

# Chapter 10

## Nutritional Value

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### 1 Introduction

Consumption of legumes is associated with physiological and health benefits, such as prevention of cardiovascular disease, obesity, diabetes mellitus and cancer, as indicated by an increasing number of studies. The growing body of research on these health benefits has stimulated interest in developing innovative technologies to expand the use of pulses in food products. Nevertheless, growing global food security challenges and protein malnutrition remain critical in many countries around the world.

Currently, nearly 870 million people are suffering from chronic undernourishment. Moreover, 100 million children under five are underweight, and this childhood malnutrition is a cause of death for more than 2.5 million children every year. Most hungry and undernourished people live on diets based on very high amounts of staple foods but few micronutrient-rich foods such as fruits, vegetables and animal and fish products. As a consequence, they lack the required protein, fat, vitamin A, iodine, zinc and iron. A particular type of malnutrition is the so-called hidden hunger, a chronic lack of vitamins and minerals as a consequence of poor dietary quality, which negatively impacts on health, cognition, function, survival and economic development. Hidden hunger mainly affects women and children from the lower-income groups in developing countries; however, it also claims victims in the developed world, where people apparently do not look hungry. Indeed, obesity or being overweight can be a sign that bodies are still hungry for crucial micronutrients. The impact of hidden hunger is serious: Globally stunted growth and anaemia

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in children become a major cause of health problems in later life, particularly the increasing prevalence of overweight/obesity and chronic diseases. This results in a double burden for the health systems, with associated massive health costs and negative impact on economic productivity.

Grain legumes (pulses) are considered an essential source of nutrients and are also recognized as a poor man's meat, showing their importance for people of developing countries, where the consumption of animal protein is limited by non-availability or is self-imposed because of religious or cultural habits. Furthermore, legume seeds contain many bioactive and/or antinutritional compounds, such as phytate, oligosaccharides, phenolic compounds, nonprotein amino acids, lectins and enzyme inhibitors that play metabolic roles in humans or animals that frequently assume these seeds. These effects may be regarded as positive, negative or both (Champ 2002).

Considerable genetic variation has been reported in the chemical composition of legume seeds, both among and within species. In addition, chemical composition can be modified by environmental factors during plant development, since many of the bioactive compounds are secondary metabolites produced during seed development and maturation. Existing data show that the balance between deleterious and beneficial effects of these compounds depends on their chemical structure, concentration, time of exposure and interaction with other dietary components. Therefore, it is important to know not only the amounts but also the types of compounds in the food and how they affect human body. The scientific understanding of how these bioactive molecules act on organisms is an important challenge for the future research and a special attention should be paid to the potential synergistic effects between/among the different classes of bioactive compounds (Rochfort and Panozzo 2007).

In this chapter, main classes of bioactive compounds, together with some species-specific ones, are described in relation to their biological activities, abundance in legume crops and role in nutrition and health.

## **2 Major Seed Proteins: Storage Proteins and Enzyme Inhibitors**

Legume seeds contain large amounts of proteins, mostly with a storage role, ranging from about 16% (dry weight) in cowpea, pigeon pea and chickpea to as much as about 50% in lupin and soybean, according to species, genotypes within species and environments (Table 10.1). Storage proteins are synthesized during seed development, stored in specific subcellular compartments, the storage vacuoles or protein bodies and then hydrolyzed during germination to provide nitrogen and carbon skeletons for the developing seedling. The major storage proteins of legume seeds are oligomeric globulins and albumins, which usually account for about 70 and 20% of the total protein, respectively.

**Table 10.1** Range of variation (% of seed weight) of principal constituents of grain legume seeds

Species	Protein	Carbohydrates			
		Total	Fibres	Starch	Oil
Common bean	20.9–30.1 <sup>a</sup>	54–64 <sup>a</sup>	10 <sup>c</sup>	41.5 <sup>c</sup>	1.3–2.5 <sup>a</sup>
Pea	21.9–31 <sup>a</sup>	52–62 <sup>a</sup>	5.9–12.7 <sup>b</sup>	18.6–54.5 <sup>b</sup>	1.3–3 <sup>a</sup>
Chickpea	16–28 <sup>a</sup>	54–66 <sup>a</sup>	2.7–9 <sup>b</sup>	42–54.9 <sup>b</sup>	3.1–7 <sup>a</sup>
Faba bean	24.3–32.2 <sup>a</sup>	57–60 <sup>a</sup>	7.5–13.1 <sup>b</sup>	37–51.5 <sup>b</sup>	1.1–4
Cowpea	16–36 <sup>b</sup>	56–68 <sup>a</sup>	6.3 <sup>b</sup>	46.84–53.63 <sup>c</sup>	1–1.3 <sup>a</sup>
Lentil	20.6–32 <sup>a</sup>	54–58 <sup>a</sup>	12–14.7 <sup>b</sup>	46–49.7 <sup>b</sup>	1–2.1 <sup>a</sup>
Pigeon pea	15.9–24.1 <sup>b</sup>	57.3–58.7 <sup>a</sup>	10 <sup>c</sup>	44.3 <sup>c</sup>	1.2–1.6 <sup>b</sup>
Mung bean	23.3–27.7 <sup>a</sup>	61–62 <sup>a</sup>	7–12.9 <sup>b</sup>	45 <sup>c</sup>	0.7–2.4
<i>Lupinus</i> spp.	28–47 <sup>c</sup>	26–47 <sup>c</sup>	3 <sup>d</sup>	0.4 <sup>b</sup>	4–15.5 <sup>c</sup>
Soybean	26.5–55.2 <sup>b</sup>	30.2–35 <sup>c</sup>	20 <sup>c</sup>	1.5 <sup>c</sup>	6.5–28.7 <sup>b</sup>

<sup>a</sup> Chibbar et al. 2010<sup>b</sup> Burstin et al. 2011<sup>c</sup> Hedley 2001<sup>d</sup> Reddy et al. 1984<sup>e</sup> Sreerama et al. 2012

## 2.1 Globulins

Globulins are classified as 7S and 11S proteins, according to their sedimentation coefficients (S), and are collectively named vicilins and legumins, respectively. Legumins are compact hexamers of about 350–400 kDa, and each monomer is made up of two disulphide-bonded subunits derived from posttranslational proteolysis of a single precursor polypeptide. Vicilins are typically trimeric proteins of 150–190 kDa that lack cysteine residues and hence cannot form disulphide bonds. Their subunit compositions vary considerably, mainly because of differences in the extent of posttranslational processing (proteolysis and glycosylation). Vicilins can be divided into two groups: In the first one, the precursor polypeptides are extensively fragmented to give rise to mature subunits in the range of 12–34 kDa. Conversely, the precursor polypeptides belonging to the second group undergo little or no posttranslational cleavage, and mature polypeptides are about 40–76 kDa. Pea vicilin is a typical example of group one vicilins, while soybean  $\beta$ -conglycinin, and common bean (*Phaseolus vulgaris* L.) are among the best characterized of the second group. Although legumins and vicilins are both present in most legumes, their relative abundance is highly variable, and some species are virtually devoid of either one or the other. Vicilins are usually less abundant than legumins, but a remarkable exception exists in *P. vulgaris* and *P. lunatus*, in which normally vicilin is the most abundant storage protein. In *P. coccineus*, the ratio between vicilin and legumin is highly variable, and seeds virtually depleted of legumin or phaseolin have been reported (Durante et al. 1989).

From a nutritional point of view, the amino acid profile of legume storage proteins reveals low amounts of the essential sulphur-containing amino acids (i.e. methionine and cysteine) and tryptophan, while lysine, another essential amino acid,

is quite abundant. Legume proteins complement very well those of cereals, which are normally rich in sulphur amino acids and poor in lysine and threonine. Besides the composition in essential amino acids, the nutritional quality of seed proteins is also largely determined by their digestibility. In fact, amino acids composition only represents the potential nutritional quality of a protein, being their bioavailability critical for the supply of amino acids in the diet. A number of data obtained with experimental approaches devised to assess the bioavailability of amino acids in foods concurrently demonstrated that seed proteins have a lower overall nutritional quality than animal proteins. This can be related to their low content in sulphur amino acids, the compact proteolysis-resistant structure of the native protein and the presence of antinutritional compounds in the seed, which may affect digestibility of proteins themselves as well as of other components. For example, the nutritive value of common bean phaseolin, the 7S globulin of this species which normally accounts for about 40–50% of total seed proteins, is limited by a low content in sulphur amino acids and by resistance to enzymatic hydrolysis, even after heat treatment (Montoya et al. 2006). The three-dimensional structures of 7S vicilins and 11S legumins have been resolved and confirmed that the two globulins are structurally related (Lawrence et al. 1994). Vicilin trimers are arranged in a disk-shaped fashion with each monomer arranged around a threefold symmetry axis and are rich of  $\beta$ -sheet and  $\beta$ -turn structures which have been proposed to be responsible of the low digestibility of globulins (Carbonaro et al. 2012; Lawrence et al. 1994).

Although the nutritional value and digestibility of globulins are not optimal, there are data showing that soybean  $\beta$ -conglycinin, the 7S globulin, is responsible for cholesterol/triglyceride-lowering activity, and it seems that the N-terminal extension domain of the  $\alpha'$  chain is responsible for inducing this biological response (Consonni et al. 2011). A similar positive role in reduction of hypercholesterolemia (and prevention of cardiovascular risk) has been shown for lupin proteins, the major role being played by  $\gamma$ -conglutin, an unusual basic 7S-type globulin specific of white lupin (Sirtori et al. 2004). Interestingly,  $\gamma$ -conglutin is also able to counteract the plasma glucose increase and improve insulin sensitivity when administered to rats, thus suggesting a potential use in the control of glycaemia in type 2 diabetes (Lovati et al. 2012).

