Revisión

Bioactive vegetable proteins and peptides in lipid-lowering; nutraceutical potential

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PROTEÍNAS Y PEPTIDOS DE ORIGEN VEGETAL EN LA REDUCCIÓN DE LIPIDOS; POTENCIAL NUTRACÉUTICO

Abstract

As the last century saw a decline in the burden of nutritional deficiency and infectious disease, the global burden of chronic disease, cardiovascular disease (CVD) in particular, is increasing. CVD is the leading cause of death in the developed countries. Significant research efforts on the prevention and treatment of this disease have identified elevated plasma cholesterol as a primary risk factor for CVD. Although CVD progresses with hypercholesterolemia, it seems possible to delay and prevent its development through improvement of diet. Recent findings demonstrate that protein concentrates, protein hydrolysates, and peptides derived from vegetables may promote a significant decrease in blood cholesterol concentration. This reduction in cholesterol and lipid levels by protein, protein hydrolysates, and peptides can be the result of dietary changes, reduced cholesterol biosynthesis, changes in bile acid synthesis, and reduced absorption of lipid cholesterol and bile acid. Combination drug/diet therapies may reduce the number of drug prescriptions, the progressive rise in “optimal” drug dosage and costs associated with pharmaceutical management of disease. These bioactive vegetable proteins, hydrolysates and peptides may be used in formulation of functional foods, nutraceuticals, and natural drugs because of their health benefit effects suggesting their use as an alternative in treatment of various dyslipidemias, and a potential agent for reducing cardiovascular diseases risk factors.

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Resumen

En el siglo pasado se observó un decaimiento tanto de la deficiencia nutricional como de las enfermedades infecciosas. Por el contrario se incrementó la mortalidad asociada a enfermedades crónicas, particularmente las enfermedades cardiovasculares. La investigación enfocada a la prevención y tratamiento de estas enfermedades ha identificado a la elevación del colesterol en plasma como un factor primario de riesgo para el desarrollo de enfermedades cardiovasculares. Sin embargo el desarrollo de las enfermedades cardiovasculares asociadas a la hipercolesterolemia puede retrasarse o prevenirse mediante mejoras en la dieta. Descubrimientos recientes han demostrado que la ingesta de concentrados proteínicos, hidrolizados proteínicos y péptidos de origen vegetal puede reducir la concentración de colesterol en sangre. La reducción de los niveles de lípidos y colesterol causada por proteínas, hidrolizados y péptidos podría deberse a la modificación en sí de la dieta, a la reducción de la síntesis de colesterol, a cambios en la síntesis de ácidos biliares o a la reducción de la absorción de colesterol y ácidos biliares. Las terapias que combinan fármacos con modificaciones de la dieta, pueden reducir significativamente la dosis de los fármacos ingeridos, el aumento progresivo de dicha dosis y los costos asociados al tratamiento de la enfermedad. Las proteínas, hidrolizados y péptidos de origen vegetal podrían ser utilizados como nutracéuticos, como parte de la formulación de alimentos funcionales o el diseño de medicamentos de origen natural, debido a sus efectos benéficos sobre la salud. Potenciado de esta forma su empleo en el tratamiento de dislipidemias y de patologías relacionadas.

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Introduction

Industrialization, urbanization and market globalization have had profound impacts worldwide on lifestyles, diets and nutritional status. Latin America has not been exempt from these transformations. Contemporary urbanization has produced declines in undernutrition in metropolitan areas, while simultaneously abetting an increase in inadequate eating habits and a decrease in physical activity. Greater intake of diets high in fat (particularly saturated fat); low in complex carbohydrates and with lower micronutrient concentrations, combined with a more sedentary life-style is largely responsible for the spread of diet-related disorders. Diseases of deficiency and excess have now become significant public health concerns. This phenomenon has been called the nutrition transition.

During the latter half of the 20th Century major health transitions occurred worldwide. These were propelled by socio-economic and technological changes that extended life expectancy and altered life-styles while creating an unprecedented human capacity to use science to prolong and enhance life. The most pervasive change among these health transitions has been the rising burden of non-communicable diseases (NCDs). Epidemics of NCDs are currently emerging or accelerating in most developing countries. Cardiovascular diseases (CVDs), cancers, diabetes, neuropsychiatric ailments and other chronic diseases are becoming major contributors to the burden of disease, even as infections and nutritional deficiencies are receding as leading contributors to death and disability. In 2002, cardiovascular disease was responsible for 17 million deaths worldwide, nearly three-quarters of these in low- and middle-income countries. It has been estimated that by 2010 CVDs will have become the leading cause of death in developing countries.

