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Fenugreek in Western Canada

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ABSTRACT

Fenugreek (*Trigonella foenum-graecum* L.) is a self-pollinating, annual leguminous crop which is well suited to growth in cool and temperate growing regions with low to moderate rainfall, making it suitable for growth within the semi-arid regions of western Canada. Best known as a spice from India, it also has a long history of use as forage feed for livestock and as a medicinal plant that can be used for treatment of a variety of ailments ranging from diabetes, heart disease, and infections, to treatment of cancer. In recent years, laboratory studies and clinical trials have focused on fenugreek as a potential nutraceutical. These studies have shown that fenugreek plants possess immunomodulatory, hypocholesterolaemic, hypoglycaemic, gastro- and hepatoprotective and antioxidative properties. Pharmacological properties of fenugreek have been explored to identify a role for the plant in diabetes management and in cardiovascular health, indicating the presence of bioactive compounds in fenugreek, which may be responsible for its health benefits. Commercial production of fenugreek in western Canada dates back to 1990 when it was introduced for growth as a spice in Saskatchewan. Fenugreek cultivars developed for use in Canada include 'AC Amber', 'CDC Quatro', 'CDC Canagreen' and 'CDC Canafen' which were developed mainly for use as a spice or for use in functional food / nutraceutical extracts of the seed, and 'AC Tristar' which has been developed primarily for use as livestock forage. In this manuscript we look at some of the major biochemical constituents of fenugreek and the physiological effects of these compounds on animal systems. We also look at processing facilities in western Canada that are supporting development of new value-added products for the emerging Canadian functional food industry. New research directions being exploited to develop novel fenugreek varieties with potential for use both domestically and to compete in the international market place are examined.

Keywords: diosgenin, forage, functional foods, galactomannan, 4-hydroxyisoleucine, medicinal plants, nutraceuticals, prairie environment

Abbreviations: **AC**, Agriculture Canada; **CDC**, Crop Development Centre; **ESPL**, Emerald Seed Products Ltd; **HACCP**, Hazard Analysis and Critical Control Points; **HDL**, high density-lipoprotein; **HFD**, high-fat diet; **HTGL**, hepatic triglyceride lipase; **IVDMD**, *in vitro* dry matter disappearance; **LDL**, low-density lipoprotein; **NADP**, nicotinamide adenine dinucleotide phosphate; **OGTT**, oral glucose tolerance test; **SDF**, soluble dietary fiber; **VLDL**, very low-density lipoprotein

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INTRODUCTION

Herbs and other natural food products are recognized for their medicinal or functional food properties because they contain biochemical components which can mitigate the effects of disease and infection or can provide dietary supplements which promote health and vitality in the consumer. One third of all medicines are extracted from plants (Gepts 2004) and in western medicine about 80% of the plant-based drugs used originate from non-western countries in which medicinal plants have been used to treat similar medical conditions (Fabricant and Farnsworth 2001). Fenugreek is one of the oldest medicinal plants known to man (Lust 1986). It has its origins in Mediterranean regions (Vavilov 1926, 1951) and commonly is grown within India and northern Africa for use as food or as a spice, but also as

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Invited Review

a forage for livestock (Petropoulos 2002; Acharya *et al.* 2006b, 2007a). It is a dry-land adapted crop that has significant potential for use as a value-added crop that can be grown in regions threatened with a decreasing water supply and drought (Lapp *et al.* 2005; Byrne *et al.* 2006) such as those found in prairie regions of western Canada (Acharya *et al.* 2008).

Fenugreek also is a medicinal plant that can lower blood glucose and cholesterol levels in animals when consumed (Petropoulos 2002; Srinivasan 2006; Acharya *et al.* 2007b). Current research has shown that it contains beneficial chemical constituents including steroidal sapogenins, fiber, galactomannans, antioxidants, and amino acids such as 4-hydroxyisoleucine which possess anti-diabetic, antioxidant, hypocholesterolemic and hypoglycemic properties which have potential for use in the treatment of obesity, diabetes and cancer (Petropoulos 2002; Acharya *et al.* 2007b). However, usefulness of natural herbs like fenugreek to consumers depends upon our ability to identify plants that contain a consistent biochemical composition capable of producing reliable physiological responses within individuals (Acharya *et al.* 2006a). While one of the primary goals of traditional breeding programs has been to develop plant varieties with consistent production capabilities from year to year under varying environmental conditions, this aspect often has been overlooked in the development of functional food and medicinal plants.

In this manuscript we look at some of the major biochemical constituents of fenugreek and the physiological effects of these compounds on animal systems that make fenugreek an ideal crop alternative for use in semi-arid regions of western Canada. We also look at fenugreek cultivars that have been developed in Canada for use by producers as well as processing facilities available that are supporting development of new value-added products for an emerging Canadian functional food industry. New research directions being exploited to develop novel fenugreek varieties with potential for use both domestically and to compete in the international market place are examined.

FENUGREEK PLANT CHARACTERISTICS

Fenugreek (*Trigonella foenum-graecum* L.) is an annual dicot (Magnoliopsida) belonging to the Leguminaceae (Fabaceae) family of flowering plants, subfamily Papilionaceae (Petropoulos 2002; Acharya *et al.* 2007a). The plants have trifoliate ovate leaves which alternate around circular herbaceous stems which become semi-woody as they mature. They grow 40 to 60 cm (16 to 24 in) in height and often exhibit extensive branching because of an indeterminate growth habit which predominates in most plants (**Fig. 1**). Flowers form within the leaf axils and mature to form long, slender pods which can twist as they age. Single pods containing up to 20 seeds typically are found on the plants. Both the plants and the seeds emanate a distinctive odour which smells somewhat like roasted coffee or maple syrup (Srinivasan 2006; Barbaro 2009).

BIOCHEMICAL CONSTITUENTS AND RELEVANCE AS A FUCTIONAL FOOD

The most important biochemicals identified in fenugreek can be categorized as 1) fiber of which galactomannan is the most important as a functional food and medicinal compound; 2) steroidal saponins of which diosgenin has significant medical applications; 3) alkaloids; 4) the amino acid 4-hydroxyisoleucine is important both as a nutritional supplement and as a medicinal compound; 5) phytochemicals and polyphenols act as antioxidants which can protect both foods and cellular components from oxidative damage; and 6) plant oils and oleoresin which can be used as additives to enhance the odour and flavour of food as well as for use in pesticides and herbicides.

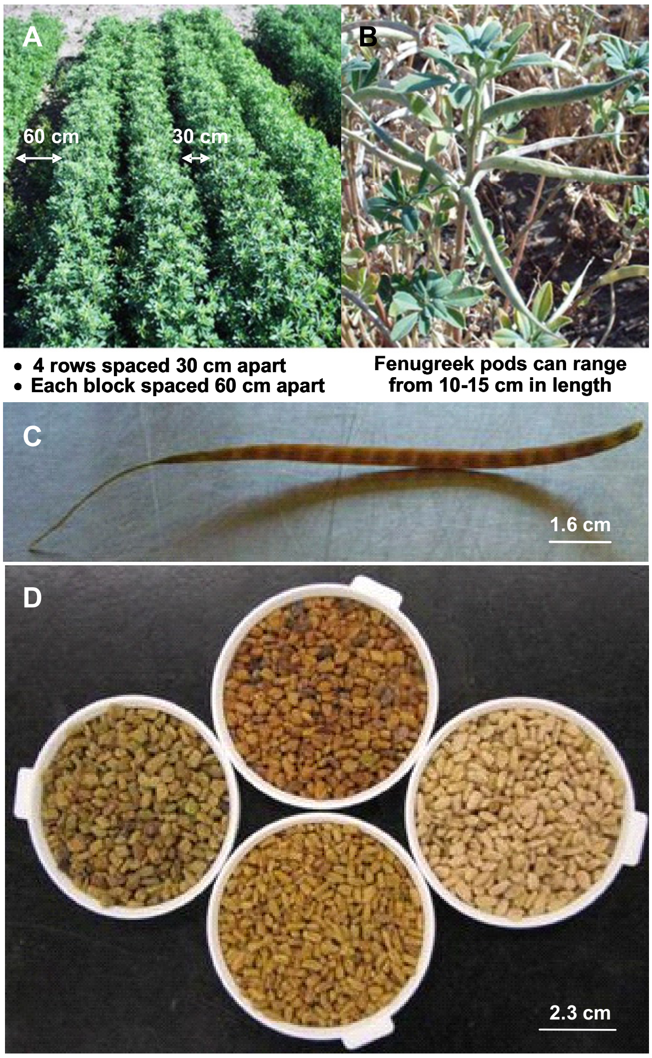

Fig. 1 Fenugreek grown in western Canada. (**A**) Plant foliage and field spacing. (**B**) Seed pods on field-grown fenugreek. (**C**) Seed pod from a harvested plant. (**D**) Color and seed size variation in harvested seeds.

