus
3.4.3 Obesity
3.4.4 Cancer

3.5 Health Benefits of Phytonutrients
3.5.1 Phytic Acid
3.5.2 Saponins
3.5.3 Oligosaccharides
3.5.4 Trypsin Inhibitors
3.5.5 Lectins
3.6 Summary

CHAPTER 4: PROXIMATE ANALYSIS

4.1 Introduction
4.2 Objective
4.3 Materials and Methods
4.3.1 Dry Bean Samples
4.3.2 Dry Bean Preparation
4.3.3 Nutrient Determination
4.4 Results
LIST OF TABLES

Table 1.1 Legume Classification and their Botanical and Common Name 5
Table 3.3.1 Nutrient Content of Selected Cooked Beans 16
Table 3.3.2.1 Vitamin Content of Selected Cooked Beans 23
Table 3.3.2.2 Mineral Content of Selected Cooked Beans 25
Table 4.4.1 Proximate Composition of Raw and Canned Navy Beans 54
Table 4.4.2 Proximate Composition of Raw and Canned Kidney Beans 55
Table 4.4.3 Proximate Composition of Raw and Canned Fava Beans 56
Table 4.4.4 Proximate Composition of Time-Treated Cooked Navy Beans 58
Table 4.4.5 Total Dietary Fiber Content in Raw and Canned Navy, Kidney and Fava Beans 62
Table 4.4.6 Total Dietary Fiber Content in Raw and Canned Navy, Kidney and Fava Beans as reported by USDA Handbook No.8 62
Table 4.4.7 Total Folate Content in Raw and Canned Navy, Kidney and Fava Beans 64
Table 4.4.8 Total Folate Content of Time-Treated Cooked Navy Beans 64
Table 5.3.1 Phytonutrient Composition of Raw and Canned Navy Beans 85
Table 5.3.2 Phytonutrient Composition of Raw and Canned Kidney Beans 86
Table 5.3.3 Phytonutrient Composition of Raw and Canned Fava Beans 87
Table 5.3.4 Phytonutrient Composition of Time-Treated Cooked Navy Beans 88
Table 6.3.1 Macronutrient Composition of Experimental Diets 105
Table 6.3.2 Micronutrient Composition of Experimental Diets 106
Table 6.4.1  Effects of Dry Bean Consumption on Body Weight, Food Intake, Protein Efficiency and Food Efficiency

Table 6.4.2  Mean Daily Food and Protein Intake

Table 6.4.3  Effect of Dry Bean Consumption on AOM-Induced ACF in F344 Fisher Rat Colon
NOTE TO USER

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Xi

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### LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3.1</td>
<td>Comparison of Macronutrient Content of Oilseed Legumes (Soy Bean) and Grain Legumes (Kidney Beans)</td>
<td>17</td>
</tr>
<tr>
<td>4.4.1</td>
<td>Percentage Decrease of Proximate Content from Raw Navy Bean Seeds at Various Cooking Intervals</td>
<td>59</td>
</tr>
<tr>
<td>4.6.1</td>
<td>Percentage of Recommended Daily Intake of Macronutrients, Fiber and Folate from One-Cup Serving of Canned Navy Beans</td>
<td>73</td>
</tr>
<tr>
<td>4.6.2</td>
<td>Percentage of Recommended Daily Intake of Macronutrients, Fiber and Folate from One-Cup Serving of Canned Kidney Beans</td>
<td>74</td>
</tr>
<tr>
<td>4.6.3</td>
<td>Percentage of Recommended Daily Intake of Macronutrients, Fiber and Folate from One-Cup Serving of Canned Fava Beans</td>
<td>75</td>
</tr>
<tr>
<td>5.3.1</td>
<td>Percentage Reduction of Phytonutrient Content of Canned Navy Beans in Tomato Sauce and Without Tomato Sauce From Raw Bean Seed</td>
<td>91</td>
</tr>
<tr>
<td>5.3.2</td>
<td>Percentage Reduction of Phytonutrient Content of Canned Kidney Beans in Water and Without Water From Raw Bean Seed.</td>
<td>92</td>
</tr>
<tr>
<td>5.3.3</td>
<td>Percentage Reduction of Phytonutrient Content of Canned Fava Beans in Water and Without Water From Raw Bean Seed.</td>
<td>93</td>
</tr>
<tr>
<td>5.3.4</td>
<td>Percentage Reduction of Phytonutrient Content of Time-Treated Cooked Navy Beans From Raw Bean Seed.</td>
<td>94</td>
</tr>
<tr>
<td>6.4.1</td>
<td>Changes in Fecal Output Between Bean-fed Diets and Control-fed Diets Over Time</td>
<td>114</td>
</tr>
<tr>
<td>6.4.2</td>
<td>Percent ACF Decrease in Bean-fed Animals from Controls</td>
<td>117</td>
</tr>
</tbody>
</table>
PUBLICATIONS ARISING FROM THESIS RESEARCH


CHAPTER 1

- INTRODUCTION -
1.1 INTRODUCTION

Beans, which are categorized as legumes, have been cultivated for thousands of years and have played an important role in traditional diets throughout the world (Messina, 1999; Deshpande et al., 1984). Bean crops have been especially important as a staple in developing areas like Africa, Latin America and Asia, providing an economical source of energy and high quality protein (Geil and Anderson, 1994; Gupta, 1983). However, in societies where the consumption of meat is quite high, such as those found in Western civilizations, bean consumption is infrequent and in small amounts. Its position as a less significant component in the Canadian diet may be a result which label beans as the “poor man’s meat”, suggesting an inverse relation between bean intake and income (Messina, 1991). Other reasons for a lower consumption of beans may be related to long cooking times and social inconveniences. Unfortunately, the lack of consumer knowledge with regard to the nutritional content in beans, may also be responsible for its low intake.

Despite their minor dietary role in Canada, beans offer nutritional value matched by few other foods (Geil and Anderson, 1994). Recent dietary guidelines suggest that excessive fat intake, cholesterol, and sodium should be avoided. On a 100 kcal basis, beans (except oilseeds, i.e. soybeans and peanuts) contain 80% less total fat than lean ground beef and are low in sodium. They contain no cholesterol and are excellent sources of complex carbohydrates and fiber. Beans also provide a good source of vegetable protein, and vitamins and minerals. In addition, beans are less expensive than animal food products
and have a considerably longer shelf life than several animal, fruit and vegetable products when stored under appropriate conditions (Haytowitz et al., 1981; Sgarbiere, 1989). A number of epidemiological studies have shown that populations consuming substantial amounts of beans in their daily diets face a lower risk of developing many chronic diseases including cancer (Correa, 1981; Jain et al., 1999; Key et al., 1997; Mills et al., 1989).

More recently beans have been shown to contain several biologically active compounds, known as phytonutrients, which include trypsin inhibitors, phytohaemagglutinins, phytic acid, saponins, oligosaccharides and others (Anderson et al., 1979; Messina, 1999; Valdemiro et al., 1982). Although traditionally these compounds have been termed ‘antinutrients’ due to their effects on the absorption and utilization of nutrients, recent research indicates that these compounds may be responsible for producing some of the health benefits in beans which may reduce the risk of various chronic diseases faced by western civilizations (Kennedy, 1995; Messina, 1991; Thompson, 1993; Wang and Wixon, 1999).

Of all the legumes, the soybean is the one that has received the greatest amount of attention. The phytonutrients in soybean have been studied extensively, and provide much supporting evidence for the role of soybean phytonutrients in the prevention of cancer, heart disease, and other degenerative diseases (Anderson et al., 1999; Kushi et al., 1999; Second International Symposium on the role of Soy in Preventing and Treating Chronic Disease, 1996). However, the legume family consists of many other varieties of
beans, and recent studies indicate that grain legumes also contain several of the same phytonutrients that have been identified in soybean for their potential health benefits.

Legumes are divided into two separate and distinct categories; the oilseeds such as soybeans, primarily grown for their protein and oil content; and the grain legumes such as lentils or kidney beans which are grown for their protein contribution to the diet (Geil and Anderson, 1999; Sgarbieri, 1989). Table 1.1 shows the various types of beans and their botanical names.

Within Canada, Ontario leads in the production of beans. Many factors including soil characteristics, climatic conditions, agricultural practices, bean varieties and methods of processing can all influence the nutritional and phytochemical composition of these beans (Deshpande, 1984; Meiners et al., 1976). However, little information is available with regard to the nutritional and phytochemical contents in the beans grown in Ontario, and their effects on chronic disease.

Cancer is one of the leading causes of mortality in North America with colon cancer being the second most common cause of death (World Cancer Research Fund, 1997). The incidence and mortality rate due to colorectal cancer is rising with more than 870,000 new cases diagnosed worldwide since 1996 (World Cancer Research Fund, 1997). As a result, the primary goal in dealing with this epidemic has focused on dietary prevention strategies (Potter, 1996). The presence of trypsin inhibitors, lectins, phytic acid, oligosaccharides and saponins in soybeans have shown to be a contributor to
Table 1.1  Legume Classification and their Botanical and Common Name.

<table>
<thead>
<tr>
<th>Legume Classification</th>
<th>Botanical Name</th>
<th>Common Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oilseeds</td>
<td><em>Glycine max</em></td>
<td>Soybeans</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peanuts</td>
</tr>
<tr>
<td>Grain Legumes</td>
<td><em>Phaseolus vulgaris</em></td>
<td>Pinto beans</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Navy beans</td>
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<tr>
<td></td>
<td></td>
<td>Red kidney beans</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Black beans</td>
</tr>
<tr>
<td></td>
<td><em>Phaseolus lunatus</em></td>
<td>Lima beans</td>
</tr>
<tr>
<td></td>
<td><em>Cicer arietinum</em></td>
<td>Garbanzo beans (chickpeas)</td>
</tr>
<tr>
<td></td>
<td><em>Vigna unguiculata</em></td>
<td>Black-eyed bean</td>
</tr>
<tr>
<td></td>
<td><em>Vicia faba</em></td>
<td>Broad beans (fava beans)</td>
</tr>
</tbody>
</table>

1: Data obtained from USDA Nutrient Database for Standard Reference (Handbook No. 8).
reduced cancer risk in those individuals who consume them (Messina, 1999; Messina and Barnes, 1991). Based on the published literature relating to the physiological role of soybeans, there is convincing evidence to suggest that beans can play an important role in the prevention of cancer. Therefore it is hypothesized that increased knowledge regarding the nutritional and phytochemical composition of Ontario grown beans and the role of beans in cancer prevention will stimulate increased consumption and generate the development of new and innovative functional foods for markets by the food industry.
CHAPTER 2

- HYPOTHESIS AND OBJECTIVE -
2.1 RATIONALE

A number of epidemiological studies have shown that populations consuming substantial amounts of beans in their daily diets are at a lower risk of many chronic diseases that are characteristic of Western countries (Jain et al., 1999; Mills et al., 1989; World Cancer Research Fund, 1997). Biologically-active compounds known as phytonutrients present in soybeans, have been extensively studied for their role in preventing and treating various chronic diseases (Messina, 1999; Second International Symposium on the role of Soy in Preventing and Treating Chronic Disease, 1996). Studies have shown that common beans such as Kidney beans, Fava beans and Navy beans, among other varieties, also contain these phytonutrient that were identified in soy beans for their potential clinical application (Second International Symposium on the Role of Soy in Preventing and Treating Chronic Disease, 1996).

In Canada, Ontario is one of the major producers and processors of a wide variety of beans, yet the nutritional and phytonutrient composition have not been studied well. A number of factors such as soil characteristics, climatic conditions, agricultural practices, and processing methods can influence the nutritional and phytonutrient composition of these beans (Deshpande et al, 1984; Meiners, 1976; Meiners et al., 1976). In spite of the health benefits of common beans, very little information is available with regards to the composition of dry and processed Ontario grown beans. Once their nutrient and phytonutrient compositions are identified and their levels established, effective strategies
can be developed to increase their consumption in Canada and provide a means for the use of these beans in clinical application.

Colon cancer is a prevalent disease worldwide with rising incidence and mortality rates (World Cancer Research Fund, 1997). Among all cancers, it is one that is highly correlated with a diet typical of Western countries, i.e. high in saturated fat, and low in dietary fiber and plant foods (Potter, 1996; Correa, 1981). Therefore, dietary intervention may be beneficial. Based on the published literature relating to the physiological role of soybeans in cancer prevention, there is convincing evidence to suggest that common beans can play a role in the prevention of cancer development as well. Therefore, bean constituents such as fiber, folate or its phytochemical composition may provide protective dietary elements within the colon.

2.2 HYPOTHESIS

Raw and canned Ontario grown beans contain substantial amounts of macronutrients, micronutrients and phytonutrients that be affected through processing and can also have an effect on colonic preneoplasia in rats.
2.3 OBJECTIVES

2.3.1 Overall Objective

To investigate the macronutrients, micronutrients and phytonutrient composition of Ontario grown beans, and their role in the prevention of colon cancer in a rat model.

2.3.2 Specific Objectives

a) To determine the macronutrient and various micronutrient composition of raw Ontario grown Kidney beans, Fava beans and Navy beans.
b) To determine the macronutrient and various micronutrient composition of commercially processed canned Ontario grown Kidney beans, Fava beans and Navy beans.
c) To determine the phytonutrient composition of raw Ontario grown Kidney beans, Fava beans and Navy beans.
d) To determine the phytonutrient composition of commercially processed canned Ontario grown Kidney beans, Broad beans and Navy beans.
e) To investigate the effect of cooking times on the macronutrient, micronutrient and phytonutrient composition of Ontario grown beans.
f) To determine if feeding commercially processed canned Navy beans prevents colonic preneoplasia in rats.
CHAPTER 3

- LITERATURE REVIEW -
3.1 HISTORY

The health benefits of beans have been known for centuries. Primitive people grew and consumed beans as a dietary staple long before modern nutrition researchers endorsed their significant health virtues (Messina, 1999). Dry or common beans are seeds that are categorized as legumes which come from a large family of plants that are distinguished by their seed-bearing pods (Sgarbieri, 1989). Of the 13,000 species of legumes, only about 20 are commonly consumed by humans (Sathe, 1984; Sgarbieri, 1989). Legumes can be separated into two classes: oil seeds such as soy beans and peanuts, which are grown for their protein and oil content; and grain legumes, such as common or dry beans (i.e. kidney beans), lentils, lima beans, cowpeas, Fava beans, chickpeas and common peas, which are grown primarily for their protein source (Geil and Anderson, 1994). Beans, peas and lentils are know as ‘pulses’ from the Latin word “pulse”, an ancient bean porridge.

Dry beans are among the oldest cultivated foods. It appears that the first cultivation of legumes occurred in Southeast Asia approximately 9750 B.C. (Sgarbieri, 1982). The next oldest sites of agriculture, which date about 8000 B.C., are those in the Middle East, in a region commonly known as the Fertile Cresent. It is suspected that this is the place of origin of chickpeas, Fava beans and lentils. The dependence of China and India on vegetarian diets, led to the development of soybean-based imitations of dairy products by the Chinese, and the utilization of legumes for food in India (Sgarbiere, 1989).
The first cultivations of legumes in the New World occurred around 4000 B.C. in both Peru and Mexico, from which the practice gradually spread to other parts of North America (Lucier, 2000). Today the world production of major legumes is estimated to be 186,503,000 metric tons annually (United Nations; Food – Domestic Utilization, 1998). In Canada, Ontario is one of the major producers and processors of a wide variety of beans.

3.2 PATTERNS OF BEAN CONSUMPTION

Beans are a dietary staple in some parts of the world, providing a significant portion of total protein intake. Pulses, together with nuts and seeds, have been estimated to provide about 5.6% total energy in economically developing countries and 2.4% in developed countries (Deshpande et al., 1984; Sgarbieri, 1989). Beans make the greatest contribution to dietary energy supply in some areas of sub-Saharan Africa, where 11-17% of total energy is derived from them, followed by the Middle East, Asia, and North Africa (Deshpande et al., 1984; Sgarbieri, 1989). In some areas of China, they provide as much as 10% total energy. In Central America, they are usually eaten with rice and provide 5% of total energy. Consumption appears to be the least in Europe, Australia, New Zealand and North America (United Nations, Food – Domestic Utilization Data, 1998; Lucier et al, 2000).

In Canada, the consumption of beans has been declining for years. According to the United Nations, Food – Domestic Utilization Data for 1998, Canadian consumption of
beans is 1.1 kg/person annually. This equates to only 0.5% of total energy intake per person. This is less than half the amount consumed by American families yearly.

It is quite possible that bean consumption is low primarily because of its label as the “poor man’s meat”, a metaphor which is consistent with the inverse relation between intake and income (Messina, 1999). Beans contain a number of components responsible for flatulence, causing social inconveniences. One likely reason for lower consumption in Western civilizations is probably related to their prolonged cooking and preparation time (Lucier, et al, 2000). Western countries are fast paced, and prefer easy-to-prepare and convenience foods (Lucier, et al, 2000). However, it is likely that the primary reason for low consumption is due to the lack of consumer knowledge regarding their nutritional content and potential contribution to a healthy diet. It should therefore be the goal of the Ontario Ministry of Agriculture, Canadian food companies and health professionals to increase the profile of beans resulting in a higher consumption of this ‘health benefiting’ food in Canada.

3.3 NUTRIENT PROFILE

Recent dietary guidelines published by Health and Welfare Canada suggest that excessive intake of total fat, saturated fat, cholesterol, sugar, and sodium should be avoided and recommend an increased consumption of complex carbohydrate and fiber (Health Canada, 1997). The nutrient composition of beans makes them ideally suited to meet these dietary recommendations for good health as they are low in fat and sodium, and are
cholesterol free (Geil and Anderson, 1994), provide an excellent source of complex carbohydrate and fiber (Anderson et al., 1984. Dry beans supply a dense source of plant protein and contain substantial sources of various vitamins and minerals. In light of their nutritional value, beans make a valuable contribution to a normal healthy diet.

3.3.1. MACRONUTRIENT COMPOSITION

The macronutrient composition of some commonly consumed beans is shown in Table 3.3.1. Beans generally provide a good source of protein, carbohydrate, fiber and vitamins and minerals (Sathe et al., 1984). Legumes are divided into two separate and distinct categories; the oilseeds such as soybeans and the grain legumes such as Kidney beans (Geil and Anderson, 1994; Sgarbieri, 1989). The nutrient contribution from each of these categories is somewhat different. The oilseed beans contain higher protein and fat, whereas the beans defined as grain legumes are higher in carbohydrate and fiber. Both categories also provide a number of vitamins and minerals, however the vitamin and mineral contribution from each category also has their differences as a result of their legume category. Figure 3.3.1 displays the macronutrient differences between the Kidney bean (grain legume) and the Soy bean (oil seed legume).
Table 3.3.1 Nutrient content of selected cooked beans (g/100g)

<table>
<thead>
<tr>
<th>Bean</th>
<th>Water (g)</th>
<th>Energy (kcal)</th>
<th>Protein (g)</th>
<th>Fat (g)</th>
<th>Carbohydrate (g)</th>
<th>Ash (g)</th>
<th>Dietary Fiber (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broad Beans (Faba beans)</td>
<td>83.70</td>
<td>62.00</td>
<td>4.80</td>
<td>0.50</td>
<td>10.10</td>
<td>0.90</td>
<td>3.60</td>
</tr>
<tr>
<td>Garbanzo Beans (chickpeas)</td>
<td>60.21</td>
<td>164.00</td>
<td>8.86</td>
<td>2.59</td>
<td>27.41</td>
<td>0.92</td>
<td>7.60</td>
</tr>
<tr>
<td>Kidney Beans (red)</td>
<td>66.94</td>
<td>127.00</td>
<td>8.67</td>
<td>0.50</td>
<td>22.18</td>
<td>1.09</td>
<td>7.40</td>
</tr>
<tr>
<td>Lentils</td>
<td>69.64</td>
<td>116.00</td>
<td>9.02</td>
<td>0.38</td>
<td>20.14</td>
<td>0.83</td>
<td>7.90</td>
</tr>
<tr>
<td>Lima Beans</td>
<td>69.79</td>
<td>115.00</td>
<td>7.80</td>
<td>0.38</td>
<td>20.89</td>
<td>1.15</td>
<td>7.00</td>
</tr>
<tr>
<td>Navy Beans</td>
<td>63.18</td>
<td>142.00</td>
<td>8.70</td>
<td>0.57</td>
<td>26.31</td>
<td>1.23</td>
<td>6.40</td>
</tr>
<tr>
<td>Peanuts, roasted (whole parts)</td>
<td>1.55</td>
<td>585.00</td>
<td>23.68</td>
<td>49.66</td>
<td>21.51</td>
<td>3.60</td>
<td>8.00</td>
</tr>
<tr>
<td>Pinto Beans</td>
<td>93.39</td>
<td>22.00</td>
<td>1.86</td>
<td>0.32</td>
<td>4.10</td>
<td>0.34</td>
<td>N/A</td>
</tr>
<tr>
<td>Soy Beans</td>
<td>62.55</td>
<td>173.00</td>
<td>16.64</td>
<td>8.97</td>
<td>9.92</td>
<td>1.91</td>
<td>6.00</td>
</tr>
</tbody>
</table>

a: Data obtained from USDA Nutrient Database for Standard Reference (Handbook No. 8).
b: N/A: data not listed
Figure 3.3.1 Comparison of Macronutrient Content of Oilseed Legumes (Soy Bean) and Grain Legumes (Kidney Bean)
3.3.1.1 PROTEIN

Bean protein can make a valuable addition to the diet. The protein content of dry beans provides between 20% to 30% of energy, with the exception of soy beans which provides 34% (Sathe et al., 1984; Sgarbieri, 1982). A serving of cooked beans (approximately 100 g or ½ cup) will provide an average of 7 to 9 grams of protein (Messina, 1999; Sathe et al., 1984; Sgarbieri, 1982). This is considerably more protein than in most other vegetables, and double the amount of protein found in 1 cup of 2% low-fat milk (Geil and Anderson, 1994).

