Review

Bioactive proteins and peptides in pulse crops: Pea, chickpea and lentil

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1. Introduction

Pulse crops belong to the family of cool season, annually grown leguminous crops (Maiti & Wesche-Ebeling, 2001). They are legume crops that are harvested for their seed only and do not include legumes which are grown for oil, such as soybean (Nwok-
2. Pea, chickpea and lentil: world production and consumption

2.1. Dry pea

Dry pea is among the world’s oldest crops, as records indicate it was grown in the Middle East approximately 9000 years ago. In addition, it has been harvested in Europe for several thousand years (Goodwin, 2003a,b), and has since been grown in 84 countries including Australia, Canada, China and the United States (Smith & Jimmerson, 2005a,b; McKay, Schatz, & Enders, 2003). Dry pea is adapted to brown, dark brown and black soil zones in semi-arid and non-irrigated areas of the world, and has been produced in the Canadian Prairies for over 100 years (Goodwin, 2003a,b). The five main types of pea grown worldwide are Austrian winter pea, green pea, maple pea, marrowfat pea and yellow pea. More than 60 varieties of pea have been developed for production in Canada. Canada primarily produces green and yellow pea, with only small quantities of maple, marrowfat and Austrian pea produced (Pulse Canada, 2008a,b). In 2004, 12 metric million tonnes of dry pea were produced worldwide. Canada was the leading country in pea production producing 28% of the total yield, followed by France and Russia at 14% and 10%, respectively (Smith & Jimmerson, 2005a,b). In Canada, Saskatchewan produces 68% of Canada’s dry pea crop, while Alberta and Manitoba produce approximately 22% and 10%, respectively (Goodwin, 2003a,b).

In addition to being the world’s largest producer of dry pea, Canada is also the leading exporter of dry pea. In 2007, Canada exported over 2 million metric tonnes of dry pea, followed by the United States and France with 469 thousand and 350 thousand metric tonnes, respectively. Conversely, in 2006, India was the largest importer of dry pea as it imported over 1.1 million metric tonnes, followed by Spain and Belgium with 663 thousand and 334 thousand metric tonnes imported, respectively. Presently, Canada and the United States are the 8th and 11th largest dry pea importing countries, importing 77 thousand and 58 thousand metric tonnes, respectively (Agriculture & Agri-Food Canada, 2008).

Pea, similar to other pulse and grain commodities, is relatively inexpensive and highly nutritious. It is high in fiber (soluble and insoluble) and protein (especially rich in the essential amino acids tryptophan and lysine), low in sodium and fat, and is an excellent source of complex carbohydrates, B vitamins, folate, and minerals such as calcium, iron, and potassium. In addition, a diet high in dry pea has been demonstrated to be effective in lowering the incidence of colon cancer, type-2 diabetes, LDL-cholesterol and heart disease (Agriculture & Agri-Food Canada, 2008). Due to the nutritional value described above, pea is considered to be an important agricultural commodity. As such, it is commonly used in soups, or processed into pea flour, pea starch, or pea protein concentrates. The processed pea products can be used in baked goods, soup mixes, breakfast cereals, processed meats, health foods, pastas and purees. Commercially, dry pea is available canned or dried in either whole or split pea varieties (Agriculture & Agri-Food Canada, 2008; Slinkard, Bhatty, Drew, & Morrall, 1990). In addition, pea is one of the most commonly utilized pulse crops in animal feed for poultry, sheep, cattle and swine feed rations, and is used as a feed additive in the aquaculture industry (Schatz, 2002). Finally, dry pea varieties such as AC Trapper can be seeded as a green manure crop as an alternative to summer-fallow in organic farming practices (Lawley & Shirtliffe, 2004).

2.2. Chickpea

The chickpea is a member of the cool season Fabaceae (Leguminosae) family of legumes (Nwokolo & Smartt, 1996). Similar to dry pea, chickpea is one of the earliest cultivated vegetables, as it is believed to have originated in the Middle East approximately 7450 years ago (Maiti & Wescbe-Ebeling, 2001). Chickpea has since been grown in temperate and semi-arid regions of the world such as Asia, Europe, Australia and North America. In 2004, 45 countries were actively producing chickpea, and together produced a total of 8.6 million metric tonnes. India was the leading producer of chickpea accounting for approximately 66% of the world’s production. Turkey was the second largest producer, producing approximately 7% of the world supply, followed by Pakistan and Iran at approximately 6% and 4%, respectively. In contrast, Canada and the United States contribute very little to the total quantity of chickpea produced worldwide, as they account for approximately 1% and less than 1% of the world production, respectively (Smith & Jimmerson, 2005a,b). The majority of the Canadian production comes from the Prairie Provinces with Saskatchewan producing approximately 81% of Canada’s chickpeas, while Alberta produces the rest.

Chickpea is the third most important pulse crop commodity in the world based on total production (Yust et al., 2003). In 2003, India was both the leading producer and importer of chickpea, with approximately 259 thousand metric tonnes imported (30% of the total amount imported worldwide). Pakistan and Bangladesh were the second and third largest importers of chickpea worldwide, with approximately 123 thousand (14%) and 84 thousand metric tonnes (10%), respectively. Similar to chickpea production, the United States and Canada only marginally contributed to the total quantity of chickpea imported worldwide. In 2003–2004, the United States imported approximately 17 thousand metric tonnes (1%) (Smith & Jimmerson, 2005a,b), while Canada imported only 2 thousand metric tonnes (Agriculture & Agri-Food Canada, 2006a,b).

