Bioactive peptides from food protein degradation: Legume peptides

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Bioactive peptides derived from food protein-processing, and specially their role in protecting against chronic diseases, are being attracting a growing attention currently. As a proof, since the first paper published in 1969, there is a huge number of publications in this field. With a turning point in the early 90s, with more than 1000 publications per year, this number continues mounting and reached more than 1600 articles in 2018, according to the Web of Knowledge ("food peptides"). Food-derived peptides that can offer health and medical benefits are of different origin (animal, marine, microbial, and plant food proteins), and can display different biological actions, including antitumor, antimicrobial, antioxidant, antiobesity, antihypertensive and anti-inflammatory activities (Figure 1).

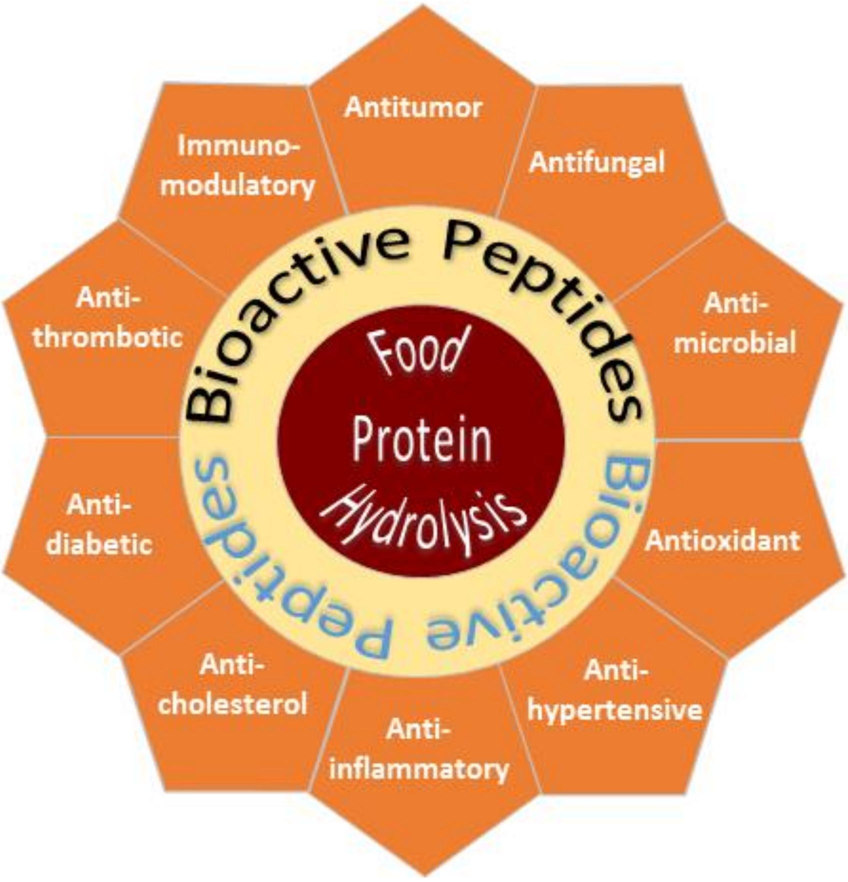

Figure 1. Main biological activities discovered for food-derived peptides

For those interested in a deeper knowledge in this field, there are a number of recent reviews that provide detailed information on food-derived bioactive peptides. These compendiums can be classified either by the type of precursor food (cereals, legumes, milk, eggs, meat, etc.)[1-5] or by the biomedical application (cancer, antihypertensive, anti-inflammatory, antiobesity, antimicrobial, etc).[2,6-10]