Currently there is much interest in bean protein worldwide as health concerns and cost of animal protein sources increase (Geil and Anderson, 1994; Sarwar et al, 1989; Sgarbieri, 1982). Even though beans have been recognized for their high protein content, the protein quality of beans have been questioned. The legume protein is relatively rich in essential amino acids such as lysine. When compared with a reference protein such as eggs, they are moderately deficient in the sulfur-containing amino acids methionine (Geil and Anderson, 1994; Messina, 1999). The significance of this problem has been exaggerated. This is because tests, such as the protein-efficiency ratio, which measures protein quality, are most often conducted with rats, which require approximately 50% more methionine for growth than humans (Messina, 1999). Consequently, because bean proteins are low in sulfur-containing amino acids, the protein-efficiency ratio of beans tend to be quite low. A mixture of legumes and a complementary source of sulfur-containing amino acids, such as those found in cereal grains, will greatly improve its
protein efficiency. Some examples of food complementary combinations are baked beans on toast and corn tortillas with refried beans.

3.3.1.2 CARBOHYDRATE

Beans provide an excellent source of complex carbohydrate. The carbohydrate content of cooked beans ranges from 5 to 23 g per 100 g serving (Gupta, 1983; Sgarbiere, 1989). The total carbohydrate component of dry beans makes up 50 – 70% by weight (Sgarbiere, 1981). The main carbohydrate component is starch ranging from 38 – 45%, with small amounts of sugars such as raffinose and starchyose which are considered indigestible and cause flatulence, and fructose-oligosaccharides (Geil and Anderson, 1994).

Fructose-oligosaccharides (FOS), have recently attracted considerable attention as nonabsorbable carbohydrates with prebiotic properties (Jenkins et al., 1999). The addition of FOS to the diet has shown to significantly increased the number of colonic bifidobacteria, which are regarded as beneficial strains in the colon. Reddy et al. (1997), found that the addition of 10% FOS to the diet significantly inhibited the formation of aberrant crypt foci formation and crypt multiplicity in the colons of azoxymethane-induced rats. Jenkins et al. (1999), suggests that the addition of FOS to the diet may play a role in reducing blood cholesterol, stimulate immune function and enhance vitamin synthesis.
Legumes seeds also contain large amounts of resistant starch (RS) which resists digestion in the small intestine and then pass into the large intestine where it is fermented by bacteria to produce short-chain fatty acids, with a high proportion of butyrate (Cummings and MacFarlane, 1992; Englyst and Cummings, 1987; Scheppach et al., 1988). Studies show foods that promote the production of butyrate are associated with a lower risk of bowel cancer (MacIntyre et al., 1993). Resistant starch may be quantitatively more important than dietary fiber as a substrate for fermentation (Ahmed et al., 2000). Starchy foods rich in RS have shown to influence the regulation of glucose metabolism by lowering postprandial hyperinsulinemia/hyperglycemia (Lehrer-Metzer et al., 1996; Raben et al., 1994).

It has also been found that RS may lower the level of plasma cholesterol (behall et al., 1989), which might be a consequence of hepatic metabolism regulation (Sacquet et al., 1983). RS also increases fecal bulk (Jenkins et al., 1998) and lowers fecal pH (Philipps et al., 1995), factors that are usually considered as markers of healthy colonic mucosa (Cummings et al., 1992).

In addition to FOS and RS, beans also contain a significant amount of dietary fiber. Legumes provide between 1 and 7 g of fiber per 100 g of cooked serving. Bean fiber is primarily comprised of soluble fiber, which contributes to 2/3 of its composition, and insoluble fiber, providing only a third (Hughes and Swanson, 1989). Fiber comprises a heterogeneous group of nonstarch polysaccharides such as cellulose, hemicellulose and pectins and non-carbohydrate substances such as phytic acid (depending on the nature
and source of fiber in the diet) (Reddy, 1999). Epidemiological and laboratory animal studies have suggested an inverse relationship between colon cancer and dietary intake of fiber-rich foods (Howe et al., 1992; Reddy, 1995; Steinmetz and Potter, 1991).

3.3.1.3 FAT

Most beans are low in fat and contain approximately 0.5 g per 100 g cooked serving. The fat content of dry beans range from 0.8 – 1.5% with the exception being soy beans and peanuts which contain 19% and 46% of fat (Geil and Anderson, 1994). This relates to 7.3 g of fat per 100g serving of cooked soy beans and 32 g of fat in ½ cup of roasted peanuts (Geil and Anderson, 1994). The fatty acid composition of dry beans is primarily unsaturated fatty-acids with α-linolenic acid present in the greatest amount (Sgarbieri, 1989). However, because the total fat composition of grain legumes is quite low, the dietary contribution of α-linolenic acid by beans is generally minor. In the case of soybeans which are high in fat, they contribute significantly to α-linolenic acid intake.

Because beans are plant foods, they are cholesterol free. The low fat content of dry beans is important in light of dietary recommendations, which suggest lower fat and cholesterol intakes to reduce the risk of developing chronic diseases (Health Canada, 1997).
3.3.2 MICRONUTRIENT COMPOSITION

3.3.2.1 VITAMINS

The micronutrient composition of commonly consumed beans is shown in table 2.3.2.1.

Dry beans provide sources of water-soluble vitamins. These include thiamin, riboflavin, niacin, and folacin, otherwise known as folate. Legumes are thought to be some of the best sources of folate (Meiners et al., 1976). The folic acid in human health has been identified in recent studies. There is convincing evidence to suggest that adequate intake of this nutrient may help prevent a number of health problems at all stages of life. This vitamin has been shown to prevent neural tube defects in the fetus (Oladapo, 2000) and lower the risk of ischemic heart disease and strokes by lowering plasma homocysteine levels in older persons (Brattstrom and Wilchen, 2000; Law, 2000).

There is also evidence to suggest that folic acid treatment reduces the incidence of cancer (Freudenheim et al., 1991; Jacob, 2000; Law, 2000; Thurmon, 2001; Willet, 2000). Folate plays a vital role in normal cell division and the repair of DNA damage (Willet, 1994). Low folate in patients with many types of cancers has been observed. The Nurses’ Health Study showed that women taking folic acid-containing multivitamins for at least 15 years were 75% less likely to develop colon cancer than women who did not. It was also found that patients with ulcerative colitis who took 1000 mcg/day of folic acid supplements had a lowered risk of developing colon cancer (Harvard Women’s Health Watch, 2001). Song et al. (2000), investigated the effects of 0, 2, 8, or 20 mg folate/kg on the
Table 3.3.2.1 Vitamin content of selected cooked beans.

<table>
<thead>
<tr>
<th>Bean</th>
<th>Thiamin (mg)</th>
<th>Riboflavin (mg)</th>
<th>Niacin (mg)</th>
<th>Folate (mcg)</th>
<th>Vitamin B-6 (mg)</th>
<th>Vitamin C (mg)</th>
<th>Vitamin A (IU)</th>
<th>Vitamin E (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broad Beans (faba beans) (^{a,c})</td>
<td>0.09</td>
<td>0.08</td>
<td>0.71</td>
<td>104.1</td>
<td>0.07</td>
<td>0.30</td>
<td>15.00</td>
<td>0.09</td>
</tr>
<tr>
<td>Garbanzo Beans (chickpeas) (^{a,c})</td>
<td>0.12</td>
<td>0.06</td>
<td>0.526</td>
<td>172.0</td>
<td>0.139</td>
<td>1.30</td>
<td>27.00</td>
<td>0.35</td>
</tr>
<tr>
<td>Kidney Beans (red) (^{a,c})</td>
<td>0.16</td>
<td>0.05</td>
<td>0.57</td>
<td>129.6</td>
<td>0.12</td>
<td>1.20</td>
<td>0.0</td>
<td>0.08</td>
</tr>
<tr>
<td>Lentils (^{a,c})</td>
<td>0.16</td>
<td>0.07</td>
<td>1.06</td>
<td>180.80</td>
<td>0.17</td>
<td>1.50</td>
<td>8.00</td>
<td>0.11</td>
</tr>
<tr>
<td>Lima Beans (^{a,c})</td>
<td>0.16</td>
<td>0.05</td>
<td>0.42</td>
<td>83.10</td>
<td>0.16</td>
<td>0.0</td>
<td>0.0</td>
<td>0.18</td>
</tr>
<tr>
<td>Navy Beans (^{a,c})</td>
<td>0.20</td>
<td>0.06</td>
<td>0.53</td>
<td>139.90</td>
<td>0.16</td>
<td>0.90</td>
<td>2.00</td>
<td>N/A</td>
</tr>
<tr>
<td>Peanuts, roasted (whole parts) (^{a,c})</td>
<td>0.44</td>
<td>0.09</td>
<td>13.52</td>
<td>145.30</td>
<td>0.25</td>
<td>0.0</td>
<td>0.0</td>
<td>7.41</td>
</tr>
<tr>
<td>Pinto Beans (^{a,c})</td>
<td>0.67</td>
<td>0.05</td>
<td>0.72</td>
<td>29.30</td>
<td>0.54</td>
<td>6.10</td>
<td>1.00</td>
<td>N/A</td>
</tr>
<tr>
<td>Soy Beans (^{a,c})</td>
<td>0.15</td>
<td>0.28</td>
<td>0.39</td>
<td>53.8</td>
<td>0.23</td>
<td>1.70</td>
<td>0.0</td>
<td>1.95</td>
</tr>
</tbody>
</table>

\(^{a}\): Data obtained from USDA Nutrient Database for Standard Reference (Handbook No. 8).
\(^{b}\): N/A: data not listed.
\(^{c}\): Values expressed per 100 g of cooked, boiled, serving.
development of intestinal polyps in mice. Their laboratory found increasing dietary folate levels significantly decreased the number of colonic ACF in a dose-dependant manner compared to the folate-deficient diet, after three months. The number of ACF was also inversely correlated with serum folate concentrations.

Recent surveys have indicated that folic acid intakes in North America are below the RNI / DRI. The average intake of folic acid from foods have been only about half of the current nutrition recommendations of 400 mcg/day. One serving of cooked beans can provide more than half the current recommendations for folate intake (Health Canada, 1997). Although some of these water-soluble vitamins are heat labile, ordinary cooking of dry beans (those cooked at home), appears to pose little problems as far as nutrient retention is concerned (Deshpande et al., 1984).

Due to the low fat content of beans they are coincidentally poor sources of fat-soluble vitamins A, and E.

3.3.2.2 MINERALS

Typical mineral contribution of commonly consumed beans is shown in Table 3.3.2.2. Beans are primarily considered a rich of essential minerals. These include calcium, iron, magnesium, phosphorus, potassium, zinc and copper (USDA Handbook No. 8, 1972). Although there is considerable variation among beans, they can provide approximately 50 mg of calcium per 90-100 g of cooked serving. This equates to
Table 3.3.2.2 Mineral content of selected cooked

<table>
<thead>
<tr>
<th>Bean</th>
<th>Calcium (mg)</th>
<th>Iron (mg)</th>
<th>Magnesium (mg)</th>
<th>Phosphorus (mg)</th>
<th>Potassium (mg)</th>
<th>Zinc (mg)</th>
<th>Copper (mg)</th>
<th>Manganese (mg)</th>
<th>Sodium (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broad Beans (Faba beans)</td>
<td>36.0</td>
<td>1.5</td>
<td>43.0</td>
<td>125.0</td>
<td>268.0</td>
<td>1.01</td>
<td>0.25</td>
<td>0.42</td>
<td>5.0</td>
</tr>
<tr>
<td>Garbanzo Beans (chickpeas)</td>
<td>49.0</td>
<td>2.8</td>
<td>48.0</td>
<td>168.0</td>
<td>291.0</td>
<td>1.53</td>
<td>0.35</td>
<td>1.03</td>
<td>7.0</td>
</tr>
<tr>
<td>Kidney Beans (red)</td>
<td>28.0</td>
<td>2.9</td>
<td>45.0</td>
<td>142.0</td>
<td>403.0</td>
<td>1.07</td>
<td>0.24</td>
<td>0.47</td>
<td>2.0</td>
</tr>
<tr>
<td>Lentils</td>
<td>19.0</td>
<td>33.3</td>
<td>36.0</td>
<td>180.0</td>
<td>369.0</td>
<td>1.27</td>
<td>0.25</td>
<td>0.49</td>
<td>2.0</td>
</tr>
<tr>
<td>Lima Beans</td>
<td>17.0</td>
<td>2.3</td>
<td>43.0</td>
<td>111.0</td>
<td>508.0</td>
<td>0.95</td>
<td>0.23</td>
<td>0.516</td>
<td>2.0</td>
</tr>
<tr>
<td>Navy Beans</td>
<td>70.0</td>
<td>2.4</td>
<td>59.0</td>
<td>157.0</td>
<td>368.0</td>
<td>1.06</td>
<td>0.29</td>
<td>0.55</td>
<td>1.0</td>
</tr>
<tr>
<td>Peanuts, roasted (whole</td>
<td>54.0</td>
<td>2.2</td>
<td>176.0</td>
<td>358.0</td>
<td>658.0</td>
<td>3.31</td>
<td>0.67</td>
<td>2.08</td>
<td>813.0</td>
</tr>
<tr>
<td>parts)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pinto Beans</td>
<td>15.0</td>
<td>0.6</td>
<td>18.0</td>
<td>30.0</td>
<td>98.0</td>
<td>0.17</td>
<td>0.11</td>
<td>0.12</td>
<td>51.0</td>
</tr>
<tr>
<td>Soy Beans</td>
<td>102.0</td>
<td>5.1</td>
<td>86.0</td>
<td>245.0</td>
<td>515.0</td>
<td>1.15</td>
<td>0.41</td>
<td>0.824</td>
<td>1.00</td>
</tr>
</tbody>
</table>

a: Data obtained from USDA Nutrient Database for Standard Reference (Handbook No. 8).
b: N/A: data not listed.
c: Values expressed per 100 g of cooked, boiled, serving.
approximately 6% of the recommended daily intake of calcium for adults. One cup of cooked beans can also provide 20 – 25% of the requirements for phosphorus, magnesium and manganese, about 20% of potassium and copper, and 10% of zinc (Geil and Anderson, 1999; Nutrition Recommendations, Health Canada, 1997). The iron content in cooked beans is approximately 1.5 – 2.0 mg per 100 grams. This compares favorably with iron recommendations of 8 – 13 mg/day for adults. However, it is important to note that the high mineral content of cooked beans may not necessarily equate to high bioavailability of some of these minerals. Minerals from plant sources are generally less bioavailable than minerals derived from animal sources (Geil and Anderson, 1999). Dry beans contain a number of components, including fiber, phenolic compounds and phytic acid which may react with these minerals to decrease their bioavailability (Thompson, 1993). Dry beans are naturally low in sodium (except for peanuts), making them nutritionally advantageous to people who are on low sodium diets.

3.4 HEALTH BENEFITS OF BEANS

Beans can be an important component of any healthy diet. The presence of their functional components enabled them to provide health benefits beyond basic nutrition (Kushi, 1999). However, future research should be directed at the protective and therapeutic role of beans in combating conditions such as coronary heart disease, diabetes mellitus, obesity, and cancer, all of which are faced by western civilizations.
3.4.1. **CORONARY HEART DISEASE**

Coronary heart disease is a major medical concern in most Western countries (Anderson et al., 1999). It accounts for more than half the deaths in North America, and it is also a significant concern in developing countries around the world (Carrol and Kurowska, 1995). Epidemiological data suggest an inverse relationship between the intake of complex carbohydrates and dietary fiber, and coronary heart disease (Anderson et al., 1984; Anderson et al., 1990). Dietary modification is necessary to lower risk factors, such as elevated serum cholesterol, in combating heart disease (Anderson et al., 1990). It has been documented that certain high fiber foods can produce significant hypocholesterolemic effects (Luken et al., 1962). Significant reduction in total cholesterol is important, as a 1% decrease in serum cholesterol results in a 2% decrease in the risk of coronary heart disease (Geil and Anderson, 1994). Although the mechanisms responsible for the hypocholesterolemic effects observed in various studies on fibre are not certain, it is hypothesized that fermentation of dietary fiber in the colon generates short-chain fatty acids, which may inhibit hepatic cholesterol synthesis (Anderson et al., 1999).

In a study conducted by Groen et al., (1962), Trappist monks consuming lactovegetarian diets, which included 100 – 150 g/day of dried beans, were shown to have serum cholesterol levels that were significantly lower than Benedictine monks who were consuming typical Western diets. Luyken et al., (1962), observed similar results using 100g/day of dried brown beans. Jenkins et al., (1983), showed a significant decrease in the total serum cholesterol levels of male hypercholesterolemic patients, consuming 140
g/day of a variety dried beans when compared to other carbohydrate sources. There have been a number of human studies which have investigated the cholesterol-lowering effects of a variety of dried beans in amounts ranging from 75 – 200 g/day (Anderson, 1984; Jenkins et al., 1985; Shutler et al., 1989). All of these studies indicated significantly lowered cholesterol levels ranging from -5.2% to -18.7%. However, it should be noted that the hypocholesterolemic effects produced by the consumption of dried beans were most noticeable in subjects whose initial serum cholesterol levels were the highest.

Canned beans account for the majority of total bean consumption (Haytowitz, 1981). Several studies have indicated that canned beans might also play a role in the management of hypercholesterolemia. Shutler et al. (1989), and Anderson et al. (1984), both demonstrated a cholesterol-lowering effect, following the consumption of canned beans in amounts ranging from 69 – 150 g/day.

3.4.2. DIABETES MELLITUS

Diet remains an important factor in controlling diabetes mellitus. Recent work on the dietary management of diabetes has focused on two factors: dietary fiber content and the nature of the carbohydrate eaten (determined by their glycemic index) (Crapo et al., 1977; Jenkins et al., 1978). A number of studies have implicated dietary fiber as a major component in certain foods, which may provide important health benefits to diabetics (Anderson and Akanji, 1991; Jenkins et al., 1978; 1983; 1987). Beans are among these
foods as they provide significant sources of dietary fiber (Geil and Anderson, 1994; Marlett, 1992). Other studies have shown that the glycemic index of foods is also an important factor in managing diabetes (Jenkins et al., 1981; 1985; 1987; 1988; Wolever et al., 1990; 1991).

The glycemic index (GI) is a system of classification in which the glucose response to foods are indexed against a standard (white bread) (Wolever, 1990). Beans have been identified as a potentially clinically useful food producing relatively flat glycemic responses. Specific incorporation of beans into the diet have been associated with reduced blood glucose and insulin levels. Jenkins et al. (1981), investigated the effect of 62 different foods on blood glucose levels of healthy fasting volunteers. Blood glucose levels were measured over 2 hours and expressed as a percentage of the area under the glucose response curve. Out of the 62 commonly eaten foods (vegetables, breakfast cereals, cereals and biscuits, fruits, dairy products and beans), beans showed the lowest rise in blood glucose.