Dyslipidemias are well-established risk factors for cardiovascular disease; in particular, hypercholesterolemia has been of concern since the 1950s, when the association was recognized between cardiovascular disease and serum cholesterol. Hypercholesterolemia currently causes 4.3 million deaths annually and 39 million disability-adjusted life years lost. Nutritional and dietary therapy, weight loss, exercise, and scientifically proven nutritional supplementation might be appropriate to manage dyslipidemia. Expense, high drug doses and low compliance to strict dietary therapies are current issues surrounding modern drug- and diet-based lipid-lowering approaches. Variable patient outcomes and suboptimal response to both drug and diet therapies are increasingly evident. The question therefore arises as to whether greater emphasis is needed on combination diet/drug therapies to reduce cholesterol levels in patients who respond suboptimally to diet and drug monotherapies.

Considerable research has explored multidrug combination therapies, but much less attention has been given combination drug/diet therapies. These combined approaches may reduce the number of drug prescriptions, the progressive rise in “optimal” drug dosage and costs associated with pharmaceutical management of disease. Future research priorities in drug/diet therapeutic approaches should not only emphasize the discovery of novel combinations but also need to address potential safety issues prior to wide-scale acceptance in clinical practice. Diets containing soy and/or milk proteins are reported to be hypocholesterolemic, with soy protein being superior to milk protein. Legumes and other pulses seeds are rich in crude protein (25%). Although many of them are widely used in animal nutrition, human consumption is lower than that of other traditionally more accepted pulses. Nevertheless, the wealth of nutrients available from these vegetable proteins and their beneficial functional properties have prompted increasing interest and demand for this legume for food preparations addressed to geriatric and infant nutrition. In the sense protease hydrolysates from soy protein have been reported as more effective at lowering cholesterol than natural soy protein, suggesting the use of hydrolyzed protein from vegetable sources as an alternative in treatment of various dyslipidemias, and a potential agent for reducing CVD risk factors.

Bioactive proteins and peptides

Each protein, apart from its basic function, is also likely to function as a reservoir for peptides regulating vital organism processes. Previously, the primary criteria applied to evaluate proteins’ effects in the organism included profiles of amino acids essential to proper organism function, the effect of proteins on body mass, their allergenic properties and their antinutritional compound content. An additional criterion now exists for addressing proteins’ value as potential bioactive peptide sources. Protein hydrolysates have reported bioactivity, and recent studies have identified single peptides with specific bioactivities.

There is a growing trend and interest in the use of food protein-derived peptides as intervention agents against chronic human diseases and for maintenance of general well-being. These peptides are produced by enzymatic hydrolysis of food proteins to release the peptide sequences, followed by posthydrolysis processing to isolate bioactive peptides from a complex mixture of other inactive molecules. These peptides are different from naturally occurring bioactive peptides, such as endorphins, because they are generated by proteolysis of native food proteins. Bioactive peptides are food protein-derived peptides that possess beneficial pharmacological properties beyond normal and adequate nutrition. Food protein hydrolysates have exhibited potent biological activities such as antihypertensive, antioxidant, immunomodulatory, anticancer, antimicrobial, and lipid-lowering activities, which are largely due to their constituent peptides.
The specific bioactivity of food peptides against various molecular disease targets depends primarily on their structural properties such as chain length and physicochemical characteristics of the amino acid residues, for example, hydrophobicity, molecular charge, and side-chain bulkiness. Data on amino acid sequences enhances understanding of the mechanisms involved in peptide bioactivities and is required to develop medical applications for bioactive peptides. Bioactive peptides are encrypted in the primary structure of plant and animal proteins as inactive amino acid sequences but they can be released by fermentation, food processing, and enzyme-catalyzed proteolysis in vitro or in the digestive tract after human consumption. In most cases, these protein hydrolysates and peptides have demonstrated better bioactivity compared to their parent proteins, and this shows that hydrolysis of peptide bonds is important in liberating the potent peptides. Several factors affect the bioactive properties of the peptides including the enzymes used for hydrolysis, processing conditions, and the size of the resulting peptides, which greatly affects their absorption across the enterocytes and bioavailability in target tissues. Most reported bioactive peptides are produced by in vitro enzymatic hydrolysis or fermentation. A challenge often faced in food protein-derived peptide research is to obtain high-yield peptide products with potent bioactivity. This limitation results in carrying out further processing of the enzymatic food protein hydrolysates. In summary, the processes commonly used for the production and processing of bioactive peptides are shown in figure 1.