Fiber and galactomannans

Fenugreek seeds are about 50% (w/w) fiber and represent one of the best sources of edible dietary fiber available (Srinivasan 2006). Of this, about 20% of the fiber content is insoluble, while 30% makes up a soluble gum consisting largely of galactomannan. Galactomannans are components of cell walls found in the plant endosperm (Meier and Reid 1997) that are structurally composed of a 1→4 β-D-mannosyl backbone substituted by a single galactose unit α-linked at the C-6 oxygen (Acharya *et al.* 2007b). Fenugreek galactomannans are unique relative to other commonly used galactomannans such as those found in guar and locust beans. They contain a galactose to mannose ratio of 1:1. This high degree of galactose substitution renders the molecule relatively more soluble compared to galactomannans from guar or locust bean, which has a galactose to mannose ratio of 1:2 and 1:4, respectively (Reid and Meier 1970; Brummer *et al.* 2003; Acharya *et al.* 2007b). The 1:1 ratio of galactose:mannose found in fenugreek galactomannans makes them highly hydrophilic and able to bind large amounts of water, making them useful as a thickener for foods as well as for production of jams and jellies (Srinivasan 2006; Acharya *et al.* 2007b).

The soluble nature of galactomannan fiber from fenugreek has been linked to numerous human health benefits, mainly in the reduction of plasma glucose levels which has an antidiabetic effect (Sharma 1986b; Madar *et al.* 1988; Madar and Shomer 1990). This has been demonstrated in animal models where diabetes was experimentally induced in dogs, rats, mice and rabbits using either alloxan or streptozocin (Zanosar®) which kill insulin-producing beta cells in the pancreas of mammals (Swanston-Flatt *et al.* 1989; Grover *et al.* 2002; Mondal *et al.* 2004; Hannan *et al.* 2007). Hannan *et al.* (2007) also have demonstrated that the soluble dietary fiber (SDF) portion of fenugreek can significantly improve glucose homeostasis in type 1 and type 2 diabetics by delaying carbohydrate digestion and absorption. They have also suggested that the SDF fraction may enhance insulin action in type 2 diabetes by improving oral glucose tolerance in afflicted individuals.

Monnier *et al.* (1978) and Jenkins (1979) reported that addition of soluble fiber to the diet of diabetics reduced blood glucose during an oral glucose tolerance test (OGTT). The OGTT is a blood test that is used to assess metabolism of sugar in test subjects. Individuals are required to fast prior to consuming a fixed amount of glucose. Their blood is later tested at designated time intervals in order to determine if it will reach unusually high glucose levels (American Diabetes Association 2008). Johnson and Gee (1980) reported that use of soluble fiber from fenugreek resulted in inhibition of glucose absorption in the intestine. It has been postulated that fenugreek galactomannan may regulate plasma glucose levels by delaying gastric emptying and by interfering with glucose absorption in the gut (Madar 1984; Al-Habori and Rahman 1998; Nahar *et al.* 2000). Sharma (1986b) suggested that the delay in carbohydrate absorption induced by a diet rich in fenugreek fiber could reduce insulin requirements. Dietary fibers such as galactomannan are able to reduce postprandial glycaemia and exhibit great potential in diabetes management (Jenkins *et al.* 2000; Basch *et al.* 2003).

In several small-scale clinical trials, fenugreek seeds have been found to reduce fasting serum glucose levels in patients exhibiting both acute and chronic hyperglycaemia (Basch *et al.* 2003). Cooked and defatted fenugreek seeds (containing soluble fiber) prevented a rise in blood glucose levels in these patients. This was attributed to hypoglycaemic properties of fenugreek gum present in the seeds which were not lost during the cooking process (Srinivasan 2006). Sharma *et al.* (1990) and Sharma and Raghuram (1990) also have reported that reduced levels of fasting blood glucose, improved glucose tolerance and reduced glucose excretion in both insulin-dependent (type 1) diabetic and non-insulin dependent (type 2) diabetic patients respectively, following a 10-day trial in which the subjects were given 100 g of defatted fenugreek powder. Pahwa (1990) conducted a study using both diabetic and non-diabetic test subjects and reported a significant hypoglycaemic effect only in diabetic individuals following fenugreek consumption prior to glucose loading (1 g kg^{-1} body weight). However, in a later study by Nahar *et al.* (1992) a similar blood glucose lowering effect was observed in healthy human subjects given the soluble fiber fraction as a single dose. A double-blind, placebo-controlled study involving 25 type 2 diabetic patients was conducted by Gupta *et al.* (2001) to evaluate the effects of fenugreek on control of blood glucose levels and insulin resistance. Diabetic and normal patients were separated into groups, and they received an alcoholic extract of fenugreek seeds and 'usual care' (dietary discretion and exercise), respectively. It was reported that both groups experienced a reduction in fasting blood glucose levels without significant differences, but significantly lower levels for blood glucose and insulin were observed in the diabetic group. This suggests that fenugreek seed extracts and a discrete diet / exercise habit may be equally effective in the control of blood glucose and insulin levels (Basch *et al.* 2003).

Amin *et al.* (1987) reported that fenugreek seeds exerted hypoglycaemic effects by inhibiting the activities of α-amylase and sucrase. More recent studies have also associated the therapeutic effectiveness of fenugreek with beneficial counter-changes in enzyme activity in glucose and lipid metabolism (Gupta *et al.* 1999; Raju *et al.* 2001). They assessed the effect of fenugreek whole seed powder administered to alloxan-induced diabetic rats on glycolytic, gluconeogenic and nicotinamide adenine dinucleotide phosphate (NADP)-linked lipogenic enzymes in liver and kidney tissues. A favourable restoration of activity of these enzymes to control levels was reported following fenugreek treatment, resulting in stabilization of glucose homeostasis. A potential therapeutic role for fenugreek in the treatment of type-1 diabetes was suggested. Recently, Hannan *et al.* (2007) looked at the inhibitory effects of a SDF fraction from fenugreek on the activity of digestive enzymes *in vivo*. Their findings suggest that the reduction in sucrose absorption seen following fenugreek treatment may be related to inhibition of disaccharidase (sucrase) activity in the gut. Although the mode of action for SDF in diabetes management is still not well understood in human test subjects, fenugreek galactomannan, as exemplified by the various animal and clinical studies, can be an effective support therapy in the control of this disease.

Fenugreek galactomannan has also been shown to have hypocholesterolaemic properties in diabetic dogs and non-diabetic rats (Sharma 1986a; Ribes *et al.* 1987). In humans, Sharma *et al.* (1991) found that treatment of 15 hyperlipidemic adults who were fed 100 g of defatted fenugreek daily over a three-week period had lower blood cholesterol levels. These test subjects reported lower than baseline values for triglyceride and low-density lipoprotein (LDL) cholesterol. Sowmya and Rajyalakshmi (1999) conducted a study to assess the effect of germinated fenugreek seed consumption in human subjects on cholesterol levels. It was revealed that germination drastically increased the soluble fiber content in fenugreek seeds. Consumption of these seeds brought about a significant reduction in total and LDL cholesterol levels, although no significant changes were observed for high density-lipoprotein (HDL), very low-density lipoprotein (VLDL) cholesterol and triglyceride levels. In a similar study by Gupta *et al.* (2001), the diabetic group showed a significant decrease in serum triglyceride levels and a similar increase in high-density lipoprotein (HDL) levels when compared to normal test subjects. A more recent study by Venkatesan *et al.* (2003) investigated the hypocholesterolemic effects of a unique dietary fiber (Fibernat®), which is a combination of fenugreek seed powder, guar gum and wheat bran, in rats. The study examined lipid metabolism and cholesterol homeostasis by determining the activity of hepatic triglyceride lipase (HTGL) and uptake of atherogenic lipoproteins (LDL and VLDL) by the hepatic apo B, E receptor. Fibernat was successful in increasing both apo B and E receptor expression in the liver and activity of HTGL, giving rise to a marked hypocholesterolemic effect and establishment of an improved cholesterol homeostasis in Fibernat-fed rats.