It should be noted that not all carbohydrate sources raise blood glucose to the same extent when fed equivalent amounts. In a study that investigated the effects of 50 g of 8 varieties of legumes and 24 common foods (grains, breads and pasta, breakfast cereals, biscuits and tuberous vegetables) found both the mean peak rise in blood glucose concentration and mean area under the curve of the subjects who ate beans were at least 45% lower than those subjects who ate the other foods (Jenkins et al, 1980).
Jenkins et al. (1984), also investigated the effect of varying the amount (25 g or 50g) and type (bread or beans) of carbohydrate in test meals on the blood glucose response. The blood glucose area after a half bread portion was 48% that of the full bread meal. Fifty grams of white pea beans gave a blood glucose response 41% that of 50 g of bread, and a combined meal of bread (25 g) and beans (25 g) gave a blood glucose response of 60% of the full bread meal.

Incorporation of low GI foods (such as beans) into the diet have also been associated with reductions in low-density lipoprotein cholesterol and triglyceride levels in hyperlipidemic patients. The substitution of 140 g of dried beans for other sources of starch in the diets of 7 male hyperlipidemic patients showed reductions in mean fasting serum triglyceride levels by 25% and a reduction in total serum cholesterol levels by 7% (Jenkins et al, 1983). Jenkins et al. (1988), also examined the effect of low GI traditional foods consumed by hyperlipidemic and non-hyperlipidemic patients for 3 months. Only subjects with raised triglyceride levels showed significant reductions in total cholesterol, LDL cholesterol and serum triglyceride with no change in HDL cholesterol.

These studies indicate that the re introduction of low GI legumes into a Western diet not only benefits individuals who have developed diabetes, but those whose lipid levels are high and are at risk for disease.
3.4.3. OBESITY

Obesity is a complex disease with serious health consequences. Dietary intervention for the purpose of weight loss is a challenge faced by dietitians and the medical community. Beans intake reduces the risk of developing obesity much in the same way it protects against diabetes and other chronic diseases because they are low in fat and high in fiber (Anderson et al., 1999; Geil and Anderson, 1994). Foods with a high fiber content may produce a feeling of satiety (Anderson et al, 1999), as they provide slower passage through the alimentary canal prolonging gastric emptying, increase insulin sensitivity, and dampen postprandial glycemia (Geil and Anderson, 1994). Therefore, high fiber, low fat foods, such as beans, can be viewed as an important component in weight-reducing diets (Rolls, 1995).

Leathwood et al. (1988), investigated the effects of bean puree versus potato puree on plasma glucose and hunger, and found beans to produce a slower, sustained rise in plasma glucose than potatoes. Through the use of a hunger rating questionnaire, the study also found delayed hunger sensations and prolonged feelings of satiety in those subjects consuming bean puree compared to those who had potato puree.

Studies also suggest that protein may be more satiating than carbohydrate or fat (Rolls, 1995). The observations of enhanced postmeal satiety associated with protein compared with other macro-nutrients may suggest that an increase in legume protein intake may provide weight-loss benefits. There is also a potential risk of greater renal stress by
increasing animal protein intake (Anderson, 1999). Therefore it would be unwise for obese individuals to consume diets with a high animal protein content since these people are more likely to be at a higher risk for developing diabetes, glucose intolerance or hypertension. Similar to soy protein which appears to lessen the workload of the kidneys when compared to animal protein (Kontessis et al., 1990), bean protein may also have advantages in treating obese individuals. They are low in fat, contain high fiber, and provide an adequate protein source, all of which serve as important factors for the management of weight loss and weight maintenance (Health Canada, 1992).

3.4.4 CANCER

It is estimated that up to 70% of all cancers are related to diet (Doll and Petro, 1981). A major protective dietary factor against cancer appears to be a diet high in fiber and low in fat, with an increased consumption of fruits and vegetables (Potter, 1996; World Cancer Research Fund, 1997). The low fat, high fiber composition of beans makes them ideally suited for this purpose (Geil and Anderson, 1994).

The role of beans in cancer prevention is unclear. Most reviews discussing epidemiological studies related to this topic found that the number of studies suggesting an inverse association are almost matched by those which found a positive association between legume intake and cancer risk (Steinmetz and Potter, 1991). In a recent report set forth by the World Cancer Research Fund, it was noted that 58 epidemiological studies had examined the association between bean consumption and cancer risk. The result of this investigation showed that of 58 studies, 29 (50%) reported a decreased risk
with higher bean intake, 22 (38%) reported an increased risk, and 7 studies (12%) indicated no association between bean intake and cancer. In an epidemiological study conducted by Jain et al. (1999), which was released following the above investigation reported a statistically significant decreased association between increased intakes of beans, lentils, and nuts and prostate cancer risk. This study is of particular interest, as it was conducted in three major geographical areas within Canada: Metropolitan Toronto, Montreal, and Vancouver. The study indicates that beans grown in Canada contain some beneficial properties. This is important as environmental conditions and processing techniques vary from country to country, which could have an effect on the nutritional and functional quality of the beans.

3.5 HEALTH BENEFITS OF PHYTONUTRIENTS

Recent interest on the health benefits of beans has focused around a number of biologically active components referred to as phytochemicals (Anderson, 1979; Kennedy, 1995; Messina, 1999; Messina and Messina, 1991; Second International Symposium on the Role of Soy in Preventing and Treating Chronic Disease, 1996; Thompson, 1993;). Although they were once referred to as ‘antinutrients’ because of their potential to reduce the availability and utilization of nutrients, and cause growth inhibition (Liener, 1994; Messina and Messina, 1991; Thompson, 1993; Wang and Wixon; 1999), they are now being looked upon as important compounds for the prevention of various chronic diseases (Second International Symposium on the Role of Soy in Preventing and Treating Chronic Disease, 1996; Thompson, 1993;).
Disease, 1996). Among these chemicals are phytic acid, lectins, saponins, trypsin inhibitors and oligosaccharides.

The clinical applications of phytonutrients have received a great deal of interest in recent years. In particular, the phytonutrients present in soybeans have been studied extensively, with a number of reviews discussing the health significance of these findings (Anderson et al., 1995; Kennedy, 1995; Kushi et al., 1999; Liener, 1993; Messina, 1999; Messina and Barnes, 1991; Thompson, 1993; Wang and Wixon, 1999). At a recently held international conference on the role of soy in preventing and treating chronic diseases, several papers were presented providing supporting evidence for the role of soy bean phytonutrients in the prevention of cancer, heart disease and other degenerative diseases (Second International Soy Symposium on the Role of Soy in Preventing and Treating Chronic Disease). Beans may also contain several of the same phytonutrients that were identified in soybeans. However, to date analysis of these phytonutrients in the beans grown in Ontario have not been performed.

3.5.1 PHYTIC ACID

Phytic acid is a storage form of phosphorus in plant seeds (Wixon and Wang, 1999). Its concentration in legumes account for approximately 1 – 3% on a dry weight basis (Deshpande et al., 1984; Reddy et al., 1982). It has been looked upon as an antinutrient because of its ability to bind to protein, starch and various minerals, thus reducing and
slowing their digestibility (Thompson, 1993; Reddy et al., 1982; Liener, 1993).

However, these properties could be considered as desirable in the prevention of diabetes, cardiovascular disease and cancer (Wang and Wixon, 1999).

Phytic acid has a clear benefit for people with diabetes mellitus by delaying glucose release from starch. Long-term exposure to phytic acid appears to be beneficial in prevention of diabetes by delaying the rise of glucose in the blood and preventing the development of tissue resistance to insulin (Wang and Wixon, 1999). Jenkins et al (1980), found legumes to have the lowest starch digestibility and blood glucose response compared to other carbohydrate foods. A number of other studies have also found benefits in improving diabetes control with the addition of phytic acid (Simpson et al., 1981; Thompson et al., 1987; Yoon et al., 1983).

The potential preventative effect of phytic acid against cardiovascular disease is likely due to its hypolipidemic and hypocholesterolemic properties (Wang and Wixon, 1999). The addition of 0.2 – 9% phytic acid to the diets of rats significantly reduced the plasma cholesterol and triglyceride levels (Klevay, 1977; Sharma, 1980, 1984; Jariwalla et al., 1990). Because phytic acid can bind to zinc, it may lower the serum zinc to copper ratio, which tends to predispose humans to cardiovascular disease (Klevay, 1977).

A number of in vitro and animal studies have demonstrated potential preventative effects of phytic acid against cancers in the colon, mammary glands and other tissues (Wang and Wixon, 1999). When phytic acid (1%) was added to the drinking water of rats one to two weeks prior to the administration of azoxymethane (AOM), a colon specific carcinogen,
and up to 5 months afterwards, significant decreases in tumor size and volume in the colons of rats were observed (Shamsuddin et al., 1988; 1989). Risk of mammary cancer risk was also reduced with the addition of phytic acid to the diet of mice, as indicated by decreased cell proliferation, nuclear aberration and intraductal proliferation (Thompson and Zhang, 1991).

The mechanisms by which phytic acid may reduce the risk of cancer have been suggested in various studies (Shamsuddin et al., 1988, 1989; Shamsuddin and Ullah, 1989; Thompson, 1989; Graf and Eaton, 1985, 1990; Thompson and Zhang, 1991).

A) Phytic acid may bind with prooxidant Fe$^{3+}$ and reduce DNA damage by free radical formation during lipid oxidation (Graf and Eaton, 1990). B) A reduction in available zinc will indirectly reduce the amount of DNA needed for cell proliferation (Thompson, 1993). The ability of phytic acid to bind with zinc may also be anticarcinogenic, as zinc is needed during DNA synthesis. C) The slowing down of starch digestion by phytic acid may cause some of the starch to escape to the colon. Fermentation of unabsorbed starch produces short-chain fatty acids (SCFA). The presence of SCFA would lower the pH environment in the colon, which in turn would provide protection against colon cancer by reducing the solubility of bile acids and neutralizing ammonia which may act as tumor promoters (Newmark and Lupton, 1990).
3.5.2 SAPONINS

Saponins are naturally occurring amphiphilic heat-stable glycosidic compounds present in many plants (Price et al., 1987). The major source of saponins in the human diet are beans (Oakenfull, 1981). They consist of a steroid or triterpene group linked to one or more sugar residues of different types. The triterpene portion is hydrophobic and the sugar portion is hydrophilic. These surface characteristics are responsible for saponins biological activities, including hypocholesterolemic (Oakenfull and Sidhu, 1990; Carroll and Kurowska, 1995), immunostimulatory (Wu et al., 1990), and anticarcinogenic (Kikuchi et al., 1991) properties.

The effects of different saponins on lipid metabolism in animals and humans were summarized by Oakenfull and Sighu (1990). In the majority of these cases, substantial reduction in plasma cholesterol concentrations was reported. This was also associated with increases in the output of feces and bile acids and neutral sterol output. Of the twenty experiments conducted, only six did not show any significant reductions in plasma cholesterol concentrations. In animal studies the evidence for hypocholesterolemic effect of saponins is strong particularly when fed in the presence of cholesterol. In human studies, controlling for other dietary substitutions, feeding saponins resulted in a significant blood cholesterol lowering effect.
The mechanism by which saponins lower cholesterol may be explained by its ability to bind with dietary cholesterol, thus preventing its absorption (Reshef et al., 1976). Saponins can also bind to bile acids, which may reduce their resorption (Oakenfull and Sidhu, 1986) and therefore more cholesterol is needed to compensate for the bile acid losses in feces, resulting in lower concentrations of serum cholesterol.

The biological characteristics of saponins suggest that they may also have some anticarcinogenic properties. In vitro studies by Rao and Sung (1995), showed soya saponins to have a dose-dependant growth inhibitory effect against human colon carcinoma cells. Koratkor and Rao (1997), investigated the effects of soya saponins on colon cancer in the mouse. They found that when they provided 3% soya saponins, by weight, to the diets of mice that were administered azoxymethane, significant reductions in the incidence of aberrant crypt foci occurred compared to those mice that were not receiving soya saponins.

Saponins appear to function as anticarcinogens by their ability to bind to primary bile acids. This in turn prevents their bacterial conversion to secondary bile acids which consequently reduces their ability to promote tumourigenesis (Oakenfull and Sidhu, 1983). In addition to the bile acids, saponins also have the ability to bind to cholesterol (Reshef et al., 1976) and prevent cholesterol oxidation in the colon, which is also known to be responsible for colon cancer promotion (Kendall et al., 1992). Saponins may also prevent carcinogenesis by functioning directly as cytotoxins to cancer cells, and or functioning as free-radical scavengers (Wang and Wixon, 1999).
3.5.3. OLIGOSACCHARIDES

A major factor which appears to limit the consumption of beans is their ability to produce gas in the gastrointestinal tract. One of the first studies to be published which showed diets containing beans markedly increased flatulence was published in 1966 (Steggerda and Dimmick, 1966). The primary reason for the gas production is due to the presence of fructooligosaccharides (FOS) present in the beans. Flatulence will occur with bean consumption because the human intestinal mucosa lacks the enzyme α-galactosidase necessary to cleave the α-1,6-galactosidic linkage. These FOS pass into the large intestine where they undergo fermentation by anaerobic bacteria, forming large amounts of carbon dioxide, hydrogen and methane (Messina, 1999; Rackis et al., 1970). Because of the discomfort and the social embarrassment associated with flatulence, many people avoid eating beans all together.

Some reports indicate that it is possible to remove substantial amounts of FOS through various processing methods (Deshpande et al., 1984). Cooking has proven to be effective in causing some reduction in the gas promoting activity of various beans (Deshpande et al., 1984). It is possible to also remove considerable amounts of FOS and reduce flatulence by changing the water in which beans are boiled one or more times (Anderson et al., 1979). There are however benefits associated with FOS consumption (Messina, 1999).

Research indicates that FOS are important nonabsorbable carbohydrates with prebiotic properties. Studies indicate that they are able to favorably stimulate the growth of
bifidobacteria, which are regarded as beneficial strains in the colon, thus promoting health and longevity of the colon (Anderson et al., 1999; Reddy et al., 1997). In diet intervention studies, Gibson et al. (1995), demonstrated that dietary administration of FOS significantly increased fecal bifidobacteria, whereas bacteriodes, clostridia and fusobacteria and or gram-positive cocci were decreased on total fecal bacterial count. These bifidobacteria, colonizing at the expense of enteropathogens, may bind the ultimate carcinogen by physically removing it via feces (Reddy, 1999).

In addition to selective modulation of bifidobacteria, FOS increase the production of short-chain fatty acids (SCFA), especially butyrate, in the colon by microbial fermentation (Reddy, 1999). Foods that promote the production of butyrate are associated with a lower risk of bowel cancer (MacIntyre et al., 1993).

Recent studies have examined the effect of feeding FOS and their ability to inhibit makers of colon cancer. Koo and Rao, (1991), reported that administration of both bifidobacteria and 5% FOS to female mice given DMH resulted in approximately 50% as many aberrant crypts (AC) as in the control animals. They also observed a decrease in the number of aberrant crypt foci (ACF) compared to the control animals. Reddy et al. (1997), also found a significant inhibition of ACF formation after feeding FOS to F344 AOM-induced rats. A number of other studies that have investigated the role of FOS consumption on the incidence of colonic preneoplastic markers in animals, are summarized in a recent review by Brady et al., (2000). Human studies have also been
summarized here, however they are more indirect and provide more circumstantial evidence than that offered by the animal studies.

Because of their growth-promoting effect on bifidobacteria and their ability to be fermented into SCFA, it has been hypothesized that FOS may promote health of the colon, increase longevity and decrease the risk of colon cancer (Benno et al., 1989; Koo and Rao, 1991; Mitsuoka, 1982).

3.5.4 TRYPSIN INHIBITORS

Protease inhibitors are abundant in raw cereals and legumes, particularly soybeans. They are protein molecules that bind with trypsins and interfere with protein hydrolysis during digestion (Wang and Wixon, 1999). The two major types of trypsin inhibitors in soybeans are the Kunitz and Bowman-Birk inhibitor (BBI). The Kunitz inhibitor is thermally unstable and is destroyed during most processing procedures, while the Bowman-Birk inhibitor is relatively heat stable and can survive normal heat treatment, with approximately 5 – 20% of the activity still remaining (Messina, 1999; Rackis and Gumbman, 1981; Hathcock, 1991; Wang and Wixon, 1999).

Although protease inhibitors have been associated with pancreatic cancer in animal studies, recent research indicates they may also act as anticarcinogenic agents. This is suggested by animal studies, in vitro cell culture work and epidemiological data, which
showed low cancer mortality rate in human populations with high intake of protease inhibitors (Troll and Kennedy, 1989; Messina and Barnes, 1991; Messina and Messina, 1991; Second International Symposium; The Role of Soy in Preventing and Treating Chronic Disease).

*In vitro*, protease inhibitors have been shown to suppress the malignant transformation of cells induced by different types of carcinogens, e.g. radiation, UV light, chemical carcinogens and steroid hormones (Kennedy, 1984; Kennedy and Billings, 1987). The BBI from soy beans has shown the greatest suppression of carcinogenesis assays in animals. These assays have indicated the BBI to be able to inhibit or prevent the development of chemically-induced cancer in the colon by 100% (St. Clair et al., 1990), suppress carcinogenesis in the liver by 71% (St. Clair et al., 1990), in the lung by 48% (Witschi and Kennedy, 1989) and the oral epithelium by 86% (Kennedy et al., 1993).

There appears to be multiple mechanisms by which protease inhibitors interfere with the development of cancer. The inhibitor may reduce the digestion of proteins, which in turn will decrease available amino acids needed to feed the growing cancer cells (Troll, 1989). Deprivation of amino acids such as leucine, phenylalanine and tyrosine have shown to prevent the growth of mouse hepatoma and mammary adenocarcinoma (Troll et al., 1987). These inhibitors may also play a significant role in stopping oncogene expression begun by carcinogen exposure. Protease inhibitors were shown to be able to suppress the formation of superoxide anion radicals (\(O_2^\cdot\)) and hydrogen peroxide (\(H_2O_2\)) formation induced by tumor promoters, (e.g TPA), in phagocytic neutrophils. These oxygen
reactive species can damage and or modify cellular DNA thus promoting oncogene expression (Kennedy and Billings, 1987; Troll, 1987, 1989). The inhibitors may also act as an anticarcinogenic agent by inhibiting the induction of the DNA polymerase a, an enzyme responsible for the amplification of virus-modified DNA (Heilbronn et al., 1985).

3.5.5 LECTINS

Lectins are proteins which have the unique ability to bind to the carbohydrates found on the surface of many mammalian cells (Wright, 1975). Also referred to as glycoproteins, they are of non-immune origin (Rhodes, 1996), which appear to have functions which vary from one lectin to another (Liener, 1983; Sgarieri and Whitaker, 1982). Their biological actions include agglutinating erythrocytes, agglutinating bacteria, inducing lymphocyte transformation, stimulating mass cell degranulation and agglutinating and damaging neoplastic cells in preference to the normal adult equivalents (Lis and Sharon, 1973). This latter property appears to provide some lectins with a protective effect against experimental carcinogenesis in laboratory animals (Inbar et al., 1972, Wright, 1975).

Lectins are ubiquitous in nature and are present in many of the food items commonly consumed in the human diet (Nachbar and Oppenheim, 1980). They are particularly plentiful in nuts and more so in seeds (Nachbar and Oppenheim, 1980; Rhodes, 1996). They are typically globular proteins which are highly resistant to digestion by
mammalian enzymes and survive passage through the digestive tract (Jaffe and Vega Lette, 1968; Brady et al., 1978; Nachbar et al., 1980).

Much of the existing literature on lectins have discussed their toxicity. Many lectins are toxic or naturally bound to toxins (Liener, 1983; Wright, 1975). The toxicity of lectins is characterized by growth inhibition in experimental animals and diarrhea, nausea, bloating and vomiting in humans (Liener, 1989). The toxic effects of lectins relate to their binding to specific receptor sites on the epithelial cell of the intestinal mucosa. This can cause lesions, disruption and abnormal development of the microvillae (Liener, 1989) thus affecting the absorption of nutrients. However, a common observation is that a high intake of vegetables (including legumes) and therefore lectins, increases intestinal motility as well as mucus and gas production, which decreases the likelihood of colon cancer (Jordinson et al., 1999; Wright, 1975). In this way, the body is protected from their toxicity (Wright, 1975).