Hypolipidemic effect of proteins and peptides

Ingestion of vegetable protein instead of animal protein is apparently associated with lower coronary heart disease risk, an effect that may reflect decreases in serum cholesterol concentrations. The lowering effects of soy protein versus animal protein have been recognized in animals for over 80 years. Ample evidence exists indicating that soy protein causes less hypercholesterolemia and less atherosclerosis in laboratory animals than animal protein. Clinical investigators have tested a variety of soy products, differing quantities of soy protein, various subject selection criteria and a variety of protocols. For example, one study found that casein or whey protein fed to piglets during the suckling period affects blood lipid levels, HMG CoA reductase activity, glucagon and cortisol levels, and weight gain. In other studies, diets with soy and milk proteins were suggested as being hypocholesterolemic, with soy protein producing a more notable effect than milk protein. A meta-analysis of the effects of soy protein intake on serum lipids in adults and children included an evaluation of changes in serum lipid concentrations in relation to initial serum lipids values. A series of variables were analyzed, including soy protein type (isolated soy protein, textured soy protein, or a combination); soy protein intake (grams per day); diet type (common Western diet or low-fat/low-cholesterol diet); age group (adults or children); and similarity of the control and soy-containing diets (specifically, weight change in subjects, and dietary fat, saturated fat and cholesterol intake). The effects of soy protein in lowering serum cholesterol concentrations were significantly linked to initial serum cholesterol values, and substitution of soy protein for animal protein produced significant decreases in serum concentrations of total cholesterol (9.3%), LDL cholesterol (12.9%), and TG (10.5%) without significantly affecting HDL cholesterol concentrations.

Effects of protein and peptides on micellar solubility of cholesterol

Food protein sources of hypocholesterolemic and hypolipidemic peptides include soy protein, milk...
Bioactive vegetable proteins and peptides in lipid-lowering

Fig. 1.—Schematic diagram showing steps toward the production and processing of food protein-derived bioactive peptides.

protein, buckwheat protein, egg white protein, and fish protein. However, the most literatures on lipid-lowering peptides are focused on soy protein hydrolysates and peptides. A soy protein peptic hydrolysate (SPH) is reported to exercise a stronger serum cholesterol lowering effect than intact soy protein in rats. Compared to casein, this SPH significantly decreased serum cholesterol levels and promoted fecal excretion of steroids, suggesting that the SPH inhibited cholesterol absorption. In the gastrointestinal system, cholesterol is rendered soluble in bile salt-mixed micelles and then absorbed. In an in vitro study, it was found that micellar cholesterol solubility was significantly lower in the presence of SPH compared to cholesterol micelles containing soy protein. In the same study, in vitro cholesterol absorption in Caco-2 cells exhibited significantly lower cholesterol uptake from SPH-containing micelles than from micelles containing soy protein. Incorporation of [H]-cholesterol into the blood, liver and intestine of rats was also significantly lower in the SPH groups than in the soy protein groups. These results indicate that soybean peptides have stronger hypocholesterolemic effects than soy protein because they inhibit cholesterol absorption by suppressing cholesterol solubility in micelles. In an effort to better define the active moiety in soy protein the LDL receptor up-regulation effects of β-conglycinin and glycinin in human hepatoma cells (HepG2) was studied, and found that β-conglycinin was markedly more effective than glycinin. In follow-up research, α + α subunits from β-conglycinin were found to have higher LDL receptor up-regulation activity than the β subunit. Incubation of HepG2 cells with purified α + α subunits sharply increased uptake and degradation of [125I]-LDL added to the culture medium, whereas the β subunit was ineffective. The α subunit probably contributed more to this difference than the α subunits. These reports led to development of an enzymatic modification process for hydrolysis of the soy β-conglycinin α subunit for use as a hypocholesterolemic agent. Administration of this hydrolysate in rats by gavage at 20 mg/kg body weight/d for 28 d resulted in a 36% decrease in plasma cholesterol; a greater effect than observed with admi-
administration of 100 mg/kg body weight/d of whole β-conglycinin. Nagaoka et al. (2001) identified a hypocholesterolemic peptide (Ile-Ala-Glu-Lys) from a milk β-lactoglobulin tryptic hydrolysate, and claimed it was the first hypocholesterolemic peptide to be isolated. A tetrapeptide (Leu-Pro-Tyr-Pro) was later isolated from soy glycinin hydrolysate. Another peptide fragment (Leu-Pro-Tyr-Pro-Arg) derived from soybean glycinin was found to reduce serum cholesterol in mice after oral administration at a dose of 50 mg/kg, for 2 d (-25.4% in total cholesterol and -30.6% in LDL cholesterol).

