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Mini-Review

Bioactive proteins from edible plants of *Solanum* genus

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ABSTRACT

Bioactive proteins have been detected in many different food sources. Beyond their nutritional value many of them exhibit functional effects both on the producing and on the consuming organisms. In recent years it has been recognized that proteins provide a rich source of biologically active peptides. The genus *Solanum* has a great variety of edible species that are an important source of protein. In this sense, some proteins might be involved in the response to wounding in plant defense mechanisms like patatine and *solamarine* and snakin 1 and 2 isolated from *S. tuberosum* tubers and *cyphomine* isolated from *S. betaceum* fruits. They have shown *in vitro* inhibitory action on hydrolases released by microorganisms involved in cell wall degradation during the invasion process. They also have *in vitro* inhibitory effect on phytopathogenic microorganism growth for both bacteria and fungi. It is thought that these proteins could be part of the so-called pathogenesis-related proteins. As they are also present in edible tissue, these proteins have been studied from a functional point of view. The purified proteins showed antioxidant or antiradical activities by a series of *in vitro* tests, including DPPH, ABTS, hydroxyl and superoxide radicals scavenging activity assays, anti-human low-density lipoprotein peroxidation tests, protections against hydroxyl radical-mediated DNA damages, peroxynitrite-mediated dihydrorhodamine 123 oxidations and β-carotene bleaching assay. A non mutagenic effect was observed in *solamarine* and *cyphomine*; *solamarine* also showed an antimutagenic effect against a direct mutagen. At present, an enzymatic hydrolysis process on proteins is being carried out in order to obtain smaller molecules (peptides and free amino acids) with improved nutritional quality and safety. Three bioactive peptides (5A, 5C and 6C) from potato protein hydrolysate fractions have recently been isolated and showed antioxidant activity. Hence, it would be possible to generate new products and carry out alternative applications for several agricultural and nutritional products.

KEYWORDS: *Solanum* genus, bioactive proteins, plant defense mechanisms, antimicrobial proteins, antioxidant proteins

1. INTRODUCTION

Biologically active proteins or peptides are those that exert a physiological effect in humans, animals or plants. Beyond their nutritional value, proteins are important ingredients of some foods such as milk, eggs, meat and fish of various kinds as well as foods from many plants. Depending on the sequence of amino acids, these proteins can exhibit opiate-like, mineral binding, immunomodulatory, antimicrobial, antioxidant, antithrombotic, hypocholesterolemic, and antihypertensive activities [1-4]. Many of the known bioactive peptides are multifunctional and can exert more than one of the effects mentioned [5-7].

The *Solanum* genus is considered to be one of the largest and most complex among the Angiosperms. It is comprised of about 1500 species and has

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at least 5000 published epithets [8]. It is widely distributed in diverse phytogeographic regions and some of its species are nutritionally important. Many *Solanum* plant species are widely used in folk medicine due to their bioactive compounds. Steroidal alkaloids were isolated from *S. umbelliferum* [9], *S. critinum* and *S. jabraense* [10]. They are also known to possess a variety of antifungal, molluscicidal, teratogenic, and embryotoxic [11-14] biological activities. Preparations containing solasodine glycosides are currently employed for the treatment of certain skin cancers [15]. Flavonoids, isoflavonoids and phenolic acids were also present in several *Solanum* species [10, 16]. In addition to organic compounds, protein fractions with biological activity obtained from aerial parts (fruits, leaves) and tubers have also been reported.

This study is a review of bioactive protein and peptides isolated from *Solanum* species with human health properties or agronomic interest.

2. Proteins from *Solanum tuberosum L.*

Potatoes are a major world crop, with an annual world production of nearly 300 million tonnes. The potato tuber is considered the most important vegetable in many developing and developed countries. Potatoes are used for several purposes like human consumption as fresh or processed produce (french fries, mashed potatoes), industrial processing (potato starch, alcohol, etc.) and re-cultivation (potato seed) [17]. Several proteins and peptides have been isolated from different tissues of *Solanum tuberosum* with a number of biological activities (Table 1).