From the before mentioned mechanism, a number of researchers have inferred that lectins in foods may play a role in protecting against intestinal cancer. A number of studies have found some dietary lectins (from legumes and mushrooms) to inhibit proliferation of colorectal carcinoma cell lines (Koninkx et al., 1992; Yu et al., 1993) and legume lectins to stimulate differentiation of undifferentiated colon cancer cells (Jordinson et al., 1999).

Several mechanisms for the body’s response to lectins as a protector against intestinal cancer have been postulated (Etzler and Branstrator, 1974; Wright, 1975): 1) mast-cell
degranulation causes inflammation and increases mucus production, thus increasing faecal bulk which may sweep away malignant cells and carcinogens; 2) lectins bind to the mucosal cells, induces mitosis and increases epithelial turnover, providing less of an opportunity for neoplastic cells to take; 3) neoplastic cells may be susceptible to damage by direct contact with lectins.

Because lectins occur in great amounts in plant foods, especially in legumes, their quantity in legumes and possible role in the prevention of colon carcinogenesis should be investigated.

Although only a few major phytonutrients present in beans are discussed, other biologically active phytonutrients are also present in beans. Several recent reviews have addressed this topic (Messina and Bennink, 1998; Sgarbieri and Whitaker, 1982; Thompson, 1993).

3.6 SUMMARY

There is considerable interest in the role that legumes may play in the prevention of chronic diseases. Much of this interest has focused recently on the role of soybeans. However, there have been relatively few studies that have focused on elucidating the effects of beans on disease risk. The epidemiological studies that have addressed this question have focused on the roles of other foods or nutrients and have collected limited information on legume intake and its relation to disease prevention. Therefore it is the
aim of this thesis to investigate the presence of various functional components that may exist in legumes, and further understand the role of legumes as a food item that may potentially bring health benefits to the diet beyond basic nutrition.
CHAPTER 4

- PROXIMATE ANALYSIS -
4.1 INTRODUCTION

Dietary guidelines for good health published by Health and Welfare Canada suggest avoiding excessive intakes of total fat, saturated fat and cholesterol, as well as sugar and sodium. On a 100 kcal basis, legumes (with the exception of oilseeds) contain 80% less total fat and only 25% of the sodium content of lean ground beef (Haytowitz et al, 1981). They contain no cholesterol and provide good sources of polyunsaturated fatty acids (Sathe et al, 1984).

Dry beans also provide a good source of complex carbohydrates (Sathe et al, 1984; Geil et al, 1994) quality vegetable protein, which is of special interest worldwide due to increased health concerns regarding high fat animal protein sources (Geil et al, 1994).

Beans are rich in dietary fiber, which has shown to lower cholesterol, blood glucose and more recently, have a protective effect against colon cancer. Dry beans are also a good source of water soluble vitamins such as folate. Epidemiological studies show that folate appears to have an inverse association to cancer risk and other chronic diseases (Rodgers, 1990; Rodgers et al, 1993; Steinmetz et al, 1993).

In spite of the recognized nutritional contribution of legumes to the diet, the annual per capita consumption of legumes in Canada is low (1.1 kg) (United Nations - Food Domestic Utilization Data, 1998). This is a result of the popularity of animal products
combined with long cooking times required to prepare beans for consumption. To compensate for low bean intakes due to excessive preparation time, food companies provide canned legumes for consumption. Lack of knowledge regarding the nutritional and health benefits of beans may also contribute to low consumption.

Although food legumes represent a large number species, only the dry beans of *Phaseolus vulgaris* have attained popularity in North America. Navy beans are used commercially in preparing canned pork with beans, baked beans and beans in tomato sauce, while kidney beans are popular in chili and similar products. Broad beans of the *Vicia faba* species appear to be more popular with Middle Eastern dishes such as Foule.

The most popular processing methods employed for dry beans in western countries include cooking and canning. These processing methods however, influence the nutritive quality of the beans consumed. Therefore it is important to obtain data on the nutritional contribution of raw, cooked and commercially processed legumes that are of nutritional and economical importance in Canada.

**4.2 OBJECTIVE**

To investigate the macronutrient, folate and total fibre composition of raw and canned (with and without liquid contents in can) Navy, Kidney and Fava beans.
4.3 MATERIALS AND METHODS

4.3.1 Dry Bean Samples

In view of their economical and nutritional importance, only Broad beans, Red Kidney beans and Navy beans and their processed equivalents were used for nutrient estimation. Dry bean seeds were obtained from the Ontario Bean Growers Association and processed samples were obtained from the H.J. Heinz Company of Canada. Three lots from three different kinds of legumes were used. One analysis was conducted on each lot.

4.3.2 Dry Bean Preparation

*Raw Bean Samples:* Raw bean samples were received and ground down into a fine powder. A Wyllie Mill with a mesh size of 40 was used to achieve the uniform particle size for all samples.

*Canned Bean Samples*

Each canned bean sample were either homogenized using the liquid contents in the can or homogenized after by rinsing the beans and draining the liquid content. With regard to the canned Kidney beans and Fava beans, the liquid content in the can was water. The liquid content in the canned Navy beans was tomato sauce. Each bean sample was then placed in flask cylinders for freeze drying. Following freeze drying, each sample was placed into the Wyllie Mill, using a mesh size of 40 to obtain uniform particle size, and ground into a fine powder.
Cooked / Time-Treated Navy Bean Samples

Triplicate lots of raw Navy bean seeds were obtained from the Ontario Bean Growers Association. The beans were soaked for three hours, blanched for 6 minutes at a temperature of 76°C, followed by cooking under pressure at 120°C for 25, 50 and 75 minutes. Most processors follow this same procedure for preparing canned beans. The beans were homogenized without the water contents, freeze dried and ground into a fine powder using a Wyllie Mill (mesh size 40) in order to achieve uniform size. The proximate composition and phytonutrient composition was obtained for the triplicate bean lots at each cooking time interval.

Storage

All raw and processed (freeze dried) beans were stored in plastic bags, in a cold room, at a temperature of 4°C.

4.3.3 Nutrient Determination of Ontario Grown Beans

Proximate Analysis

AOAC Official Methods of Analysis were used to determine proximate analysis (percent moisture, total proteins, total lipids, and ash) of raw and processed beans. Total carbohydrate content of the products were determined by the difference.

Dietary Fiber Determination

Total dietary fiber content of three selected beans was measured by the method described by Englyst et al, 1982.
Folate Determination

Samples were analysed for folate within the Laboratory Services Division at the University of Guelph, Ontario. The analysis was conducted using current standard published procedures for folate determination.
4.4 RESULTS

Proximate analysis of raw and cooked Navy beans, Kidney beans and Fava beans are shown in Tables 4.4.1. to 4.4.3.

The data from the present analysis was found to be in close agreement with other available data with regard to the raw legumes. Moisture values for uncooked legumes were generally in good agreement with data from the USDA Handbook No. 8, except for the Fava beans, which showed slightly higher values than the published data (Meiners, 1976; Watt and Merril, 1972). The protein, fat and ash values determined for the samples in the current analysis were also in good agreement with the data tabulated in Handbook No. 8.

With regard to the processed samples, data for moisture, protein, fat, ash and carbohydrate for Fava and Kidney beans (homogenized with liquid contents in the can) are generally in good agreement with data for these components as given in Handbook No. 8 (Appendix A). With regard to the samples which were rinsed and drained from the liquid contents in the can, the proximate values for protein, fat, carbohydrate and ash (with the exception of moisture) appear to be greater than that of the beans analyzed with the liquid contents in the can.
### Table 4.4.1 Proximate Composition of Raw and Canned Navy Beans

<table>
<thead>
<tr>
<th></th>
<th>CHO (g/100g)</th>
<th>Protein (g/100g)</th>
<th>Fat (g/100g)</th>
<th>Ash (g/100g)</th>
<th>Moisture (g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Raw Bean</strong></td>
<td>61.13 ± 0.08</td>
<td>21.93 ± 0.08</td>
<td>1.26 ± 0.07</td>
<td>3.31 ± 0.24</td>
<td>12.37 ± 0.08</td>
</tr>
<tr>
<td><strong>Processed Navy</strong></td>
<td>23.06 ± 0.51</td>
<td>6.19 ± 0.09</td>
<td>0.41 ± 0.29</td>
<td>2.02 ± 0.06</td>
<td>69.38 ± 0.25</td>
</tr>
<tr>
<td><strong>Beans</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>(Baked Beans, homogenized with tomato sauce)</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Processed Navy</strong></td>
<td>22.73 ± 0.60</td>
<td>6.08 ± 0.13</td>
<td>0.40 ± 0.03</td>
<td>2.01 ± 0.05</td>
<td>68.20 ± 0.82</td>
</tr>
<tr>
<td><strong>Beans</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>(Baked Beans, homogenized without tomato sauce)</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a: each value represents the mean ± standard error of the mean for three analysis from each of three purchase lots (N=3).*
Table 4.4.2 Proximate Composition of Raw and Canned Kidney Beans

<table>
<thead>
<tr>
<th></th>
<th>g / 100g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CHO</td>
</tr>
<tr>
<td>Raw Bean</td>
<td>62.45</td>
</tr>
<tr>
<td></td>
<td>± 0.76</td>
</tr>
<tr>
<td>Processed Kidney Beans (Beans homogenized with water contents in can)</td>
<td>14.07</td>
</tr>
<tr>
<td></td>
<td>± 0.38</td>
</tr>
<tr>
<td>Processed Kidney Beans (Beans homogenized without water contents in can)</td>
<td>18.77</td>
</tr>
<tr>
<td></td>
<td>± 0.36</td>
</tr>
</tbody>
</table>

a: each value represents the mean ± standard error of the mean for three analysis from each of three purchase lots (N=3).
Table 4.4.3 Proximate Composition of Raw and Canned Fava Beans

<table>
<thead>
<tr>
<th></th>
<th>g / 100g</th>
<th>CHO</th>
<th>Protein</th>
<th>Fat</th>
<th>Ash</th>
<th>Moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Raw Bean</strong></td>
<td></td>
<td>59.92</td>
<td>25.03</td>
<td>1.42</td>
<td>2.45</td>
<td>11.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 0.13</td>
<td>± 0.58</td>
<td>± 0.01</td>
<td>± 0.12</td>
<td>± 0.36</td>
</tr>
<tr>
<td><strong>Processed Fava Beans (Beans homogenized with water contents in can)</strong></td>
<td></td>
<td>12.67</td>
<td>9.82</td>
<td>0.26</td>
<td>1.12</td>
<td>75.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 0.27</td>
<td>± 0.18</td>
<td>± 0.02</td>
<td>± 0.06</td>
<td>± 0.59</td>
</tr>
<tr>
<td><strong>Processed Fava Beans (Beans homogenized without water contents in can)</strong></td>
<td></td>
<td>23.82</td>
<td>9.13</td>
<td>0.58</td>
<td>2.82</td>
<td>63.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 1.04</td>
<td>± 0.53</td>
<td>± 0.02</td>
<td>± 0.11</td>
<td>± 1.66</td>
</tr>
</tbody>
</table>

a: each value represents the mean ± standard error of the mean for three analysis from each of three purchase lots (N=3).
The proximate analysis of Navy beans cooked at various time intervals to determine the effects of cooking on nutrient retention are provided in Table 4.4.4.

These results indicate that as the cooking continued, the beans took on more water. As they took on more water, their macronutrient composition declined. Figure 4.4.1 illustrates the changes in percent composition of Navy beans as they continue to be cooked.
Table 4.4.4. Proximate Composition of Time-Treated Cooked Navy Beans

<table>
<thead>
<tr>
<th></th>
<th>CHO</th>
<th>Protein</th>
<th>Fat</th>
<th>Ash</th>
<th>Moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blanched for 6 minutes (75.9°C)</strong></td>
<td>29.01</td>
<td>9.61</td>
<td>0.52</td>
<td>1.02</td>
<td>60.88</td>
</tr>
<tr>
<td></td>
<td>± 0.83</td>
<td>± 0.20</td>
<td>± 0.004</td>
<td>± 0.03</td>
<td>± 0.68</td>
</tr>
<tr>
<td><strong>Pressure Cooked for 25 minutes (119.9°C)</strong></td>
<td>21.22</td>
<td>8.07</td>
<td>0.43</td>
<td>1.12</td>
<td>70.33</td>
</tr>
<tr>
<td></td>
<td>± 0.43</td>
<td>± 0.12</td>
<td>± 0.02</td>
<td>± 0.06</td>
<td>± 0.47</td>
</tr>
<tr>
<td><strong>Pressure Cooked for 50 minutes (119.9°C)</strong></td>
<td>21.08</td>
<td>6.99</td>
<td>0.40</td>
<td>0.84</td>
<td>70.13</td>
</tr>
<tr>
<td></td>
<td>± 0.54</td>
<td>± 0.21</td>
<td>± 0.02</td>
<td>± 0.09</td>
<td>± 1.12</td>
</tr>
<tr>
<td><strong>Pressure Cooked for 75 minutes (119.9°C)</strong></td>
<td>19.06</td>
<td>6.36</td>
<td>0.38</td>
<td>0.85</td>
<td>73.06</td>
</tr>
<tr>
<td></td>
<td>± 0.18</td>
<td>± 0.05</td>
<td>± 0.01</td>
<td>± 0.06</td>
<td>± 0.18</td>
</tr>
</tbody>
</table>

a: each value represents the mean ± standard error of the mean for three analysis from each of three purchase lots (N=3).
b: samples are drained from water after cooking.
Figure 4.4.1. % Decrease of Proximate Content From Raw Navy Bean Seeds at Various Cooking Intervals
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60

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The total fiber content of raw and cooked Navy beans, Kidney beans and Fava beans are shown in Table 4.4.5. Values reported for fiber content in Handbook No. 8 are provided in Table 4.4.6.

Values found in the current analysis for raw bean total dietary fiber were much less than those values reported in Handbook No. 8. Considerable differences were noted in the raw Navy and Fava beans. The raw Kidney beans value reported here was in closer proximity to the value reported in Handbook No. 8.

With regard to the canned beans, the values are generally in good agreement to those reported in Handbook No.8.
Table 4.4.5. Total Dietary Fiber Content in Raw and Canned Navy, Kidney and Fava Beans.

<table>
<thead>
<tr>
<th></th>
<th>g / 100g</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw Beans</td>
<td></td>
<td>Canned beans</td>
</tr>
<tr>
<td>Navy</td>
<td>14.50 ± 0.8</td>
<td>3.55 ± 0.10 (with tomato sauce)</td>
<td>2.83 ± 0.60 (without Tomato sauce)</td>
</tr>
<tr>
<td>Kidney</td>
<td>13.10 ± 1.0</td>
<td>3.60 ± 0.10 (without water from can)</td>
<td></td>
</tr>
<tr>
<td>Fava</td>
<td>14.92 ± 0.6</td>
<td>5.67 ± 0.40 (without water from can)</td>
<td></td>
</tr>
</tbody>
</table>

a: each value represents the mean ± standard error of the mean for three analysis from each of three purchase lots (N=3).
b: samples are drained from water after cooking.

Table 4.4.6. Total Dietary Fiber Content in Raw and Canned Navy, Kidney and Fava Beans as Reported By USDA Handbook No. 8.

<table>
<thead>
<tr>
<th></th>
<th>g / 100g</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw Beans</td>
<td></td>
<td>Canned beans</td>
</tr>
<tr>
<td>Navy</td>
<td>24.40</td>
<td>5.10</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>15.20</td>
<td>6.40</td>
<td></td>
</tr>
<tr>
<td>Fava</td>
<td>25.00</td>
<td>3.70</td>
<td></td>
</tr>
</tbody>
</table>

a: Data obtained from USDA Handbook No. 8.
The folate content of raw and cooked Navy, Kidney and Fava beans are shown in Table 4.4.7 and 4.4.8.

Folate values for raw and processed Navy, Kidney and Fava beans were found to be considerably low in this study compared to those values provided in Handbook No. 8. The percentage of vitamin retention following processing was very low. With the exception of the Fava bean, virtually 100% of folate was lost after processing of canned beans. The Fava beans showed a 77% reduction of the folate content in those beans not homogenized with the liquid content in the can, and only a 61% reduction in folate of those beans that were homogenized with the liquid content in the can.

With regard to the time / treated cooked navy beans, a 94%, 96%, 97.6% and 99% reduction in folate composition was seen in the 6 minute blanched, 25, 50 and 75 minute pressure cooked beans respectively.
Table 4.4.7. Folate Content of Raw and Canned Navy, Kidney and Fava Beans

<table>
<thead>
<tr>
<th></th>
<th>Navy Bean</th>
<th>Kidney Bean</th>
<th>Fava Bean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw Bean</td>
<td>99.54 ± 4.08</td>
<td>103.93 ± 19.63</td>
<td>85.44 ± 26.25</td>
</tr>
<tr>
<td>Canned Beans (homogenized with liquid content in can)</td>
<td>0.26 ± 0.014</td>
<td>0.18 ± 0.02</td>
<td>32.93 ± 7.16</td>
</tr>
<tr>
<td>Canned Beans (homogenized with liquid content in can)</td>
<td>0.01 ± .002</td>
<td>0.09 ± 0.003</td>
<td>19.48 ± 6.78</td>
</tr>
</tbody>
</table>

* Each value represents the mean ± standard error of the mean for three analyses from each of three purchase lots (n=3).

Table 4.3.8. Folate Content of Time-Treated Cooked Navy Beans

<table>
<thead>
<tr>
<th></th>
<th>mcg / 100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw Bean</td>
<td></td>
</tr>
<tr>
<td>Blanched for 6 minutes (75.9°C)</td>
<td>99.54 ± 4.08</td>
</tr>
<tr>
<td>Pressure Cooked for 25 minutes (119.9°C)</td>
<td>5.60 ± 0.11</td>
</tr>
<tr>
<td>Pressure Cooked for 50 minutes (119.9°C)</td>
<td>3.32 ± 0.22</td>
</tr>
<tr>
<td>Pressure Cooked for 75 minutes (119.9°C)</td>
<td>2.34 ± 0.013</td>
</tr>
<tr>
<td></td>
<td>0.125 ± 0.007</td>
</tr>
</tbody>
</table>

* Each value represents the mean ± standard error of the mean for three analyses from each of three purchase lots (n=3).

b: Samples are drained from water after cooking.
4.5 DISCUSSION

Proximate Composition of Raw Beans

Data on proximate composition (moisture, protein, fat, carbohydrate and ash) values are provided for raw, canned and cooked legumes in Tables 4.4.1 through 4.4.3. With regard to the raw legumes, the data from the present analysis was found to be in close agreement with other available data. Moisture values for uncooked legumes were generally in good agreement with data tabulated in the USDA Handbook No. 8, except for the Fava beans, which showed slightly higher values than the published data (Meiners, 1976; Watt and Merril, 1972). Meiners (1976), explained that the moisture content of dried legumes can be greatly affected by relative humidity of the surrounding atmosphere at harvest and during storage. Any differences in moisture content between the current analysis and data from other published sources may be due to past environments and sources of the samples (Meiner et al., 1976).

The protein, fat and ash values determined for the samples in the current analysis were also in good agreement with the data tabulated in Handbook No. 8. Variations among lots of raw legumes are shown by the standard errors reported in Tables 4.4.1 – 4.4.3 for each of the proximate components which were determined by laboratory analysis. Results of triplicate analysis for the different market lots of the legumes analysed were in remarkably good agreement. The slight variation displayed by the SE may be primarily caused by differences among bean samples. These differences, while not great (~ ≤ 1%) were seen in the carbohydrate, protein and ash of the Fava beans; carbohydrate and
protein in Kidney and Navy beans. As previously mentioned, these differences may be caused by past environments, i.e., varied climatic conditions.

*Proximate Composition of Canned Beans*

One of the most popular methods for preparing beans in western countries is canning. Canned beans contain water, which may or may not be used in preparing beans dishes depending on cultural or personal preferences. Some cooking practices prefer to drain the water from the canned beans, while others choose to include it in their finished product.

Meiners et al. (1976), examined the cooking water of legumes to determine the proximate composition remaining when different varieties of legumes were cooked. Their study found that the water in which the beans were cooked contained some residues. The cooking water contained about 1 to 3% protein, a small amount of ash and between 4 to 10% other materials, of which was suspected to be primarily carbohydrate. Therefore, in the current study, the analysis included examining the bean samples homogenized with the liquid contents in the can and also the bean samples rinsed and drained from the liquid content in the can.