Based on their ability to lower micellar cholesterol solubility, Zhong et al. (2002) evaluated the in vitro hypocholesterolemic activity of soy protein protease hydrolysates prepared with different enzymes and at different degrees of hydrolysis (DH). In vivo, suppression of micellar cholesterol solubility is linked to inhibition of cholesterol absorption in the jejunum. Nagaoka et al. (2001) suggested that suppression of micellar cholesterol solubility may be closely related to lowering of serum cholesterol. The mechanisms responsible for soy protein’s effects on serum lipoproteins are unknown. Lovati et al. (1998) reported that monocyte LDL-receptor activity is eight times greater in human subjects receiving soy protein than in those eating control diets. Recently, studies in rats have shown that Lupinus mutabilis proteins have remarkable efficacy in reducing both plasma cholesterol and triglyceride levels, confirming the hypolipidemic potential of legume proteins. Rigamonti et al. (2010) reported that Pisum sativum proteins exert hypotriglyceridemic activity, mainly through downregulation of fatty acid synthesis. Results from other authors suggest a similar mechanistic explanation for the hypotriglyceridemic effect exerted by lupin proteins. These results suggest that common pathways may explain the hypolipidemic effect of legume proteins. Other vegetable proteins also have hypolipidemic effects. Vioque et al. (2006) evaluated the hypocholesterolemic effect of Helianthus annuus hydrolysates isolated with pepsin and Alcalase and Brassica carinata hydrolysates isolated with pepsin, pancreatin and carboxypeptidase. In this study, two Helianthus annuus hydrolysates obtained after 5 and 20 min hydrolysis with Alcalase caused a significant reduction of cholesterol incorporation into micelles. These authors suggested the hydrolysates may contain hydrophobic peptides, since this trait is required to interact with micelles.

According to Hosomi et al. (2010) dietary peptides are more effective in decreasing serum cholesterol and LDL-C contents than dietary protein. Two main causes

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**Fig. 2.—Proposed mechanisms for hypolipidemic and hypocholesterolemic properties of proteins, hydrolysates and peptides in cell culture, animal models, and humans.**

Abbreviations: TAG, triacylglycerol; LDL, low density lipoprotein; LDL-R, LDL receptor; SREBP, sterol regulatory element binding proteins; FAS, fatty acid synthase; SCD1, steroyl-CoA desaturase-1.

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\[ \text{Protein, hydrolysates or peptides} \]

\[ \downarrow \text{Cholesterol and fatty acids synthesis} \]

\[ \uparrow \text{Lipoproteins and TAG catabolism} \]

\[ \downarrow \text{Gene expression: SREBP, FAS, SCD1} \]

\[ \uparrow \text{Lipoproteins and TAG lipases activity} \]

\[ \downarrow \text{HMG-CoA Reductase enzyme and gene expression} \]

\[ \uparrow \text{LDL-R activity and gene expression} \]

\[ \downarrow \text{Cholesterol synthesis} \]

\[ \uparrow \text{Intestinal cholesterol absorption} \]

\[ \downarrow \text{Cholesterol + bile acid binding activity} \]

\[ \downarrow \text{Cholesterol micelle formation} \]

\[ \downarrow \text{Cholesterol uptake in CaCo, cells} \]