It has been suggested that formation of a physical barrier in the gut by the viscous fraction of fenugreek may aid in inhibition of bile salt absorption in the intestine or may possibly cause intra-luminal binding of cellular receptors, resulting in increased fecal extraction of bile acids and neutral sterols. In both cases, existing cholesterol is converted to bile acids, hence decreasing plasma cholesterol levels (Madar and Shomer 1990; Venkatesan *et al.* 2003). This has been demonstrated in a recent study by Dakam *et al.* (2007) which looked at the physiological properties of fenugreek galactomannan in rats. Using male albino Wistar rats, they divided the animals into three groups according to their diet over a period of 4 weeks; *i.e.*, galactomannan (gum), gum + sodium bicarbonate, gum + albumin. The researchers reported a significant decrease in plasma total cholesterol in all three groups, with the greatest decrease in LDL-cholesterol for the gum + albumin group. They hypothesized that the combination of albumin (protein) with galactomannan contributed to the viscosity associated with the gum solutions administered, and consequently may offer better protection against coronary heart disease.

Steroidal saponins

Fenugreek contains various steroidal sapogenins with diosgenin (Δ^5, 25α-spirostan-3β-ol) being the major component. Sapogenins are the aglycone portions of the of plant-based steroid-derivative saponins, containing 6-C rings with 2 to 3 side chains substitutable either with methyl or hydroxyl groups (Skaltsa 2002). Saponins are amphipatic glycosides, containing both hydrophilic glycoside and lipophilic triterpene moieties, therefore capable of producing soap-like foaming properties. Diosgenin, a 27-C steroidal compound is currently used as a raw material for the manufacture of oral contraceptives and sex hormones by the pharmaceutical industry (Skaltsa 2002). It is traditionally extracted from wild Mexican and Asian yam species, *Dioscorea.* Utilization of fenugreek seeds as an alternative source of diosgenin was proposed in the 1950s in recognition of the increasing demand for raw steroids (Marker *et al.* 1947; Fazli and Hardman 1968 cited in Skaltsa 2002; Bhatnagar *et al.* 1975). It is important to note that fenugreek seeds contain no free sapogenins (Sauvaire and Baccou 1978), and that they occur as complex glycosides (saponins) which are released following enzymatic treatment or acid hydrolysis (Blunden and Hardman 1963 cited in Skaltsa 2002). Fenugreek sapogenins also occur in various forms due to stereochemistry and functional groups substititution. Those that have been reported in extracts from fenugreek seeds are diosgenin, yamogenin, tigogenin, neotigogenin, yuccagenin, lilagenin, gitogenin, neogitogenin, sarsapogenin and smilagenin (Gupta *et al.* 1986; Cornish *et al.* 1983; Skaltsa 2002). Yamogenin is the (25S)-epimer of diosgenin and occurs at a ratio of 2:3 with diosgenin in the seed, acid-hydrolyzed extract (Skaltsa 2002).

Diosgenin is a 27-carbon steroidal compound with the chemical name (25R)-spirost-5-en-3-β-ol and is one of the many sapogenins found in fenugreek seed, also derived from yams (*Dioscorea* spp.) (Sauvaire and Baccou 1978). It is currently an important raw material for the manufacturing of pharmaceutical hormones and steroids such as estrogen, progesterone, testosterone and glucocorticoids (Skaltsa 2002). Diosgenin occurs naturally as a glycosylated compound in fenugreek, and can be liberated by acid hydrolysis (which removes three carbohydrate residues) of the steroidal saponin, dioscin (Dewick 1997; Sauvaire *et al.* 1991).

Diosgenin is synthesized as part of the melavonate pathway in the biosynthesis of steroids (C_{18}-C_{30}). Steroids are modified triterpenoids which contain a tetracylic ring structure of lanosterol but lack the three methyl groups at C-4 and C-14 (Dewick 1997). Lanosterol is a precursor to the biosynthesis of plant cholesterol, which in turn is the most fundamental structure of a plant steroid. Steroidal diosgenin is formed by modification of the side chain of cholesterol, in which a spiroketal structure is formed at C-22, yielding a non-polar compound with 6 carbon rings.

Hydrolyzed chemical extracts (*i.e.*, in ethanol/ methanol) of fenugreek seeds yield a mixture of steroidal sapogenins (Marker *et al.* 1947 cited in Skaltsa 2002). These extracts contain major sapogenins such as diosgenin, yamogenin, tigogenin, neotigogenin, smilagenin and sarsapogenin, and minor compounds such as yuccagenin, gitogenin and neogitogenin (Fazli and Hardman 1971; Gupta *et al.* 1986; Sauvaire *et al.* 1996; Taylor *et al.* 1997; Petropoulos 2002). Depending upon biogeographic origins, genotypes and environmental factors, reported diosgenin contents in fenugreek vary between 0.3 and 2.0% (Fazli and Hardman 1968 cited in Petropoulos 2002; Puri *et al.* 1976; Sharma and Kamal 1982; Taylor *et al.* 2002). It has been demonstrated that fenugreek with similar genotypes grown in different environments and geographical locations, can possess significantly different plant chemical compositions (Taylor *et al.* 1997; Acharya *et al.* 2006a; Thomas *et al.* 2006) resulting from genotype x environment interactions.

Atherosclerosis (the narrowing of blood vessels) is associated with high levels of LDL- and VLDL-cholesterol and is a major cause of cardiovascular disease (Srinivasan 2006). Therefore, a reduction in LDL-cholesterol is considered essential to reducing the risk of a heart disease. Laguna *et al.* (1962) reported that diosgenin treatment was able to lower plasma cholesterol concentrations in chickens and rabbits fed with cholesterol. In another study, diabetic rats were fed with steroidal saponin extracts from fenugreek and a reduction in both VLDL and LDL total cholesterol was observed (Sauvaire *et al.* 1996). Ribes *et al.* (1987) treated diabetic dogs with extracts of germinated fenugreek seeds containing large amounts of protein (52.8%) and saponins (7.2%), and reported a significant decrease in triglycerides and plasma cholesterol concentrations in these animals. Further analysis using extracts containing a saponin concentration as high as 22% (w/v) produced a sharp decrease in LDL-cholesterol levels whereas a high protein-containing extract (70.5%) did not produce similar effects. Steroidal saponins and/or sapogenins (diosgenin) have been studied by Sauvaire *et al.* (1991) and were specifically implicated, either alone or synergistically in the hypocholesterolemic effect of fenugreek seeds in diabetic dogs.

Stark and Madar (1993) investigated the hypocholesterolemic potential of an ethanol extract from defatted fenugreek seeds *in vitro* as well as *in vivo* in hypercholesterolemic rats. *In vitro*, the purified ethanol extract exhibited the ability to inhibit bile salt absorption in a dose-dependent manner. Reductions in plasma cholesterol levels (18-26%) and lower concentrations of liver cholesterol were observed *in vivo*. This study attributed the decrease in cholesterol absorption observed to an interaction of saponins with bile salts in the digestive tract. These findings seem to agree with those of Bhat *et al.* (1985) and Sharma (1984), in which an increase of both fecal weight and bile acid excretion was observed in treated subjects following consumption of a fenugreek-enriched diet.

It has been suggested that the hypocholesterolemic effect of diosgenin can be attributed to inhibition of cholesterol absorption, and its capacity to increase biliary cholesterol secretion and to increase neutral sterol excretion in feces (Cayen and Dvornik 1979 cited in Al-Habori and Raman 2002; Uchida *et al.* 1984; Ulloa and Nervi 1985). It is also postulated that formation of large mixed micelles containing bile salts and saponins in the gut inhibits the absorption of these molecules; hence they are lost in the feces. This would lead to a subsequent increase in conversion of cholesterol to bile acids in the liver, thereby lowering blood and hepatic cholesterol levels (Al-Habori and Raman 2002).

Obesity and the onset of type-2 diabetes often are associated with development of insulin resistance in animals, an increase in the size of white adipocytes or fat cells, and chronic inflammation into the adipose tissue by macrophages (Kadowaki 2000; Flier 2004; Dandona *et al*. 2004). Uemura *et al.* (2010) showed that obese diabetic mice given a high fat diet supplemented with 2.0% fenugreek for 4 wk exhibited a significant decrease in plasma glucose and insulin levels, along with a smaller increase in the amount of adipose tissue. Their studies showed that a hydrolyzed saponin fraction from fenugreek was able to induce cell differentiation in cultured adipocytes, resulting in reduced cell size. Purification of the active agent in these experiments showed that diosgenin, the most abundant bound saponin in fenugreek, was able to promote adipose cell differentiation, enhance insulin-dependent glucose uptake and increase intracellular lipid accumulation in treated 3T3-L1 cultured cells. Diosgenin treatment also increased levels of mRNA production during early adipocyte differentiation and decreased levels of mRNA expression of inflammatory genes. They suggest that fenugreek and diosgenin could be used to help control insulin resistance in animals and reduce levels of inflammation in adipose tissues associated with obesity and diabetes.