Data for proximate composition of canned legumes on Navy, Kidney and Fava are provided in tables 4.4.1 – 4.4.3. Data for moisture, protein, fat, ash and carbohydrate for
Fava and Kidney beans (homogenized with liquid contents in the can) are generally in good agreement with data for these components as given in Handbook No. 8 (page 14).

Discrepancies in data of the current analysis and that of Handbook No. 8 are easily distinguishable with regard to the canned navy beans (homogenized with tomato sauce). It is important to point out that the canned navy beans in the current study contained tomato sauce, whereas those reported by Handbook No. 8 are prepared with water. However, the moisture content of the canned navy beans is in close proximity to that of Handbook No. 8 which may suggest that the tomato sauce present in the current analysis may compensate for the water content found in other canned navy beans. The greater differences are seen in the protein, carbohydrate and ash content, all of which may be explained by the presence of tomato sauce.

With regard to the samples which were rinsed and drained from the liquid contents in the can, the proximate values for protein, fat, carbohydrate and ash (with the exception of moisture) appear to be greater than that of the beans analyzed with the liquid contents in the can. On a g/100g basis, the lack of water content in the can would make the serving of beans more concentrated with macronutrients. This could explain why the proximate values for the beans homogenized without water are slightly higher than those with water.

Cooking such as boiling, is also a popular method of preparing beans. The length of time required to cook beans may bring out several changes in physical and nutritional qualities. In addition, prolonged cooking may even reduce the nutritive quality of beans
Several workers have studied the changes in cooking methods on proximate composition of dry beans (Carpenter, 1981; Deshpande, 1984; Halaby et al., 1981; Meiners et al., 1976; Sgarbieri, 1986; Sathe et al, 1984¹; Sathe et al, 1984²), but none have examined the nutrients lost during the cooking procedure at various time intervals.

With the need for accurate tables of food composition, most attention has focused on the effects of cooking on nutrient content and nutrient retention. In the current study, proximate analysis was conducted to obtain the nutrient composition of Navy Beans at various cooking time intervals to determine the effects of cooking on nutrient retention. The results of this experiment are shown in Table 4.3.4.

Table 4.4.4 indicates that as cooking continues, the beans take on more water. As they take on more water, their macronutrient composition declines. Figure 4.4.1 illustrates the changes in percent composition of navy beans as they continue to be cooked.

### Fiber Composition of Raw, Canned and Cooked Beans

Values for total dietary fiber content in selected beans are reported in Table 4.4.5. The values for total fiber content reported in the USDA Handbook No. 8 are provided in Table 4.4.6.
In contrast to the generally good agreement between data from this research and that which is provided in Handbook No. 8 for protein, fat and ash contents, values reported here for total dietary fiber for raw beans were much less than total dietary fiber values reported in Handbook No. 8. Considerable differences were noted in the raw Navy and Fava beans. The raw Kidney beans value reported here was in closer proximity to the value reported in Handbook No. 8. With regard to the canned beans, the values are generally in good agreement to those reported in Handbook No. 8.

The methods used for measuring dietary fiber are intended to mimic the enzymatic and chemical reactions of food in the mouth, stomach and small intestine. Discrepancies in published values for some raw and canned foods could be explained by the characteristics of the enzymes used and, to some extent, by the principle of the method used (Baker and Holden, 1981; Englyst, 1982; Mongeau and Brassard, 1982; Paul and Southgate, 1978). The value for canned baked beans in tomato sauce reported by Englyst et al. (1982), is 3.87g/100g. This is in good agreement with the current reported value of Navy beans in tomato sauce, which also used the Englyst method for fiber determination.

Folate Composition of Raw, Canned and Cooked Beans

Augustine et al (1981), reported the mineral and vitamin composition of raw and cooked samples of nine classes of Paseolus vulgaris species. Among those finding were the results of the folate content within the legumes. On a 100g dry weight basis, the mean
Folate values for raw beans was 0.30 mg. After cooking, the value fell to 0.22 mg, indicating that 73% of the vitamin was retained. In the current study however, the percentage of vitamin retention following processing was much lower. With the exception of the Fava bean, virtually 100% of folate was lost after processing of canned beans. The Fava beans showed a 77% reduction of the folate content in those beans not homogenized with the liquid content in the can, and only a 61% reduction in folate in those beans that were homogenized with the liquid content in the can.

Although folate values provided by Handbook No. 8 are much higher than those found in this study, it is possible that commercial processing techniques have a significant effect on folate retention. Legumes are generally thought to be good sources of folate, however, it appears that not all legumes will retain nutrients to the same extent under commercial processing techniques.

With regard to the time / treated cooked navy beans, a 94%, 96%, 97.6% and 99% reduction in folate composition was seen in the 6 minute blanched, 25, 50 and 75 minute pressure cooked beans respectively.

Although the values reported for folate in this study is much lower than those reported in other published works, the folate value for canned navy beans and the final time / treated value (which is intended to mimic the cooking method of processed canned beans) are in good agreement, indicating almost a 100% reduction in folate content.
It is difficult to speculate why the reported values in this study are so different than those in published work (Augustine et al, 1981; USDA Handbook No. 8). It may be a result of different geographical growing location, growing seasons, processing techniques and analytical techniques.

4.6 SUMMARY

The nutrient contents of selected beans, from the current study, suggest they are ideally suited to meet major dietary recommendations for good health. The fat content found in canned Navy beans (in tomato sauce), Kidney and Fava beans (combined with the water content from the can) in the current study is very low in fat. On a 2000 kcal per day diet, a one-cup serving (250 ml) of Navy, Kidney or Fava beans will contribute only 2%, 1.8% and 1.3% calories from fat. This is approximately 80% less total fat than that provided by lean ground beef and because beans contain a much higher ratio of unsaturated to saturated fats, a serving of beans will provide a higher quality fat.

Beans are generally thought to provide a high quality plant protein. The protein contribution by the Navy beans (with tomato sauce), Kidney and Fava beans (with water content from the can) in this study (based on a 2000 kcal per day diet) will provide 23%, 16% and 20% of the recommended nutrient intake, respectively. The protein content of legumes is of special interest worldwide due to high cost and the increased health
concerns regarding high fat animal protein sources. Hence, bean protein is a good alternative source.

Dietary guidelines recommend consuming increased amounts of starches and complex carbohydrates, which also makes beans ideally suited for Canada's Guide to Healthy Eating. A one-cup serving (based on a 2000 kcal diet) of canned Navy beans (in tomato sauce), Kidney and Fava beans (with the water contents from the can) provide 21%, 13% and 11.5% of the recommended intake of total carbohydrates.

Based on the current analysis, a one-cup serving of canned Navy beans (in tomato sauce) Kidney and Fava beans (with out the water content from the can) will provide between 28-39%, 17-24% and 27-38% (respectively), of the current recommendation of 25 – 35g/day of fiber. A number of articles have implicated dietary fiber as a major component in certain foods which may provide important health benefits in diabetes, hypercholesterolemia and cancer (Anderson and Akanji, 1991; Jenkins et al, 1980; Saperstein et al, 1978) making beans functionally beneficial in the diet.

The following figures illustrate the percentage of the recommended nutrient intake obtained from consuming a one-cup serving of canned Navy, Kidney and Fava beans, based on a 2000 kcal diet.
Figure 4.6.1 Percentage of Recommended Daily Intake of Macronutrients, Fiber and Folate From One-Cup Serving of Canned Navy Beans

- W/TS: represents canned Navy beans in tomato sauce
- WO/TS: represents canned Navy beans with tomato sauce removed
Figure 4.6.2 Percentage of Recommended Daily Intake of Macronutrients, Fiber and Folate From One-Cup Serving of Canned Kidney Beans

- W / H2O: represents canned Kidney beans with water content in can
- WO / H2O: represents canned Kidney beans without water content in can
Figure 4.6.3 Percentage of Recommended Daily Intake of Macronutrients, Fiber and Folate From One-Cup Serving of 
Canned Fava Beans

- W / H2O: represents canned Fava beans with water content in can
- WO / H2O: represents canned Fava beans without water content in can
Figures 4.6.1 to 4.6.3 indicate that a small portion of the macronutrients, folate and fiber are leached out into the liquid content of the can. Preferences to consume beans with or without the liquid content found in canned beans appear to alter the percentage of macronutrients, folate and fiber contributed to the diet per serving. Consuming the canned beans with the liquid portion of the can will provide a slightly higher nutrient intake.
CHAPTER 5

- PHYTONUTRIENT COMPOSITION -
5.1 INTRODUCTION

Recent studies have suggested that dry legumes may contain a number of biologically active compounds referred to as phytonutrients. These phytonutrients which include trypsin inhibitors, phytates, haemagglutinins, saponins and oligosaccharides, have traditionally been labeled "anti-nutrients" because of their ability to reduce the availability and utilization of nutrients (Thompson, 1993). They are predominantly present in raw legumes seeds. Cooking and or processing may alter the levels of these heat-labile phytonutrients (Deshpande et al., 1984). Studies now indicate that as a result of the compositional changes that occur during processing, these phytonutrients may occur at levels that may exert significant beneficial properties (Anderson and Wolf, 1995; Deshpande et al., 1984; Liener, 1993; Thompson, 1993; Wang and Wixon, 1999).

Much research has been conducted on the phytonutrients found in soybeans. These studies have shown their beneficial effects of include lowering serum cholesterol, decreasing the risk of heart disease, maintaining bone density, and lowering the frequency of breast, colon and prostate cancer (Kennedy, 1995; Messina and Barnes, 1991; Second International Symposium on the Role of Soy in Preventing and Treating Chronic Disease, 1996; Thompson, 1993; Wang and Wixon, 1999).

Although soybeans have been studied extensively with regard to its phytochemical composition and their role in disease prevention, legumes being from the same family may also contain a similar profile of the beneficial phytonutrients.
Very little information about the phytonutrient composition of beans is available in the literature. Therefore this study was undertaken with the objective of analyzing the phytonutrient composition of commonly consumed beans grown in Ontario.

5.2 MATERIALS AND METHODS

5.2.1 Dry Bean Samples and Preparation

See Chapter 4 (page 46).

5.2.2 Phytonutrient Determination

Fat free samples were used for the estimation of trypsin inhibitor activity, saponins, isoflavones, phytic acid and lectins.

Preparation of Fat Free Dry Samples

The dry powder after preliminary processing was weighed accurately into a thimble and plugged with a metal cap and filter paper. The magnetic thimble was placed into a Soxtec Fat Analyzer and fat was extracted using petroleum ether for 20 minutes. The fat free residue was collected in glass cups, cooled and weighed.

Trypsin Inhibitors Determination

Trypsin Inhibitory Activity was determined by the method of Roy and Rao (1971). The activity of the enzyme, trypsin, was assayed using casein as substrate and inhibition of this activity was measured in the bean extracts.
500 mg of defatted, pulverized bean material was treated with 20 ml of 0.1M sodium phosphate buffer (pH 7.5). The samples were shaken for 1 hour and centrifuged at 2200 rpm for 30 min at 15°C. The supernatants were diluted in such a way that inhibition was observed at 60, 80, and 100% of control enzyme activity.

The incubation mixture consisted of 0.1ml of trypsin (0.05 mg/ml in 0.001 M HCL), 0.2 ml 2% casein solution in phosphate buffer, 0.06ml of bean extract and 0.04ml of sodium phosphate buffer (pH 7.5). incubation was carried out at 37°C for 20 min. The treatment was stopped by the addition of 0.6%ml 5% TCA. Corresponding blanks were run concurrently and absorbance measured at 280 nm in a spectrophotometer.

One trypsin unit (TU) is arbitrarily defined as an increase of 0.01 absorbance units at 280nm under the conditions described. Trypsin inhibitor activity is defined as the number of trypsin units inhibited (TUI).

*Phytic Acid Determination*

Phytic acid was estimated by the method of Davies and Reid (1979).

One gram of ground, fat free sample was extracted with 25ml of 0.5N HNO3 for 3 hours by continuous shaking, followed by centrifuging at 12000 rpm for 20 min. The supernatant was collected and made up to suitable volume. 0.28ml of the filtrate was
combined with 0.2ml of ferric ammonium sulphate solution (21mg in 100ml of H2O) and placed in a boiling water bath for 20 min.

The contents were cooled and 1ml of isoamyl alcohol was added and mixed. 0.02ml of 10% ammonium thiocyanate solution was added and shaken thoroughly, and centrifuged at 3000 rpm for 10 min. The alcohol layer was then separated and the color intensity was read at 465nm against isoamyl alcohol blank after 15 min. Sodium phytate standard (4 – 40 ug) was run simultaneously with the samples and results were expressed as mg phytic acid / 100g.d.wt.

*Saponin Determination*

Saponin estimation was determined using method of unpublished work by Gurfinkle and Rao, 2001.

Finely ground, defatted beans material was extracted in methanol (70ml) for 4hr at 60C, with gentle shaking. The saponin containing methanol extract was filtered and the bean residue washed with additional methanol bringing the total volume of methanol extract to 100 ml. A clean-up procedure was then applied to separate the saponin from non-saponin constituents of the methanol extract. 5 ml aliquot was mixed in a 1:1 ratio with 0.4 M ammonium sulfate and left overnight at room temperature. This resulted in the formation of non-saponin precipitate. The precipitate was removed by centrifugation at 2000g for
30 min. The precipitate was then washed three times with 1:1 mixture of methanol and 0.4 M ammonium sulphate to release any entrapped saponins. The saponin-containing centrifugate was then slowly passed through an Oasis HLB extraction cartridge (Waters Corporation; 500 mg adsorbent, 12-ml barrel). The saponins were retained by the adsorbent. The cartridge was washed with 10 ml of water and the saponins were extracted from the cartridge with 6 ml of methanol. 5 ul were applied to a TLC plate for saponin determination by direct densitometry.

*Haemagglutination Activity Determination (Lectin Activity)*

Haemagglutinin activity (HU) was estimated by the method described by Weder et al., 1997. One gram of finely ground bean sample was combined with 5ml of PBS (pH 7.2). Extraction took place by continuous shaking for 24 hours at 4C. the mixture was centrifuged at 16000 rpm for 20 min at 0C. The supernatant was collected and diluted serially with PBS for a total of 6 dilutions (6,250ug/ml to 200,000ug/ml). 0.025ml of each dilution was lightly shaken with 0.025ml of 2% erythrocyte suspension (0.4ml of erythrocyte pellets with 19.6ml of PBS). The mixture was left for 1 hour and shook slightly to detect agglutination activity.

One unit of haemagglutination activity (HU) is defined as the amount of material per ml in the last dilution giving visible haemagglutination.
Oligosaccharide Determination

Samples were analysed for fructo-oligosaccharide content at the ORAFTI Company in Belgium. The analysis was conducted using the method described by Prosky and Hoebregs (1999).
5.3 RESULTS

The selected phytonutrient content in Navy, Kidney and Fava beans are shown in Tables 5.3.1. to 5.3.4.

The literature suggests that cooking generally inactivates the heat sensitive factors such as trypsin inhibitors, lectins, phytates and oligosaccharides, while the heat stable factors such as saponins, may only be slightly reduced during heat processing (Deshpande et al, 1984; Reddy et al, 1982). In the current study, lectin activity (HA) is still present following processing (Tables 5.3.1 to 5.3.3). The HA in the current study suggests a reduction by 96% in canned kidney beans with water and 97.4% reduction in canned Kidney beans without water. It also indicates a 77.4% decrease in Fava beans with water, and 84.87% without water. The Navy beans in tomato sauce showed a 98.8% reduction in HA and a 98.9% reduction when the tomato sauce was removed (Figure 5.3.1). The HA of the time / treated Navy beans appear to be increasingly inactivated as processing continued (Table 5.3.4.). This is to be expected as lectins are a heat sensitive factor. The appreciable decrease in HA from the raw Navy bean seed at 6, 25, 50 and 75 minutes were 57.8%, 96.5%, 98% and 98.9%, respectively.

The trypsin inhibitory activity (TIA), in the current study, appears to be somewhat affected by commercial heat processing. The loss of TIA in Navy beans cooked at different time intervals (Figure 5.3.4.) also suggests the presence of heat labile and non-labile inhibitory components in the bean seed (Singh & Jambunathan, 1981). In the current study, only 69.21%, 55.85% and 62.56% of TIA of Kidney beans with water,
Table 5.3.1. Phytonutrient Composition of Raw and Canned Navy Beans

<table>
<thead>
<tr>
<th></th>
<th>TIU (x10^3)</th>
<th>Saponin</th>
<th>Pytic Acid</th>
<th>Fructo-Oligo-Saccharide (g)</th>
<th>HU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw Bean</td>
<td>3827.24</td>
<td>0.38</td>
<td>2.41</td>
<td>3.41</td>
<td>146302.94</td>
</tr>
<tr>
<td></td>
<td>± 322.6</td>
<td>± 0.04</td>
<td>± 0.01</td>
<td>± 133.5</td>
<td></td>
</tr>
<tr>
<td>Processed Navy</td>
<td>2137.57</td>
<td>0.27</td>
<td>1.32</td>
<td>1.36</td>
<td>1633.06</td>
</tr>
<tr>
<td>Beans (Baked Beans,</td>
<td>± 93.9</td>
<td>± 0.03</td>
<td>± 0.03</td>
<td>± 13.7</td>
<td></td>
</tr>
<tr>
<td>homogenized with</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tomato sauce)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Processed Navy</td>
<td>1063.43</td>
<td>0.26</td>
<td>1.19</td>
<td>0.7</td>
<td>1573.69</td>
</tr>
<tr>
<td>Beans (Baked Beans,</td>
<td>± 42.2</td>
<td>± 0.07</td>
<td>± 0.03</td>
<td>± 40.1</td>
<td></td>
</tr>
<tr>
<td>homogenized with</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>out tomato sauce)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a: each value represents the mean ± standard error of the mean for three analysis, one from each of three purchase lots (N=3).
Table 5.3.2. Phytonutrient Composition of Raw and Canned Kidney Beans

<table>
<thead>
<tr>
<th></th>
<th>TIU (x10^3)</th>
<th>Saponin</th>
<th>Pytic Acid</th>
<th>Fructo-Oligo-Saccharide (g)</th>
<th>HU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw Bean</td>
<td>11196.60</td>
<td>0.29</td>
<td>2.33</td>
<td>3.27</td>
<td>72405.59</td>
</tr>
<tr>
<td></td>
<td>± 174.3</td>
<td>± 0.02</td>
<td>± 0.02</td>
<td>± 179.6</td>
<td></td>
</tr>
<tr>
<td>Processed Kidney</td>
<td>7749.28</td>
<td>0.25</td>
<td>1.31</td>
<td>N/A</td>
<td>2233.39</td>
</tr>
<tr>
<td>Beans (Beans</td>
<td>± 819.2</td>
<td>± 0.11</td>
<td></td>
<td>± 56.6</td>
<td></td>
</tr>
<tr>
<td>homogenized with</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>water contents in</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>can)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Processed Kidney</td>
<td>6307.59</td>
<td>0.24</td>
<td>1.20</td>
<td>0.55</td>
<td>1818.38</td>
</tr>
<tr>
<td>Beans (Beans</td>
<td>± 564.7</td>
<td>± 0.05</td>
<td>± 0.01</td>
<td>± 34.1</td>
<td></td>
</tr>
<tr>
<td>homogenized with</td>
<td></td>
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<tr>
<td>out water contents</td>
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<tr>
<td>in can)</td>
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<td></td>
</tr>
</tbody>
</table>

a: each value represents the mean ± standard error of the mean for three analysis, one from each of three purchase lots (N=3).
Table 5.3.3 Phytonutrient Composition of Raw and Canned Fava Beans

<table>
<thead>
<tr>
<th></th>
<th>TIU (x10³)</th>
<th>Saponin</th>
<th>Pytic Acid</th>
<th>Fructo-Oligo-Saccharide (g)</th>
<th>HU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw Bean</td>
<td>2466.77</td>
<td>0.00</td>
<td>1.96</td>
<td>3.19</td>
<td>9006.84</td>
</tr>
<tr>
<td></td>
<td>± 134.6</td>
<td></td>
<td>± 0.02</td>
<td>± 0.04</td>
<td>± 36.8</td>
</tr>
<tr>
<td>Processed Fava Beans (Beans homogenized with water contents in can)</td>
<td>1543.41</td>
<td>0.00</td>
<td>1.54</td>
<td>N/A</td>
<td>2035.49</td>
</tr>
<tr>
<td></td>
<td>± 81.3</td>
<td></td>
<td>± 0.04</td>
<td></td>
<td>± 49.8</td>
</tr>
<tr>
<td>Processed Fava Beans (Beans homogenized without water contents in can)</td>
<td>702.58</td>
<td>0.00</td>
<td>1.45</td>
<td>0.55</td>
<td>1361.88</td>
</tr>
<tr>
<td></td>
<td>± 58.6</td>
<td></td>
<td>± 0.85</td>
<td>± 0.08</td>
<td>± 63.9</td>
</tr>
</tbody>
</table>

*a: each value represents the mean ± standard error of the mean for three analysis, one from each of three purchase lots (N=3).*
Table 5.3.4. Phytonutrient Composition of Time-Treated Cooked Navy Beans

<table>
<thead>
<tr>
<th></th>
<th>TIU (x10^3)</th>
<th>Saponin %</th>
<th>Pytic Acid %</th>
<th>Fructo-Oligo-Saccharide (g)</th>
<th>HU</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blanched for 6 minutes (75.9°C)</strong></td>
<td>3739.82 ± 73.2</td>
<td>0.33 ± 0.09</td>
<td>1.59 ± 0.09</td>
<td>N/A</td>
<td>61717.42 ± 1697.2</td>
</tr>
<tr>
<td><strong>Pressure Cooked for 25 minutes (119.9°C)</strong></td>
<td>2997.36 ± 98.7</td>
<td>0.32 ± 0.02</td>
<td>1.24 ± 0.02</td>
<td>N/A</td>
<td>5114.96 ± 88.6</td>
</tr>
<tr>
<td><strong>Pressure Cooked for 50 minutes (119.9°C)</strong></td>
<td>2551.72 ± 68.8</td>
<td>0.28 ± 0.01</td>
<td>1.16 ± 0.01</td>
<td>N/A</td>
<td>2860.69 ± 84.9</td>
</tr>
<tr>
<td><strong>Pressure Cooked for 75 minutes (119.9°C)</strong></td>
<td>2366.77 ± 33.77</td>
<td>0.27 ± 0.02</td>
<td>1.14 ± 0.02</td>
<td>N/A</td>
<td>1561.21 ± 5.35</td>
</tr>
</tbody>
</table>

*a: each value represents the mean ± standard error of the mean for three analysis, one from each of three purchase lots (N=3).*
Navy beans with tomato sauce and Fava beans with water (respectively), still remained after commercial processing. When TIA was measured for the same beans without the liquid contents from the can, 56.33%, 27.78% and 28.48% TIA remained (respectively). The commercially mimicked time / treated navy bean samples indicate an appreciable loss of TIA also. As cooking progressed under the various time intervals, TIA decreases by 2.28%, 21.68%, 33.32% and 38.15%, in the beans cooked for 6, 25, 50 and 75 minutes (respectively), from the raw bean. The final time interval indicates 61.85% still remaining after pressure cooking.