There are two main commercially available types of chickpea grown worldwide: the desi and the kabuli chickpea. Desi chickpea seed is small with a dark irregular-shaped seed coat and is grown on semi-arid land. Kabuli chickpea (Garbanzo beans) is larger than desi chickpea, has a thin light-colored seed coat and is normally grown in temperate regions of the world (Agriculture & Agri-Food Canada, 2008). A variety of desi and kabuli chickpeas have been
developed and the characteristics of these cultivars may vary depending on the producing region. For example, some varieties such as CDC Frontier (Kabuli variety) have been developed for improved performance in the brown and dark soil zones of the Canadian Prairies. These varieties were specifically designed to express certain traits such as early maturation and the ability to resist particular diseases, as this region in Canada has a short growing season and a high incidence of the soil-borne disease Ascochyta. Certain traits such as early maturation and the ability to resist pathogens have been associated with reducing the incidence of colon cancer, whereas soluble fiber has been demonstrated to have a beneficial effect on weight loss and weight management (Agriculture & Agri-Food Canada, 2006a,b). The nutritional benefits of chickpea have led to its use in various culinary applications such as hummus. In addition, chickpea is used in stews, soups and salads, and can be processed into flour.

2.3. Lentil

Lentil (also known as red dahl, masur, massar, masuri tillseed and splint pea) is grown annually on a variety of soil types ranging from sand to clay loam soils in semi-arid regions of the world. Lentil production originated in the Near East more than 8500 years ago and has since spread to the Mediterranean, parts of Asia and was subsequently introduced into North America by the early 1900s (Oplinger, Hardman, Kaminski, Kelling, & Doll, 1990). Specifically, Canada began producing lentil in 1970 (Agriculture & Agri-Food Canada, 2006a,b). Lentil is now produced in over 48 countries. In 2002, India, Turkey and Canada were the first, second, and third largest producers, of lentil, accounting for approximately 33%, 16%, and 12%, respectively of the world's production (Johnson & Jimmerson, 2003). Saskatchewan produces approximately 95% of the Canadian lentil crop, with the remaining 5% produced in Manitoba and Alberta (Agriculture & Agri-Food Canada, 2006a,b).

In 2001, Canada was the largest exporter of lentil with over 490 thousand metric tonnes exported, which was approximately 70% of the total quantity of lentil produced in Canada that year. Egypt was the largest importer of lentil with over 113 thousand metric tonnes. Turkey and Sri Lanka were the second and third largest importers, with over 98 and 90 thousand metric tonnes imported, respectively in 2001 (Agriculture & Agri-Food Canada, 2006; Johnson & Jimmerson, 2003).

Six types of lentil are commonly grown worldwide: Eston class, French green, Laird class, red, Richlea class and Spanish brown. These six types can be further subdivided into different varieties, which are adapted to various growing environments and conditions. Some varieties are better able to adapt to certain geographical areas than others. For example, lentil varieties adapted to the Western Canadian Prairies mature earlier due the short growing season (Pulse Canada, 2008a,b). Canada produces mainly green lentil (Laird varieties) accounting for approximately 70% of the world’s green lentil production. However, it is estimated that 70% of the lentil produced worldwide is of the red lentil type, with 25% being of the green type and 5% of the brown and other types (Agriculture & Agri-Food Canada, 2006a,b).

The nutritional value of lentil and its use in a variety of culinary applications make it an important commodity in terms of production, and trade. Lentil is high in protein especially rich in lysine and leucine, low in fat, and is an excellent source of dietary fiber and complex carbohydrates. Lentil also contains vitamins and minerals such as B vitamins, calcium, phosphorous and potassium, along with oleic, linoleic and palmitic acid (Adsule, 1996; Agriculture & Agri-Food Canada, 2006a,b). The nutritional characteristics of lentil have been associated with cholesterol and lipid lowering effects in humans, along with reducing the incidence of colon cancer and type-2 diabetes (Agriculture & Agri-Food Canada, 2006a,b). Lentil is not commonly used in animal feed rations; however lentil straw is frequently used as animal feed (Saskatchewan Pulse Growers, 2000). Similar to some dry pea varieties, lentil such as Indian Head black lentil, is commonly grown as a green manure crop in organic farming practices (Lawley & Shirliffe, 2004).

3. Protein content of pulses

Pulse seeds accumulate protein throughout their development, hence mature pulses seeds are normally high in protein. Chickpea, lentil, and dry pea contain approximately 22%, 28.6%, and 23.3% protein, respectively on a dry weight basis (DW) (Pulse Canada, 2004; Sotelo & Adsule, 1996). However, these percentages may vary slightly depending on plant species, variety, maturity and growing conditions.

The majority of the protein found within pulse seeds is in the form of storage proteins, which are classified as albumins, globulins, and glutelins based on their solubility properties. Globulins, soluble in salt-water solutions, represent approximately 70% of the total protein found in pulses. One of two types of globulin usually predominates in pulses based on their sedimentation coefficient, vicilin or legumin which sediments at 7S or 11S, respectively. Albumins, soluble in water, account for 10–20% of the total protein in pulses. Finally, glutelins, soluble in dilute acid and base, account for 10–20% of the total protein found in the seeds of pulses (Duranti, 2006; Nwokolo & Smartt, 1996). Nutritionally, pulse storage proteins are relatively low in sulfur-containing amino acids, such as methionine, cysteine and tryptophan. However, the lysine content is relatively high compared to cereal crops. For this reason, combining pulse crops and grains such as rice, in the diet provides the essential amino acids required for proper human nutrition (Duranti, 2006).