2.1. *Solamarine*

In a previous work we isolated and purified *solamarine* [18-19], a low molecular weight (17 kDa) polypeptide from *S. tuberosum* tubers. This Mr 18 kDa protein is on the cell wall [19] and *in vitro* assays exhibited an inhibitory action on soluble invertases and invertases associated to the cell wall [20]. Furthermore, it is not species-specific, because it can act on other invertases isolated from different plant species [21]. In addition, *solamarine* was active on other hydrolases, such as polygalacturonases, pectinase, pectin lyase, α-arabinofuranosidase and β-glucosidase, all of them derived from phytopathogenic fungi [21]. *Solamarine* was also able to inhibit the growth of phytopathogenic bacteria like *Erwinia carotovora*, *Pseudomonas solanacearum*, *P. corrugate, P. syringae and Xanthomonas campestris*. Isla *et al.* [21] proposed that this protein *solamarine* would be involved in plant defense mechanisms and could be useful for the management of diseases caused by these microorganisms in plants. It has recently been demonstrated that *solamarine* inhibited uric acid formation (IC_{50} values between 55 and 60 μg/mL), and reduced oxidative damage

Table 1. Principal peptides and proteins isolated from different *Solanum* species.

Source of protein	Protein name	Biological activity	Ref.
Solanum tuberosum Tubers	*Solamarine*	Antimicrobial against phytopathogenic bacteria and fungi and antioxidant activities	[18, 19-20]
	Patatine	lipid acyl hydrolase acyltransferase and antioxidant activities	[22-23]
	Snakin-1 and Snakin-2	Antimicrobial against phytopathogenic fungi	[25-26]
	5A, 5C, 6C peptides	Antioxidant activity	[27]
Leaves	AP1	Antifungal activity against crop pathogens	[28]
Solanum stramonifolium Seed	---	Antibacterial activity against human pathogen Gram (+) and Gram (-) bacteria	[29]
Solanum betaceum fruit	*Cyphomine*	Antimicrobial against phytopathogenic bacteria and fungi and antioxidant activities	[20-21, 30-32]

by scavenging hydroxyl radicals and superoxide anion in a dose-dependent manner [22]. Before *Solamarine* might be considered as a natural antioxidant, Ordóñez *et al*., evaluated the toxicity of this protein. *Allium cepa* and Salmonella/ microsome assays showed no genotoxic and mutagenic effects. Also, *solamarine* showed an antimutagenic effect against a direct mutagen (4-nitro-o-phenylenediamine) [22]. Hence, this protein could optimally be exploited for human nutrition and health.

2.2. Patatin

Patatin is the trivial name given to a family of glycoproteins that make up >40% of the total soluble protein in potato (*Solanum tuberosum*) tubers and serves as a storage protein. The major protein had Mr 45 kDa. It was proved that patatin exhibited both lipid acyl hydrolase and acyltransferase activities that might be involved in tuber tissue response to wounding [23]. Recently, the purified patatin showed antioxidant or antiradical activity by a series of *in vitro* tests like 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (half-inhibition concentration, IC_{50}, was 0.582 mg/mL) scavenging activity assays, anti-human low-density lipoprotein peroxidation tests, and protections against hydroxyl radical-mediated DNA damages and peroxynitrite-mediated dihydrorhodamine oxidations [24]. Through modifications of patatin by iodoacetamide or N-bromosuccinimide, the antioxidant capacity was modified; these results suggest that cysteine and tryptophan residues might contribute to its scavenging activity [24]. Bartová and Barta obtained a potato fruit juice concentrated with proteins by ethanol precipitation [25]. The fraction of patatin proteins (39-43 kDa) presented an essential amino-acids index (EAAI) value of 86.1% in the nutritionally improved protein component. The lipid acyl hydrolase activity of the patatin family was not negatively affected by cooled ethanol precipitation. It may thus be suggested that biological and enzymatic activities of this protein family are utilizable after this type of precipitation [25].

2.3. Snakin-1 and Snakin-2

A new type of antimicrobial peptide, snakin-1 (SN1), has been isolated from potato tubers [26]. SN1 was characterized molecularly and has 63 amino acid residues (Mr 6,922 Da), 12 of which are cysteines. This peptide was found to be active against bacterial and fungal pathogens for potato and other plant species (conc <10 μM). SN1 and potato defensin PTH1 properties were synergistic against *Clavibacter michiganensis* subsp. *sepedonicus* bacterium and additive against *Botrytis cinerea* fungus. Otherwise, Snakin-1 causes aggregation of both gram-positive and gram-negative bacteria. This peptide was developmentally accumulated in different tissues of potato plants and the expression of its corresponding gene was unaffected by a variety of abiotic or biotic challenges [26]. Subsequently, Berrocal-Lobo *et al.* [27] described the isolation of a new potato peptide (StSN2) that represents a widely divergent snakin subfamily. The StSN2 peptide is also active *in vitro* against bacterial and fungal plant pathogens and, in contrast with StSN1, expression of the corresponding gene is affected by certain external treatments like pathogen infection [27].