The range of phytate reduction from the raw seed in Navy, Kidney and Fava beans drained from the liquid contents in the can was 50.62%, 48.49% and 26.02%, respectively. When the liquid contents of the canned beans was not removed, the phytic acid reduction was only 45.22%, 43.77% and 21.42% respectively (Figure 5.3.1. – 5.3.3). With regard to the time / treated cooked Navy beans, phytate content appreciably decreased by 34.02%, 48.54%, 51.86% and 52.69%, as cooking continued.

Oligosaccharide content for the 3 canned samples without the liquid contents from the can showed a 79.47%, 83.18% and 82.75% decrease respectively. Although oligosaccharide content for canned Kidney and Fava beans with water contents are not reported, the Navy bean sample with tomato sauce shows only a 60.11% reduction.

With regard to the saponin analysis, the current study showed a 28.94% and 31.57% reduction in saponin content of Navy beans with and without tomato sauce (respectively).
Canned Kidney beans showed a higher level of saponin retention with the water content (13.79%) and without the water content (17.24%) from the can compared to the canned Navy beans in tomato sauce. When viewing the effect of cooking Navy beans at different time intervals, saponin content decreased gradually. The reduction ranged from 13.15% to 28.94%, which is in good agreement with previously mentioned, published work.

Figures 5.3.1 – 5.3.4. illustrate the phytonutrient content remaining in canned beans with or without the liquid contents combined with the beans. These figures appear to suggest that the removal of the heat stable factors in canned beans is primarily due to leaching losses (when the water or tomato sauce in the can is removed). When the liquid contents from the can is not removed, a lower loss of heat stable phytonutrients are observed.
Figure 5.3.1 % Reduction of Phytonutrient Content of Canned Navy Beans in Tomato Sauce and Without Tomato Sauce From Raw Navy Bean Seed
Figure 5.3.2  % Reduction of Phytonutrient Content of Canned Kidney Beans in Water and Without Water From Raw Kidney Beans Seed
Figure 5.3.3. % Reduction of Phytonutrient Content of Canned Fava Beans With Water and Without Water From Raw Fava Bean Seed
Figure 5.3.4. % Reduction of Phytonutrient Content of Time Treated Cooked Navy Beans From Raw Navy Bean Seed
5.4 DISCUSSION

Dry beans contain a number of components once referred to as antinutrients, including trypsin inhibitors, saponins, heamagglutinins (lectins), Phytates, and oligosaccharides, among others. Processing and heat treatment are required to prepare grain legume seeds for food use. Cooking or processing will alter the antinutrient levels in beans and their biological properties, permitting more efficient digestion and assimilation of protein and starch.

Cooking generally inactivates the heat sensitive factors such as trypsin inhibitors, lectins, phytates and oligosaccharides, while the heat stable factors such as saponins, may only be slightly reduced during heat processing (Deshpande et al, 1984; Reddy et al, 1982). Complete inactivation of the heat sensitive factors cannot be achieved. Thompson et al (1983), found that heat processing or cooking can only reduce the levels of lectins. De Mulelenaere (1964), found lectins to also be resistant to inactivation by dry heat. Jaffe (1980), reported that lectins in beans were heat resistant and were not completely destroyed.

In the current study, lectin activity, otherwise known as heamagglutiation activity (HA), is still present following processing (Tables 5.3.1 to 5.3.3). A number of studies have reported almost complete inactivation of HA in *Phaseolus vulgaris* stain of legumes (Liener, 1977; 1979). There is generally good agreement with HA of processed Kidney beans and Navy beans in the current study. However, the Fava bean retains 15 – 20% of
HU after processing. This could be explained by the different variety from which the Fava bean is derived (Vicia faba) and the presence of more heat stable isomeric forms in this bean.

The HA of the time / treated Navy beans appear to be increasingly inactivated as processing continues (Table 5.3.4.). This is to be expected as lectins are a heat sensitive factor. The appreciable decrease in HA from the raw Navy bean seed at 6, 25, 50 and 75 minutes were 57.8%, 96.5%, 98% and 98.9%, respectively.

To date no studies have indicated at what level lectins will exhibit their potential beneficial effects. However, Noah et al (1980), have suggested that through adequate cooking, resulting in almost complete inactivation of HA, will not bring about symptoms of lectin toxicity reported in humans.

Trypsin inhibitors, being protein are subject to denaturation and inactivation by heat. In the current study, trypsin inhibitory activity (TIA) appears to be reasonably destroyed by commercial heat processing. The loss of TIA in Navy beans cooked at different time intervals (Figure 5.3.4.) also suggests the presence of heat labile and non-labile inhibitory components in the bean seed (Singh & Jambunathan, 1981). The resistance to complete destruction of TIA in legumes by heat is reported by a number of researchers (Anderson et al, 1995; Ellenrieder et al, 1980; Kennedy, 1995).
In the current study, TIA in Kidney and Fava beans with water and Navy beans with tomato sauce still remained after commercial processing. When TIA was measured for the same beans without the liquid contents, less TIA remained in all three samples. This suggests that a substantial amount of TIA is leached out into the water. Depending on individual cooking practices and preferences (cooking and consuming canned beans with or without the liquid contents in the can), this could have a significant impact on the amount of trypsin inhibitor units one consumes, when eating canned legumes.

The commercially mimicked time / treated navy bean samples indicate an appreciable loss of TIA also. As cooking progressed under the various time intervals, TIA decreased gradually from the raw bean. The final time interval of the pressure cooked samples indicated a somewhat higher TIA than the canned Navy beans with or without tomato sauce. This suggests that TIA is destroyed more readily through commercial processing rather than pressure cooking, as done within the home.

In general, two types of TI are recognized: the heat labile Kuntz Inhibitor (KI) and the heat stable Bowman Birk Inhibitor (BBI). Levels of TI in these beans and in processed beans may be a reflection of relative amounts of these inhibitors presence.

Reduction in phytates and oligosaccharides have been observed in Navy, Kidney and Pinto beans after cooking for 90 min at 100°C (Tyer et al, 1980). Other researchers have also reported similar findings (Deshpande et al, 1984; Reddy et al, 1982; Tinsley et al,
1985). The range of phytate reduction from the raw seed in Navy, Kidney and Fava beans appeared to follow the same pattern as that suggested in the literature. With regard to the time / treated cooked Navy beans, phytate content appeared to be more readily destroyed within the initial cooking period and then ultimately reached some sort of threshold point during prolonged cooking (Figure 5.3.4.). The results therefore indicated that even after prolonged cooking times, approximately 50% of the phytic acid content will be retained in the bean.

Oligosaccharide content for the 3 bean samples without the liquid contents from the can showed a decrease. Although oligosaccharide content for canned Kidney and Fava beans with water contents are not reported, the Navy bean sample with tomato sauce shows only a 60.11% reduction. This showed a considerably different phytic acid content remaining compared to Navy beans without tomato sauce, suggesting a substantial amount of leaching into the tomato sauce.

Saponins are heat stable factors and may not be reduced significantly during heat processing. Although there was no saponin detection in the raw or processed Fava bean, the analysis indicated their presence in both the navy and kidney beans.

There have been very few studies on the saponin content of cooked or processed beans. However, Price et al (1987), suggest that cooking and canning have little effect on the saponin content of various beans. The current study showed a 28.94% and 31.57% reduction in saponin content of Navy beans with and without tomato sauce (respectively).
Canned Kidney beans showed a higher level of saponin retention with the water content (13.79%) and without the water content (17.24%) from the can compared to the canned Navy beans in tomato sauce. When viewing the effect of cooking Navy beans at different time intervals, saponin content decreased gradually. The range of saponin losses were in good agreement with previously mentioned, published work.

5.5. SUMMARY

The current study indicates that certain phytonutrients are present in Ontario grown beans. Traditionally, these same phytonutrients were considered toxic. However, recent studies now suggest that they may be responsible for exerting beneficial effects against various chronic diseases. In fact, these same studies have indicated that in many cases, the same interactions that make them antinutritive are also responsible for their beneficial effects. However, these health benefits are only possible at certain levels of phytonutrient intake.

To date, much of the clinical applications with regard to phytonutrients in beans have focused on those found in soy beans, with a number of reviews discussing the health significance of these findings (Anderson et al., 1995; Kennedy, 1995; Kushi et al., 1999; Liener, 1993; Messina, 1999; Messina and Barnes, 1991; Thompson, 1993; Wang and Wixon, 1999). Dry beans also contain several of the same phytonutrients identified in
soybeans. Whether they exert the same protection as what was seen with soybean consumption, is still to be determined.

Observations from the current study showed that the phytonutrient levels from processed beans where generally lower than soy beans. The reason for this is not clear, however the fact that soy-beans are oilseed, as opposed to grain legumes, may explain the differences. It is also possible that through commercial processing and canning beans with water, grain legumes undergo a higher level of leaching than oilseeds and are therefore unable to retain their phytonutrients to the same extent as soybeans.

Attention should focus on finding ways to preserve phytonutrients in canned grain legumes. Additionally, further studies should also investigate whether the phytonutrients in grain legumes can exert the same physiological benefits as those observed from soy beans.
CHAPTER 6

- THE EFFECT OF NAVY BEAN CONSUMPTION ON
AZOXYMETHANE-INDUCED COLONIC PRENEOPLASIA -
6.1 INTRODUCTION

Colon cancer is one of the leading causes of cancer related-deaths in the world (World Cancer Research Fund, 1997). In the mid 1990’s, more than 870,000 new cases were diagnosed worldwide (WHO, 1997), which accounted for almost 10% of all new cancers (World Cancer Research Fund, 1997). Colon cancer is second only to lung cancer in North America, and its incidence and mortality rates are rising (Pappalardo et al, 1996).

Epidemiological data suggests that colon cancer is the form of cancer most associated with diet (Potter, 1996) accounting for up to 90% of geographical differences in colorectal cancer incidence (Pappalardo et al, 1996; World Cancer Research Fund, 1997). This has caused speculation that significant decreases in colon cancer incidence could be achieved through dietary modification (Doll and Peto, 1981). It is suggested that a major protector against cancer appears to be a diet high in fiber, low in fat, with generous intakes of plant foods that are rich in nutrients and phytochemicals (Anderson et al, 1999). Foods that possess these characteristics are fruits, vegetables and legumes. From the legume family, beans (*Phaseolus vulgaris*), are low in fat, contain a good source of dietary fiber and a wide range of phytochemicals that have been linked to the reduction in colon cancer (Hughes et al, 1997). However, bean consumption has received little attention with regard to their potential role in reducing the incidence of colon cancer.

To date, among the legume family, soy beans have received the most amount of attention for their role in the prevention of cancer. Phytochemical contents of soy beans have been suggested as playing an important role in disease prevention (Messina, 1991; 1999).
Many researchers have used animal models to evaluate the capacity of phytochemicals to reduce the onset and progression of colon carcinogenesis. Inositol hexaphosphate (Ullah and Shamsuddin, 1990), saponins (Koratkar and Rao, 1997), polyphenols (Kim et al, 1994), protease inhibitors (Kennedy, 1994; St. Clair et al, 1990) and oligosaccharides (Koo and Rao, 1991) are among phytochemicals that have shown to protect against or prevent carcinogenesis in animals.

Recent studies reveal that dry beans also contain several of the same components found in soy beans suggested to have anticarcinogenic effects, yet very little information exists on dry bean consumption and the incidence of colon cancer.

6.2 OBJECTIVE

In a rat model, the objective of this study, therefore, was to investigate the role of bean consumption on chemically-induced preneoplastic lesions in the colon of rats.

6.3 MATERIALS AND METHODS

Animals

A total of 48 male Fisher rats (F344) at 3 weeks of age weighing approximately 58 grams from Charles River, Inc. Montreal, Canada, were used in this study. The animals were individually housed in plastic cages with corncob bedding under a 12:12-hour light-dark cycle and controlled temperature (22°C) and humidity (50°C) conditions. Food and water
were provided ad libitum. The care and housing for the rats complied with the guidelines under the Canadian Council on Animal Care. The protocol was approved by the Ethics Committee for the use of animals, University of Toronto.

Preparation and Composition of Diets

The macro- and micro- nutrient composition of all four experimental diets fed to the rats are outlined in Table 6.3.1 and 6.3.2.

All diets prepared for this study were isocaloric. The bean flour was quantitatively used to replace the AIN-93M diet. Rats in the control group consumed 100% bean- free, AIN-93M diet with casein (Dyets Inc., Bethelhem, PA) while all other rats consumed one of three prepared mixtures of AIN-93M diet and canned navy beans without tomato sauce. The three diet mixes were as follows:

a) Test group 1: 10% of the diet supplied by navy beans and 90% AIN93M diet.

b) Test group 2: 25% of the diet supplied by navy beans and 75% AIN93M diet.

c) Test group 3: 50% of the diet supplied by navy beans and 50% AIN93M diet.

Navy beans in tomato sauce were obtained from the H.J. Heinz Company of Canada, Ltd. The beans were drained, rinsed and freeze dried. The dry beans were then ground into a fine flour to achieve the same consistency as the AIN-93M diet. The diets were mixed and prepared according to the composition percentage of the bean diet. Fresh diets were prepared every month, and all diets were refrigerated to prevent spoilage.
Table 6.3.1 Composition of Experimental Diets Fed to Rats
(g/100g)

<table>
<thead>
<tr>
<th></th>
<th>AIN-93M Casein Diet</th>
<th>10% Bean-fed Diet</th>
<th>25% Bean-fed Diet</th>
<th>50% Bean-fed Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIN-93M Beans</td>
<td>100</td>
<td>90</td>
<td>75</td>
<td>50</td>
</tr>
<tr>
<td>CHO</td>
<td>72.07</td>
<td>72.11</td>
<td>72.16</td>
<td>72.27</td>
</tr>
<tr>
<td>Protein</td>
<td>14</td>
<td>14.42</td>
<td>15.04</td>
<td>16.08</td>
</tr>
<tr>
<td>Fat</td>
<td>4</td>
<td>3.73</td>
<td>3.32</td>
<td>2.63</td>
</tr>
<tr>
<td>Fiber</td>
<td>5</td>
<td>5.39</td>
<td>5.98</td>
<td>6.95</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.32</td>
<td>0.28</td>
<td>0.24</td>
<td>0.16</td>
</tr>
</tbody>
</table>

a: 0% rat diet consists of 100% AIN-93M diet mix
b: 10% bean diet consists of 10% canned navy beans and 90% AIN-93M diet mix
c: 25% bean diet consists of 25% canned navy beans and 75% AIN-93M diet mix
d: 50% bean diet consists of 50% canned navy beans and 50% AIN-93M diet mix
e: Canned Navy beans supplied by the H.J. Heinz Company of Canada
f: AIN-93M Diet mix supplied by Dyets Inc., Bethlehem, Pennsylvania
Table 6.3.2 Contribution of AIN 93M Diet to the Vitamin and Mineral Content of the Rat Diet (35g/kg)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>0% Beans Diet</th>
<th>10% Bean Diet</th>
<th>25% Bean Diet</th>
<th>50% Bean Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vitamin Mix</strong> (mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Niacin</td>
<td>300.00</td>
<td>270.0</td>
<td>225.01</td>
<td>150.02</td>
</tr>
<tr>
<td>Thiamine</td>
<td>60.00</td>
<td>54.01</td>
<td>45.03</td>
<td>30.07</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>60.00</td>
<td>54.05</td>
<td>45.81</td>
<td>30.22</td>
</tr>
<tr>
<td>Folic Acid (mcg)</td>
<td>20.00</td>
<td>18.21</td>
<td>15.34</td>
<td>10.01</td>
</tr>
<tr>
<td>Pantothenic acid</td>
<td>160.00</td>
<td>144.02</td>
<td>120.23</td>
<td>80.67</td>
</tr>
<tr>
<td>Vitamin B6</td>
<td>70.00</td>
<td>63.10</td>
<td>52.5</td>
<td>35.71</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>250.00</td>
<td>225.23</td>
<td>187.50</td>
<td>112.50</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>80.00</td>
<td>72.01</td>
<td>60.02</td>
<td>40.04</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>1500.00</td>
<td>1350.66</td>
<td>1125.81</td>
<td>750.01</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>25.00</td>
<td>22.50</td>
<td>18.50</td>
<td>11.20</td>
</tr>
<tr>
<td><strong>Mineral Mix</strong> (mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>357.00</td>
<td>321.35</td>
<td>267.86</td>
<td>178.74</td>
</tr>
<tr>
<td>Potassium</td>
<td>324.60</td>
<td>292.43</td>
<td>244.17</td>
<td>162.44</td>
</tr>
<tr>
<td>Sodium</td>
<td>74.00</td>
<td>67.10</td>
<td>56.72</td>
<td>39.44</td>
</tr>
<tr>
<td>Magnesium</td>
<td>24.00</td>
<td>21.65</td>
<td>18.12</td>
<td>12.24</td>
</tr>
<tr>
<td>Iron</td>
<td>6.06</td>
<td>5.45</td>
<td>4.54</td>
<td>3.04</td>
</tr>
<tr>
<td>Zinc</td>
<td>1.65</td>
<td>1.48</td>
<td>1.24</td>
<td>0.83</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.63</td>
<td>0.56</td>
<td>0.48</td>
<td>0.32</td>
</tr>
<tr>
<td>Selenium</td>
<td>0.30</td>
<td>0.02</td>
<td>0.006</td>
<td>0.00</td>
</tr>
</tbody>
</table>

a: 0% rat diet consists of 100% AIN-93M diet mix  
b: 10% bean diet consists of 10% canned navy beans and 90% AIN-93M diet mix  
c: 25% bean diet consists of 25% canned navy beans and 75% AIN-93M diet mix  
d: 50% bean diet consists of 50% canned navy beans and 50% AIN-93M diet mix  
e: Canned Navy beans supplied by the H.J. Heinz Company of Canada  
f: AIN-93M Diet mix supplied by Dyets Inc., Bethelhem, Pennsylvania
**Study Design**

Animals were acclimatization for a period of one week, where they consumed laboratory Purina rat chow. The animals were then randomly assigned to one of the four groups, which contained 12 rats in each treatment. Following acclimatization, rats were placed on their respective diets for 4 weeks to determine the protein efficiency ratio of the dry beans used in this study. After the rats had consumed their respective diets for 4 weeks, treatment with carcinogen began. Rats from all groups received 3 intraperitoneal injections of azoxymethane (AOM) (Sigma-Aldrich Canada LTD., Ontario, Canada) each consisting of 5 mg/kg of body wt, over the course of one week, totaling 15 mg/kg body wt for each rat. AOM was dissolved in 0.9% saline to a concentration of 5 mg/ml a day prior to injections. Body weights and food intake were monitored every week for the duration of the experiment. Fecal weights were obtained the week following the last AOM injection and the final week of the experiment. Upon completion of the experiment (100 days after AOM injections) rats were euthanized by cardiac puncture and cervical dislocation under CO₂.