There are many other types of protein found in legumes including various enzymes, protease inhibitors and lectins, which are collectively known as antinutritional compounds (ANCs). The majority of these proteins are within the water-soluble albumin class of legume proteins (Nwokolo & Smartt, 1996). In addition, they are distinct from other storage proteins with respect to functionality, as they have evolved within the seed as a protective mechanism. The interest in the biological activity of pulse crop ANCs and their potential use as a nutraceutical for promoting health and wellness and preventing or managing certain diseases has increased in recent years.

4. Antinutritional compounds of pulse crops

Antinutritional compounds (ANCs) are molecules that disrupt the digestion process when raw seed or flour is consumed by monogastric species rendering the seed unpalatable (Domoney,
The agglutination activity of lectins from chickpea, lentil and field pea.

<table>
<thead>
<tr>
<th>Common name</th>
<th>Scientific name</th>
<th>Part tested</th>
<th>Agglutination activity on blood type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chickpea</td>
<td>Cicer arietinum</td>
<td>Seed</td>
<td>A</td>
</tr>
<tr>
<td>Lentil</td>
<td>Lens culinaris</td>
<td>Seed</td>
<td>B</td>
</tr>
<tr>
<td>Field pea</td>
<td>Pisum sativum</td>
<td>Seed</td>
<td>AB</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>O</td>
</tr>
</tbody>
</table>


Animals fed a soybean-rich diet can develop enlarged spleen, reduced serum insulin levels and have disruption of the normal protein, fat and carbohydrate intermediary metabolism due to the activity of lectin (Sharon & Lis, 2004). The lectin activity of pea, chickpea and lentil has not been shown to produce the same pathological effects as raw soybean and kidney bean, although mild self-limiting ailments may arise in human populations and livestock. However, there are conflicting reports on their toxicity. Peumans and Van Damme (1996) reported that lectins from lentil and field pea may be harmful to humans if consumed raw, while others have found that lectins from lentil, pea and chickpea are non-toxic (Gonzalez, Mejia, & Prisecaru, 2005). A study on the lectin activity of several leguminous crops demonstrated that chickpea and pigeon pea had lectin activity, although the activity was reported to be below toxic levels (Singh, 1988). Although there is a risk of food poisoning from consuming raw pulse seeds, it should be emphasized that under standard operating processing conditions the antinutritional effect of lectins is relatively minor.

4.3. Nutraceutical potential of pulse lectins in human health

Lectins present in pulses have long been considered antinutritional components, which must be denatured by thermal processing prior to human consumption. Over the past several years, scientific data has demonstrated that lectins may play a key role in preventing certain cancers, and in the activation of certain innate defense mechanisms and can be utilized as a therapeutic agent for preventing or controlling obesity (Ewen, Bardocz, Pusztaiz, & Pryme, 2006; Hartmann & Meisel, 2007; Lima, Sampaio, Henriques, & Barja–Fidalgo, 1999; Pusztaiz & Bardocz, 1996; Sames et al., 2001; Wang, Ng, Ooi, & Liu, 2000). The potential use of pulse lectins as a nutraceutical for controlling obesity can be attributed to their ability to resist gastric digestion and by their ability to be absorbed into the blood stream while remaining biologically active. These properties have also resulted in increased research activity in the use of pulse lectins as a cancer preventative. Although research remains in its infancy, several animal models have been described. Mice with non-Hodgkins lymphoma fed 10 mg of mistletoe lectin per day were shown to have tumors with 75% decrease in mitotic activity and 21% reduction in the nuclear area (Ewen et al., 2006). Lentil lectins have a strong effect on reducing the onset of human hepatoma (H3B) (Wang et al., 2000). Lentil agglutinins are also known to have considerable effect on human Merkel skin carcinomas (Sames et al., 2001). *Pisum sativum* agglutinin (PSA) has high affinity for tumor cells, which may in turn reduce the tumorigenicity of these cells (Bures, Molycka, Bostik, Slavik, & Jirasek, 1986). Several factors can affect the efficacy of lectins in reducing the onset of certain types of tumors.

The mode of action of lectins on tumor cells depends on the type of tumor, the source of lectin and its biological activity. They are thought to bind to cell membranes or cell receptors causing cytotoxicity and apoptosis. Some theories suggest agglutinin causes reduction in cell division, increase in the number of macrophages which increases the susceptibility of tumor cells to macrophage attack, and improves the host immunocompetence (Barac, Stanoevic, & Pasic, 2005). Lectins may also penetrate the cell, resulting in cancer cell agglutination and many other antitumor properties, such as the activation of certain protein kinases (Gonzalez et al., 2005). Most research on lectins/agglutinins as a potential adjunct to cancer treatment use lectin sources other than pea, lentil and chickpea. Bean varieties, amaranth, wheat, potatoes, mistletoe, vetch, soybean and many animal lectins are commonly studied. For further information regarding the use of lectins from sources other than pulse crops, Gonzalez et al. (2005) provide a detailed review on the uses of important plant lectins as a potential cancer treatment in their paper entitled, *Lectins as biactive plant proteins: a potential in cancer treatment*. Most studies investigating anticancer effects of lectins found in pulse crops have been performed with lectins from lentil and various pea varieties. In general, pea, lentil and chickpea are not well studied sources of lectins used as cancer therapeutic agents; however, these pulse lectins may have great potential as a nutraceutical used to decrease the risk of certain cancers.