Saponins also appear to selectively inhibit the growth of tumor cells by arresting their growth within the cell cycle and inducing them to initiate apoptosis or programmed cell death (Raju *et al*. 2004; Liu *et al.* 2005; Li *et al.* 2010).

Some of these studies have specifically linked diosgenin with cancer prevention (Raju *et al*. 2004; Liagre *et al.* 2005; Aggarwal and Shishodia 2006). It was shown that diosgenin can inhibit and/or activate key proteins (*i.e*., bcl-2 and caspase-3) involved in mediation of apoptosis in human colon cancer cells (Raju *et al.* 2004). Liagre *et al.* (2005) has elucidated a mechanism of diosgenin-induced apoptosis in human erythroleukemia cells, and Shishodia and Aggarwal (2006) have identified a chemopreventative role for diosgenin against bone cancer through growth suppression and apoptosis induction in these cells. Li *et al.* (2010) also showed that treatment of hepatocellular carcinoma cells with diosgenin resulted in inhibition of both constitutive and inducible activation of STAT3 (a Signal Transducer and Activator of Transcription**)** signaling pathways which are involved in regulation of JAK (Janus Kinase). Treatment of cells resulted in down-regulation of STAT3-regulated gene products including cyclin D1, the anti-apoptotic proteins bcl-2, bcl-xL, survivin, mcl-1 and the angiogenic gene product VEGF, resulting in inhibition of cell division and induction of apoptosis. In addition, Srinivasan *et al.* (2009) were able to show that treatment of human breast cancer cells with diosgenin resulted in inhibition of phosphorylated Akt (also known as protein kinase B) kinase activity, resulting in inhibition of NF-κB, bcl-2, survivin and XIAP (X-linked Inhibitor of Apoptosis Protein). The treatment also resulted in inhibition of the Raf/MEK/ERK phosphorylation cascade and signaling pathway in cells containing the estrogen receptor and downregulation of cyclin D1, cdk-2 and cdk-4 (cyclin dependent kinases) resulting in arrest of tumor cells in G1 of the cell cycle. Diosgenin also has been reported as a potential therapeutic chemical that can be used to treat dementia in drug abusers with HIV infection and a history of intravenous drug abuse (Turchan-Cholewo *et al.* 2006).

Alkaloids

Trigonelline, a methylbetaine derivative of nicotinic acid (Skaltsa 2002) is one of the major alkaloids found in fenugreek seeds. This compound (chemical formula $C_7H_7NO_2$) has been reported to exert mild hypoglycemic (Shani *et al.* 1974; Marles and Farnworth 1994) and anti-pellagra [pellagra is a disease caused by lack of dietary niacin (vitamin B3) and the amino acid tryptophan] effects (Bever and Zahnd 1979).

Amino acids

4-Hydroxyisoleucine is the most abundant free amino acid (up to 80% of the total free amino acids) in fenugreek seeds (Fowden *et al.* 1973; Sauvaire *et al*. 1984). The stereochemistry of this rare amino acid was subsequently described by Alcock *et al.* (1989) and is only found in specific plants such as those of the genus, *Trigonella*.

4-Hydroxyisoleucine exists in two isomeric forms; the major isomer has a (2S, 3R, 4S) configuration which accounts for 90% of the total 4-hydroxyisoleucine found in the seeds, while the minor isomer has a (2R, 3R, 4S) configuration (Broca *et al.* 2000). Under specific conditions, such as high acidity, the linear form of 4-hydroxyisoleucine may cyclicize into the lactone form (Broca *et al.* 2000). This unique amino acid has been shown to possess both hypoglycemic and insulinotropic properties *in vitro* and *in vivo* using animal and human models, making it a potential candidate as a anti-diabetic agent (Sauvaire *et al.* 1998; Broca *et al.* 1999).

Branched-chain amino acids such as valine, leucine and isoleucine are produced exclusively in plants. Since humans lack the enzymes needed to synthesize these amino acids, they represent essential amino acids that must be consumed as part of the human diet (Binder *et al.* 2007). 4-Hydroxyisoleucine is a branched-chain amino acid that is produced almost exclusively in plants of the genus *Trigonella*. As its name suggests, 4-hydroxyisoleucine is formed through the hydroxylation of isoleucine (Haefelé *et al.* 1997). Formation of this amino acid in fenugreek is attributed to the activity of isoleucine hydroxylase, a dioxygenase that catalyzes the attachment of one atom of an oxygen molecule to C-4 of the amino acid, while the other oxygen atom is incorporated into 2-oxoglutarate to yield succinate and carbon dioxide (Haefelé *et al.* 1997). The same study also identified 2-oxoglutarate, ascorbate, iron (II) and oxygen as essential co-factors and co-substrates that contribute towards the functionality of the enzyme. It is important to note that the linear form of 4-hydroxyisoleucine is necessary for it to be biologically active. The lactone form, which has a significantly altered chemistry, is inactive (Broca *et al.* 2000). Also it has been indicated that full methylation (full branching along the carbon skeleton), at carbon-α in the S-configuration and carbon-γ hydroxylation are required for the insulinotropic activity of 4-hydroxyisoleucine (Broca *et al.* 2000). A recent study by Hajimehdipoor *et al.* (2008) determined the content of 4-hydroxyisoleucine to be 0.4% in Iranian fenugreek seeds, while a previous publication reported a content of just 0.015% in Indian fenugreek seeds (Narender *et al.* 2006).

Treatment of subjects with 4-hydroxyisoleucine has been shown to result in insulin stimulating effects in animals, hence giving it anti-diabetic properties (Hillaire-Buys *et al*. 1993; Petit *et al*. 1995; Sauvaire *et al.* 1996). Sauvaire *et al.* (1996) reported that 4-hydroxyisoleucine treatments produced a concentration dependent insulin response both *in vitro* in cell cultures and *in vivo* in fasted dogs. They also showed that 4-hydroxyisoleucine treatments were effective after oral administration and improved oral glucose tolerance. The amino acid was shown to stimulate pancreatic β cells to produce insulin directly in rats and human subjects. It was found that this effect was glucose-dependent and occurred only at moderate (8.3 mM) to high concentrations (16.7 mM) (Sauvaire *et al*. 1998). Broca *et al.* (1999) studied the effects of 4-hydroxyisoleucine in normal and type-2 diabetic rats through intravenous and oral glucose tolerance tests. A single intravenous administration of 4-hydroxyisoleucine partially restored a glucose-induced insulin response, while a subchronic administration of the compound reduced basal hyperglycemia and basal insulinemia, and significantly improved glucose tolerance in type 2 diabetic rats. They concluded that 4-hydroxyisoleucine imparted anti-diabetic effects through direct pancreatic β cell stimulation.

Obesity is a condition in which lipid accumulates excessively in the adipose tissue. Aside from genetic factors, this disease is also caused by various environmental factors such as a high-fat diet (Weiser *et al.* 1997). If uncontrolled, obesity may be a risk factor for chronic diseases such as diabetes, hyperlipidemia and hypertension. Sharma *et al.* (1990) first reported a lipid lowering effect for fenugreek in type 1 diabetic patients. They found a significant reduction in total serum cholesterol, LDL- and VLDL-cholesterol and triglycerides, indicating that fenugreek can be effective in the management of diabetes. In a placebo-controlled study by Bordia *et al.* (1997), fenugreek was found to significantly reduce blood lipids without affecting HDL-cholesterol in patients with both coronary heart disease and type 2 diabetes. In another study, whole fenugreek seed powder fed to alloxan-treated diabetic rats was shown to improve glucose homeostasis by altering glucose and lipid metabolic enzyme activities, indicating a possible therapeutic potential of fenugreek for type 1 diabetes (Raju *et al.* 2001). Hannan *et al.* (2003) used the soluble dietary fiber fraction of fenugreek to study its effect on lipidemia in type 2 diabetic models. A lack of 4-hydroxyisoleucine in these extracts may have contributed to failure in this study to identify a lipid lowering principle, even though positive therapeutic effects for fenugreek in the control of heart disease were identified.