**Colon Preparation**

Colons were immediately removed from the rats, washed free of fecal material with 0.9% saline, cut longitudinally and spread out on filter paper. The colons were then fixed flat in 10% buffered Formalin (VWR Canlab Inc., Ontario, Canada) between two filter papers for one week before ACF determination.
**ACF Enumeration**

The method used for ACF enumeration was followed by that outlined by Bruce et al. (1993) and Mclellan (1990). Fixed colons were stained with 0.2% methylene blue for 15-20 min in a petri dish. The colons were then scored for Aberrant Crypt Foci (ACF) and Aberrant Crypts (AC) on the mucosal surface of the colon with a light microscope under a magnification of x40. ACF and AC were distinguished from normal crypts by their darker staining, enlarged and slightly elongated size, thick epithelial lining, slightly elongated cryptal opening, and increased pericryptal zone. The total number of ACF and AC were recorded for all colons. Total ACF were divided into 3 separate groups to reflect foci of different aggressive states. ACF which consisted of 1 – 3 AC’s were classified as small, 4 – 6 AC’s; medium and ACF that consisted of 7 AC’s or greater were regarded as large.

**Statistical Analysis**

All statistical analysis was done using unpaired two-tailed students t-test in the Excel 5.0 program (Microsoft Corp., Redmond, WA). P values of <0.05 were considered statistically significant. Results were expressed as mean±SEM.

**6.4 RESULTS**

All animals remained healthy throughout the experimental period. Body weights, food intake, food efficiency ratio (FER) and protein efficiency ratio (PER) of rats in various groups are shown in Table 6.4.1 and 6.4.2.
Table 6.4.1 Effects of Dry Bean Consumption on Body Weight, Food Intake, Protein Efficacy and Food Efficacy in Rats.

<table>
<thead>
<tr>
<th>Week</th>
<th>Groups</th>
<th>Body Weight (g)</th>
<th>Food Intake (g/week)</th>
<th>FER (g)</th>
<th>PER (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1</td>
<td>Control</td>
<td>91.34±3.17</td>
<td>74.65±1.87</td>
<td>0.44±0.01</td>
<td>3.15±0.03</td>
</tr>
<tr>
<td></td>
<td>10% bean-fed</td>
<td>93.55±2.96</td>
<td>76.58±1.35</td>
<td>0.46±0.01</td>
<td>3.19±0.02</td>
</tr>
<tr>
<td></td>
<td>25% bean-fed</td>
<td>93.50±2.83</td>
<td>77.35±1.25</td>
<td>0.45±0.01</td>
<td>3.08±0.02</td>
</tr>
<tr>
<td></td>
<td>50% bean-fed</td>
<td>90.77±2.79</td>
<td>75.95±1.42</td>
<td>0.44±0.01</td>
<td>2.72±0.03*</td>
</tr>
<tr>
<td>Week 6</td>
<td>Control</td>
<td>208.23±6.31</td>
<td>109.48±1.87</td>
<td>0.27±0.01</td>
<td>1.92±0.07</td>
</tr>
<tr>
<td>(Following AOM injections)</td>
<td>10% bean-fed</td>
<td>210.11±4.02</td>
<td>110.01±1.35</td>
<td>0.17±0.01</td>
<td>1.22±0.09</td>
</tr>
<tr>
<td></td>
<td>25% bean-fed</td>
<td>206.10±3.16</td>
<td>111.00±1.25</td>
<td>0.17±0.01</td>
<td>1.18±0.05*</td>
</tr>
<tr>
<td></td>
<td>50% bean-fed</td>
<td>191.87±4.67*</td>
<td>110.40±1.42</td>
<td>0.16±0.01*</td>
<td>1.12±0.20*</td>
</tr>
<tr>
<td>Week 20</td>
<td>Control</td>
<td>345.73±4.73</td>
<td>119.38±1.87</td>
<td>0.05±0.01</td>
<td>0.35±0.05</td>
</tr>
<tr>
<td>(Prior to euthanasia)</td>
<td>10% bean-fed</td>
<td>348.15±4.85</td>
<td>123.82±1.27</td>
<td>0.04±0.01</td>
<td>0.32±0.03</td>
</tr>
<tr>
<td></td>
<td>25% bean-fed</td>
<td>339.49±4.70</td>
<td>131.05±1.63*</td>
<td>0.06±0.01*</td>
<td>0.34±0.04</td>
</tr>
<tr>
<td></td>
<td>50% bean-fed</td>
<td>320.72±5.75*</td>
<td>128.40±1.28*</td>
<td>0.07±0.01*</td>
<td>0.34±0.05</td>
</tr>
</tbody>
</table>

a: FER, food efficiency ratio (body weight gain (g) / food intake (g/day));
PER, protein efficiency ratio (body weight gain (g) / protein intake (g/day)).
b: Values are Means ± SEM.
c: * Significantly different from control P<0.05 (n=12 for each group).
Table 6.4.2 Mean Daily Food and Protein Intake.

<table>
<thead>
<tr>
<th>Week</th>
<th>Groups</th>
<th>Mean Daily Food Intake (g)</th>
<th>Mean Daily Protein Intake (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1</td>
<td>Control</td>
<td>10.66±0.25</td>
<td>1.49±0.03</td>
</tr>
<tr>
<td></td>
<td>10% bean-fed</td>
<td>10.94±0.27</td>
<td>1.57±0.07</td>
</tr>
<tr>
<td></td>
<td>25% bean-fed</td>
<td>11.05±0.23</td>
<td>1.66±0.05</td>
</tr>
<tr>
<td></td>
<td>50% bean-fed</td>
<td>10.71±0.25</td>
<td>1.72±0.03</td>
</tr>
<tr>
<td>Week 6 (Following AOM injections)</td>
<td>Control</td>
<td>15.64±0.26</td>
<td>2.18±0.02</td>
</tr>
<tr>
<td></td>
<td>10% bean-fed</td>
<td>15.72±0.28</td>
<td>2.26±0.02</td>
</tr>
<tr>
<td></td>
<td>25% bean-fed</td>
<td>15.85±0.31</td>
<td>2.38±0.01*</td>
</tr>
<tr>
<td></td>
<td>50% bean-fed</td>
<td>15.77±0.29</td>
<td>2.51±0.01*</td>
</tr>
<tr>
<td>Week 20 (Prior to euthanasia)</td>
<td>Control</td>
<td>17.04±0.33</td>
<td>2.39±0.060</td>
</tr>
<tr>
<td></td>
<td>10% bean-fed</td>
<td>17.68±0.25</td>
<td>2.55±0.05</td>
</tr>
<tr>
<td></td>
<td>25% bean-fed</td>
<td>18.72±0.23*</td>
<td>2.82±0.03*</td>
</tr>
<tr>
<td></td>
<td>50% bean-fed</td>
<td>18.34±0.36*</td>
<td>2.95±0.02*</td>
</tr>
</tbody>
</table>

a: Values are Means ± SEM.
b: * Significantly different from control (P<0.05).
c: n=12 for each group.
**Body Weights**

Body weights did not significantly (P<0.05) differ between the control group and those rats consuming the 10% and 25% bean diets throughout the study period. However, there was a significant (P<0.05) decrease in body weight in rats that received 50% of their diet from beans following AOM injections, and prior to euthanasia.

**Food Intake**

Mean weekly and daily food intakes did not significantly (P<0.05) differ between all groups except within the latter period of the study where significant increases were observed in rats that were receiving 25% and 50% of their diet from navy beans. All groups continued a consistent food intake throughout the study suggesting that the bean diets were well tolerated.

**FER**

FER was calculated by determining the body weight gain (g/week) of the rats divided by their food intake (g/week). There was a significant decrease (P<0.05) in the FER in rats consuming the 50% bean diet, following AOM, compared to the control diet. There was however a significant increase in the FER in the 25% and 50% bean groups compared to control within the latter period of the study. This could be explained by their significantly higher food intakes during the same time.
PER

PER was calculated by determining the body weight gain (g/week) of the rats divided by their protein intake (g/week). The results indicate a significantly lower (P<0.05) PER value in the 50% bean-fed rats at the end of week one. This is surprising since there was no significant difference (P<0.05) in the mean daily protein intake during the same time period. Week six also showed significantly (P<0.05) lower PER values for the 25 and 50% bean-fed groups compared to controls. However, the daily protein intake for the same time frame indicates significantly (P<0.05) higher levels of protein for these two groups. This may suggest that the quality of protein in these diets may be questionable.

Week twenty shows significantly (P<0.05) higher levels of daily protein intake for the 25 and 50% bean-fed groups, but no significant differences were seen in the PER value. This is of great interest as food intakes were significantly (P<0.05) higher in these groups compared to the control group. Furthermore, the macronutrient composition of the diets (Table 6.3.1) indicate higher protein contribution to the diet from the 25 and 50% bean diets. Increased food intake coupled with a higher protein diet should ideally produce significant increases in PER values. However, this is not the case, which further supports the possibility that the quality of bean protein in this diet is poor.

Fecal Output

Fecal output was measured at the time following AOM injections and once again prior to euthanasia. There were significant (P<0.05) increases in fecal output in the 25% and 50% bean-fed groups compared to the control group following the AOM injections.
Significant (P<0.05) increases in fecal output were also observed in all three bean-fed groups compared to the control group at the point prior to euthanasia. All three bean-fed groups also showed significant (P<0.05) increases in fecal output from the time AOM injections were given to the time the animals were euthanized, but no significant differences were observed in fecal output in the control group during the same time period. This is illustrated in Figure 6.4.1.
Figure 6.4.1 Changes in Fecal Output between Bean-Fed Diets and Control-Fed Diets Over Time.

a: * Significant difference from control following AOM injections.
b: † Significant difference from control prior to euthanasia.
c: ‡ Significant difference from time AOM injections were given to time at which animals were sacrificed.
Aberrant Crypt Foci and Aberrant Crypt Development

The dose response effect of bean consumption on AOM-induced ACF incidence is shown in Table 3. Although there were no statistical differences in the total number of ACF between the control group and the bean-fed groups, some protective trends were noticed. A reduction of 14.14% (65.83±6.26), 19.03% (62.08±8.73) and 5.11% (72.75±6.59) in ACF development was observed in rats consuming 10, 25, and 50% bean diets respectively, over the control group (76.67±9.55). There were significant (P<0.05) decreases in the total number of ACF development in the 10% and 25% bean-fed groups compared to control animals (175±15.61, 170.17±25.37 and 254.83±33.69, respectively). A 17.8% reduction in the total number of ACF was observed in the 50% bean-fed group, but this difference was not significant. When ACF were classified according to their size (1-3 ACF’s or small, 4-6 ACF’s or medium and 7 or greater ACF’s or large) no significant differences were seen among all bean-fed groups and the control group with regard to small size ACF. The 10% bean-fed group showed significant (P<0.05) decreases in medium size ACF compared to control, and although no significant differences were observed in the 25% and 50% bean-fed groups with regard to medium size ACF, there was a 38.9% and 21.47% respective reduction in medium size ACF over the control group. All bean-fed animals showed significant (P<0.05) decreases in large size ACF over the control.
<table>
<thead>
<tr>
<th>Group</th>
<th>Total ACF</th>
<th>Total AC</th>
<th>Small ACF</th>
<th>Medium ACF</th>
<th>Large ACF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>76.67±9.55</td>
<td>254.83±33.69</td>
<td>46.92±5.33 (61%)</td>
<td>24.83±4.03 (32%)</td>
<td>4.92±0.85 (6.40%)</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% Bean-Fed</td>
<td>65.83±6.26</td>
<td>175.00±15.61*</td>
<td>50.00±5.50 (76%)</td>
<td>15.17±1.15* (23%)</td>
<td>0.67±0.30* (1.00%)</td>
</tr>
<tr>
<td>25% Bean-Fed</td>
<td>62.08±8.73</td>
<td>170.17±25.37*</td>
<td>46.25±6.32 (75%)</td>
<td>15.18±2.80 (24%)</td>
<td>0.58±0.24* (0.93%)</td>
</tr>
<tr>
<td>50% Bean-Fed</td>
<td>72.75±6.59</td>
<td>209.42±22.55</td>
<td>52.33±4.83 (72%)</td>
<td>19.50±2.35 (27%)</td>
<td>1.25±0.52* (1.72%)</td>
</tr>
</tbody>
</table>

a: ACF, aberrant crypt foci; AC, Aberrant crypt.
b: Size of ACF: small; 1-3 AC/foci, medium; 4-6 AC/foci, large; 7 or greater AC/foci.
c: Values are means ± SEM.
d: * Significant difference from control (P<0.05).
e: (%) Percentage of total ACF
Figure 6.4.2. Percent ACF Decrease in Bean-fed Animals from Controls.

*Percent ACF decrease in bean-fed animals from controls. ACF were classified according to their size as small (1-3 ACF/foci), medium (4-6 ACF/foci) and large (>7 ACF/foci). n=12.
6.5 DISCUSSION

Dry beans supply a good source of protein, fiber, essential vitamins and minerals, are low in fat and are regarded as a healthy food item (Geil and Anderson, 1994). Dry bean intake has been shown to have protective and therapeutic effects against conditions such as coronary heart disease, diabetes mellitus and obesity (Geil and Anderson, 1994), however, to date few data exist on the intake of dry beans and the incidence of colon cancer. Although there is limited epidemiological data which has investigated the consumption of beans and the incidence of cancer, some researchers have reported a decrease in colon cancer incidence in areas of the world where dry bean intake is high, i.e. Latin America and Mexico, and a higher incidence in colon cancer where dry beans consumption is low such as the U.S. and Canada (Correa, 1981; Lucier et al, 2000).

The results of this study show that canned Navy beans without tomato sauce can significantly inhibit the incidence of AOM-induced colonic preneoplastic lesions in rats. AOM-induced colon cancer in male Fisher rats at a dose of 15mg/kg of body weight is a commonly used model for evaluating cancer promoting or inhibiting capacity of dietary components. (Holt et al, 1996). Colon cancer begins with the appearance of ACF, which sequentially develop into polyps and adenomas and ultimately carcinomas. Therefore ACF are reliable endpoint biomarkers of colon cancer for short-term animal studies (Bruce et al, 1993; Bird, 1995).

In this study, protective trends were noticed in those animals that consumed the various percentages of a bean diet. Although only the 10% and 25% bean-fed groups showed a
significant decrease in total AC, all bean-fed groups showed reductions in the incidence of total ACF and AC over the control-fed animals. The results suggest a stronger protective effect against colon cancer in those groups consuming 10% and 25% of their diet as beans followed by the 50% bean-fed diet.

With regard to the size of the ACF, no significant reductions were made in those ACF which were classified as small. The 10% bean-fed group showed a significant decrease in the medium size ACF, and although not significant, the 25% bean-fed group showed the next greatest protective effect, followed by the 50% bean-fed group against the control. All three bean-fed groups showed a significant decrease in the large size ACF, with the greatest reduction occurring in the 10% and 25% bean-fed groups, followed by the 50% bean-fed group. This is of great importance because large foci are suggested to have more growth potential over smaller foci, hence they are more aggressive (Bird, 1995). Aggressive foci respond differently to growth factors or promoters than less aggressive foci allowing them to get promoted more rapidly to the next step in carcinogenesis. While the decrease in total ACF in the bean-fed groups is not significant, the pronounced reductive effect seen in ACF of increasing size (Fig. 6.4.2) suggests that beans may slow the growth of ACF detaining them from growing into bigger foci.

The results of the data appear to suggest that a greater protective effect against preneoplastic biomarkers of colon cancer occurred within the 10% and 25% bean-fed group followed by the 50% bean-fed group. This is surprising since (Hughes et al 1997), found that rats fed a 59% bean (pinto) diet had significantly fewer colon
adenocarcinomas and tumors than rats that were fed a casein diet. A number of components within beans have been suggested for playing a role in the prevention of cancer, which may also provide some explanation for the protective trend that was observed.

Dry beans are generally considered to be a good source of vegetable protein, however legume proteins have some limitations. They are in general deficient in the sulfur-containing amino acid methionine (Sgarbieri, 1989 and Gupta, 1983). In this study, the group that showed the least protective effect compared to the control was the 50% bean-fed group. The mean body weight of this group was also significantly lower than that of the control group, following the AOM injections, which continued until the end of the study. Since bean flour quantitatively replaced AIN-93M diet, it is possible these rats suffered from a methionine deficiency. Methionine deficiency may produce the alteration in DNA methylation, which is characteristic of tumors and premalignant tissue (World Cancer Research Fund, 1997). It has been reported that rats actually have a methionine requirement that is ~50% higher than humans (Sarwar et al, 1989). Therefore the combination of lower methionine intake coupled with higher requirement for methionine could explain why the least protective effects were seen in the 50% bean-fed group.

Hughes study supplemented the 59% pinto bean diet with methionine and saw no significant differences in body weight between the bean group and control, and also saw
a significant decrease in the development of colonic adenocarcinomas and tumors in rats consuming the bean diet compared to the control.

Despite the decrease in body weight in the 50% bean-fed group, the 10% and 25% bean-fed groups indicated no significant differences in body weight throughout the study. This is important to note because a 10% and 25% inclusion of beans into the daily diet is more realistic for human consumption and easier to achieve that 50% of the daily diet supplemented by beans.

Dry beans also contain some of the richest sources of total dietary fiber (Hughes et al, 1997; Hughes and Swanson, 1989), and have been among the more frequently investigated dietary factors in studies of the etiology of colon cancer (Kushi et al, 1999). Hughes et al (1997) suggests that the dietary fiber in dry beans is a good candidate for the anticarcinogenic properties of dry beans. This is because dry bean fiber contains considerable amounts of insoluble fiber known for diluting carcinogens or bile acid promoters (Jenkins et al, 1987). Insoluble fiber also results in increases in fecal output.

In this study, there was a significant increase in fecal output in all bean-fed groups at the end of the study compared to the control-fed group. It is suggested that small fecal masses may be associated with bowel disease which can weaken the intestinal wall, and allow components such as mutagens or carcinogens to become more highly concentrated in the luminal contents (Burkitt, 1973). Insoluble fibers cause increases in fecal output and reduce the length of time toxic components remain in the colon (Fleming et al, 1985).
Dry beans also contain soluble fiber which have shown to be fermented into SCFA such as acetate, butyrate and propionate, thus lowering colonic pH and influencing bacterial metabolism (Fleming et al, 1985).

In addition to a good source of dietary fiber, dry beans also contain a number of biologically-active compounds referred to as phytochemicals. Phytochemicals such as saponins, phytic acid, protease inhibitors, polyphenols, lectins and oligosaccharides all have demonstrated to have anticarcinogenic properties (Thompson, 1993). To date much of the research that has investigated the anticarcinogenic effects of legume phytochemicals has focused on those found in soy beans (Messina, 1999). Very little is known about the phytochemicals in dry beans, however these phytochemicals have been investigated for their anticarcinogenic properties using various other sources.