\[ \uparrow \text{Fecal excretion of neutral sterols and bile acids} \]

\[ \uparrow \text{LDL uptake and degradation} \]

\[ \uparrow \text{LDL-R activity and gene expression} \]

\[ \downarrow \text{Gene expression: SREBP, FAS, SCD1} \]

\[ \downarrow \text{HMG-CoA Reductase enzyme and gene expression} \]

\[ \downarrow \text{Cholesterol synthesis} \]

\[ \downarrow \text{Cholesterol + bile acid binding activity} \]

\[ \downarrow \text{Cholesterol micelle formation} \]

\[ \downarrow \text{Intestinal cholesterol absorption} \]

\[ \uparrow \text{Fecal excretion of neutral sterols and bile acids} \]
have been put forth in regard to the decreased serum and liver cholesterol contents related to the dietary protein. One hypothesis is that it relates to the amino acid composition of the protein in particular, the ratio of lysine/arginine, and the content of specific amino acids, namely, methionine, cysteine, and glycine. The other hypothesis involves an intradigestive tract effect, namely, that the digestibility of dietary protein and the physicochemical properties of digestion products in the digestive tract are related to cholesterol metabolism. Nagaoka et al. (2001) found that in animal models the degree of serum cholesterol lowering depends on the extent of fecal excretion of steroids. To clarify this mechanism Hosomi et al. (2010) evaluated the decrease in serum and liver cholesterol contents in rats related to the fish protein and peptides diet. Compared to rats fed casein, rats fed fish protein and peptides had decreased serum and liver cholesterol contents as a result of the suppression of steroids absorption. Previous studies have suggested that soy protein and egg ovomucin suppressed the micellar solubility of cholesterol and enhance bile acid binding capacity in vitro resulting in increased fecal steroid excretion. Proteins that are insoluble digestion products to mammalian digestive enzymes are known as resistant proteins that act to decrease blood cholesterol levels.

Impact of protein and peptides on genes regulating hepatic lipid metabolism

Rigamonti et al. (2010) investigate a possible impact of pea proteins on the expression of genes involved in cholesterol metabolism, the relative mRNA concentration of sterol regulatory element-binding protein (SREBP)-2 and that of its target genes such as hydroxymethyl-glutaryl-CoA (HMG-CoA) reductase and LDL receptor was determined, together with the hepatic gene expression of cholesterol 7a-hydroxylase (CYP7A1). The major focus this kind of studies is the investigation of potential mechanisms explaining the impact of proteins on circulating plasma total cholesterol and triglycerides. In order to examine the hypocholesterolemic effect of pea proteins, Rigamonti et al. (2010) measured the hepatic mRNA concentrations of SREBP-2, its target genes HMG-CoA reductase and LDL receptor, as well as CYP7A1. These authors did not observed relevant variations of SREBP-2, HMG-CoA reductase and CYP7A1, the LDL-receptor expression was significantly elevated in pea protein-fed animals. The LDL receptor is a major regulator of circulating LDL-cholesterol levels, and increased hepatic LDL receptor expression results in accelerated clearance of LDL particles. The observed elevation of hepatic LDL-receptor mRNA concentration in pea protein-fed animals may therefore result in an increased LDL catabolism and contribute to the observed plasma cholesterol reduction in these animals. According to the same authors the pea protein-based diet provided to rats did not influence triglyceride secretion or hydrolysis, whereas an effect was observed on fatty acid synthesis. In this study, a modest, not significant reduction of SREBP-1c gene expression was observed in pea protein-fed rats compared with casein-fed animals. SREBP-1c is a key regulator of fatty acid and triglyceride synthesis in the liver, an increase of the nuclear concentration of SREBP-1c, occurring through an increased gene expression or enhanced proteolytic activation, leads to transcription activation of genes encoding fatty acid synthesis enzymes. Whereas mRNA concentrations of SREBP-1c target genes (FAS, fatty acid synthase; SCD1 and SCD2, stearoyl-CoA desaturase 1 naad 2) were markedly lower in pea protein-fed animals.