Handa *et al.* (2005) conducted a study using obese mice that were fed a high-fat diet supplemented with 0.3-1.0% fenugreek seed extract (containing 20% 4-hydroxyisoleucine). It was found that fenugreek seed extract significantly reduced total adipose tissue and liver weights and resulted in a decrease in liver triglyceride levels. They repeated the experiments with similar parameters using 4-hydroxyisoleucine alone and reported a decrease in plasma triglyceride levels following corn oil administration (a method that would promote an increase in blood triglyceride levels in the test subjects). Their results identified 4-hydroxyisoleucine as an effective agent in fenugreek seed extracts that has potential for use in obesity prevention. In another study, Narender *et al.* (2006) used a high-fat diet (HFD) fed to dyslipidemic hamsters to examine the anti-dyslipidemic properties of fenugreek. The HFD caused a 2 to 5-fold elevation in plasma triglycerides, total cholesterol, HDL-cholesterol, glycerol and free fatty acids (FFA) in the animals. However, following treatment with 4-hydroxyisoleucine (50 mg kg^{-1} body weight), a significant decrease in plasma triglycerides (33%), total cholesterol (22%) and an increase in HDL-cholesterol (8.7%) was observed. These results were similar to those obtained when fenofribrate, a triglyceride lowering drug was used under similar conditions (Koyama *et al.* 2004). A 14% reduction in FFA and a 4.9% decrease in glycerol levels were also reported. The study concluded that 4-hydroxyisoleucine possessed both hyperglycemic as well as anti-dyslipidemic properties, which allowed it to decrease plasma triglycerides, cholesterol and free fatty acid levels in the test subjects.

Phytochemicals and polyphenols

Polyphenols possess anti-oxidative attributes, which may prevent some forms of chronic disease. Gupta and Nair (1999) reported that fenugreek is generally rich in polyphenols (100 mg g^{-1}). The phenylpropanoid pathway occurs ubiquitously across the plant kingdom and has been shown to be involved in the biosynthesis of important secondary plant metabolites such as flavonoids and lignins (Dixon and Sumner 2003). Flavonoids consist of several classes; *i.e.*, the flavones, flavonones, flavonols, flavanols (flavan-3-ols), isoflavones, proanthocyanidins and anthocyanins. They exhibit strong antioxidative effects *in vitro* and have been largely associated with prevention of oxidative damage in biological systems, which can confer protection against cardiovascular disease and cancer (Erdman *et al.* 2007).

Flavonoids have a basic three ring chemical structure; *i.e.*, two aromatic rings (A and B) coupled with a three-carbon oxygenated heterocyclic ring (C). Among the many flavonoids reported in fenugreek, quercetin, luteolin, vitexin and kaempferol appear to be the most common (Parmar *et al.* 1982; Jain *et al.* 1992; Huang and Liang 2000; Skaltsa 2002). Quercetin and kaempferol are flavonols; luteolin is a flavone while vitexin occurs as a glycosylated flavone.

Isoflavanoid phytoalexins are also reported to occur in fenugreek in the form of the pterocarpans, medicarpin and maackiaian (Ingham 1981). These compounds play a key role in maintaining plant health in the event of microbial invasion; they are absent in healthy plants and their production is only induced upon microbial attack (Skaltsa 2002).

Polyphenolics are also potent antioxidants that scavenge for free radicals and protect against oxidation. Common phenolic compounds isolated from fenugreek are scopoletin, coumarin, chlorogenic and caffeic and p-coumaric acids (Skaltsa 2002; Acharya *et al*. 2007b). Aqueous extracts of germinated fenugreek seeds containing 17.5 mg mL^{-1} quercetin and 64.4 mg mL^{-1} gallic acid equivalents per gram of fenugreek powder and was shown to exhibit significant antioxidant activity and offered greater protection against oxidation compared to other extracts (Dixit *et al.* 2005). The protective action of fenugreek seed polyphenols has been investigated in rats on lipid peroxidation using aqueous extracts and was found to exert gastroprotective effect on gastric ulcer (Pandian *et al.* 2002) and prevent ethanol-induced toxicity in the liver and the brain (Thirunavukkarasu *et al.* 2003). Kaviarasan *et al.* (2004) also reported that a polyphenolic extract of fenugreek seeds can have a cytoprotective effect on ethanol-induced damage in human Chang liver cells. The protective effects were comparable to those of silymarin, a known hepato-protective agent.

Plant oils and oleoresin

Volatile constituents contribute to the aroma and flavor of fenugreek. Anethol, an aromatic compound found mostly in anise, camphor and fennel, also occurs in fenugreek and produces a licorice-like aroma (Aggarwal and Shishodia 2006; Acharya *et al.* 2007b). Mazza *et al.* (2002) have identified 175 volatile constituents in Sicilian fenugreek seeds, which include carbonyls, sesquiterpene hydrocarbons, alcohols, heterocyclic, and furan compounds. Sotolone (3-hydroxy-4, 5-dimethyl-2(5H)-furanone has been identified as the principal component contributing to the flavor of fenugreek (Hatanaka 1992; Blank *et al.* 1997). These compounds together impart the burnt sugar, curry or maple syrup flavour, which makes up the oleoresin that is characteristic of fenugreek (Monastiri *et al.* 1997).

Plant safety and toxicity

Due to its documented historical and traditional use as a spice and medicinal herb in various parts of the world, fenugreek has been granted "Generally Recognized As Safe" status by the U.S. Food and Drug Administration (U.S. Food and Drug Administration 2006). However, caution is warranted in patients with allergies to fenugreek (Patil *et al.* 1997). Ohnuma *et al.* (1998) have reported that a patient with an allergy to curry powder containing fenugreek responded with severe bronchospasms, wheezing and diarrhea. Tiran (2003) reported that a significant number of patients had an allergenic reaction to fenugreek in a skin test-patch. Transient diarrhea, flatulence and dizziness have also been reported as side effects of fenugreek consumption (Sharma *et al.* 1996a; Abdel-Barry *et al.* 2000). Bartley *et al.* (1981) suggested that fenugreek consumption can elicit a maple syrup-like odor in urine and may potentially cause the misdiagnosis of maple syrup urine disease.

There also is potential for chemical components in fenugreek to interact with other drugs during patient medication (Ernst *et al.* 2001). Bioactive components in fenugreek such as dietary fiber (*i.e.*, galactomannan) and steroids (*i.e.*, diosgenin), which have the potential to affect glucose and cholesterol levels in humans, could pose risk to individuals with health problems. Consumption of fenugreek can lead to hypoglycemia; hence proper blood glucose monitoring may be necessary for patients prior to use of fenugreek as a dietary supplement but significant clinically harmful adverse effects due to consumption of fenugreek as a food or medicinal supplement have not been reported (Basch *et al.* 2003).

Most reports on the toxicity of fenugreek consumption were obtained from studies with laboratory animals. Shlosberg and Egyed (1983) reported that ruminants that consumed fenugreek appeared to become myopic. Nakhla *et al.* (1991) also reported finding pathological abnormalities in the liver and kidney, along with reduced body weight and increased concentrations of uric acid in the blood in Sudanese chicks that had been fed fenugreek. Panda *et al.* (1999) have reported a decrease in body weight due to the inhibition of triiodothyronine (T_3) production in mice and rats. Fenugreek preparations contain coumarin or its derivatives, which are anti-coagulants that prevent blood clotting (Lee *et al.* 2000; Lambert and Cormier 2001). This will increase the risk of internal bleeding in patients who are taking blood-clotting drugs such as warfarin, as the coumarin in fenugreek may increase the activity of these drugs. Use of fenugreek during pregnancy is also discouraged as saponins in the plant may stimulate uterine contractions leading to premature abortion of the fetus as observed in early animal studies (Abdo and al-Kafawi 1969; Basch *et al.* 2003). In an animal study, an acute oral LD_{50} was reported to be more than 5 g kg^{-1} in rats and an acute dermal LD_{50} was found to be more than 2g kg^{-1} in rabbits (Opdyke 1978; Basch *et al.*

2003). In another animal study, the diets of mice and rats were supplemented with 1-10% debitterized fenugreek powder, an approach which likely removes many of the saponins. No signs of toxicity or mortality in laboratory animals that received acute and subchronic regimens were observed (Muralidhara *et al.* 1999). Rao *et al.* (1996) conducted a nutritional and safety evaluation of fenugreek over a short term in weanling rats. The rats' diet was supplemented with 5-20% fenugreek powder over a 90-day period; the studies concluded that fenugreek appeared to be non-toxic in doses administered to the animals, within that time frame. However, a recent evaluation conducted by Kassem *et al.* (2006) reported a potential anti-fertility effect of fenugreek in rabbits. Through a diet containing 30% fenugreek seeds, these researchers reported a significant anti-fertility effect in female rabbits and a toxicity effect in male rabbits. A significant reduction in developing fetuses was observed in females at 20 days of gestation and a histo-pathological assessment of the testis in male rabbits revealed damaged seminiferous tubules and interstitial tissues.

ADAPTATION OF FENUGREEK FOR USE IN PRAIRIE REGIONS OF CANADA

Fenugreek is thought to be a ideal crop for introduction to semi-arid regions of the prairies in western Canada largely because of its adaptation to dry-land conditions and its potential for use as a functional food for humans, feed for animals and as a source of value-added chemicals which can be marketed as a means to diversify agricultural opportunities within the region (Acharya *et al*. 2006b, 2008). Seed originating from Mediterranean regions is adapted to a similar day length to that found in western Canada and can produce tall, leafy plants that are easy to harvest as forage for animal feed or can be combined at the end of the season for its seed (Slinkard *et al*. 2009).