Different approaches have been undertaken to improve the nutritional quality of legume storage proteins, and many have been directed towards increasing the content of sulphur amino acids and/or change in relative abundance/type of storage proteins. An example strategy, applied in common bean, consisted in the selection and breeding for highly digestible phaseolin types (Montoya et al. 2010). In fact, comparison of the degree of hydrolysis of 43 different phaseolin types showed variability ranging from 11 to 27% for uncooked phaseolin and from 57 to 96% for heat-treated one (Montoya et al. 2008). An alternative approach is the manipulation of seed protein compositions by decreasing the percentage of those types with low or limiting amino acid content. Using three common bean lines differing for major storage proteins content (devoid of phaseolin and/or major lectins), Taylor et al. (2008) evaluated the impact of storage protein deficiency on protein accumulation and amino acid composition, especially those containing sulphur, in mature seeds.

They found that deficiency of phaseolin and major lectins was associated with a progressive and compensatory increase of the content of other proteins, mainly legumin,  $\alpha$ -amylase inhibitor ( $\alpha$ AI) and mannose lectin FLT-3 receptor interacting lectin (FRIL; Marsolais et al. 2010). However, the most interesting finding was that the deficiency of some classes of storage proteins caused a modulation of sulphur amino acid content. The deficient lines showed a decrease of *S*-methyl-Cys and  $\gamma$ -Glu-*S*-methyl-Cys (both nonprotein amino acids) that were compensated with an increase of the Cys (70%) and Met (10%) pools, and the combined content raised from 18.9 to 26.8 mg/g protein, a value slightly above Food and Agriculture Organization (FAO) guidelines of 25 mg/g protein for human nutrition.

Albumins are the second most abundant class of legume storage proteins. They are water-soluble proteins comprising most of the bioactive polypeptides, such as lectins, protease inhibitors and  $\alpha$ AI. The abundance of these bioactive molecules is quite variable in the different legumes. Lectins are widespread in many legume seeds, while  $\alpha$ AI activity has been detected only in few legume species. Remarkably, the majority of these proteins have evolved within the seed as a protective mechanism against insects, fungi, predators and a number of stress conditions (Chrispeels and Raikhel 1991). On the other hand, very often, the biological activity of these proteins is also responsible for the nutraceutical and health properties of legumes, thus the interest for the potential uses of these molecules has increased in recent years.

## 2.2 Lectins

Lectins are a family of highly homologous glycoproteins that exhibit specific and reversible carbohydrate-binding properties. As a result, lectins can bind to specific sugars and glycoproteins on the surface of cells in the gut wall, thereby interfering with nutrient breakdown and absorption.

Many lectins are able to agglutinate red blood cells, thus their presence is traditionally measured by their haemagglutinating activity (HA). Lectins' abundance and their biological activity in legume seeds vary among species as well as among genotypes of the same species (Table 10.2). Null or very-low lectin activity/presence has been reported for chickpea, lupin and *Vigna* genus, on the contrary, seeds of *Phaseolus* species have the highest content, although common bean genotypes devoid of lectins have been identified (Campion et al. 2009a; Confalonieri et al. 1992).

Growth suppression, diarrhoea and bloating are the most common effects of raw lectin ingestion by humans and livestock (Vasconcelos and Oliveira 2004). The toxicity of lectins is very often due to their high resistance to proteolysis and stability over a large range of pH. Even though some lectins are heat sensitive, they are not always completely destroyed by cooking because of the use of gentle cooking methods, such as dry heat and short cooking times. Lectin activity can, to various degrees, be removed from foods by different technological processes. For

**Table 10.2** Comparison of haemagglutinating activity of different legume seed protein extracts towards rabbit or human erythrocytes. One unit of haemagglutinating activity (HU) is defined as the amount of seed extract per ml (range from 12,500 to 0.1 µg/ml) in the last serial dilution giving 50% of agglutination (the lowest the HU value, the highest is the haemagglutinating activity of the sample). (Data adapted from Grant et al. 1983)

Species	Haemagglutinating activity range	
	Rabbit erythrocytes	Human erythrocytes (AB type)
<i>Phaseolus vulgaris</i> (white kidney beans)	6–24	12–390
<i>Phaseolus vulgaris</i> (pinto beans)	6250–12,500	12,500
<i>Phaseolus coccineus</i>	1.5–12	98–390
<i>Phaseolus acutifolius</i>	1.5–12	24
<i>Phaseolus lunatus</i>	12,500	98
Lentil	49–780	1560–6250
Pea	49–195	3120
Chickpea	12,500	12,500
Cowpea	12,500	12,500
Pigeon pea	12,500	12,500
Mung bean	12,500	12,500
Faba bean	49–3120	3120–12,500
Soybean	24–390	12,500

example, soaking, autoclaving and toasting completely destroyed the lectin in *P. lunatus* (Adeparusi 2001). Apart from common bean, microwave heating adequately destroys haemagglutinins and trypsin inhibitors in legume seeds without affecting protein quality, and irreversible lectin denaturation is achieved using boiling water (Hernandez-Infante et al. 1998).

Active lectins, that survived cooking and/or passage in the gastrointestinal tract, may induce changes in some or all of the digestive, absorptive, protective or secretory functions of the whole digestive system and affect cellular proliferation and turnover. For example, phytohaemagglutinin (PHA), the common bean lectin, binds to the gastric mucosal and parietal cells inhibiting the gastric acid secretion in conscious rats (Kordas et al. 2001), while in pigs it causes an increase in stomach weights and thickness (Radberg et al. 2001).

Although lectins are considered antinutrients, positive and beneficial roles for human health and nutrition have also been reported. Studies have revealed that oral administration of low doses of lectins can produce beneficial effects on the digestive/absorptive efficiency of the gut, its immune system and bacterial ecology and that, by modulating the secretion of gut hormones, some lectins can influence the body's endocrine system with beneficial consequences for general metabolism (Pusztai and Bardocz 1996). Some lectins may play a key role in preventing certain cancers (De Mejia and Prisecaru 2005) or can be used as therapeutic agents for preventing or controlling obesity (Bardocz et al. 1996).

### 2.3 *Protease Inhibitors*

Many legume seeds also contain inhibitors of proteolytic enzymes. These are considered antinutritional molecules interfering with protein digestion, due to their ability to irreversibly inhibit, if not properly inactivated, the action of digestive enzymes, such as trypsin, chymotrypsin, carboxypeptidases and elastase. However, once inactivated, protein inhibitors may even play a positive nutritional role, due to their high content of sulphur-containing amino acids compared to the majority of the seed proteins.

Most protease inhibitors belong to two major classes, the Kunitz trypsin inhibitors, particularly abundant in soybean seeds, and the Bowman–Birk inhibitors, that are widely found in the other legume seeds (Clemente et al. 2011; Oliva et al. 2011). Kunitz-type inhibitors have a molecular mass of about 20 kDa, with two disulphide bridges, and act specifically against trypsin. The Bowman–Birk inhibitors are double-headed inhibitors of 8–9 kDa with a high proportion of disulphide bonds. They usually comprise two distinct binding loops responsible for the inhibition of two identical or different proteases (chymotrypsin and/or trypsin) per inhibitor molecule. Differences in inhibitor concentrations and activity have been reported among legume species as well as varieties (Guillamon et al. 2008) and may be affected by environmental conditions during seed maturation (Piergiovanni and Pignone 2003). Trypsin inhibition (measured as trypsin inhibitor units per mg, TIU/mg) can range from negligible, as in the *Lupinus* spp., to very abundant in soybean (43–84 TIU/mg) and common bean (17–51 TIU/mg). The TIU content of different *Lathyrus* cultivars is in the range 19–30 TIU/mg sample, and this is higher than in chickpea (15–19 TIU/mg) and pea (6–15 TIU/mg). Most lentil and faba bean cultivars have lower values (3–8 and 5–10 TIU/mg sample, respectively) (Guillamon et al. 2008).

Kunitz and Bowman–Birk inhibitors' antinutritional effects are not only a consequence of inhibition of intestinal protein digestion for their presence in a diet consisting of free amino acids still has adverse effects resulting in decreased growth (Lajolo et al. 2004). It has been proposed that these inhibitors act suppressing the negative feedback regulation of pancreatic secretions through the release of hormone cholecystokinin from the intestinal mucosa (Liener 1994). The consequence is the stimulation of pancreas enlargement and hypersecretion of digestive enzymes (sulphur-rich proteins), causing a loss of sulphur-rich endogenous proteins. This would depress growth, as legume seed proteins are deficient in sulphur amino acids. On the other hand, the presence of trypsin inhibitors in the diet has been linked also to health-promoting properties. Bowman–Birk inhibitors are effective in preventing or suppressing carcinogen-induced transformation in vitro and carcinogenesis in animal assays. Anti-inflammatory properties of protease inhibitors have also been demonstrated (Ware et al. 1999). A number of patents on the use of Bowman–Birk inhibitors to combat obesity (Defreitas et al. 2003), degenerative and autoimmune diseases (Kennedy and Rostami 2004) and, in general, skeletal muscle atrophy (Sweeney et al. 2005) have been released.