Lectins from various sources have been identified as active immunomodulatory agents that can enhance the immune system, such as lymphocyte proliferation, natural killer cell activity, antibody synthesis and cytokine regulation (Hartmann & Meisel, 2007). Lectins from mistletoe induce cytokine activity by mononuclear cells which can improve the effect of chemotherapeutic drugs (Ewen et al., 2006). The use of mistletoe lectin as an adjunct during cancer treatment is currently commercially available in Europe. This has inspired research to discover lectins with immunomodulatory properties from various other sources such as soybean, fungi and fruit. Soybean agglutinin lectins induce neutrophil and lymphocyte migration in vivo and activate mononuclear cells (Benjamin, Figueiredo, Henriques, & Barja–Fidalgo, 1997). Recently, lectins from mushrooms (Dalloul, Lillehoj, Lee, Lee, & Chung, 2005; Wang, Liu, Ng, Ooi, & Chang, 1996) and Jackfruit (*Artocarpus integrifolia*) (Coltri, Casabona–Fortunato, Panuto–Castello, 2004) have been demonstrated to have immunomodulatory properties. The current knowledge regarding pulse crops containing lectins with immunomodulatory properties is based on pea. *Pisum sativum* agglutinin (PSA) studies (Lima et al., 1999) demonstrated that PSA induces immunomodulatory effects, activating spleen lymphocytes in mice. More research is needed to characterize the effects of PSA in humans, as well as the use of lentil and chickpea agglutinins as potential immunomodulatory compounds.

Obesity is often considered the root cause of other illness such as heart disease, cancer and diabetes. Pulse seed lectins may also be efficacious in treating obesity. Pusztaiz and coworkers (Pusztaiz et al., 1998) demonstrated that lectins from kidney bean could decrease fat accumulation in rats, which was attributed to a decrease in insulin levels secondary to lectin activity. They also suggest that it may be possible to use bean lectin as a dietary adjunct or primary therapeutic agent in human trials to stimulate gastrointestinal function and reduce the incidence of obesity, if a safe and effective dose range were to be established (Pusztaiz & Bardocz, 1996). The main source of lectin used for treating/preventing obesity appears to be from bean. To the authors’ knowledge there are no reports suggesting that lectins isolated from chickpea, dry pea or lentil have a beneficial role in treating obesity. However, since lectins isolated from closely related pulse crops such as bean have been demonstrated to treat and/or prevent obesity there may be a potential for the use of lectins isolated from other pulses as nutraceutical components.

4.4. Protease inhibitors

Similar to lectins, protease inhibitors are protein ANCs that have been isolated from a wide variety of fruit, vegetable and pulse crops including pea, chickpea and lentil. In the 1970s and 1980s, considerable attention was given to protease inhibitors in pulse-based feed, as they interfered with digestion, growth and performance in domestic livestock (Champ, 2002). The level of protease inhibitors within pulses has since been considered an important parameter in determining the quality of feed and food. Two well-characterized protease inhibitors found in pulse seeds are trypsin and chymotrypsin inhibitors, belonging to the Bowman–Birk inhibitor (BBI) family. Protease inhibitors from the BBI family con-
tain two active sites which inhibit the proteolytic enzymes trypsin and chymotrypsin. Due to the double inactivation of trypsin and chymotrypsin, BBIs have been termed double-headed inhibitors (Domoney, Welham, & Sidebottom, 1993). When isolated from pigeon pea, trypsin and chymotrypsin inhibitors had an average molecular mass of 10,500 and 15,000 Da, respectively (Godbole, Krishna, & Bhatia, 1994). However, the molecular mass may vary between different species and environmental growing conditions. Ragg et al. (2006), Ferrasson, Quillien, and Gueguen (1997) and Smirnoff, Khalef, Birk, and Applebaum (1976), provide detailed information on the characteristics of trypsin and chymotrypsin inhibitors from pea, chickpea and lentil for further review of this subject.

All protease inhibitors present in pea, chickpea and lentil belong to the BBI family, whereas protease inhibitors from soybeans may belong to the BBI or Kunitz family of protease inhibitors (Guillamon et al., 2008). The main difference between the BBI and the Kunitz inhibitors is the polypeptide make-up. The polypeptide in Kunitz inhibitors consists of 181 residues with only two disulfide bridges and one active site. The BBI polypeptide consists of 70 residues and seven disulfide bridges with a double-headed inhibition mechanism (Laskowski & Kato, 1980).

The differences between the BBI and the Kunitz families of protease inhibitors have a bearing on their respective inhibitory activities. As protease inhibitors from pea, chickpea and lentil are from the BBI family, their inhibitory characteristics are relatively similar. However, differences in the concentration of inhibitor and their activity exist between pulse crop species (Champ, 2002) and varieties (Alonso, Orue, & Marzo, 1998). The concentration and inhibitory activity of trypsin and chymotrypsin inhibitors in three pea varieties grown in Spain were found to vary (Table 2). Protease inhibitor concentrations also demonstrated varying degrees of susceptibility to various processing treatments. This indicates that the processing technique and conditions used to denature protease antinutritional components may vary depending on the seed variety being processed (Alonso et al., 1998). A similar study (Morisson, Savage, Morton, & Russell, 2007) also demonstrated variable trypsin inhibitory activity between cultivars of raw peas grown in New Zealand. This study also illustrated that the decrease in activity varied between cultivars after thermal processing. Mean values of trypsin and chymotrypsin inhibitors in chickpea have also been shown to vary. It was found that the concentration of both trypsin and chymotrypsin was higher in desi than in kabuli chickpea (Singh & Jambunathan, 1981).