The most important issue facing producers of fenugreek in western Canada is time available to them in the field to produce high quality seed. The growing season is constrained by disappearance of snow and frost in the spring, which can vary from early to late May depending on the region, and onset of the first killing frost, typically in early to late September. Consequently the growing season may be as short as 90 days, but might extend up to 150 days in a good year. Variation in temperature and rain within the region also influence growth of the plant producing a significant impact on productivity, biochemical content and quality of materials produced both regionally and on a year by year basis (Taylor *et al*. 2002; Basu *et al.* 2009; Lee 2009).

Most fenugreek has an indeterminate growth habit. These plants exhibit apical dominance, producing new shoots, branches and leaves at the apex of the plant instead of directing resources into maturation of seed already produced (Acharya *et al*. 2007). Consequently, much of the seed originating from Mediterranean and east Asian regions require from 105 to 171 days in order to complete flowering and produce high quality seed (McCormick *et al.* 2009; Slinkard *et al*. 2009). Southern prairie regions of Canada can only depend upon ~ 100 frost free days for growth, tying fenugreek yield closely to maturity duration of the plant. Although seed pods produced by fenugreek are resistant to shatter, and can be harvested after a killing frost (Slinkard *et al*. 2009), the crop is subject to low seed set, making it difficult to maintain within the region. Consequently, much of the breeding for development of new fenugreek cultivars in western Canada has focused on increasing seed productivity within a limited maturation duration for the plant.

FENUGREEK CULTIVARS DEVELOPED IN CANADA

In Canada, commercial production of fenugreek began around 1990 and generally has been focused within southern prairie regions of Saskatchewan where about 400 ha (1000 ac) were planted in 2009 (Slinkard *et al*. 2009). Total seed production under dryland conditions within this region ranges from 150 to 2800 kg/ha (130-2500 lb/ac), with an average of 1500 kg/ha (1300 lb/ac). To date, five cultivars of fenugreek have been released for use in Canada (Slinkard *et al.* 2009). Of these, three were developed to address a broad range of applications; *e.g.*, seed production for use as a spice and for processing of functional food and nutraceutical extracts, as well as for food flavoring:

1) 'AC Amber', the first fenugreek cultivar developed, was released in 1992 by Agriculture and Agri-Food Canada, Morden Research Station, Manitoba.

2) 'CDC Quatro', released in 1995 by the Crop Development Centre (CDC) in Saskatoon, is a double-podded variety with increased plant vigor, height and seed yield.

3) 'CDC Canagreen', released in 2002 by the CDC in Saskatoon also was selected for increased seed yield and produces about 28% more seed than 'CDC Quatro'.

These three cultivars produce pods containing ~ 10 to 20 rectangular seeds 4 to 6 mm long and 2 to 3 mm wide; the plants possess light purple flowers that develop mature yellow to gold colored seeds. Cultivars mature within 105–140 days, although development can be delayed during cool, moist conditions (Slinkard *et al.* 2009). Selection for the double pod trait is known to be linked to selection of plants with high diosgenin contents and higher seed yields (Petropoulos 2002).

Two specialty cultivars of fenugreek also have been developed:

1) 'CDC Canafen', released in 2002 by the CDC in Saskatoon was developed primarily for use in production of functional foods and nutraceuticals. 'CDC Canafen' is a white flowered cultivar with a translucent seed coat and colorless endosperm that lacks much of the bitter taste and pungent odour normally associated with fenugreek plants and seed (Slinkard *et al.* 2009).

2) 'AC Tristar' was developed by Agriculture and Agri-Food Canada, Lethbridge, Alberta as an animal forage and released in 2004 (Acharya *et al*. 2006b, 2008).

Rainfall in southern Alberta is limited and irrigation is used extensively. 'AC Tristar' is the first fenugreek cultivar that has been selected under both dryland and irrigated conditions. In field trials 'AC Tristar' produced 2 to 11% more biomass than 'AC Amber' under dryland and irrigated conditions respectively, and 26% more seed under irrigated conditions (Acharya *et al*. 2007c). Fenugreek plants maintain a consistently high protein content (16 to 18%) throughout the growing season and in short term rotations, making it ideally adapted for use as a forage for livestock (Slinkard *et al.* 2009). In field studies 'AC Tristar' produced as much high quality biomass as two cuts of alfalfa, a forage plant typically grown in support of the intensive livestock and beef industry found regionally (Acharya *et al*. 2007c, 2008). When used as cattle feed alfalfa can cause bloat as it is digested in the rumen, while fenugreek is bloat–free (Acharya *et al.* 2007c; McCartney and Fraser 2010). However, care must be taken in use of fenugreek as a feed for pregnant animals. It contains naturally occurring steroidal saponins which are hydrolysed to diosgenin when eaten, and can stimulate premature abortion in pregnant animals. However, this steroid also can support weight gain in beef cattle and milk production in dairy cattle, adding to its value as a specialty crop for the industry (Mir *et al.* 1998; Okine *et al.* 2001; Shah and Mir 2004; Acharya *et al.* 2006b, 2009).

Thomas *et al.* (2011) looked at the biochemical composition of 10 different fenugreek lines grown at three different locations in western Canada under rainfed and irrigated conditions. Genotype contributed most to variability in thousand seed weight (63%) and total seed yield (76%) of fenugreek grown under different field conditions, and played a major role in determining the biochemical content for seed galactomannan (70%) and 4-hydroxyisoleucine (78%). By contrast, variation in the environment contrib.-uted most (78%) to variation in diosgenin contents of seed obtained during the experimental trials. In general there was only a weak positive correlation ($R^2 = 0.07$) between total seed yield (T ha^{-1}) and thousand seed weight (g) for fenugreek grown under both rainfed and irrigated conditions. Still, high seed yields tended to be associated with either irrigated plots or received high levels of rainfall. These conditions typically resulted in high productivity in seed galactomannan (400 to 800 kg ha^{-1}), diosgenin (10 to 25 kg ha^{-1}) and 4-hydroxyisoleucine (18 to 34 kg ha^{-1}) relative to plants grown with less water. High seed yielding genotype x environment combinations also tended to exhibit a more stable biochemical composition compared to low seed yielding combinations where variation in the biochemical constituents of the seed was more pronounced.

FENUGREEK PROCESSING IN WESTERN CANADA

North American markets for fenugreek spice, functional food and nutraceutical extracts are at present limited (Basu *et al.* 2007). This has resulted in vertical integration within the marketplace, where seed production is closely tied to processing of established market products (Slinkard *et al.* 2009). Moreover, it has been recommended in Canada that fenugreek not be grown for functional food / nutraceutical markets unless producers have a contract for seed production. While some fenugreek seed is grown for use as a spice, in Saskatchewan most seed production takes place under contract to Emerald Seed Products Ltd. (ESPL), a privately owned company based in Regina. ESPL has been involved in the development and production of fenugreek value-added products since 1995 (Saskatchewan Nutraceutical Network 2001). In 2001 it partnered with Schouten USA, a subsidiary of the Royal Schouten Group N.V. of the Netherlands to help market its seed and functional food products which now are marketed to over 30 countries internationally.

Governments for each of the Prairie Provinces in Canada have taken a leadership role in promotion and development of expertise in functional food and nutraceutical processing. In Saskatchewan success of the industry has in part, been attributed to the presence of an industrial scale pilot plant and supporting laboratories situated at the University of Saskatchewan in Saskatoon, Saskatchewan (Industry News 1982; Emerald Seed Products 2010). The facility opened in 1977 in order to support grain and oilseed market development within the province and specializes in extraction, fractionation, purification and modification of biological materials (POS Pilot Plant Corp. 2010). It currently operates within a 54,000 sq. ft. facility fully equipped to handle large scale primary, solvent-based, oil-based and secondary manufacturing. The plant is ISO certified, is licensed to produce health products by the government of Canada, and utilizes Hazard Analysis and Critical Control Points (HACCP) and traceability systems to help guarantee quality assurance and regulatory compliance. Development of fenugreek processing technologies by ESPL was done in close association with this facility, and in 2004 the business was able to erect a new processing plant in Avonlea, where its value-added products are now produced. ESPL also holds exclusive licenses to the fenugreek cultivars 'CDC Quatro', 'CDC Canagreen' and 'CDC Canafen'. Another fenugreek cultivar, 'CDC Amber' has been exclusively licensed to G. H. Schweitzer Enterprises (Slinkard *et al.* 2009).