Koratkar and Rao (1997), found soy bean saponins to play a role in inhibiting the incidence of ACF in the colons of mice. Similarly, Pretlow et al (1992), found protective effect in rat colon carcinogenesis from phytate obtained from corn or rice. Kennedy et al (1993) and St. Claire et al (1990), demonstrated the effectiveness of trypsin inhibitors on reducing the incidence of cancer, and Kim et al (1994) showed the ability of phenolics to inhibit colon cancer in Fisher rats. Future investigations should examine the individual effectiveness of dry bean phytochemicals on the incidence of colon cancer, since very little is know with regard to their capacity to prevent carcinogenesis.
In conclusion, the results of this study suggest that dry beans (navy beans) have a beneficial effect on AOM-induced colonic preneoplastic markers of colon cancer. However, dry bean acceptance in Western diets still remains low. Further clinical trials are required not only to revive dry beans consumption as a healthy part of the daily diet, but to also encourage its inclusion into the diet as a healthful food known for its protective effects against disease.
CHAPTER 7

- GENERAL DISCUSSION -
7.1 DISCUSSION

Among all cancers, colon cancer is the one that is highly influenced by the diet and has
great potential to be prevented (Potter, 1996). A diet which includes fruits, vegetables
and whole grains, including legumes appear to play a significant role in the prevention of
this disease. Insufficient data from human and animal studies exists for the purpose of
determining the effect of relatively high consumption on cancer risk. The only other
evidence with regard to bean consumption and cancer risk, comes mainly from
experiments that have investigated individual constituents of pulses that have biological
properties and may prevent cancer in experimentally induced animals. No studies have
investigated the cumulative effect of macronutrients, micronutrients, fiber and certain
phytonutrients on the incidence of preneoplasia in rats.

Therefore the overall objective of this study was to investigate the effect of consuming
commercially processed Navy beans on chemically-induced colonic preneoplasia in rats.
To accomplish this objective an investigation of the nutrient and phytonutrient content of
beans was undertaken. Although this information is already available in published
literature, environmental conditions and industrial processing techniques vary, which may
result in different nutrient and phytonutrient values. This was observed as a result of the
current analysis.

The first phase of this analysis showed Kidney beans, Fava beans and Navy beans,
all grown and processed in Ontario, contain the nutritional and phytonutrient components found to be beneficial in preventing chronic diseases of the Western countries.

Cooking or processing is required for legume consumption. It is necessary not only to tenderize the seed, develop aroma, acceptable flavour and texture, but to also to improve the bioavailability and utilization of nutrients and phytonutrients. However, as a result of the cooking process, compositional changes occur within the legume nutrient and phytonutrient content.

Data for moisture, protein, fat, ash and carbohydrate for canned Fava and Kidney beans (homogenized with liquid contents in the can) were generally in good agreement with data provided in Handbook No. 8 (Appendix). There were discrepancies noted from current analysis and that of Handbook No. 8 with regard to the canned Navy beans (homogenized with tomato sauce). These discrepancies may be a factor of the beans being cooked in tomato sauce instead of water. These beans may also require slightly different processing conditions than beans processed and canned in water, which too may alter the proximate values for moisture, fat, ash and carbohydrates.

For the samples that were rinsed and drained from the liquid contents in the can, the proximate values for protein, fat, carbohydrate and ash (with the exception of moisture) appear to be slightly greater than that of the beans analyzed with the liquid contents from the can. This suggests a portion of the macronutrients were leached out into the liquid
content of the can. As a result, it would appear that the utilization of the liquid content within a can of beans would provide more nutrients to the diet.

In contrast to the generally good agreement between data from this research and that which is provided in Handbook No. 8 for protein, fat and ash contents, values reported here for total dietary fiber for raw beans were much less than total dietary fiber values reported in Handbook No. 8. Considerable differences were noted in the raw Navy and Fava beans. The raw Kidney beans value reported here was in closer proximity to the value reported in Handbook No. 8. The methods used for measuring dietary fiber are intended to mimic the enzymatic and chemical reactions of food in the mouth, stomach and small intestine. Discrepancies in published values for some raw and canned foods could be explained by the characteristics of the enzymes used and, to some extent, by the principles of the various methods available for use (Baker and Holden, 1981; Englyst, 1982; Mongeau and Brassard, 1982; Paul and Southgate, 1978).

The literature suggests that approximately 73% of folate is retained after cooking (Augustine et al, 1981). In the current study however, the percentage of vitamin retention following processing was much lower. With the exception of the Fava bean, virtually 100% of folate was lost after processing of the canned Navy and Kidney beans. Although folate values provided by Handbook No. 8 are much higher than those found in this study, it is possible that commercial processing techniques have a significant effect on folate retention. Legumes are generally thought to be good sources of folate, however, it appears that not all legumes will retain nutrients to the same extent under commercial
processing techniques. It may also be a result of different geographical growing location, growing seasons, processing techniques and analytical techniques. Further investigation of this is warranted as folate is a vitamin that can provide many benefits to the diet.

Preferences to consume beans with or without the liquid content found in canned beans will alter the percentage of macronutrients, folate and fiber contributed to the diet per serving. Consuming the canned beans with the liquid portion of the can will provide a slightly higher nutrient intake.

Recent studies suggest that dry legumes may contain a number of biologically active compounds referred to as phytonutrients. These phytonutrients which include trypsin inhibitors, phytates, haemagglutinins, saponins and oligosaccharides, have traditionally been labeled “anti-nutrients” because of their ability to reduce the availability and utilization of nutrients (Thompson, 1993). In the current study, these biologically active phytonutrients were predominantly seen in the raw legume seeds. Cooking and or processing reduced the levels of these phytonutrients. As a result, more efficient digestion and utilization of nutrients from the diet may occur. Various studies have indicated that as a result of the compositional changes that occur during processing, these phytonutrients occur at levels that may posses significant beneficial properties (Anderson and Wolf, 1995; Deshpande et al., 1984; Liener, 1993; Thompson, 1993; Wang and Wixon, 1999).
In the current study, the presence of phytonutrients was observed in both the raw and selected canned beans. Different varieties of beans, and the form in which the canned beans may be consumed, showed varying phytonutrient levels.

The clinical applications of phytonutrients have received a great deal of interest in recent years. In particular, the phytonutrients present in soybeans have been studied extensively, with a number of reviews discussing their findings (Anderson et al., 1995; Kennedy, 1995; Kushi et al., 1999; Liener, 1993; Messina, 1999; Messina and Barnes, 1991; Thompson, 1993; Wang and Wixon, 1999).

The current study indicates that certain phytonutrients are present in Ontario grown beans that were identified in soybeans for exerting potential benefits. Results showed that the phytonutrient levels from the current study are generally lower than those found in soybeans. The reason for this is not clear, however the fact that soy beans are oilseed, as opposed to grain legumes, may explain the differences. It is also possible that through commercial processing and canning beans with water, grain legumes undergo a higher level of leaching than oilseeds and are therefore unable to retain their phytonutrients to the same extent as soybeans. As there are a variety of methods for determining certain phytonutrients, it is possible that methods used in this analysis were different than those used in the literature for phytonutrient determination of soybeans. This could also explain compositional differences between soybeans and the beans analysed in this study. Whether they exert the same protectiveness as what has been observed with soybean consumption, is still to be determined.
The second phase of this study was to investigate the effect of commercially processed bean consumption, at different levels of intake, on the incidence of colonic preneoplasia in rats. The results of this study show that canned Navy beans (rinsed from the tomato sauce) can significantly inhibit the incidence of AOM-induced colonic preneoplastic lesions in rats. AOM-induced colon cancer in male Fisher rats at a dose of 15mg/kg of body weight is a commonly used model for evaluating cancer promoting or inhibiting capacity of dietary components (Holt et al, 1996).

Colon cancer begins with the appearance of ACF, which sequentially develop into polyps and adenomas and ultimately carcinomas. Therefore ACF are reliable endpoint biomarkers of colon cancer for short-term animal studies (Bruce et al, 1993; Bird, 1995).

In this study, protective trends were noticed in those animals that consumed the bean diets. Although only the 10% and 25% bean-fed groups showed a significant decrease in total AC, all bean-fed groups showed reductions in the incidence of total ACF and AC over the control-fed animals. The results suggest a stronger protective effect against colon cancer in those groups consuming 10% and 25% of their diet as beans followed by the 50% bean-fed diet.

The reason for this is not clear. It is possible that the nutritional composition of each diet may be, in part, responsible for the observed results. Table 6.3.1 outlines the macronutrient composition of each diet. Although the protein content of the 50% bean diet is higher, the mean body weight of this group was significantly lower than the
control, and lower than the other bean groups. This may suggest that as the bean flour was quantitatively replacing the AIN diet, the protein quality in the diet was becoming compromised. This would be in good agreement with published literature, which reports bean protein to be somewhat deficient in sulfur-containing amino acids, such as methionine.

The possibility of a poor quality protein intake in the 50% bean fed group is further supported by significantly higher mean PER and food intake values, at the end of the study. Based on this information, the 50% bean-fed group ideally should not have experienced significantly lower body weights. If in fact, the 50% bean-fed group did suffer a methionine deficiency, this would help to explain why the least protective effect was observed in this group, as a methionine deficiency may produce the alteration in DNA methylation, which is characteristic of tumors and premalignant tissue (World Research Fund, 1997).

Another possibility that may help to explain why the 50% bean-fed group showed the least protective effect against AOM-induced colonic preneoplasia may be related to the micronutrient composition of the diets. Table 6.3.2. (page 98) outlines the micronutrient composition of each diet. As the bean flour was quantitatively replaced by the AIN diet, the micronutrient content of the bean diets lessen, with the most pronounced differences seen in the 50% diet.

The current analysis of folate indicates that the folate in canned beans is almost completely eliminated during processing. Therefore, the only folate being supplied to the
animals is that which was provided by the AIN diet. As a result of the AIN diet being substituted for bean flour, the 50% bean-fed group consumed a substantially lesser amount of folate. Low folate intake has been associated with increased risks of colon adenoma and colon cancer (Freudenheim et al, 1991).

Animal studies have also shown that calcium may be a promising agent to reduce the risk of colon cancer (Hyman et al, 1998). Hyman et al (1998), showed that calcium supplements modestly reduced recurrence of colon adenomas in a recently reported randomized trial. The calcium composition of the 50% bean-fed group was considerably lower than all other groups. This may suggest that the lack of sufficient calcium intake may have contributed to the lesser protective effect.

It is important to point out that the animals were placed on their respective diets at only 5 weeks of age. The 50% bean fed group consumed a considerable lower micronutrient intake during their growth stage and in adulthood compared to the other groups. Perhaps the low micronutrient intake during their growth stage and adulthood had some sort of an effect on the animals natural ability to ward off disease.

The current analysis found that the Navy beans used to quantitatively replace the AIN diet contained various phytonutrients. Studies have shown that very high intakes of these components may produce adverse effects by reducing the availability of nutrients and thus causing growth inhibition (Thompson, 1993). The higher level of phytonutrients in
the 50% bean diet coupled with already compromised micronutrient intakes, may have also been responsible for the 50% bean-fed group to show the least protective effect.

All three bean-fed groups showed a significant decrease in the large size ACF, with the greatest reduction occurring in the 10% and 25% groups, followed by the 50% bean-fed group. This is of great importance because large foci are suggested to have more growth potential over smaller foci, hence they are more aggressive (Bird, 1995). Aggressive foci respond differently to growth factors or promoters than less aggressive foci allowing them to get promoted more rapidly to the next step in carcinogenesis.

While the decrease in total ACF in the bean-fed groups is not significant, the pronounced reductive effect seen in ACF of increasing size (Fig. 6.4.2.) suggests that beans may slow the growth of ACF detaining them from growing into bigger foci.

A number of components within legumes have been suggested for playing a role in the prevention of cancer, which may also provide some explanation for the protective trend that was observed.

Dry beans also contain some of the richest sources of total dietary fibre (Hughes et al, 1997; Hughes and Swanson, 1989). Bean fibre contains considerable amounts of insoluble fibre known for diluting carcinogens or bile acid promoters (Jenkins et al, 1987). Insoluble fibre also results in increases in fecal output.
In this study, there was a significant increase in fecal output in all bean-fed groups at the end of the study compared to the control-fed group. It is suggested that small fecal masses may be associated with bowel disease, which can weaken the intestinal wall, and allow components such as mutagens or carcinogens to become more highly concentrated in the luminal contents (Burkitt, 1973). It is possible that the insoluble fibres from the beans used in the current study may have in part been responsible for their protective effect. Dry beans also contain soluble fibre, which appears to be fermented into SCFA such as butyrate, thus lowering colonic pH and influencing bacterial metabolism (Fleming et al, 1985). Because the current study did not analyze the insoluble and soluble fibre components of the beans used in this study, we can only speculate that they are present, and are exerting some sort of beneficial effect.

In addition to a good source of dietary fibre, dry beans also contain a number of biologically-active compounds referred to as phytochemicals. Phytochemicals such as saponins, phytic acid, protease inhibitors, polyphenols, lectins and oligosaccharides all have demonstrated to have anticarcinogenic properties (Thompson, 1993). Very little is known about the phytochemicals in dry beans and the levels at which they may exert protective effects against chronic disease.

In conclusion, the results of this study suggest that canned Navy beans have a beneficial effect on AOM-induced colonic preneoplastic markers of colon cancer. However, bean consumption in Western diets still remains low. Further clinical trials are required not only to revive dry beans consumption as a healthy part of the daily diet, but to also
encourage its inclusion into the diet as a healthful food known for its protective effects against disease.
CHAPTER 8

- SUMMARY -
8.1 SUMMARY

Overall Objective:

The overall objective of this thesis was to investigate the nutritional and phytonutrient composition of selected Ontario grown beans, and study their effect in azoxymethane-induced preneoplasia in rats.

Results form the current study found canned Navy beans to contain anticarcinogenic phytonutrient compounds capable of inhibiting chemically-induced preneoplastic lesions in the rat colon.

Specific Objectives:

a) Nutritional composition of raw Ontario grown Kidney, Fava and Navy beans.

With regard to the raw legumes, the data from the present analysis was found to be in close agreement with other published data. Moisture values for raw legumes were generally in good agreement with data tabulated in the USDA Handbook No. 8, except for Fava beans, which showed slightly higher values than the published data. The protein, fat and ash values determined for the samples in the current analysis were also in good agreement with the data provided in Handbook No. 8. The slight variations among lots of raw legumes may be primarily caused by differences among legume samples and environmental conditions.
ii) Values reported here for total dietary fiber for raw beans were much less than total dietary fibre values reported in Handbook No. 8.

b) Nutritional composition of canned Ontario grown Kidney, Fava and Navy beans.

i) Data for moisture, protein, fat, ash and carbohydrate for canned Fava and Kidney beans (homogenized with liquid contents in the can) were generally in good agreement with data provided in Handbook No. 8 (Appendix). There were discrepancies noted from current analysis and that of Handbook No. 8 with regard to the canned navy beans (homogenized with tomato sauce). These discrepancies may be a factor of the beans being cooked in tomato sauce instead of water. For the samples that were rinsed and drained from the liquid contents in the can, the proximate values for protein, fat, carbohydrate and ash (with the exception of moisture) appear to be slightly greater than that of the beans analyzed with the liquid contents from the can.

ii) The fiber content of the selected canned beans were generally in good agreement to those reported in Handbook No.8.

iii) The percentage of folate remaining following processing was much lower then values provided by Handbook No. 8. With the exception of the Fava bean, which showed a 61-77% reduction in folate, virtually 100% of folate was lost after processing of canned Navy and Kidney beans.
c) **Phytonutrient composition of raw Ontario grown Kidney, Fava and Navy beans.**

i) The current study indicated that the phytonutrients trypsin inhibitors, phytates, lectins, saponins and FOS were present in the selected raw Ontario grown beans chosen for this study.

d) **Phytonutrient composition of canned Ontario grown Kidney, Fava and Navy beans.**

i) The current study indicated that phytonutrients remained following commercial processing. Results from the this analysis also showed that the phytonutrient levels from canned Navy, Kidney and Fava beans where generally lower than literature values provided for Soy beans. The values from the current study also indicate a higher loss of phytonutrients when water contents from the can was removed, suggesting leaching of phytonutrients during processing.

e) **Nutritional and phytonutrient composition of raw and canned Ontario grown Kidney, Fava and Navy beans cooked at different time intervals.**

i) The time / treated analysis of Navy beans in the current study indicated that as cooking continued, beans took on more water, and the macronutrient and phytonutrient contribution progressively declined.

f) **The effects of canned Navy beans on colonic preneoplasia in rats.**

i) With regard to the effect of canned Navy bean consumption on AOM induced colonic preneoplasia in rats, protective trends were observed in those animals that consumed the bean diets.
ii) Although only the 10% and 25% bean-fed groups showed a significant decrease in total AC, all bean-fed groups showed reductions in the incidence of total ACF and AC over the control-fed animals.

iii) With regard to the size of the ACF, all three bean-fed groups showed a significant decrease in the large size ACF, with the greatest reduction occurring in the 10% and 25% bean-fed groups.

iv) In this study, there was a significant increase in fecal output in all bean-fed groups at the end of the study compared to the control-fed group. One possible explanation may be a result of the increased amount of insoluble fibre in the bean-containing diets.

v) It is possible that the phytonutrient components in beans may have contributed to the lower incidence of preneoplasia in the animals which consumed the bean diets over the control.
CHAPTER 9

- CONCLUSION -
9.1 CONCLUSION

It was hypothesized that raw and canned Ontario grown beans contain substantial amounts of macronutrients, micronutrients and phytonutrients that can be affected during processing and may also have an effect on colonic preneoplasia. In order to test this hypothesis, the following specific objectives were undertaken: A) to investigate both the nutritional and phytonutrient composition of raw and commercially processed canned Navy, Kidney and Fava beans and B) investigate the effects of canned Navy bean consumption on the development of preneoplastic lesions in the colons of azoxymethane-treated F344 Fisher rats.

The macro- and micro- nutrient and phytonutrient content of canned beans, homogenized with and without the liquid content found in the can, still contained a respectable amount of these components even after processing, when compared to their raw equivalents. The nutrient and phytonutrient content of canned beans homogenized with the liquid content from the can appeared to be slightly higher than those beans that were not homogenized with the liquid contents, suggesting leaching. From this we can conclude that the utilization of beans with the liquid content from the can will provide a slightly higher level of nutrients and phytonutrients to the diet.

Although the liquid content from the canned Navy beans (tomato sauce) were rinsed from the beans fed to the rats, the consumption of these Navy beans still showed a significant protective effect against the development of aberrant crypt foci and aberrant crypts in chemically induced colonic preneoplasia in the rats.
In conclusion, this study supports the hypothesis as the results demonstrate that Navy, Kidney and Fava beans contain anticarcinogenic phytonutrient compounds capable of inhibiting chemically-induced preneoplastic lesions in the rat colon.
CHAPTER 10

- FUTURE DIRECTIONS -
10.1 FUTURE INVESTIGATIONS

Legumes are globally important dietary components which contain appreciable quantities of nutrients and phytonutrients. It is therefore important to develop a thorough understanding of the nutritional disease preventing benefits of their nutrients and phytonutrients that could not be answered in this study. To accomplish this, several aspects need to be investigated and evaluated.

A) Focus on individual functional components in beans to determine which components are specifically producing beneficial effects and the extent of their benefits.

B) Evaluate the protein quality (amino acid profile) of beans and understand their role in preventing chronic diseases.

C) Evaluate the micronutrient composition of beans and determine their role in the prevention of various chronic diseases.

Other areas for future studies are also listed:

D) Study the effect of agricultural practices and indicate differences in phytonutrient composition of beans.
E) Analyze and compare the nutrient and phytonutrient composition of beans processed using traditional and modern methods.

F) Dietary intervention studies using clinical models to investigate the role of beans in the prevention of cardiovascular disease.

G) Animal studies to further understand the role of beans in initiation, progression and tumorogenesis stages of colon cancer.

H) Long term animal studies using cancer as the end point to study the role of beans in the prevention of colon cancer.

I) Short and long term human intervention studies to investigate the role of beans in the prevention of colon cancer, cancer of other sites and cardiovascular disease.

J) Establish recommended daily intake levels of beans based on the nutrient and phytonutrient composition from animal and human studies.
CHAPTER 11

- REFERENCES -
11.1 REFERENCES


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