## 2.4 $\alpha$ -Amylase Inhibitors

Presence of  $\alpha$ AI differs greatly among legume species and the best described and most abundant are those found in *Phaseolus* species. Grant et al. (1995), analyzing the  $\alpha$ AI levels in seeds of a number of legumes found in Europe, detected the highest contents of  $\alpha$ AI in *Phaseolus* species (2–4 g/kg). Much lower levels (0.1–0.2 g/kg) were found for lima bean, cowpea, chickpea, faba bean and sweet lupin, while no  $\alpha$ AI activity was found in seeds of adzuki bean, lentil, mung bean, pea, soybean and winged bean. In common bean,  $\alpha$ AIs belong to a group of evolutionarily related seed proteins, comprising lectins and arcelins, whose presence has been frequently associated to resistance against phytophagous insects (Pueyo and Delgado Salinas 1997; Zaugg et al. 2013).

$\alpha$ AIs do not inhibit plant amylases, while they are active against a different type of amylases, such as human (saliva and pancreatic), porcine, fungal and, most interesting, insect amylases (Ishimoto et al. 1995). Hence, it is not surprising that most of the studies looking for  $\alpha$ AI presence in seeds have been motivated by the protective role of this molecule against predatory insects. The best characterized  $\alpha$ AI is that of common bean; it is a quite stable molecule being relatively heat resistant (it is still active after 30 min at 80 °C). In native conditions, it can resist tryptic digestion up to 24 h at 37 °C (Adeparusi 2001); however, proper thermal treatment, such as heating for at least 10 min at 100 °C, is sufficient for complete inactivation and loss of resistance to trypsin (Sparvoli et al. 1999). These properties could in part explain the biological effects of  $\alpha$ AI. In clinical studies, purified  $\alpha$ AI inhibited intraduodenal amylase. High dietary intakes of  $\alpha$ AI can cause a number of potentially deleterious alterations in the metabolism of experimental animals. Starch digestion in the rat small intestine was also inhibited, with occasional blockage of the caecum, particularly at daily intakes of  $\alpha$ AI higher than 20 mg, leading to losses of body nitrogen, lipids and carbohydrates and growth depression (Pusztai et al. 1995). In humans,  $\alpha$ AI consumption decreases postprandial plasma glucose and insulin levels (Jain et al. 1989; Layer et al. 1986), thus the starch-blocking properties of  $\alpha$ AI have prompted several studies to exploit the use of this molecule to control obesity as well in the prevention and treatment of diabetes (Obiro et al. 2008).

## 3 Starch, Fibres and Oligosaccharides

Starch and fibres, along with carbohydrate derivatives, such as oligosaccharides, constitute the major components of seed carbohydrates, making up to 30–40% of seed dry matter in those species with higher protein content, such as lupin and soybean, and up to 50–65% in less protein-rich legumes species (Table 10.1). There is increasing evidence that the addition of fermentable fibre to the diet alters the function and structure of the gut, modifies the production of gut-derived hormones, and is associated with improved whole-body glucose homeostasis even in the absence of disease.



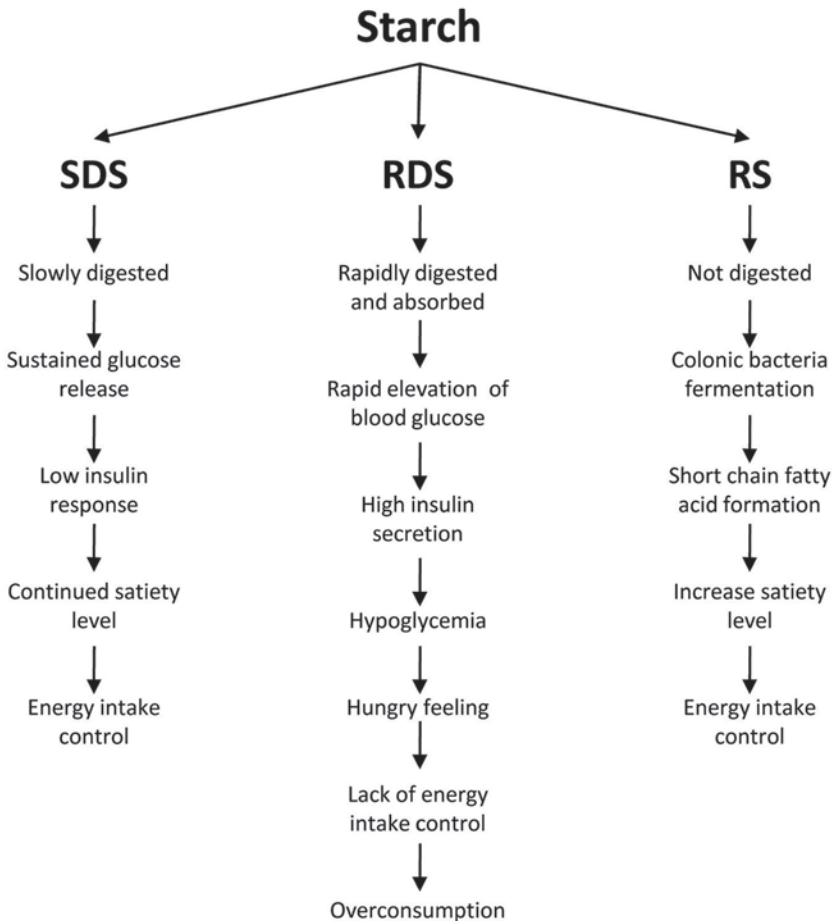
### 3.1 *Starch*

Generally, starch constitutes the largest part of carbohydrate fraction, accounting for 35–45% of seed dry weight, the only exceptions being soybean and lupin in which oil replaces starch as the main energy storage source (Table 10.1). Amylose and amylopectin are the two basic components of starch; amylose is a linear molecule with molecular weight ranging between 70 and 200 kDa, whereas amylopectin is a highly branched molecule consisting of main chains of (1–4)- $\alpha$ -D-glucose with short chains of (1–6)- $\alpha$ -D-glucose-linked branches with molecular weight greater than  $2 \times 10^4$  kDa.

Starch composition is one of the determinants that define legume seed nutritional value and health effects. On the basis of its susceptibility to amylases and consequent digestibility profile, starch is classified as rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). RDS is rapidly digested and absorbed in the duodenum and proximal regions of the small intestine leading to a rapid elevation of blood glucose and usually a subsequent episode of hyperglycaemia. SDS is slowly digested in the small intestine to provide sustained glucose release with a low initial glycaemia and subsequently a slow and prolonged release of glucose. On the contrary, RS is not hydrolyzed in the small intestine and is fermented by the colonic microflora in the large intestine, producing short-chain fatty acids (SCFA) that provide additional energy to the body along with butyrate that is beneficial to colonic health (Aller et al. 2011; Fig. 10.1). Due to the different method used to evaluate the content of starch fractions, it is difficult to make a meaningful comparison of the levels of SDS, RDS and RS starches among legume seeds. Factors such as amylose content, crystallinity and amylopectin structure have been shown to influence SDS and RS levels (Hoover et al. 2010). Most starches from grain legumes have a relatively high amylose content (30–40%) compared to those from cereals or tubers. These characteristics may lead to increase in RS content after processing, hence having important effects on human physiology (Guillon and Champ 2002), such as promoting slow and moderate postprandial glucose and insulin responses (Sievenpiper et al. 2009).

### 3.2 *Dietary Fibre (DF)*

DFs consist of chemically heterogeneous molecules such as cellulose, and non-cellulosic polysaccharides like hemicellulose, pectins, oligosaccharides and lignin derived from structural carbohydrates of the plant cell walls. DFs resist digestion and absorption in the small intestine and are partially or completely fermented in the large intestine, thus exerting various physiological effects with health implications (Tharanathan and Mahadevamma 2003). Depending on their water solubility, total DF (TDF) are classified into insoluble DF (IDF) and soluble DF (SDF). The first class (IDF) is made up by cellulose, hemicellulose and lignin, and its consumption reduces intestinal transit time, thereby improving laxation. The second class



**Fig. 10.1** Scheme representing starch classification and its main postprandial effects. *SDS* slowly digestible starch, *RDS* rapidly digestible starch, *RS* resistant starch. (Adapted from Aller et al. 2011)

(SDF) consists of oligosaccharides, glucans, gums and pectins, and its action is mainly in helping lowering blood cholesterol and regulating glucose. The composition and concentration of DF in legume seeds vary depending on their localization: the seed coat or the cotyledons, with the first having higher DF concentrations. DFs from seed coats contain large amounts of cellulose (35–57%) and less amounts of hemicellulose and pectins, while the major polysaccharides in cotyledons are pectin compounds (about 55%), cellulose (9%) and non-starchy glycans (ranging between 6 and 12%; Guillon and Champ 2002). Cellulose has been found to be the major constituent of crude fibre in pea and common bean, while hemicellulose is more abundant in lentil, faba bean, pigeon pea and mung bean (Reddy et al. 1984).

### 3.3 Oligosaccharides

The most common oligosaccharides in legume seeds are  $\alpha$ -galactosides, which are soluble low-molecular-weight sugars mostly represented by the raffinose family oligosaccharides. They are  $\alpha$ -(1 $\rightarrow$ 6)-galactosides linked to carbon C-6 of the glucose moiety of sucrose and include raffinose (trisaccharide), stachyose (tetrasaccharide) and verbascose (pentasaccharide). Their relative and total abundance vary among species and cultivars. Appreciable levels of these oligosaccharides, ranging between 0.4 and 16.1% of dry matter, are accumulated in the seeds of lentil, chickpea, lupin, pea and faba bean. Lupins contain higher levels of stachyose and raffinose compared to peas and faba beans, while verbascose is more abundant in peas and faba beans compared to lupin, chickpea and common bean (Muzquiz et al. 2012); stachyose is the most abundant  $\alpha$ -galactoside in common bean (Diaz-Batalla et al. 2006). Interestingly, in chickpea there is a marked difference in oligosaccharides content between desi-type and kabuli-type chickpeas with the last having 16.8% higher content than the former (Saini and Knights 1984).