A review by Champ (2002) compared the trypsin (trypsin inhibitory units; TIU/mg DM) and chymotrypsin (chymotrypsin inhibitory activity; CIA/g) inhibitory activity of various pulses. The results indicated that there was no TIA activity in chickpea or CIA activity in lentil or chickpea. However, other researchers found that the trypsin and chymotrypsin inhibitory activity in lentil seed meal was 1.2 and 0.9 mg/g, respectively (Weder, Hegarty, Holzner, & Kern-Dirndorfer, 1983). Winter pulse crop varieties may also have more protease inhibitory activity than spring varieties (Champ, 2002). The results reported are variable when comparing species due to irregularities in units used and methods of analysis. A more comprehensive research is needed to analyze the activity of protease inhibition between species and varieties to further improve processing conditions and our understanding of the beneficial and detrimental effects of protease inhibitors in human and animal nutrition. To date, the best characterized and studied BBIs are found in pea cultivars (Duranti & Gius, 1997), therefore more research is needed on lentil and chickpea varieties.

### 4.5. Deleterious effects of protease inhibitors on human health

Although concentration and activity of protease inhibitors in pulse seeds are variable, detrimental effects of their inhibitory properties have been demonstrated (Champ, 2002; Duranti, 2006; Messina, 1999). Similar to lectins, protease inhibitors interfere with digestion when pulse seeds or pulse flour are consumed raw. Protease inhibitors are resistant to pepsin and the acidic pH of the human digestive tract and, therefore, interfere with digestion by inhibiting trypsin and chymotrypsin through irreversible binding to the enzymes. The mechanism by which BBI and Kunitz inhibitor act has been described by Guillamon et al. (2008) as “suppressing the negative feedback regulation of pancreatic secretions through the release of the hormone cholecystokinin from the intestinal mucosa”. The negative feedback regulation may stimulate pancreatic hypertrophy and decrease growth and performance of the host due to reduced ability of the digestive enzymes to properly hydrolyze dietary protein, thereby decreasing the amino acid absorption and de novo proteins synthesis. The reduction in growth and performance can also be attributed to the loss of the sulfur-rich components of trypsin and chymotrypsin due to their inhibition by protease inhibitors. In addition to pancreatic enlargement secondary to the presence of protease inhibitors in raw pulse seeds, chemically induced pancreatic tumors have also been reported in some animal species from soybean trypsin inhibitors (Messina, 1999). Unlike lectins, gastrointestinal ailments such as diarrhea, bloating and vomiting associated with protease inhibitors have not been reported, and the only harmful effects on humans occurred when seeds were consumed raw or processed inadequately. The deleterious effects of protease inhibitors on humans are usually performed in in vitro trials (Duranti, 2006), however the potential benefits of denatured trypsin and chymotrypsin inhibitors are being studied in in vivo models (Kennedy, 1993). In addition to affecting the host, protease inhibitors can negatively affect the properties of foodstuffs where protease enzymes are commonly used. Gel-formation, water-holding capacity, foaming, and the whipping ability of a product are all negatively affected by protease inhibitors from pulse seeds (Garcia-Cerreno, 1996).

### Table 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Trypsin inhibitor (IU mg⁻¹ DM)</th>
<th>Chymotrypsin inhibitor (IU mg⁻¹ DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Renata</td>
<td>Solara</td>
</tr>
<tr>
<td>Raw seed</td>
<td>3.80 ± 0.24</td>
<td>2.80 ± 0.09</td>
</tr>
<tr>
<td>Dehulling</td>
<td>4.02 ± 0.08</td>
<td>2.82 ± 0.06</td>
</tr>
<tr>
<td>Soaking</td>
<td>3.74 ± 0.09</td>
<td>2.52 ± 0.06</td>
</tr>
<tr>
<td>Germination (24 h)</td>
<td>3.57 ± 0.11</td>
<td>1.18 ± 0.02</td>
</tr>
<tr>
<td>Germination (48 h)</td>
<td>3.32 ± 0.08</td>
<td>1.18 ± 0.02</td>
</tr>
<tr>
<td>Germination (72 h)</td>
<td>2.76 ± 0.11</td>
<td>0.70 ± 0.03</td>
</tr>
<tr>
<td>Extrusion</td>
<td>0.19 ± 0.02</td>
<td>0.16 ± 0.06</td>
</tr>
</tbody>
</table>

DM = dry matter.
IU = international units.
 Adapted from: Alonso et al. (1998).
4.6. Methods for reducing protease inhibitory compounds in pulse seeds

As previously mentioned, denaturation of antinutritional protease inhibitory compounds is dependent on processing conditions, pulse species and variety. Germination, extrusion cooking, dehulling and hydrothermal processing are common commercial processes used to inactivate protease inhibitors (Table 2). Studies on chick fed processed soybean demonstrated that extrusion cooking of soybean meal at temperatures between 104 °C and 120 °C for 30–60 s resulted in an increase in growth rates in chicks while pancreas weights remained low. The increased growth rate and the absence of pancreatic hypertrophy indicated that a specific time and temperature range was sufficient to denature the protease inhibitor in that particular soybean variety. Combining soaking with heat treatment has also been successful in decreasing the concentration of protease inhibitors. After soaking 17 different pea varieties for 18 h and then boiling them for 20 min, trypsin inhibitor content was reduced in all of the varieties by 42–92% (TIU/mg DM), with an average reduction of 78% (TIU/mg DM) (Morrison et al., 2007). Other studies demonstrate variability in protease inhibitor activity after utilizing different time and temperature combinations, depending on the species, cultivar and treatment method (Alonso et al., 1998; El-Adawy, Rahma, El-Bedawy, & Sobihah, 1999; Marquez & Alonso, 1999).