In Alberta functional food and nutraceutical product development is supported by efforts to link field production to value-added processing (Government of Alberta 2010). The Food Science and Technology Centre which operates out of the Crop Diversification Centre South in Brooks, Alberta is equipped to run field trials and has some pilot-scale capabilities. This group has been running field trials to develop new fenugreek cultivars, and has been doing biochemical analysis of fenugreek seed in order to identify its potential for use in niche markets (Lee 2009). The facility also is equipped to evaluate color, texture, viscosity and product composition, largely focusing on bench-top to prototype design and demonstration. Larger scale product development is done at the Alberta Food Processing Development Centre located at Leduc, Alberta. This facility was established in 1984 and contains both laboratory and pilot plant product development laboratories. In addition, it provides support for integration of regulatory and quality control/quality assurance standards with product development. Other support for the industry is being provided through field crop development studies being done at Agriculture and Agri-Food Canada, Lethbridge, as well as through university and college laboratory research, often done on a collaborative basis.

In Manitoba, the Canadian Food Products Development Centre began operations in 1975 at a laboratory located at the Agriculture Canada Research Center located in Morden, Manitoba (Manitoba Agriculture, Food and Rural Initiatives 2007). The objective of the facility was to provide a technological link with industry, university and government in order to support provincial economic development; focus of its research efforts was on animal feed development and increasing the value of primary and secondary agriculture in the province. In 1977 the facility moved to Portage la Prairie. Now called the Manitoba Food Development Centre, it functions as a specialized agency of Manitoba Agriculture, Food and Rural Initiatives. The center's mandate is to assist the agri-food industry in the development and commercialization of conventional and functional foods and natural health products (Manitoba Agriculture, Food and Rural Initiatives 2010). It has the ability to participate in product testing, scale-up and marketing, in particular of grains and oils. Updates to space and plant infrastructure were made in 2005.

VALUE ADDED PRODUCTS MADE IN CANADA

Ground fenugreek seed grown in western Canada is marketed as a food for use in curries, spice mixes, teas and herbal blends (Emerald Seed Products 2010; Manitoba Business Directory 2010). Cracked, roasted whole and ground seed also is sold to enhance taste in food recipes requiring a nutty roasted flavour. In addition, extracts of food grade fenugreek gum, consisting of at least 75% soluble fiber (largely galactomannan) are marketed as Canafen® for use as an emulsifier and thickening agent to provide viscosity, stability and texture to food products. A less purified gum fraction, marketed as Fen-Gum® is sold as a cost effective industrial polymer able to increase solution viscosity, reduce drag and increase floatation in liquids.

Fenugreek fiber, marketed as Fenfibre® is sold as a functional food product to help lower blood glucose levels and control body weight (Emerald Seed Products 2010). Development of specialty seed such as CDC Canafen has facilitated development of a low odor, high fiber (85%), debittered galactomannan extract which is marketed as FenuLife Extract® (Saskatchewan Nutraceutical Network 2001) and sold through Acatris Inc., a division of Frutarom Industries Ltd. (Frutarom 2010); because the extract is both tasteless and odorless it has potential for use in a broader range of applications than most fenugreek extracts. For example Shirani and Ganesharanee (2009) have shown that a combination of up to 2% fenugreek flour and 15% fenugreek polysaccharide (FenuLife® supplemented) can be used to develop extruded chickpea-rice snack products with acceptable colour, flavour, texture and quality, that also possess a low glycaemic index for health conscious individuals. Fenugreek containing samples possessed significantly lower levels of radial expansion but increased levels of longitudinal expansion when extruded, compared to a chickpea and rice (70:30) flour blend.

Fenugreek extracts also are sold as a source of concentrated phytonutrients which can serve as antioxidants to protect cells from premature aging, ethanol toxicity and accumulated cellular damage that can result in cancer (Acharya *et al.* 2007b). The Fenugreek Phyto Extract® sold by

Emerald Seed Products (Emerald Seed Products 2010) contains a minimum of 10% oleoresin, an oily product which contains the concentrated flavour of the plant. This product can be used both as an antioxidant to help prevent food spoilage as well as for food flavouring (Armitage *et al.* 2002). Fenugreek extracts are used to flavour butterscotch, licorice, rum, vanilla and maple syrup (STAT Communications Ltd. 2004).

Ground cotyledons from fenugreek seed also are processed and sold as a feed supplement for livestock, poultry and pet food manufacturers (Emerald Seed Products 2010) which is marketed as Nutrifen®. The extract contains more than 30% protein as well as being enriched in phytonutrients and fenugreek oil. The fiber/galactomannan content is reduced in order to remove any compounds that can bind nutrients in the intestine and interfere with nutritional benefits of the feed. Horses appear to show a preference for feed flavored with fenugreek (Goodwin *et al.* 2005). In addition fenugreek seed has been shown to stimulate feed intake in rats (Petit *et al.* 1993) and to stimulate feelings of hunger in humans (Abdel-Barry *et al.* 2000). It has the potential to increase milk production in dairy cattle and weight gain in beef cattle (Acharya *et al.* 2008; Montgomery 2009).

FENUGREEK AS AN ALTERNATIVE ANIMAL FEED

Fenugreek has a long history of use as animal feed (Petropoulos 2002). Its species name "foenum-graecum" means "Greek hay" indicating that the plant was used as a forage during ancient Greek and Roman times. Field trials with fenugreek in prairie regions of Canada show that it is able to provide high quality forage through all stages of its growth (Acharya *et al.* 2008). It maintains a high protein content of 16 to 18% throughout the growing season and in short term rotations (Slinkard *et al.* 2009).

'AC Tristar' fenugreek is the first forage cultivar developed for use in prairie regions of western Canada (Acharya *et al*. 2007c). The plant has an indeterminate growth habit where it branches extensively to produce a high biomass in the field. The cultivar is well suited for intensive production of silage and hay in western Canada and in short-term rotations it produces as much biomass as two cuts of alfalfa (Acharya *et al*. 2007c). As forage, 'AC Tristar' fenugreek is harvested once at the mature stage and then again near the end of the growing season. However, we note that the plant is subject to low seed set when the growing season is short, or cold and wet.

Fenugreek plants do not compete well with common prairie weeds and an in-crop treatment with herbicide is recommended (Moyer *et al*. 2003). In field trials using 'AC Amber', weeds contributed 37 to 86% to the total dry matter harvested if no herbicide was used. However when fenugreek was treated post emergence with herbicides such as imazamox, imazethapr and ethalfluralin applied at the three to four trifoliate leaf stage weeds only made up 5% of the total dry matter. In cases where the herbicide did damage the plants, only a slight yellowing and stunting of the initial growth was observed; *i.e.*, typically 0 to 6% damage was noted in herbicide treated plants as compared to a hand weeded check. Weed control was required to produce forage fenugreek with a low fiber content (few weeds), high protein and digestible dry matter. Herbicide treatment did not affect quality of the forage fenugreek harvested. The main weeds found contaminating the crop in this study were green foxtail, wild oats and redroot pigweed. As a legume fenugreek needs to be seeded with *Rhizobium* bacteria in order for it to reach its maximum growth potential (Acharya *et al.* 2008). However because of its ability to fix nitrogen and replenish the soil it also can be continuously cropped using conservation tillage systems or used as a "green manure". In addition, integration of fenugreek into crop rotations will not only help to replenish the soil, but also will help to minimize plant disease and pests (McCartney and Fraser 2010).

Much of the effort to develop fenugreek as an alternative forage for use in western Canada has been done in support of the beef industry. Fenugreek contains the natural steroid diosgenin which can promote weight gain in cattle and has the added benefit of not causing bloat in cattle fed a diet containing it (Acharya *et al.* 2006b, 2008). Steers fed on silage made up solely from fenugreek alone or alfalfa exhibited similar weight gain over a period of 105 days (Mir *et al.* 1996; Acharya *et al.* 2008). Supplementation of these diets with 0, 15 and 30% barley silage resulted in an additional weight increase. In steers fed fenugreek, total in vitro dry matter disappearance (IVDMD) was higher when the animals were fed 15 and 19 wk old greenhouse grown fenugreek than when they were fed on a diet of alfalfa ($P < 0.05$). Although 9 week old fenugreek contained higher crude protein levels than alfalfa, mature fenugreek 15 and 19 wk old had a similar protein content. The volatile fatty acid production and composition in steers fed fenugreek were similar to that found in steers fed early bloom alfalfa.