From a nutritional point of view,  $\alpha$ -galactosides are considered antinutritional factors as they are not hydrolyzed by mucosal enzymes in the small intestine of monogastric animals and are then fermented in the lower gut by resident anaerobic bacteria with the consequent production of carbon dioxide and hydrogen gases that are responsible for digestive discomfort (Rochfort and Panozzo 2007). On the other hand,  $\alpha$ -galactosides have also proven to exert prebiotic effects by promoting the beneficial activity of specific members of the intestinal microflora, thus improving gut health by suppressing intestinal putrefaction, reducing constipation and diarrhoea, stimulating the immune system and increasing resistance to infection (Buddington et al. 2002).

## 4 Minerals and Phytic Acid

Legume seeds are an excellent source of essential minerals, particularly iron, zinc and calcium (Campos-Vega et al. 2010). The highest levels of iron can be found in seeds of common bean, faba bean, mung bean and lentil. High zinc contents have been reported for *Lupinus* spp., lentil and chickpea, while the highest calcium content is found in seeds of common bean, lupin, faba bean and chickpea (Table 10.3).

The increasing concerns about food security, together with a widespread “hidden hunger”, have stimulated research on crop biofortification, that, in many cases, has been translated into wide screenings of natural variability to identify donor genotypes with high mineral content. These genetic materials have been further used for breeding high Fe and high Zn varieties as well as for the identification of useful molecular markers to assist breeding (Amarakoon et al. 2012; Blair et al. 2013; DellaValle et al. 2013; Nair et al. 2013).

**Table 10.3** Range of variation of essential minerals ( $\mu\text{g/g}$  seed dry weight) and phytic acid content (mg/g seed dry weight) in different legume species

Species	Fe	Zn	Ca	PA	Reference
Mung bean	44.5–107	23.3–48	273	1.8–5.8	Sompong et al. 2010; Taunk et al. 2012
Pea	46–73	39–63	622–1219	3.1–7.1	Amarakoon et al. 2012; Muzquiz et al. 2012; Trinidad et al. 2010
Lentil	64.6–90	44–73.1	480–1280	2.5–12.2	Cabrera et al. 2003; Karakoy et al. 2012; Muzquiz et al. 2012; Thavarajah et al. 2009
Chickpea	46–77	37–74	517–1974	2.8–13.6	Grant et al. 2003; Konietzny and Greiner 2003; Muzquiz et al. 2012; Thavarajah and Thavarajah 2012; Wang et al. 2003
Pigeon pea	54	61	514	3.5–17.5	Chitra et al. 1995; Sompong et al. 2010; Trinidad et al. 2010
<i>Lupinus</i> spp.	24–108	29–176	1350–2225	6–8.9	Muzquiz et al. 2012; Porres et al. 2007; Trugo et al. 1993
Cowpea	106	65	209	5.4	Chitra et al. 1996; Grant et al. 2003; Konietzny and Greiner 2003
Common bean	62–280	10–42	562–4065	3.4–28.7	Cabrera et al. 2003; Doria et al. 2012; Grant et al. 2003; Guzman-Maldonado et al. 2000; Konietzny and Greiner 2003; Muzquiz et al. 2012; Trinidad et al. 2010
Faba bean	55–110	20.5–58	610–1973	5.9–15	Cabrera et al. 2003; Campos-Vega et al. 2010; Konietzny and Greiner 2003; Muzquiz et al. 2012; Oomah et al. 2011; Uzun et al. 2011
Soybean	161	66	1502	4.8–20.1	Muzquiz et al. 2012; Trinidad et al. 2010

Although legume seeds have a good content in essential minerals, they also accumulate significant amounts of compounds that lower their nutritional value by lowering nutrient bioavailability. Phytic acid (*myo*-inositol-1,2,3,4,5,6-hexa-kisphosphate,  $\text{InsP}_6$ , PA) and its lower phosphorylated derivatives ( $\text{InsP}_5$  and  $\text{InsP}_4$ ) are some of such compounds. Phytic acid is the main phosphorous storage form in seed and is stored as a mineral complex (phytate salts) in specific subcellular structures, called globoids, in the protein vacuole of embryo and cotyledonary cells. It accounts for an average of 75 % of total seed P and constitutes 1–3 % of dry weight. However, PA and its less abundant derivatives,  $\text{InsP}_5$  and  $\text{InsP}_4$ , are well recognized antinutrients, as, during gastrointestinal passage, they bind trace elements (e.g. Fe, Zn, Ca and Mg) and reduce their absorption leading, under certain dietary circumstances, to mineral (mostly Fe, Zn, Ca) deficiencies (Gibson et al. 2006). Phytic acid also interferes with other nutrient absorption: Its ability to complex with proteins decreases their solubility, therefore, impacting on digestive enzyme activity (Urbano et al. 2000). Recent studies have also identified PA as an antioxidant and have demonstrated that it possesses anticarcinogenic/antineoplastic properties, can reduce or prevent kidney stone formation, and plays important roles in many physiological processes (Raboy 2003; Vucenik and Shamsuddin 2006). The amount of PA accumulated in seeds varies among species, varieties and soil P availability; however, accumulation of very low PA levels has been detected only in induced mutants. Among legumes, mung bean, pea, lentil and chickpea have relatively lower levels of PA compared to common bean, faba bean and soybean (Table 10.3).

Mutations that reduce the level of seed PA (low phytic acid, *lpa*) have been identified in major crops such as maize, rice, wheat, common bean, pea and soybean (Campion et al. 2009b; Rasmussen et al. 2010; Warkentin et al. 2012; Wilcox et al. 2000). Decreased accumulation of seed PA varies depending on the type of mutation and generally ranges between 30 and 90%. The highest reductions are found associated to mutations affecting a multidrug resistance-associated protein (MRP)-type adenosine triphosphate (ATP)-binding cassette (ABC) transporter, which is a high-affinity  $\text{InsP}_6$  transporter (ABCC5) necessary for PA vacuolar storage (Maroof et al. 2009; Panzeri et al. 2011). Since PA and inositol phosphate derivatives play key roles in plant and cell functions, reduction in plant agronomic performance and fitness has been reported for a number of *lpa* mutants. Moreover, the extent of the negative pleiotropic effects of the mutation appears to correlate to the level of PA reduction (Panzeri et al. 2011). Despite this, there are indications that in some cases low PA levels in the seed are compatible with good plant performance and seed viability, as shown in common bean (Campion et al. 2013), or there is a potential for breeding to obtain acceptable performances, as shown for other crops (Israel et al. 2007). From a nutritional point of view, recent papers demonstrated that *lpa* mutants are effectively biofortified, being able to provide more micronutrients to humans than their wild type (WT) counterparts (Petry et al. 2013).

## 5 Phenolic Compounds: Tannins and Anthocyanins

Phenolic compounds are mainly represented by tannins and flavonoids and are mostly accumulated in the seed coats where they contribute to the determination of the color. Total phenolic compounds vary in composition and contents across different species, tissues, stages of development and in response to environmental factors (Caldas and Blair 2009; Diaz-Batalla et al. 2006; Marles et al. 2010; Oomah et al. 2011). A survey on phenolic compounds content in legume species can be found in the US Department of Agriculture (USDA) flavonoid, isoflavone and proanthocyanidin databases (USDA, 2013).

In nutritional terms, the major effect of tannins is the reduction of protein digestibility by inhibition of proteolytic activity and/or formation of indigestible complexes with dietary protein. Tannins also form complexes with polysaccharides and iron in the gastrointestinal lumen; therefore, they reduce the efficiency of carbohydrate absorption and the bioavailability of the minerals in the grain. Despite these concerns, tannins may function as anticarcinogenic compounds and antioxidants (Serrano et al. 2009), thus the balance between health benefits and antinutritional effects is important when planning breeding work for this trait. Moreover, since some legume seeds, such as faba bean and peas, are also used as a protein source for feeding monogastric animals, tannin-free varieties are considered superior to tannin-containing ones (Crepon et al. 2010).

Flavonoids have been shown to exert many beneficial roles on human health since they possess diverse biological activities such as antioxidation, antiageing, anticancer, antiinflammation, antiatherosclerosis, cardiovascular protection, improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities. In legumes, the highest polyphenolic contents are found in dark, highly pigmented seed varieties, mostly belonging to *Phaseolus* and *Vigna* species. Xu and Chang (2007) made a comparative analysis of phenolic composition in a number of widely cultivated legume species and showed that lentil, black and red varieties of common bean and black soybean have the highest total phenolic content (TPC), total flavonoid content (TFC) and condensed tannins content (CTC). These high phenolic contents correlate with the highest antioxidant activities, as assessed with different evaluation methods (2,2-diphenyl-1-picrylhydrazyl radical scavenging assay DPPH; ferric reducing antioxidant power, FRAP; and oxygen radical absorbance capacity, ORAC; Table 10.4). In a recent work, black chickpea genotypes with high TPC and TFC content as well as FRAP levels have been described (Segev et al. 2010).