Here, I would like to focus the attention on a few recent examples (last 5 years) of bioactive peptides coming from legumes, crucial elements of healthy and balanced Mediterranean and Asian diets. For instance, soybeans do not only provide essential nutrients, but many bioactive proteins (lectins, protease inhibitors) and peptides. One of the first bioactive peptides isolated from legumes was Lunasin, a 43-mer peptide initially identified in soybean, but found also in different cereals, and endowed with antitumor, anti-inflammatory and antioxidant activities, among others (Table 1, entry 1).[11] The antitumor activity of Lunasin is ascribed to the presence of two domains involved in histone binding and antimitotic function, namely the 23-31 domain and the C-terminal fragment 35-43, respectively (Figure 2), exerting an epigenetic mechanism of action and modulating cell growth and apoptosis. In addition, the 31-34 RGD fragment is the adhesion module responsible of internalization. Lunasin is orally bioavailable and different preparations containing lunasin or soy protein precursors are currently commercialized as dietary supplements claiming for different health benefits.

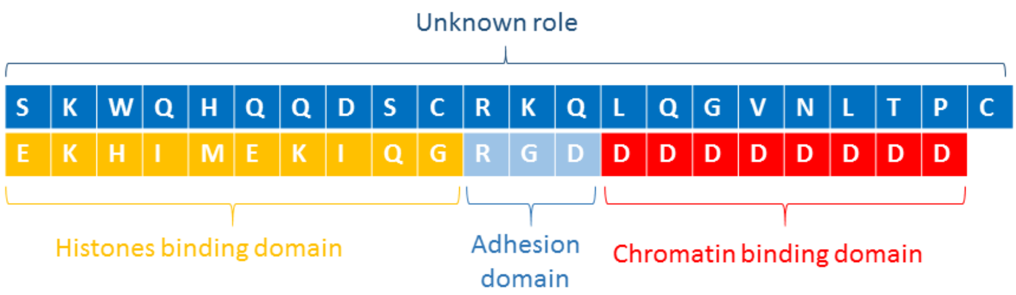

Figure 2. Primary structure of Lunasin and recognized domains responsible for its anticancer activity.

After Lunasin, other shorter peptides from soybeans have been isolated and demonstrated to have interesting hypercholesterolemic or hypoglycemic activities (Table 1, entry 2). Thus, IAVPGEVA and IAVPTGVA have been isolated after soybean protein glycinin hydrolysis, and demonstrated their ability to modulate the cholesterol metabolism, through the inhibition of the β-hydroxy-β-methylglutaryl-coenzime A reductase (HMGCoAR).[12] In addition, these peptides can also modulate the glucose metabolism, enhancing glucose uptake through its transporters GLUT1 and GLUT4.[13] These results suggest the use of these peptides in the prevention of metabolic syndrome and cardiovascular diseases. A peptide as short as Phe-Leu-Val (FLV, Table 1, entry 3), also derived from soybean proteins, was found to decrease inflammatory responses and insulin resistance in adipocytes by inhibiting the release of inflammatory cytokines (TNFα, MCP-1, and IL-6).[14] As adipose inflammation is crucial in the development of certain metabolic disorders like insulin resistance and type 2 diabetes, this peptide could be of interest in preventing obesity.

Several short and medium size peptides were identified from broad bean (Vicia faba) hydrolysates, and then synthesized to evaluate their biological potential.[15] One of these peptides, LSPGDVLVIPAGYPVAIK (Table 1, entry 4), showed a nice radical scavenging activity in the DPPH assay. In addition to the antioxidant properties, this peptide is able inhibit *P. aeruginosa* biofilm formation, an activity that was related to its ability to chelate iron. A second peptide from the above mentioned hydrolisates, VESEAGLTETWNPNHPELR, with lower antioxidant and antibiofilm properties compared to the previous one, displayed an interesting activity as tyrosinase inhibitor. Since tyrosinase is a crucial enzyme in melanin production, this latter peptide could have applicability in cosmetics, i.e. for pigmentation disorders.