Agricultural practices may also have an effect on the protease inhibitor content of pulse seeds. Direct versus two-phase harvesting of different variety of pea, chickling vetch, lentil and soybean showed that two-phase harvesting reduced the trypsin inhibitory content in pea by up to 44% and up to 25% in chickling vetch. The reduction in trypsin inhibitor concentration in lentil and soybean was too small to be considered statistically significant (Pisulewska & Pisulewski, 2000). The reduction in protease inhibitor concentrations in two-phase harvesting may be an indication that the seed accumulates protease inhibitory compounds as it reaches maturity. In a two-phase harvesting system, the plant is cut and then left in the field to dry before the plant seed has an opportunity to accumulate the inhibitory compounds.

Plant breeding programs have attempted to decrease protease inhibitor levels in pulses through genetic manipulation. France has established a protease inhibitor threshold and does not permit the registration of pea varieties with levels of trypsin inhibitors two units higher than the two official control cultivars (Morrison et al., 2007). Although this policy decreases the antinutritional content in the seeds, the crops may not perform as well, resulting in decreased yields, since protease inhibitors also protect the plant from predation. Similarly, protease inhibitors also protect the seed post-harvest by protecting against fungi and other microorganisms during storage (Garcia-Cerreno, 1996). Therefore, decreasing the protease inhibitor content of pulse crops through plant breeding programs may actually be more detrimental than growing plants with a higher protease inhibitory content and subsequently inactivating the inhibitors post-farm gate through established processing techniques.

4.7. Beneficial properties of denatured protease inhibitors on human health

Despite the negative effects of protease inhibitors, denatured protease inhibitors have several beneficial properties on human health. In 1992 BBI proteases from legume crops achieved investigational new drug status by the FDA due to their health-promoting benefits in a denatured form (Kennedy, 1995). Since then numerous reports have been published on the potential health-promoting benefits of protease inhibitors, such as their potential use as an anti-inflammatory agent. Most research on the health benefits of protease inhibitors has been performed with soybean. A soybean extract containing soybean BBI that suppressed carcinogenesis in several animal models was developed with chymotrypsin protease inhibitor as the active component responsible for the anticarcinogenic behaviour (Kennedy, 1993). Soybean BBI suppressed colon, esophageal, liver, lung and oral carcinogenesis, and it has also been proven efficient in suppressing radiation and chemically induced carcinogenesis in in vitro transformation assays (Moy & Bilings, 1994). Studies in mice have confirmed that BBI activity was widely distributed in various tissues (blood, kidney, liver and lungs) in mice after ingestion. Human and animal fibroblast and epithelial cells have also been shown to internalize protease inhibitors (Moy & Bilings, 1994). These findings indicate that BBIs are able to resist digestion and be transported to various tissues in the host.

Anti-inflammatory properties of protease inhibitors in pulses have also been demonstrated. Mice fed BBI concentrate demonstrated decreased inflammation of the colon when ulcerative colitis was induced in comparison to mice on a standard diet (Ware, Wan, Newberne, & Kennedy, 1999). The beneficial effects of BBI or other protease inhibitors as an anti-inflammatory or therapeutic cancer agent depend on the pulse species and seed variety used. Protease inhibitors are suggested as potential drugs for treating various diseases such as human immunodeficiency virus (HIV), hypertension and neurodegenerative disease, along with various infectious diseases, however these are often treated with synthetic peptidomimetic inhibitors rather than with natural protease inhibitors from pulses. To the best of our knowledge there have been no reports confirming the successful use of protease inhibitors from pulse crops in the treatment of the above diseases. Currently, published information regarding the use of protease inhibitors from pulse seeds as therapeutic agents is limited to the treatment of certain cancers or as an anti-inflammatory agent. Further research is needed to determine if protease inhibitors derived from pulse crops can also be used in the treatment of various other diseases.

Once the protease inhibitor reaches the target tissue through the blood stream, the precise mechanism of cancer and inflammatory suppression is not well understood. Moy and Bilings (1994) suggest that the protease inhibitors “suppress malignant transformation by inhibiting cellular enzymes involved in the induction and/or expression of the transformed phenotype”. Other researchers have hypothesized that “protease inhibitors suppress carcinogenesis by altering the levels of certain types of proteolytic activities, hydrolyzing activities and the expression of certain types of oncogenes that play an important role in carcinogenesis” (Kennedy, 1993). Therefore, further research is needed to characterize the precise mechanism of cancer and inflammation suppression.