Common beans, and in general *Phaseolus* species, exhibit a wide variety of seed coat colours and patterns, thus they have been the subject of most of the published studies regarding composition and abundance of the different classes of phenolic compounds in legume species. Extensive genetic analyses have identified specific loci, controlling seed coat colour (*P*, *C*, *R*, *J*, *D*, *G*, *B*, *V* and *Rk*, that regulate flavonol and anthocyanin synthesis) and pattern (*T*, *Z*, *L*, *J*, *Bip* and *Ana*), and 12 quantitative trait loci (QTL) controlling condensed tannin concentration. Among them,

**Table 10.4** Phenolic contents in seeds of different legume species. (Adapted from Xu et al. 2007)

Species	Market class	TPC (mg GAE/g)	TFC (mg CAE/g)	CTC (mg GAE/g)	DPPH ( $\mu\text{mol Trolox eq/g}$ )	FRAP ( $\text{mmol Fe}^{2+}\text{eq/100 g}$ )	ORAC ( $\mu\text{mol Trolox eq/g}$ )
Pea	Yellow pea	0.86–1.14	0.09–0.17	0.22–0.58	0.57–2.65	0.62–0.82	3.26–12.8
	Green pea	0.65–0.99	0.05–0.15	0.23–0.61	0.98–2.25	0.43–0.86	1.73–9.95
Lentil	Black bean	4.86–9.6	3.04–4.54	3.73–10.2	19.07–19.87	8.75–12.44	59.55–95.19
	Common bean	3.37–6.99	2.51–3.3	4.09–5.73	14.49–18.95	6.05–9.70	48.91–92.73
Soybean	Navy bean	0.57	0.92	0.47	1.48	1.27	13.3
	Small red bean	5.76	4.24	5.16	17.9	4.53	70.58
Chickpea	Black soybean	5.57	4.04	1.96	18.44	9.43	131.34
	Yellow soybean	1.74–1.82	1.06–1.24	0.37–0.79	0.92–1.83	1.09–1.49	35.1–44.23
		0.98	0.72	0.52	1.26	0.8	9.26

TPC total phenolic content, TFC total flavonoid content, CTC condensed tannins content, GAE gallic acid equivalent, CAE catechin equivalents, DPPH 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay, FRAP ferric reducing antioxidant power, ORAC oxygen radical absorbance capacity

the *P* gene plays a key role in the regulation of seed colour, since it is epistatic on the expression of *C*, *D* and *J*, and it is considered the controlling factor for the presence or absence of flavonoids in the seed coat (Caldas and Blair 2009). An example of the variability of phenolic compounds in common bean has been reported by Diaz-Batalla et al. (2006) who quantified the concentrations of flavonoids (kaempferol, quercetin) and phenolic acids (p-hydroxybenzoic acid, vanillic acid, p-coumaric acid and ferulic acid) in a collection of ten cultivated and four wild varieties of Mexican bean seeds. A similar analysis was performed on a collection of Italian common bean landraces (Doria et al. 2012) and the content of genistein and daidzein isoflavones was also assessed. Only some genotypes contained this class of compounds and the highest values were 101 µg/g for daidzein (average value 36.7 µg/g) and 21.6 µg/g for genistein (average value 9.14 µg/g). However, these values are very far from those reported in soybean, which is a well-known good font of daidzein (470 µg/g) and genistein (740 g/g; Rochfort and Panozzo 2007).

## 6 Saponins

Saponins are naturally occurring compounds widely distributed and particularly abundant in legume seeds. Saponins consist of a lipid-soluble nucleus, having either a steroid or a triterpenoid aglycone structure, with one or more side chains of water-soluble carbohydrates. Based on their aglycone structure, saponins are generally categorized into three main groups as groups A, B and E. The main saponin components in legumes are the group B saponins, which contain the aglycone, soyasapogenol B. Many saponins are bitter and reduce the palatability of livestock feeds and have long been considered antinutrients due to toxicity and their haemolytic activity (Khalil and El-Adawy 1994). However, only a few are toxic since an enormous structural diversity within this chemical class exists, depending on the aglycone structure, the attachment of the glycosidic moieties and the nature of the glycosides. Saponins are attracting considerable interest as a result of their diverse properties. Clinical studies have suggested that they are health-promoting components that affect the immune system in ways that help to protect the human body against cancer, and also lower cholesterol levels. Saponins decrease blood lipids, lower cancer risks and lower blood glucose response. A high saponin diet can be used in the inhibition of dental caries and platelet aggregation, in the treatment of hypercalciuria in humans, and as an antidote against acute lead poisoning. In epidemiological studies, saponins have been shown to have an inverse relationship with the incidence of renal stones (Shi et al. 2004).

Presence of saponins has been reported in many edible legumes such as lupin, lentil, chickpea, faba bean, as well as soybean, bean and pea. Contents vary from 10 µg/g in pea up to 5000–6000 µg/g in soybean and chickpea (Campos-Vega et al. 2010). A study on a group of Italian landraces of common bean has reported an average soyasapogenol B content of 304 µg/g, and values were ranging from 105 to 454 µg/g (Doria et al. 2012). Another study on Spanish varieties showed a variation



from 890 up to 2050  $\mu\text{g/g}$  and a third study on navy bean seeds reported even higher values up to 7620  $\mu\text{g/g}$  (Burbano et al. 1999; Shi et al. 2009).

## 7 Other Minor Antinutritional/Bioactive Compounds: Vicine, Convicine, L-DOPA and ODAP

The nutritional quality of some legume seeds may be affected also by other more species-specific molecules of different chemical origin. The seeds of faba bean accumulate vicine and convicine, two pyrimidine glycosides, whose aglycone forms, divicine and isouramil, respectively, are the causative agent of favism. This is a haemolytic anaemia that affects male individuals carrying a specific genetic defect in the gene coding for erythrocyte-located glucose-6-phosphate dehydrogenase. Vicine and convicine have antinutritional effects also in the diet of monogastric animals, and several efforts, related to breeding as well as to processing treatments, have been undertaken to reduce their amounts in seeds (Crepon et al. 2010).

Faba bean, together with *Mucuna pruriens* (a tropical legume also known as velvet bean), is one of the best plant sources of L-3,4-dihydroxyphenylalanine (L-DOPA), a naturally occurring nonprotein isomer of the amino acid 3,4-dihydroxyphenylalanine, which is potentially toxic if ingested in large amounts. L-DOPA has been reported to cause serious hallucinations in addition to gastrointestinal disturbances, such as nausea, vomiting and anorexia. Despite this, a lot of interest exists for this compound, since it is the major ingredient in medicines used to treat Parkinson disease (PD) patients. In fact, L-DOPA is a substrate of L-DOPA decarboxylase, which converts L-DOPA to the biologically active catecholamine dopamine, a compound that is depleted in the brain of people affected by PD. In faba bean, L-DOPA accumulates mostly in embryo axis of germinating seeds, and levels of around 75 mg/g dry weight have been detected after 9 days of germination, while much lower amounts (0.34 mg/g dry weight) have been found in the seed (Goyoaga et al. 2008). On the contrary, L-DOPA levels in the seeds of *Mucuna* species are around 3.1–6.7% dry weight, and can reach up to 9% (Pras et al. 1993).

Another nonprotein amino acid, the neuroexcitatory,  $\beta$ -N-oxalyl-L- $\alpha$ ,  $\beta$ -diaminopropionic acid (ODAP), is found in *Lathyrus sativus* seeds. It is responsible for neurolathyrism, a disease associated to prolonged overconsumption of this protein-rich seed in a monotonous diet and consisting in the degeneration of upper motor neurons and the irreversible paralyzing of the legs in up to 6% of the affected individuals. Assessment of ODAP content in *L. sativus* has shown that germplasm from South Asia contained relatively high amounts of ODAP (0.7–2.4% dry weight), whereas those from North Africa, Syria, Turkey and Cyprus had significantly lower quantities of ODAP (0.02–1.2%). No accessions were found to be free of the toxin (Hillocks and Maruthi 2012).

## 8 Health Benefits of Grain Legumes

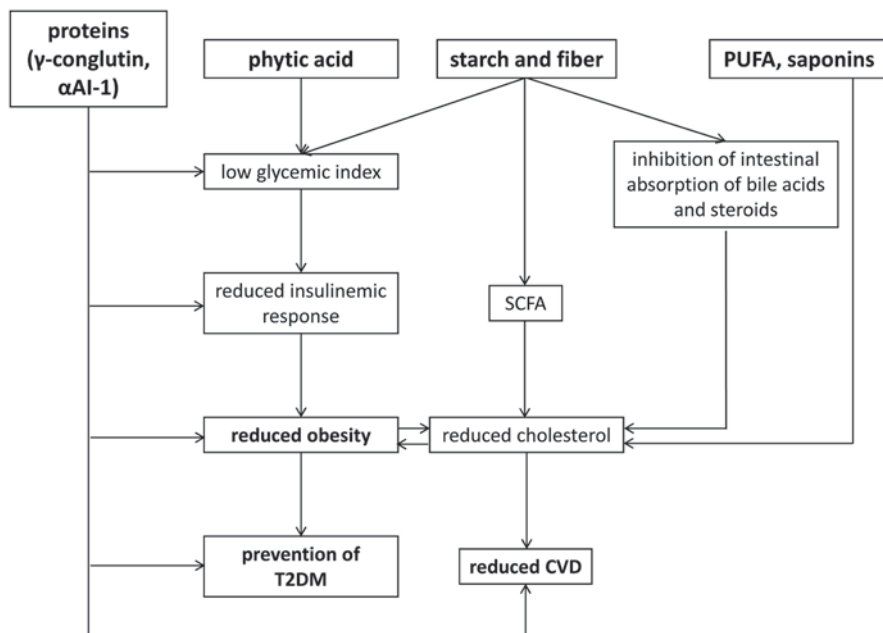
Legumes have been consumed for thousands of years for their nutritional qualities, but only during the past few decades the potential impact of pulses on human health has been revived. Many different studies have reported that the consumption of pulses have beneficial physiological effects in the prevention and control of a broad range of chronic and degenerative diseases such as obesity, cardiovascular diseases (CVD), diabetes and cancer which are typical of industrialized societies (Bazzano et al. 2011). Pulses could potentially be considered as “functional foods” in addition to their accepted role of providing proteins and fibres. The consumption of pulses is in fact recommended as part of healthy eating by governments and health organizations globally. A high consumption of pulses is also one of the eight components of the highly lauded Mediterranean diet. However, according to FAO, the current consumption ratio of cereal grains to pulses in the diet is 8:1, which is considerably different from the ideal consumption ratio of 2:1. Particularly, the consumption of pulses in the Western world remains quite low, at less than 3.5 kg/capita per year, while in other parts of the world annual pulse consumption can range from 10 kg/capita (South America and India) to 40 kg/capita (Burundi). The role of pulses in the prevention and control of different pathologies is summarized below.