There are numerous examples of protein-derived peptides acting on the renin-angitensin system and coming from different sources, including legumes.[7] For instance, a series of lentil-derived peptides were described to possess dual antioxidant and angiotensin converting enzyme (ACE) inhibitory activity (Table 1, entry 5).[16] More interestingly, further digestion of these peptides increased the dual activities, characterizing heptapeptide LPILRYL as the most potent peptide in the series. Molecular modeling studies were used to rationalize the binding of this peptide to ACE, and suggested interactions with ACE Y520, K511 and Q281, also involved in the binding of angiotensin II, the natural substrate. In a similar manner, the inhibition of renin and ACE by lima bean protein hydrolysates has been described in a 2018 recent paper.[17] Different hydrolysates with significant ability to inhibit both enzymes were able to lower more than 50% systolic and diastolic blood pressure in an *in vivo* animal model, although no information about the structure of peptide components have been disclosed yet.

Table 1. A selection of recently described bioactive peptides from legumes

Entry	**Peptide sequence**	**Source**	**Activity**	**Reference**
1	SKWQHQQDSCRKQLQGVNLTPC- EKHIMEKIQGRGDDDDDDDDD (Lunasin)	Soybean	Anticancer Antioxidant Anti-inflammatory	11
2	IAVPGEVA IAVPTGVA	Soybean	Hypocholesterolemic Hypoglycemic	12,13
3	FLV	Soybean	Anti-inflammatory in adipocytes	14
4	LSPGDVLVIPAGYPVAIK VESEAGLTETWNPNHPELR	Faba bean (*Vicia faba*)	Antioxidant Antibiofilm Tyrosinase inhibitor	15
5	NSLTLPILRYL LPILRYL	Lentils	Antioxidant ACE inhibitor	16
6	VFVRN	Chickpea	Hypolipidemic	18
7	PGTAVFK IKAFKEATKVDKVVVLWTA	Soybean	Antimicrobial	19

The use of a computer-assisted pharmacophore model allowed the identification of the short pentapeptide VFVRN (Table 1, entry 6) from chickpea peptides, which inhibits the activity of the 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) *in vitro*, and the synthesis of cholesterol and the expression of the liver X receptor (LXRa) in cell cultures, all important players in the control of lipid metabolism.[18] In the same paper, the authors demonstrated that fractions of peptides obtained by hydrolysis of chickpea isolated proteins, significantly decreased the content in total serum cholesterol and total triglycerides. These peptides behave also as HMGR and fatty acid synthase (FAS) inhibitors, and modulate the expression of peroxisome proliferator-activated receptors (PPARα), low-density lipoprotein receptor *(*LDLR) and regulatory element binding protein (SREBP-2), among others, in high-fat diet rats.

Finally, peptides from legumes like soybean and chickpea also display antimicrobial activity. For example, following a high throughput assay, soy peptides PGTAVFK and IKAFKEATKVDKVVVLWTA were identified as effective against *L. monocytogenes*, and *P. aeruginosa* and *L. monocytogenes*, respectively (Table 1, entry 7).[19] Taking into account the antibiotic resistance crisis, these type of peptides could constitute a foundation for alternative antimicrobial agents.

In conclusion, food peptidomics is an emerging field within food science that has evolved enormously in last years, with important advances in methodologies for food peptide isolation, purification, characterization, and the identification of important bioactivities, as shown above for legume peptides. However, it should be noted here that not all food-derived peptides are beneficial for health. In fact, glutamine and proline-rich gluten proteins from cereals are partially hydrolyzed in the gastrointestinal tract to several proteolytically-resistant peptides that are behind gluten intolerance and celiac disease.[20] Within the beneficial bioactive peptides, further research is essential to evaluate the real physiological efficacy of these food-derived peptides in humans. As modulators of therapeutically relevant proteins and endowed with important activities in prevalent diseases, these food-derived peptides could not only be of interest as ingredients of functional foods, but as invaluable starting points in drug discovery programs. However, despite the large number of identified food-bioactive peptides, the structure-activity relationship studies are really scarce and the exact molecular mechanism is unknown in many cases. Therefore, there is a huge gap in the field that need to integrate multidisciplinary studies for future innovation, including genomics, proteomics, bioinformatics, food and medicinal chemistry, and pharmacology.

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