5. Angiotensin I-converting enzyme (ACE) inhibitory peptides

Angiotensin I-converting enzyme (ACE) is widely distributed in mammalian tissues, predominantly as membrane bound eetoinzymes in vascular and endothelial cells, along with several other cell types such as absorptive epithelial, neuroepithelial and male germinal cells. ACE is a zinc metalloepitidase, which is activated by chloride and has broad in vitro substrate specificity (Li, Le, Shi, & Shrestha, 2004). ACE causes high blood pressure by converting the biologically inactive angiotensin I to the potent vasoconstrictor angiotensin II, and also inactivates the vasodilator bradykinin (Hong et al., 2008) (Fig. 1). Drugs containing ACE inhibitory peptides are commonly used for treating hypertension and regulating blood pressure, however they are costly and have been associated with numerous side effects (Erdmann, Cheung, & Schroder, 2008).

Since the discovery of ACE inhibitory peptides from snake venom in 1971, there has been a renewed interest in isolating ACE
ACE inhibitory peptides have proven to be effective in the prevention and treatment of hypertension, heart failure, myocardial infarctions and diabetic nephropathy in both human and animal models (Meisel et al., 2005). The efficacy of ACE inhibitory peptides is dose dependent and, therefore, dependent on their potency, which is normally measured as an IC$_{50}$ value (concentration of inhibitory peptide needed for 50% inhibition of ACE activity). For example, inhibitory peptides isolated from rice, mung bean, and pea have IC$_{50}$ of 18,200, 26.5 and, 0.15–0.23 μM, respectively (Hong et al., 2008). However, the inhibitory activity of these peptides in vitro may not have the same potency in vivo. When ingested orally, some ACE inhibitory peptides within the food matrix may be latent and must be fully or partially digested by gastrointestinal enzymes such as trypsin, chymotrypsin and papain. The digestion of ACE inhibitors by these enzymes may result in either inactivation or activation of the inhibitory peptide, or the peptide may remain in the latent form. Therefore, the potency measured in vitro may be vastly different from the potency in vivo. Li et al. (2004) discovered that inhibitory peptides may not always have antihypertensive activities. Peptides with a high inhibitory activity did not always translate to a decrease in blood pressure (Li et al., 2004). In addition, the amino acid sequence and the length of the inhibitory compound's antihypertensive properties. Inhibitory peptides with short peptide chains, usually 2–5 amino acids, with C-terminal proline or hydroxyproline residues have stronger inhibitory effects, as they have been shown to bind the ACE more strongly. Proline, lysine and arginine are the preferred C-terminal substrate for ACE, contributing to a more potent ACE inhibition (Erdmann et al., 2008). In addition, short peptides are more readily absorbed by the cardiovascular system than free amino acids. Larger peptides (10–51 amino acids) are readily absorbed, however their ACE inhibitor potency is greatly reduced (Erdmann et al., 2008).

Many food peptides and proteins derived by enzymatic hydrolysis with antihypertensive properties in vitro and in vivo have been discovered. Many in vivo studies have been performed in humans and spontaneous hypertensive rats (SHR) by intravenous and oral administration of the bioactive peptides and/or hydrolysate. Most studies have focused on the use of peptides derived from dairy products, fish, sesame seed, eggs and mung bean, among many others (Hong et al., 2008). For example, a group of hypertensive humans consuming 95 ml of Calpis sour milk daily showed significant reduction in blood pressure compared to placebo (Hata, Yamamoto, Ohni, Nakajima, & Takano, 1996). Fujita and Yoshikawa (1999) isolated two different peptides from thermolysin-digest of “Katsuo-bushi”, a traditional Japanese dish created from dried bonito. The LKP(KM) (100 mg/kg) and LKP (30 mg/kg) peptides decreased blood pressure in SHR by 30 and 50 mm Hg, respectively, after intravenous administration. Minimum effective doses of LKP(KM) and LKP were 8 and 2.25 mg/kg. Purified peptides from sesamene peptide powder have been shown to substantially decrease systolic blood pressure in SHR after a single orally administered dose of either 1 or 10 mg/kg (Nakano et al., 2006).

Several studies continue to demonstrate the antihypertensive characteristics of various peptides isolated from a wide variety of foods and food ingredients (Erdmann et al., 2008; Hong et al., 2008; Li et al., 2004; Meisel et al., 2005). However, to date a comparison of the ACE inhibitory properties of peptides derived from pea, chickpea and lentil to that of dairy proteins has not been adequately investigated, and further research is warranted. Investigations of peptides as a potential source of ACE inhibitory peptides has primarily focused on soybean (Kuba, Tana, Tawata, & Yasuda, 2005; Mallikarjun, Gowda, Appu, & Prakash, 2006; Wu & Ding, 2002) and mung bean, to a lesser extent (Li, Shi, Liu, & Le, 2006). However, recent research has focused on pea and chickpea (Aluko, 2008; Humiski & Aluko, 2007; Pedroche et al., 2002; Vermeirssen et al., 2005). The treatment of legumin from chickpea with alcalase produced a hydrolysate with ACE inhibitory (IC$_{50}$) activity of 0.18 mg/ml. Fractionation of the hydrolysate by reverse phase chromatography also yielded five inhibitory peptides ranging in inhibitory activity (IC$_{50}$) from 0.011 to 0.021 mg/ml (Yust et al., 2003). A study by Vermeirssen et al. (2005) evaluated the antihypertensive effect of pea and whey digests and investigated the success of ACE inhibitory compounds in reaching the systemic circulatory system in vitro. After intravenous administration of 50 mg protein kg$^{-1}$ body weight in SHR, ACE inhibitory compounds from pea showed a transient but strong antihypertensive effect of 44.4 mm Hg, while whey digest had no effect at an equivalent dose. Peptides derived from whey were more susceptible to digestion by gastric enzymes than pea-derived ACE inhibitory peptides. In addition, pea ACE inhibitory peptides were converted to other active peptides upon gastrointestinal digestion with similar ACE inhibitory activity rather than being denatured (Vermeirssen et al., 2005). Research on the isolation and characterization of ACE inhibitory compounds from lentil varieties is limited. Work is currently ongoing in the author's laboratory to identify ACE inhibitory peptides in lentil. Presence of ACE inhibitor peptides in chickpea and pea cultivars has provided a solid foundation for the potential use of pulse seeds or pulse seed extracts in treating and preventing hypertension. However, more research is needed regarding the isolation and characterization of ACE inhibitory compounds derived from pulse crops using both in vitro and in vivo models.