### 8.1 *Metabolic Syndrome*

The metabolic syndrome includes different risk factors of chronic diseases such as CVDs and diabetes, abdominal obesity, atherogenic dyslipidemia (high level of serum triglycerides and LDL cholesterol and low blood concentrations of HDL cholesterol), raised blood pressure, insulin resistance, proinflammatory state and prothrombotic state.

Results of two meta-analyses showed the long-term benefits of pulse consumption (2–5 cups per week for 3–12 weeks) on risk factors of the metabolic syndrome (Bazzano et al. 2011; Sievenpiper et al. 2009). Another study demonstrated that frequent consumption (5 cups/week over 8 weeks) of different legumes (yellow peas, chickpeas, navy beans and lentils) in an ad libitum diet reduced risk factors of metabolic syndrome in overweight and obese adults. These effects were similar or even stronger, depending on the different parameters analyzed, to the ones obtained with an energy-restricted diet (by 2093 kJ/day, corresponding to 500 kcal/day) implemented by counselling (Mollard et al. 2012).

The effects of consumption of grain legumes in the reduction of CVDs, obesity and diabetes mellitus are very strictly correlated and dependent on the different nutritional and nutraceutical components present in pulses, as recently reviewed (Hayat et al. 2014) and shown in the model reported in Fig. 10.2. Particularly, due to their slow release of carbohydrates, pulses are considered as low glycemic index foods (Atkinson et al. 2008), contributing to a reduction in the insulinemic responses. On the other hand, the consumption of pulses, through different mechanisms, reduces serum total cholesterol and LDL cholesterol (Anderson and Major 2002).



**Fig. 10.2** A simplified model representing how different pulse compounds can protect from diseases associated with metabolic syndrome. *PUFA* polyunsaturated fatty acids, *SCFA* short-chain fatty acid, T2DM type 2 diabetes mellitus, *CVD* cardiovascular disease (Adapted from Hayat et al. 2014)

## 8.2 Diabetes Mellitus

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder, resulting from insulin resistance, a condition in which cells fail to use insulin properly. This disorder leads to several secondary complications, including cardiovascular disease, chronic renal failure, diabetic retinopathy, hypertension, atherosclerosis, coronary artery disease and hyperlipidaemia. Approximately 150 million people worldwide are affected by T2DM, with a projection of 300 million people being affected by 2025. Diabetes has become a serious public health problem, particularly in developed countries.

The glycemic index (GI) of a food is defined as the incremental area under the blood glucose curve following ingestion of a test food, expressed as a percentage of the corresponding area following an equivalent load of a reference carbohydrate, either glucose or white wheat bread. After consumption of high GI foods, there is a large, rapid increase in blood sugar levels (glycemic response) and in response a rapid increase in insulin levels, while the consumption of low GI foods is correlated to a reduction in postprandial glucose and insulin elevations. Pulses are foods with low GI (Atkinson et al. 2008). Epidemiological studies have suggested that the consumption of foods with low GI protects against the development of T2DM and is useful also in the management of T2DM patients (Campos-Vega et al. 2010; Venn

and Mann 2004). Particularly, people consuming about three portions per week of whole grain foods have lower probability (risk reduction of 20–30%) to develop T2DM than low consumers (<3 servings per week; Venn and Mann 2004). Different short-term studies have shown that consumption of seeds of common beans and other pulses typically reduces postprandial glucose elevations in nondiabetic and diabetic individuals compared with most starchy foods. Moreover, pulses combined with a high GI food produce a glycaemic response that is intermediate between the high- and low GI foods, but it is not clear if the nature of the effect is additive or linear (Tappy et al. 1986; Thompson et al. 2012). A clear association between a higher intake of legumes and a reduced risk of T2DM was particularly evident from results of a large, prospective, population-based study of middle-aged Chinese women. In this study, 64,227 women with no history of T2DM, cancer or cardiovascular disease at study recruitment, were followed up for an average of 4.6 years. An inverse association between quintiles of total legume intake of three mutually exclusive legume groups (peanuts, soybeans and other legumes) and T2DM incidence was observed (Villegas et al. 2008).

Different mechanisms of action have been proposed to explain the low glycemic response to legumes consumption (Hutchins et al. 2012). The high content of viscous fibres in pulses contributes to the low glycemic response, as they form a gel-like substance along the digestive tract, slowing down absorption rate of nutrients. The inclusion of a viscous fibre with a test meal is able to reduce the blood glucose response by an average of 44% (Wolever and Jenkins 2001). However, the viscous fibre component of legumes is not sufficient to determine the low glycemic and insulin response to legumes, as the addition of bean fibre to a potato meal is significantly less effective in lowering glucose and insulin response than a bean meal alone (Tappy et al. 1986).

Legumes are particularly rich in amylose; its lower molecular weight, smaller surface area and linear structure make it subject to slower digestion than amylopectin. The presence of RS in legumes results in the lower availability of glucose with the consequent slow entry of glucose in the bloodstream, the reduction of the demand for insulin, the lowering of the GI and the insulinemic postprandial response (Tappy et al. 1986). The protein fraction of pulses may also reduce starch digestibility and consequently glycemic response by directly interacting with starch (Alli and Baker 1980).

Moreover, proteins from pulses with a specific role in the prevention and management of diabetes have been extensively studied, such as  $\gamma$ -conglutin and  $\alpha$ -amylase inhibitor isoform 1 ( $\alpha$ AI-1; Barrett and Udani 2011; Lovati et al. 2012). The use of seeds decoctions of white lupin as “antidiabetic” is well known in the old pharmacopoeia. In the past years,  $\gamma$ -conglutin has been identified as the molecule conferring hypoglycemic properties to lupin. It was demonstrated that it has a glucose-lowering effect in normal rats upon glucose overload trials. This effect is very similar to that of metformin, a well-known antidiabetic drug. Moreover, the chronic oral  $\gamma$ -conglutin treatment in rats, in which hyperglycaemia had been induced, attenuated the rise in plasma glucose and insulin (Lovati et al. 2012).  $\gamma$ -conglutin is able to interact in vitro with mammalian insulin (Magni et al. 2004). To explain

its antidiabetic effect, it has been hypothesized that it acts as an insulin-like agent. These data suggest the potential use of this protein in the control of glycaemia in patients with manifest or preclinical diabetes as well as for applications as functional foods and dietary supplements (Lovati et al. 2012; Magni et al. 2004). Another pulse protein with a well-characterized role in lowering the postprandial increases in blood sugar level is  $\alpha$ AI-1 from common bean, also referred to as phaseolamin in starch blockers preparations (Barrett and Udani 2011). Starches are digested into sugars by  $\alpha$ -amylase secreted in the saliva and by the pancreas and consequently absorbed in the small intestine. The use of purified forms of  $\alpha$ AI-1 as a dietary supplement reduces the postprandial spikes of glucose and insulin following a high-GI meal (Obiro et al. 2008).

The glycemic response is influenced also by phytic acid. It was demonstrated that the consumption of unleavened bread made from navy bean flour, containing phytic acid, significantly reduced blood glucose area compared with that of bread made with wheat flour, while the opposite effect was obtained removing phytic acid. Moreover, phytic acid is also able to directly interact with starch and inhibit in this way starch digestibility (Thompson et al. 1987). As phytic acid binds cations such as  $\text{Ca}^{2+}$ , its presence may also reduce the stability of  $\alpha$ -amylase, dependent on  $\text{Ca}^{2+}$  (Yoon et al. 1983).

Another possible mechanism of action responsible for the low glycemic response to pulses consumption is independent on components but depends on the fact that beans are commonly consumed in their whole form or minimally processed with little or no grinding. The integrity of the cell wall is then maintained after eating. Moreover, the cell wall of pulses is generally more resistant to digestion than the cell wall of cereal grains. These aspects may contribute to slow digestion and consequent low glycemic response (Noah et al. 1998).