2.4. Peptides obtained from potato tuber extracts

Three peptides called 5A, 5C and 6C were obtained from potato protein hydrolysate fractions [28]. These three peptide fractions were sequenced and identified as Phe-Gly-Glu-Arg, Phe-Asp-Arg-Arg and Phe-Gly-Glu-Arg-Arg, respectively and characterized in relation to their biological activity. All fractions demonstrated antioxidant activity and repressed lipid peroxidation by different assays. Oral administration of the 5A, 5C and 6C chemically synthesized peptides reduced ethanol-induced gastric mucosal damage. According to these results, these peptides show real biological activity as antioxidants and could further be investigated for potential use as food additives [28].

2.5. Other proteins from *S. tuberosum*

An antimicrobial protein called **AP1** was recently purified from potato leaves. AP1 had a good inhibition activity against five different strains of *Ralstonia solanacearum*, harmful for potato or other crops, and two potato fungal pathogens, *Rhizoctonia solani* and *Alternaria solani*. Studies on C-terminal and N-terminal sequence of the cloned protein demonstrated that AP1 shared homology with an acid phosphatase. This protein most likely belongs to a new group of proteins with *in vitro* antimicrobial characteristics. It works through phosphorylation and plant energy metabolism [29].

3. Protein from *Solanum stramonifolium*

Solanum stramonifolium Jacq. fruit is a commonly found vegetable and herb from Asia. The seed aqueous extracts of *S. stramonifolium* exhibited a very good antibacterial activity against human pathogen gram-positive bacteria, such as *Stapyllococcus aureus, Bacillus licheniformis*, *B. subtilis* and *Xanthomonas* sp. and against gram-negative bacteria like *Pseudomonas aeruginosa* and *Salmonella typhi* [30]. Small proteins or peptides with Mr less than 14.4 kDa might be involved in the antibacterial activity of this species so that seed proteins from this plant species might be a good source of antibacterial agents.

4. Protein from *Solanum betaceum*

4.1. *Cyphomine*

A new proteinaceous inhibitor from *Solanum betaceum* Sendt (ex *Cyphomadra betacea* Sendt.) mature fruits, called *cyphomine,* was isolated and characterized [31]. The inhibitor structure involves a single polypeptide with Mr of 19 kDa and produces erythrocyte agglutination *in vitro*. *Cyphomine* is able to inhibit activity of invertases isolated from different species, genera and even family of plants. Furthermore, it is not invertase specific: fungal, bacterial and higher plant hydrolases (such as polygalacturonase and pectinase) are also inhibited [21]. Ordóñez *et al.* [32] demonstrated that this polypetide inhibited the growth of xylophagous and phytopatogenic fungi (*Ganoderma applanatum, Schizophyllum commune, Lenzites elegans, Pycnoporus sanguineous, Penicillium notatum, Aspergillus niger, Phomopsis sojae* and *Fusarium mango*) and phytopathogenic bacteria (*Xanthomonas campestris* pvar vesicatoria CECT 792, *Pseudomonas solanacearum* CECT 125, *Pseudomonas corrugata* CECT 124, *Pseudomonas syringae* pv. syringae and *Erwinia carotovora* var carotovora). The concentration required to completely inhibit the growth of all studied fungi ranged from 7.8 to 62.5 µg/mL. Phytopatogenic bacteria were the most sensitive [32]. Antifungal and antibacterial activities can be associated with their ability to inhibit hydrolytic enzymes [21]. Consequently, a possible participation of *cyphomine* in the plant defense mechanism and a potential application as a biocontrol agent against phytopathogenic fungi and bacteria are proposed. Recent reports demonstrated that *cyphomine* had antioxidant activity: this protein was able to scavenge free radicals (DPPH, hydroxyl and superoxide anion) and this activity was not affected by heat [22]. Furthermore, *Cyphomine* was not genotoxic at the concentration that shows the biological activity. Therefore this protein could be used as a natural antioxidant in the development of functional food.