Fenugreek forage also is being promoted for use by the dairy industry. Because of its consistent high quality and ability to compete with alfalfa when grown in the same geographical region of the country, fenugreek is a good alternative feed for use by the dairy industry (Montgomery 2009). Digestion studies in dairy cattle using 'CDC Quatro' as feed have shown that its digestibility is comparable to alfalfa. Presence of diosgenin in the feed also should help sustain milk production in a manner similar to that seen in humans (Acharya *et al.* 2007b; Montgomery 2009).

Use of appropriate agronomic practices is necessary to maximize forage and seed yield for fenugreek (Basu *et al.* 2008). In the field, plants should be planted in well-drained loam soils (pH 8-8.5) and spaced 20 to 30 cm apart to provide good conditions for the crop to mature; heavy and wet soils limit crop growth (Rosengarten 1969). Potash can be used to adjust soil pH in order to increase nutrient uptake of fenugreek (Yadav and Kumawat 2003 cited in Basu *et al.* 2008) and application of organic and inorganic fertilizers, farmyard manure, nitrogen and phosphorus has been effective in increasing fenugreek yield (Verma *et al.* 1990; Detoroja *et al.* 1995; Khiriya and Singh 2003). In southern Alberta, Canada optimization of seed yield for both 'AC Tristar' and 'CDC Amber' required an application of 40 to 50 kg/ha of phosphate to support good growth of the plant while high forage yield was obtained with 50 to 60 kg/ha of phosphate (Basu *et al.* 2008). Combining seed after swathing yielded significantly more seed with 'AC Tristar' fenugreek than direct combining ($P < 0.001$).

The small acreage of fenugreek planted so far in Canada has not resulted in extensive pest problems (Basu *et al.* 2006a, 2006b; Basu 2009). In field trials run in southern Alberta, Canada with 'AC Tristar' and 'CDC Amber' general insect damage in the field was low and as a 'rule of thumb', pests that affected growth of alfalfa in the field also affected fenugreek. Potential insect pests of fenugreek are *Lygus* bugs and to a lesser extent alfalfa plant bugs. In the greenhouse western flower thrips can be a problem. Powdery mildew (*Erysiphe polygoni* DC) can be a serious problem which can affect biomass and seed yield under warm and moist agro-climatic conditions.

RESEARCH DIRECTIONS

With accumulation of experimental evidence in support of the nutraceutical properties of fenugreek, there is growing interest in marketing it as a natural health product in Canada. Therefore, there is a need to evaluate the biochemical productivity of the plant and its seed to allow for selection of suitable genotypes that may be further developed into cultivars specific for the natural health product industry. Field studies performed within a multi-location trial in southern Alberta (Lee 2009) show that different cultivars of fenugreek can possess significantly different biochemical compositions (**Table 1**). Galactomannan contents for 'CDC Quatro' and 'AC Tristar' were statistically similar, while those of 'AC Amber' and 'Indian Temple', a fenugreek cul-

Table 1 Mean performance of eight selected traits of three Canadian and two foreign cultivars of fenugreek from India and Afghanistan grown in southern Alberta, Canada.

Genotype	Traits*							
	TSW (g)	**Seed Yield (kg ha^{-1})**	**GLM (%)**	**GLM-P (kg ha^{-1})**	**DIOS (%)**	**DIOS-P (kg ha^{-1})**	**4-OH-ILE (%)**	**4-OH-ILE-P (kg ha^{-1})**
Canadian Cultivars								
AC Amber	17.6^{g}	1198^{a}	14.6^{a}	186^{a}	0.81^{g}	9.8^{cd}	0.95^{cd}	10.5^{a}
CDC Quatro	13.1^{b}	1733^{b}	16.5^{d}	301^{b}	0.66^{e}	11.6^{e}	0.91^{c}	14.8^{d}
AC Tristar	13.7^{b}	1586^{b}	16.6^{d}	277^{b}	0.53^{a}	8.4^{c}	0.99^{d}	14.7^{d}
Foreign Cultivars								
Indian Temple (India)	15.3^{e}	959^{a}	15.5^{a}	150^{a}	0.62^{d}	5.9^{a}	0.99^{d}	8.7^{a}
F75 (Afghanistan)	12.9^{a}	1814^{c}	17.6^{f}	325^{c}	0.59^{d}	10.9^{de}	0.98^{cd}	16.6^{e}

* TSW, thousand seed weight; GLM, galactomannan content; GLM-P; galactomannan productivity; DIOS, diosgenin content; DIOS-P, diosgenin productivity; 4-OH-ILE, 4-hydroxyisoleucine content; 4-OH-ILE-P, 4-hydroxyisoleucine productivity. Means that share similar superscripts within the same column under either genotype main effect or environment main effect are not significantly different from each other (Tukey's HSD at $P \leq 0.05$).

tivar obtained from India, had similar but less galactomannan within the seed. 'F75', a cultivar originating from Afghanistan had significantly higher galactomannan contents than all other cultivars tested ($P \leq 0.05$). Galactomannan contents for the seed cultivars developed in Canada ranged from 14.6 to 16.6% of the total seed contents. By contrast, diosgenin contents were significantly different ($P \leq 0.05$) in four of the five cultivars looked at. 'AC Amber' had the most diosgenin per seed while 'AC Tristar' had the least. 'CDC Quatro' which produces double pods, a trait thought to be linked to high diosgenin contents (Petropoulas 2002), had an intermediate diosgenin content. Diosgenin contents for the seed cultivars developed in Canada ranged from 0.53 to 0.81% of the total seed contents. Both foreign cultivars (*i.e.*, 'Indian Temple' and 'F75') had statistically similar diosgenin seed contents which were intermediate in value within the test results. 'AC Amber', 'AC Tristar', 'F75' and 'Indian Temple' all had statistically similar ($P \leq 0.05$) 4-hydroxyisoleucine contents per seed; 'CDC Quatro' had numerically less, although statistically 'CDC Quatro', 'AC Amber' and 'F75' had statistically similar contents. This anomolous result was due to the high level of variation in amino acid content seen among seed obtained from different test plots, suggesting that there is considerable opportunity for selection of this trait among test cultivars being developed. 4-Hydroxyisoleucine contents for the seed cultivars developed in Canada ranged from 0.91 to 0.99% of the total seed contents. While these differences in biochemical composition may look small in some cases, they often were compounded by differences in seed size, seed weight and total productivity. In all cases the seed produced by cultivars selected for growth in western Canada were more productive than 'F75' seed from Afghanistan, and in most cases it was more productive than the 'Indian Temple' seed from India, both of which had not undergone extensive selection for growth in Canada (**Table 1**). Although the thousand seed weight for 'Indian Temple' was intermediate in weight for the cultivars examined, its total productivity, measured as seed yield tended to be lower than the Canadian cultivars tested; *i.e.*, 'Indian Temple' produced only 959 kg ha^{-1} of seed while the western Canada-adapted cultivars produced from 1198 to 1733 kg ha^{-1}. In general this resulted in higher biochemical productivities in the western Canada-adapted cultivars as well. By contrast seed from 'F75' exhibited the highest seed yields and levels of biochemical productivity identified in these field trials, suggesting that the germ plasm from this seed is ideal for development of new fenugreek cultivars in western Canada.

In addition, studies on fenugreek grown in western Canada (Taylor *et al.* 2002; Acharya *et al.* 2006a; Lee 2009; Basu *et al.* 2009; Thomas *et al.* 2011) have identified significant genotype × environment interactions affecting morphology, growth habit, biomass and seed production, as well as the content of chemical constituents such as steroidal sapogenins within plant cultivars developed for growth in Canada and from seed initially obtained from Asian markets; e.g., differences in plant and seed properties were observed for fenugreek cultivars grown both at different locations in western Canada and for plants grown at the same location over a two year period. Biodiversity in production of active agents by natural systems including plants can lead to variation in anticipated physiological response within the user. Consequently, there is need to characterize concentrations of bioactive agents in different sources of these plants and how they are regulated through genotypic and environmental influences in order to ensure efficacy of these products as reliable medicinal extracts and functional foods.

It should be noted that the diversity in phenotypic expression often seen in plants when they are introduced into a new environment offers opportunity to breeders to produce new cultivars with unique morphological and biochemical properties. Recent developments in nutrigenomics and phenotypic mapping of traits in complex systems will allow us to expand our ability to select new plant variants with unique functional food and nutraceutical properties. Fenugreek's broad range of biochemical constituents with potential for use in multiple applications will allow development of the plant for sale into diverse markets. This approach should promote diversification of agriculture for both producers and the industrial sector. As an environmentally friendly crop that can be used as a "green manure" to replenish soils, that can help reduce the need for fertilizers, and that uses reduced amounts of water relative to traditional crops grown on the prairies, fenugreek appears to be an ideal crop for continued development in western Canada.

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