### **8.3 Obesity and Overweight**

Based on data from the World Health Organization (WHO), in 2008 approximately 1.4 billion adults worldwide were overweight and at least 500 million were obese. Increased consumption of foods rich in DFs, such as pulses, is associated with a lower body mass index (BMI), defined as the individual's body mass divided by the square of their height. Moreover, intake of foods with a high-fibre content helps in reaching satiety faster, and this effect is maintained longer as fibre-rich foods require a longer time to chew and digest in the intestinal system (Marlett et al. 2002). Different epidemiological studies demonstrated the efficacy of combined diets containing wholegrains and pulses in conferring a lower average BMI, a smaller waist circumference and demonstrated the negative correlation of this kind of diet with waist-to-hip ratio (Koh-Banerjee and Rimm 2003). Only a few published studies have specifically measured the effects of pulses consumption on body weight and satiety. Papanikolaou and Fulgoni (2008) reported on the association of consumption of beans with dietary quality and obesity risk in >8000 adult participants in the

US National Health and Nutrition Examination Survey (1999–2002). Compared to nonconsumers, bean consumers ( $N=1475$ ) had a lower body weight and a smaller waist size. Additionally, consumers of beans had a 23% reduced risk of increased waist size and a 22% reduced risk of being obese (Papanikolaou and Fulgoni 2008). Experimental studies in humans to evaluate the effects of pulse consumption on weight loss were performed during intentional energy restriction or without energy restriction. The first group of studies showed that, when pulse consumption is coupled with energy restriction, there is a beneficial effect on weight loss and on other parameters important to evaluate obesity risk. One of these trials was performed on 30 overweight and obese participants consuming a reduced energy intake diet (30% energy restriction based on initial energy requirement) for 8 weeks eating either four servings/week of pulses or none. Results showed significantly greater decreases in BMI and body weight, expressed as percent of initial value, in the pulse-consuming group compared with the control group (22.0 vs 20.9 kg/m<sup>2</sup> and 27.8 vs 25.3%, respectively). However, percentage body fat and waist circumference decreases did not differ significantly between groups (Hermsdorff et al. 2011). Similar results were obtained from other studies (Abete et al. 2009; Karlström et al. 1987). On the other hand, randomized controlled trials performed without energy restriction did not support a beneficial effect of pulses on weight loss, as reviewed by McCrory et al. (2010).

There is available evidence in support of pulse grains' ability to induce satiety. Subjects consuming at least 1200 g/week canned chickpeas for 12 weeks reported a significant increase in satiety compared with subject consuming their habitual diet (Murty et al. 2010). Similar effects on reducing appetite were described for navy beans (Wong et al. 2009). In both cases, despite an increase in DF intake, no differences in total energy intake were observed.

RS and DF are mainly responsible of the pulse effects on the control of appetite through increased satiety for their low digestibility. The fermentation of fibre and RS by bacteria in the large intestine generates specific SCFA, mainly butyric acid (Marinangeli and Jones 2012). This compound is the main product of indigestible fractions of black beans, lentils and chickpea by human microbiota, as demonstrated by *in vitro* fermentation (Hernández-Salazar et al. 2010). Rats fed high diets containing 25% adzuki beans or two varieties of common beans significantly increased cecal butyric acid concentrations, compared with rats fed control cornstarch diet (Han et al. 2003). Butyrate was shown to increase hepatic and muscle expenditure as well as fat oxidation, mitochondrial oxidation and biogenesis when supplemented to mice diet (Gao et al. 2009). Thus, increased production of SCFA by fermentation of RS and fibres is an underlying reason for the protective benefits by the consumption of pulses (Finley et al. 2007). Moreover, high-fibre foods are believed to stimulate and prolong cholecystokinin secretion, a gastrointestinal peptide acting as hunger suppressant (Holt et al. 1992). Therefore, it is reasonable to hypothesize that appropriate dosages of pulse grain fibres can stimulate cholecystokinin release (de Graaf et al. 2004).

Protein component of pulses also plays an important role in weight management. Proteins, compared to carbohydrates, produce the highest thermic effect of food,

which depends on the energetic costs associated with dietary peptide catabolism, protein synthesis and gluconeogenesis (Robinson et al. 1990). Amino acid composition could facilitate increase in energy expenditure (Marinangeli and Jones 2012). Arginine and glutamine, present at high level in pulses, have been shown to possess thermogenic properties (Iwashita et al. 2006; McKnight et al. 2010).

A specific antiobesity role for some proteins has also been described. Extracts of the already-cited phaseolamin have an antiobesity effect, as shown by different studies, although some conflicting results have been reported (Barrett and Udani 2011; Obiro et al. 2008). The  $\alpha$ AI-1 inhibitory action results in the mobilisation of body fat reserves, due to energy restriction. In different studies, the efficacy of a commercial  $\alpha$ AI-1 extract, referred to as Phase 2® (Pharmachem Laboratories, Inc., Kearny, NJ, USA), in reducing obesity was reported. Celleno et al. examined the effects of a dietary supplement containing 445 mg of Phase 2® on body composition of overweight human subjects in a 30-day study. They found greater reduction of body weight, BMI, fat mass, adipose tissue thickness and waist/hip/thigh circumferences, while maintaining lean body mass in subjects treated with Phase 2® compared to subjects receiving placebo (Celleno et al. 2007). Similar results were obtained in other studies, as reviewed by Barrett and Udani 2011. On the other hand, other reports did not confirm the efficacy of this starch blocker (Chokshi 2006). The effect of the extracts depends on a given manufacturer's methods of extraction, as regards the maintenance of high anti-amylase activity and purity (Obiro et al. 2008). As raw beans contain the lectin PHA, a highly toxic protein if consumed in native conditions, the protocol for the preparation of starch blockers requires a specialized process to inactivate haemagglutinin activity. To overcome this problem, common bean genotypes not able to accumulate PHA in the seed have been developed (Bollini et al. 1999). On the other hand, it was suggested that PHA as a dietary adjunct or therapeutic agent may be efficacious to stimulate gut function and ameliorate obesity if a safe and effective dose range can be established. The effects of inclusion of different levels of raw kidney bean of high lectin content (27 g/kg meal) in the diet of obese Zucker rats and their lean littermates in comparison with pair-fed controls were tested. It was shown that the growth of both obese and lean rats on bean diets was retarded by the daily bean intake in a dose-dependent manner, and most of this decrease was because bean-fed rats contained less body fat than the controls after 10 days (Pusztai et al. 1998). Consumption of bean-derived lectins was shown to increase cholecystokinin secretion, compared with controls fed lactalbumin, contributing to induce satiety (Herzig et al. 1997).

#### **8.4 Cardiovascular Disease**

CVDs are the number one cause of death, globally accounting for 30% of all deaths, and are projected to remain the single leading cause of death by 2030.

In general, increased consumption of soluble fibre from foods results in reduced serum total cholesterol and LDL-cholesterol and has an inverse correlation with

coronary heart disease (CHD) mortality (Noakes et al. 1999). Legume consumption has been associated with lower risks of CVD and CHD in observational epidemiologic studies (Bazzano et al. 2001; Kushi et al. 1999). For example, a study involving a total of 9632 men and women revealed a significant inverse relationship between legume intake and risk of CHD and CVD. In fact, legume consumption four times or more per week, compared with less than once a week, was associated with a 22% lower risk of CHD and an 11% lower risk of CVD (Bazzano et al. 2001).

Among the different controlled trials that have examined the potential hypocholesterolaemic effects of a diet rich in non-soy legumes, such as peas, lentils, different market classes of common beans, lima beans, chickpeas and faba beans, the majority identified positive effects, particularly in some cases (Anderson et al. 1984; Nervi et al. 1989; Sowmya and Rajyalakshmi 1999), while in a very few studies no effect was identified (Cobiac et al. 1990; Mackay and Ball 1992; Winham et al. 2007). A meta-analysis of randomized controlled trials was conducted to quantify the direction and magnitude of the potential effect that consumption of non-soy legumes may have on serum cholesterol concentrations (Bazzano et al. 2011). From 140 reports on the subject, the authors selected ten publications including studies performed on a total of 268 participants, and in which a comparison between a non-soy and a control diet was carried out for at least 3 weeks. This meta-analysis study provided a strong evidence that non-soy legume consumption lowers serum total cholesterol (Bazzano et al. 2011). Very recently, another study confirmed the efficacy of a diet rich in pulses (two servings daily of beans, chickpeas, peas or lentils, about 150 g/day) for reducing CVD risk factors in individuals 50 years or older, an age group who are at increased risk of this disease and on which a few studies had focused before (Abeysekara et al. 2012).

Low glycaemic index of pulses is important for lowering the risk of CVD (Duranti 2006). Moreover, several components of pulses are likely to contribute to their cholesterol-lowering effects. Soluble fibre is thought to bind to bile acids in the intestines and prevent reabsorption into the body. Consequently, an increase in the production of bile acids reduces the liver pool of cholesterol, triggering uptake of serum cholesterol by the liver, thereby lowering circulating cholesterol in the blood (Galisteo et al. 2008).

Another mechanism for the reduction of serum cholesterol depends on the activity of SCFA, particularly propionate, which alters metabolic pathways resulting in reduced serum cholesterol (Venter et al. 1990).

Chickpea contains a higher amount of fat than other pulses, and it is a relatively good source of nutritionally important polyunsaturated fatty acids (PUFAs), oleic acid and linoleic acid, constituting almost about 50–60% of chickpea fat (Jukanti et al. 2012). It was shown that the intake of PUFA such as linoleic acid has a beneficial effect on serum lipids, insulin sensitivity and haemostatic factors, thereby it could be helpful in lowering the risk of CHD (Hu et al. 2001).