The significant downregulation of FAS, SCD1 and SCD2 in spite of an almost absent reduction of SREBP-1c expression could be explained by a reduced proteolytic activation of SREBP-1c and a consequent reduced nuclear concentration of the activated transcription factor. Altogether, these results clearly indicate that pea proteins exert a hypotriglyceridemic activity mainly through downregulation of fatty acid synthesis. Results from other authors suggest a similar mechanistic explanation for the hypotriglyceridemic effect exerted by lupin proteins. These observations again suggest that common pathways may explain the hypolipidemic effect of legume and other pluses proteins. These studies demonstrates a marked hypocholesterolemic and hypotriglyceridemic effect of vegetables protein-based diet in hypercholesterolemic rats and suggests that these effects may occur, respectively, through upregulation of LDL receptor and downregulation of fatty acid synthesis.

Cholesterol proteins and peptides as nutraceutical ingredients

Two soybean protein-derived products, Lunasoy™ and WildlasiXP™ have been commercialized as suitable ingredients for the formulation of cholesterol-lowering foods. The two products are made from lunasin a bioactive soy protein component. The first product is commercialized as a protein complex suitable for the formulation of functional foods and beverages. The second product is commercialized as a peptide extract formulated for use as a dietary supplement. Lunasin is a peptide composed of 43 amino acid residues with an MW of 5.5 kDa. It contains 9 aspartic acid residues on its carboxyl end, a cell adhesion motif composed of arginine-glycine-aspartic acid residues, and a predicted helix with structural homology to a conserved region of chromatin-binding proteins. Earlier studies on animals showed that lunasin is not fully digested in the gastrointestinal system but is absorbed intact, reaching target tissues. Figure 3 presents the predicted secondary structure of lunasin, its 43 amino acids, and the motif.
This peptide has been shown to be responsible for the cholesterol-lowering effects associated with consumption of soybean products. Lunasin acts by reducing the level of HMGCoA reductase, which is similar to the action of statins, the popular cholesterol-lowering drugs. The cellular mechanism of action of lunasin involves reduction in the rate of gene expression for HMGCoA reductase, therefore less enzyme protein is made by the liver, which leads to reduced production of cholesterol. In addition lunasin increases the transcription levels of LDL receptor mRNA which enhances clearance of plasma LDL cholesterol. Another soy peptide, CSPHP (C-fraction soy protein hydrolysate with bound phospholipids) has been granted Generally Recognized As Safe (GRAS) status, allowing it to be sold as an ingredient for the formulation of cholesterol-lowering foods (functional foods and beverages) or dietary supplements. In human clinical trials, daily consumption of 3 g of CSPHP for three consecutive months leads to reductions in total cholesterol by about 38 mg/dL and LDL-cholesterol level by 46 mg/dL in hypercholesterolemia patients. Also important is the finding that CSPHP did not reduce cholesterol levels in people with normal cholesterol levels. The mechanism of action is believed to involve suppression in absorption of dietary cholesterol from intestinal tract, which enhances lowering of plasma cholesterol levels. An advantage presented by these three products is that no side effects have been reported related to consumption.

Conclusion

Dyslipidemias, particularly hypercholesterolemia, are well-established risk factors for cardiovascular disease. Expense, high drug dose, and low compliance to strict dietary therapies are current issues surrounding modern drug- and diet-based lipid-lowering approaches. Furthermore, variable patient outcomes and suboptimal response to both drug and diet therapies are increasingly evident. The question arises as to whether more emphasis should be placed on combination diet/drug therapies to reduce cholesterol levels in patients who respond suboptimally to current diet and drug monotherapies. Vegetable proteins contain bioactive peptides with diverse and unique health benefits. Many of these peptides hold promise for use in the prevention of age-related chronic disorders such as cardiovascular disease, cancer, obesity and decreased immune function. A large and growing body of evidence indicates that vegetable protein isolates, hydrolysates and peptides can reduce blood cholesterol concentrations in experimental animals and humans. Indirect evidence also suggests that some peptides can be absorbed by the gastrointestinal system and exert their action on specific target organs, while other peptides (e.g. hypocholesterolemic peptides) do not require absorption and act directly at the intestinal level. Proteins, hydrolysates and peptides with hypocholesterolemic bioactivity are potential nutraceutical ingredients with promising applications in development of functional foods for use in diet/drug therapies aimed at reducing cholesterol levels in the population at risk for cardiovascular risk disease.

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Bioactive vegetable proteins and peptides in lipid-lowering