In addition to possessing bioactive properties that reduce hypertension, the onset of heart failure, the risk of myocardial infarctions and diabetic nephropathy, ACE inhibitors have been demonstrated to have antioxidative properties. Limited studies have demonstrated that antihypertensive drugs containing ACE inhibitors such as enalaprilat, lisinopril and captopril have antioxidative properties. These three drugs showed oxygen free radical scavenging properties by the chemiluminescence assay of oxidation of hypoxanthine by xanthine oxidase in the presence of luminol (Mira, Silva, Queiroz, & Manso, 1993). The structure of these drugs (Fig. 2) illustrates that the compounds have functional groups that react with oxygen, resulting in the scavenging of reactive hydroxyl ions through a decarboxylation reaction of the carbonyl group. Furthermore, enalaprilat and lisinopril have aromatic rings which may contribute to the compound's antioxidative actions, whereas some of the oxygen scavenging properties
from captopril may result from the oxidation of its sulfur group (Mira et al., 1993). In a related study of six antihypertension drugs (captopril, enalapril, fosinopril, perindopril, quinapril and ramipril), only captopril had significant antioxidative properties when assayed by the ferric reducing antioxidant power (FRAP) assay (Benzie & Tomlinson, 1998).

The comparison of antioxidative properties found in ACE inhibitor drugs to those found in raw pulse seeds or pulse flour may be difficult, as ACE inhibitor potencies of pharmaceutical compounds are generally expressed in IC50 values rather than concentration. Therefore, it is difficult to definitively determine if pharmaceutical ACE inhibitors are more potent antioxidants than those derived from pulse seeds based on current information. However, it can be hypothesized that ACE inhibitor containing drugs have a higher concentration of ACE inhibitors compared to chickpea, pea and lentil, as pharmaceutical compounds are sold in capsules in a concentrated form. Furthermore, the antioxidative properties of pharmaceuticals may have a higher potency and may be metabolized more readily, as pharmaceutical compounds are typically encapsulated to protect the active ingredient from adverse conditions such as microbial activity, protease enzymes and low stomach pH. In addition, the antioxidative properties of the ACE inhibitor peptides derived from pulses may be lost due to modifications to the peptide prior to being absorbed by the host.

There are other components of pulse seeds, such as genistein, daidzein and other phytoestrogens, which also have been demonstrated to have strong antioxidative properties. Therefore, some of the antioxidative effects in vivo may be predominantly the result of other phenolic components and not ACE inhibitors (Mitchell, 2001). Although a small number of studies have identified certain ACE inhibitor containing drugs as having antioxidative properties, further research is needed to characterize ACE inhibitors isolated from pulse seeds. Moreover, most of the antioxidative studies described above have been performed in vitro. Further research should be undertaken to examine antioxidative potencies in vivo, with and without encapsulation. In conclusion, additional research is needed to further investigate the antioxidative properties of pulse seeds and their potential role in human health and disease.

6. Conclusion

The abundance and availability of pulse crops worldwide, coupled with their high nutritional value, have resulted in pulse crops being one of the most widely produced and consumed agricultural commodities. Pulse crops have long been known for their nutritional and health-promoting properties, such as being an excellent source of protein, fiber, carbohydrates, and for their role in decreasing the risk of certain cancers, managing obesity, lowering cholesterol and type-2 diabetes. Recently, the bioactive properties of proteins and peptides derived from pulse seeds have gained increased recognition in the areas of food science and nutrition for their potential benefits in treating and/or reducing the onset of disease. Lectins and protease inhibitors which were traditionally considered as protein antinutritional compounds have shown potential in the treatment and/or prevention of various cancers, obesity and hypertension which has necessitated a reconsideration of the use of the term “antinutritional”. Additionally, the ACE inhibitory properties of pulse peptides could make them primary therapeutic agents or adjuncts to treatment for certain cardiovascular diseases. Pulse seeds may, therefore, be potentially excellent sources of beneficial bioactive proteins and peptides, and techniques for the efficient extraction and fractionation of these proteins and peptides are needed. Further research is also needed to improve our understanding of the mechanisms involved in the absorption into the blood stream, target sites and activity in various tissues of biologically active compounds derived from dry peas, chickpeas and lentils. Another area requiring research is comparative studies of the bioactivities of various pulse cultivars of the same species and between different pulse crops, in order to determine the cultivar of a specific pulse that has the greatest concentration and activity of a desired biologically active compound.

References