Recent evidence suggests that legume saponins, in addition to their anticancer activity may also be beneficial for hyperlipidemia (Shi et al. 2004) and in reducing the risk of heart diseases in humans (Geil and Anderson 1994).



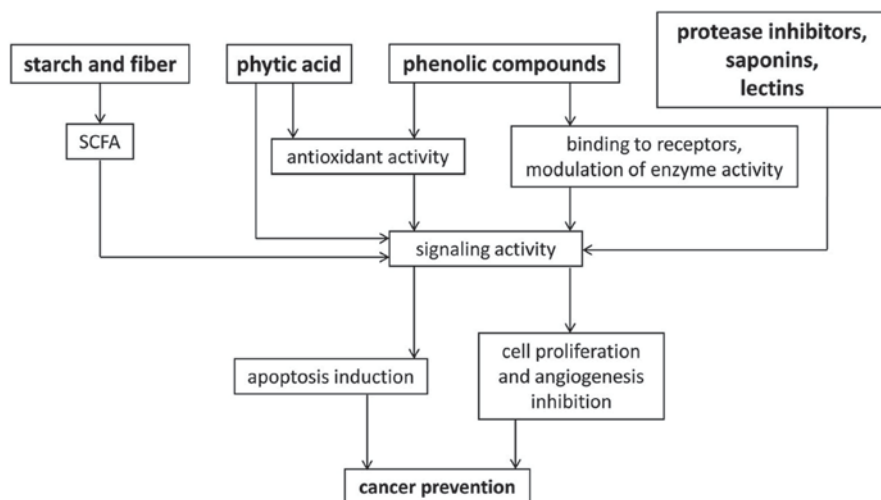
## 8.5 Cancer

Cancer is a leading cause of death, mainly in industrialized countries, in the USA, for example, it is second only to CVD. In many developing countries cancer incidence appears much lower, but it is expected to raise due to increased control over infectious diseases and control of childhood diseases, leading to rise in life expectancy and in proportion of elderly people (Khatib and Aljurf 2008).

Different epidemiological analyses indicated a decreased risk of death associated with colon, breast and prostate cancer in countries with higher consumption of pulses (Mathers 2002). Moreover, experiments performed on laboratory animals have confirmed these results. One such study showed that feeding black or navy beans to rats exposed to the carcinogen azoxymethane reduced both the incidence and number of colon tumours by 50% (Hangen and Bennink 2002). Most of these studies were focused on common beans, while a few investigations have been performed on other pulses. For example, a 64% suppression of azoxymethane-induced aberrant cryptic foci was shown in mice fed with 10% chickpea flour (Murillo et al. 2004). It was reported a systematic comparative study on antiproliferation effects of hydrophilic extracts from 13 commonly consumed food legumes (green pea, yellow pea, chickpea, lentil, yellow soybean, black soybean, pinto bean, black bean, small red bean, red kidney bean, mung bean, adzuki bean and black-eyed pea), using nine *in vitro* cultured human cancer cell lines (Xu and Chang 2012). Among the legume tested, lentil, the four common beans, mung bean and adzuki bean exhibited dose-dependent inhibitory effects against cell proliferation of all cancer cell lines. In particular, adzuki bean exhibited the strongest antiproliferative properties in a dose-dependent manner against seven of the nine cancer cell lines. Moreover, other legumes tested showed antiproliferative effects only against some cancer cell lines (Xu and Chang 2012). These results indicate that commonly consumed legumes may serve as an excellent dietary source for cancer prevention and further studies are warranted to characterize the potentiality of different legumes in cancer protection.

There are several bioactive food components of pulses that could be responsible for the cancer-preventive effect, as shown in the model reported in Fig. 10.3.

It is recognized that a major role in the anticancer effects of food is played by phenolic components. Beneficial effects of isoflavonoids in preventing breast and prostate cancers have been extensively studied (McCue and Shetty 2004). The already-mentioned paper from Xu and Chang (2012) showed that the total phenolic content of 13 food legumes exhibited significant linear correlation with the overall antioxidant activities. Coloured common beans, black soybean, lentil, adzuki bean and mung bean exhibited stronger antioxidant capacities and cancer cell proliferation inhibitory effects on different human cancer cell lines, as compared to green and yellow peas, chickpea, yellow soybean and black-eyed pea. Although the antioxidant properties of polyphenols have been extensively investigated, more recently their real impact as antioxidants has been reconsidered and questioned. In fact, there is an emerging view that these molecules may act not only by scavenging reactive



**Fig. 10.3** A simplified model representing how different pulse compounds can protect from cancer. *SCFA* short-chain fatty acids. (Adapted from Hayat et al. 2014)

oxygen and nitrogen species or suppressing their production but also by enhancing the endogenous antioxidant capacity of cells/tissues (e.g. glutathione synthesis) or by influencing signalling pathways through interaction with proteins, enzymes and nuclear receptors, as recently reviewed (Martin et al. 2013). The anticancer activity of polyphenols has been associated with lower leukocyte immobilization, apoptosis induction, cell proliferation and angiogenesis inhibition (Garbisa et al. 2001; Nijveldt et al. 2001).

Phytic acid is a broad-spectrum antineoplastic agent, playing an important role in cancer prevention as well as in control of experimental tumour growth, progression and metastasis. Phytic acid seems to be responsible for the epidemiological link between high-fibre diets (high phytic acid content) and low incidence of some cancers. Phytic acid, after its rapid intake and dephosphorylation, enters the pool of inositol phosphates and acts as a strong antioxidant, enhances immune function, elicits anti-inflammatory activity, modifies phase I and II metabolizing enzymes, modulates oncogene expression, normalizes abnormal cell proliferation, induces cell differentiation, induces apoptosis and inhibits angiogenesis (Vucenik and Shamsuddin 2006).

Saponins are another class of non-nutrient bioactive compounds for which epidemiological studies suggest anticancer activity (Shi et al. 2004). Soyasaponin I properties have been mainly investigated, and its molecular activity was identified. This compound is able to inhibit the transfer of sialic acids to the nonreducing terminal positions on sugar chains of glycoconjugates, a process correlated with oncogenic transformation and metastatic potential (Chang et al. 2006). Moreover, saponins are able to regulate the apoptosis pathway enzymes, leading to programmed cell death of cancer cells (Zhu et al. 2005).

Also, lectins present in legumes may play a key role in preventing certain cancers (Campos-Vega et al. 2010). In vitro studies demonstrated, for example, that *Vicia faba* agglutinin (VFA) stimulated the morphological differentiation and reduced the malignant phenotype of colon cancer cells (Jordinson et al. 1999). The inclusion of PHA from raw kidney bean in the diet of a murine model for non-Hodgkin lymphoma tumour greatly reduced, in a dose-dependent manner, the growth rate of the tumour, either as an intraperitoneal ascites tumour or as a solid subcutaneous one (Pryme and Bardocz 2001). The number of Krebs II lymphosarcoma tumour cells in the ascitic fluid of mice fed a PHA diet for 8 days was three times lower than in mice fed a control diet (Bardocz et al. 1997). There is scientific evidence for different anticarcinogenic mechanisms of action of lectins, including binding to tumoural cell membranes, cytotoxic effects of lectins on tumour cells (decrease in protein synthesis and induction of apoptosis), reduction of cell proliferation and stimulation of the immune system (De Mejia and Prisecaru 2005).

Different in vitro and in vivo experiments have shown that protease inhibitors have anticarcinogenic properties. Although the majority of these studies were performed with soybean, as reviewed by Roy et al. (2010), more recently, the antiproliferative effects on human colon cancer cells of two recombinant wild-type Bowman-Birk inhibitors from pea seeds has been reported (Clemente et al. 2005).

All the molecules present in pulses having anticancer properties described so far are soluble in aqueous-alcohol extracts, while RSs, present in high amount in pulses, together with nonstarch polysaccharides, are primarily insoluble residues from aqueous-alcohol extracts. Colon carcinogenesis was induced by azoxymethane treatment in obese *ob/ob* mice fed with diet containing cooked navy beans (whole beans), the insoluble or soluble fraction of aqueous-alcohol extracts or a standard diet (Bobe et al. 2008). In comparison to control-fed mice, the incidence rates of various types of colon lesions were detected in fewer mice on either bean fraction diet. Moreover, no significant differences in incidence rate of various types of colon lesions between mice-fed diets containing bean residue or bean extract were observed. These results suggest that the insoluble fraction, containing RS, contributes in a similar way of the soluble fraction to the cancer-protective effect of cooked navy beans (Bobe et al. 2008). The cancer-preventive effect of RSs and nonstarch polysaccharides was previously shown (Bauer-Marinovic et al. 2006). As already discussed, SCFA, mainly butyric acid, are the products of the bacterial fermentation of resistant starches. They have protective effect against colon cancer as against the majority of tumours developed in the distal colon. Butyrate was reported to induce apoptosis, growth arrest and differentiation in colon cancer cell lines (Barnard and Warwick 1993); its effect is due to its activity in histone hyperacetylation and down-regulation of epidermal growth factor receptor (Archer et al. 1998). The low GI of legumes attenuates the postprandial insulin response contributing to their cancer-preventive effect as hyperinsulinaemia and hyperglycaemia are reported to increase the risk of colon cancer (Jenkins et al. 2002).

**Acknowledgment** This work was partially supported by the Programme FILAGRO “Strategie innovative e sostenibili per la filiera agroalimentare”, as part of the activities defined within the Accordo Quadro Consiglio Nazionale delle Ricerche and Regione Lombardia.

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