REFERENCES

1. Hartmann, R. and Meisel, H. 2007, Curr. Opin. Biotechnol., 18, 163-9.
2. Kitts, D. D. and Weiler, K. 2003, Curr. Pharm. Des., 9, 1309-23.
3. Kovacs-Nolan, J., Phillips, M. and Mine, Y. 2005, J. Agric. Food Chem., 53, 8421-31.
4. Meisel, H. 1997, Biopolymers, 43, 119-28.
5. Korhonen, H. and Pihlanto, A. 2003, Curr. Pharm. Des., 9, 1297-308.
6. Meisel, H. 2004, Biofactors, 21, 55-61.
7. Meisel, H. and FitzGerald, R. J. 2003, Curr. Pharm. Des., 9, 1289-95.
8. Nee, M. 1999, Solanaceae IV: Advances in biology and utilization. Nee, M., Symon, D. E., Lester, R. N. and Jessop, J. P. (Eds.) Royal Botanic Gardens, Kew, pp.285-333.
9. Kim Youn, C., Qing-Ming Ch., Leslie Gunatilaka, A. A. and Kingston David, G. I. 1996, J. Nat. Prod., 59(3), 283-285.
10. Esteves-Souza, A., Sarmento da Silva, T., Fernandes Alves, C., de Carvalho, M., Braz-Filhob, R. and Echevarria, A. 2002, J. Braz. Chem. Soc., 13, 838-842.
11. Rowan, D. D., Macdonald, P. E. and Skipp, R. A. 1983, Pytochem., 22, 2102.
12. Kusano, G., Takahashi, A., Sugiyama, K. and Nozol, S. 1987, Chem. Pharm. Bull., 35, 4862.
13 Alzerreca, A. and Hart, G. 1982, Toxicol. Lett., 12, 153.
14. Friedman, M., Rayburn, J. R. and Bantle, J. A. 1992, J. Agric. Food Chem., 40, 1617.

15. Cham, B. E., Daunter, B. and Evans, R. A. 1991, Cancer Lett., 59, 183.
16. Lewis, Christine E., Walker, John R L., Lancaster, Jane E. and Sutton, Kevin H. 1998, J. Sci. Food Agric., 77, 45-57.
17. Talburt, W. F. 1987, History of potato processing, Talburt, W. F. and Smith, O. (Eds.), Potato Processing (pp. 1-10). New York: Van Nostrand.
18. Isla, M. I., Vattuone, M. A. and Sampietro, A. R. 1991, Phytochem., 30, 739-743.
19. Isla, M. I., Leal, D. P., Vattuone, M. A. and Sampietro, A. R. 1992, Phytochem., 31, 1115-1118.
20. Isla, M. I., Vattuone, M. A., Ordóñez, R. M. and Sampietro, A. R. 1999, Phytochem., 50, 525-534.
21. Isla, M. I., Ordoñez, R. M., Nieva Moreno, M. I., Sampietro, A. R. and Vattuone, M. A. 2002, J. Enz. Inh., 17, 37-43.
22. Ordóñez, R. M., Sayago, J. E., Zampini, I. C., Rodríguez, F., Cattaneo, F. and Isla, M. I. 2011, J. Agric. Food Chem., 59, 8655-8660.
23. Andrews, D. L., Beames, B., Summers, M. D. and Park, W. D. 1988, Biochem. J., 252, 199-206.
24. Liu, Y. W., Han, CH. H., Lee, M. H., Hsu, F. L. and Hou, W. Ch. 2003, J. Agric. Food Chem., 51, 4389-4393.
25. Bártová, V. and Bárta, J. 2009, J. Agric. Food Chem., 57, 9028-9034.
26. Segura, A., Moreno, M., Madueño, F., Molina, A. and García-Olmedo, F. 1999, Mol. Plant-Microbe Interact, 12, 16-23.
27. Berrocal-Lobo, M., Segura, A., Moreno, M., López, G., García- Olmedo, F. and Molina, A. 2002, Plant Physiol., 128, 951-961.
28. Kudoa, K., Onoderab, S., Takedab, Y., Benkebliac, N. and Shiomib, N. 2009, J. Func. Food, 1, 170-176.
29. Feng, J., Yuan, F., Gao, Y., Liang, Ch., Xu, J., Zhang, Ch. and He, L. 2003, Biochem. J., 376, 481-487.
30. Sarnthima, R. and Khammuang, S. 2012, Int. J. Agric. Biol., 14, 111-115.
31. Ordóñez, R. M., Isla, M. I., Vattuone, M. A. and Sampietro, A. R. 2000, J. Enz. Inh., 15, 583-596.
32. Ordoñez, R. M., Ordoñez, A. A. L., Nieva Moreno, M. I., Sayago, J. E. and Isla, M. I. 2006, Peptides, 27, 1187-1191.