BioTechnologia vol. 103 (4) • pp. 397–408 • 2022
Journal of Biotechnology, Computational Biology and Bionanotechnology REVIEW PAPERS
https://doi.org/10.5114/bta.2022.120708

Next-generation nutraceuticals: bioactive peptides from plant proteases

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Abstract
Bioactive peptides are short and specific fragments of proteins with a wide range of biological activities that provide health benefits to the host. These natural peptides are safe and nontoxic and do not show any side effects. Nowadays, the production and characterization of bioactive peptides have been a key area of research as they show great potential as nutraceuticals and functional foods. Thus, bioactive peptides are considered next-generation therapeutic agents that can replace pharmaceutical products with profound adverse effects in the near future. So far, proteolytic hydrolysis has been used as the method of choice for the large-scale production of bioactive peptides. Studies have reported that peptides with specific characteristics can be generated using a particular type of protease. Microbial proteases are the predominantly used ones because of the ease in their production and purification. However, recently, plant proteases have gained a renewed interest as they offer diversity and better specificity compared with other proteases. This review highlights the potential of plant proteases for the production of bioactive peptides and also describes the benefits of bioactive peptides as nutraceuticals.
Key words: ACE inhibitory; antioxidant; bioactive peptides; nutraceuticals; papain; plant proteases

Introduction

In recent years, bioactive peptides have been extensively used as an active component in food as they contribute to diverse functional and biological properties. They are often regarded as nutraceuticals as they can be used in the prevention and treatment of various diseases and can also be incorporated into dietary food to compensate for nutritional deficiencies (Manzoor et al., 2022; Bhandari et al., 2020). Of late, nutraceuticals are gaining a great deal of attention due to the dissatisfaction and harmful effects produced by pharmaceutical drugs. A nutraceutical can be defined as a part of a food or whole food that provides medical or health benefits to an individual. Short peptide sequences are present in structures of many food proteins, which exhibit functional properties (Chew et al., 2019). These peptides can be released during gastric digestion in animals or microbial fermentation or industrial processing of food or can be produced by hydrolyzing food proteins using a range of proteases (Sanchez and Vázquez, 2017). They can also be synthesized using chemical reactions (Sanchez and Vázquez, 2017). To exhibit a positive health effect, bioactive peptides must cross the gastrointestinal barrier and survive enzyme degradation (Mora et al., 2018). Proteases are one of the largest groups of enzymes that catalyze the hydrolysis of peptide bonds in proteins and peptides (Gurumallesh et al., 2019). All plants, animals, and microorganisms synthesize a wide range of proteases, which participate in various physiological reactions in and out of the cell. Commercially, proteases contribute to 60% of the worldwide enzyme sale, being utilized in detergents and leather, textile, food, and pharmaceutical industries (Matkawala et al., 2021). Microbial proteases contribute to approximately 70% of the entire protease market, followed by animal (rennin, trypsin, and pepsin) and plant proteases (papain and bromelain) (Protease Market Report). According to the world enzyme market report published in 2021, the global pro-

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tease market is expected to achieve a compound annual growth rate (CAGR) of nearly 5.4% during the forecast period of 2021–2031 and may reach $5762.7 million by the end of 2031 (Protease Market Report). In the food industry, one of the most common methods used in the production of bioactive peptides is the application of commercial proteases in protein hydrolysis (Agyei and Danquah, 2011). However, growing concern about consumer health issues has fueled the use of natural enzymes in the food industry. Therefore, the demand for plant proteases has increased in recent years. In 2019, the papain market size was valued at $169.37 million, which is expected to grow at a CAGR of 4.67% during the forecast period of 2020–2025 (Papain Market Research Report). Similarly, the global bromelain market size was valued at $37.6 million, which is expected to grow at a CAGR of 7.2% from 2020 to 2027 (Bromelain Market Report). This review aimed to shed light on the utilization of plant proteases in the production of bioactive peptides, describing their potential applications in the food and pharmaceutical industries (Fig. 1).

Bioactive peptides

Bioactive peptides are short-chain peptide sequences usually composed of 2–20 amino acid residues. They have a relatively low molecular weight of less than 6 kDa (Chew et al., 2019). They remain inactive in the native protein but exhibit various physiological and biological activities as a result of protein hydrolysis by enzymatic or chemical reactions. The functionality of a bioactive peptide depends on its sequence and amino acid composition. Several bioactive peptides are shown to exhibit antimicrobial, antioxidative, antihypertensive, antithrombotic, immunomodulatory, and anticarcinogenic activities (Chew et al., 2019; Mazorra-Manzano et al., 2017). Bioactive peptides can be synthesized from a vast repository of plant and animal proteins (Sanchez and Vázquez, 2017). They are produced by enzymatic or chemical hydrolysis of endogenous proteins such as those synthesized within the host or exogenous proteins present in food. Several reports have illustrated that bioactive peptides are present in bioactive proteins, and to date, dairy proteins are the biggest source of bioactive peptides (Shivanna and Natraj, 2020). However, researchers have reported the synthesis of bioactive peptides from other food proteins, such as proteins derived from animals, plants, and marine sources. Yang et al. (2020) synthesized antioxidative peptides from duck plasma using microbial proteases. Ballatore et al. (2020) synthesized antioxidative and cytoprotective peptides by the hydrolysis of whey protein concentrate using an animal protease. Sonklin et al. (2020) produced antihypertensive peptides from mung bean protein using a plant protease. Zhang et al. (2020) developed peptides with angiotensin-converting enzyme (ACE) inhibitory activities from wheat gluten using a microbial protease. In addition, bioactive peptides with antioxidative and dipeptidyl peptidase (DPP-IV) inhibitory and functional properties have been synthesized from marine protein sources, viz. common carp, Atlantic salmon, and Chinese sturgeon, respectively (Jin et al., 2020; Delgado-Garcia et al., 2019; Noman et al., 2019).

Plant proteases in the production of bioactive peptides

The industrial production of bioactive peptides is confined to the hydrolysis of proteins using proteases, as these enzymes have rich diversity and good catalytic specificity and reaction control. Moreover, hydrolyzates produced by proteases show a higher degree of hydrolysis. Studies have demonstrated that peptides with a high degree of hydrolysis and low molecular weight exhibited improved functional properties compared with intact proteins or those with a low degree of hydrolysis (Singh et al., 2018). Microbial proteases are preferred in the large-scale production of bioactive peptides from food proteins as they are less expensive than proteases from other sources (Agyei and Danquah, 2011). However, in recent years, there has been a renewed interest in plant proteases since several countries have reported allergies from using animal and microbial proteases and discontinued the use of recombinant proteases (Mazorra-Manzano et al., 2017). In addition, the demand for products extracted from natural sources has increased. Plants produce a plethora of proteases that play important roles in physiological and developmental processes such as mobilization of storage proteins during seed germination, maintaining protein turnover, posttranslational modification of proteins, photoinhibition in the chloroplast, defense mechanisms, degradation of misfolded proteins, and initiation of cell death and senescence programs (Martinez et al., 2019; Mazorra-Manzano et al., 2017). Therefore, plants are an important source of proteases and produce a diverse range of proteases throughout their life cycle. Most of the plant proteases

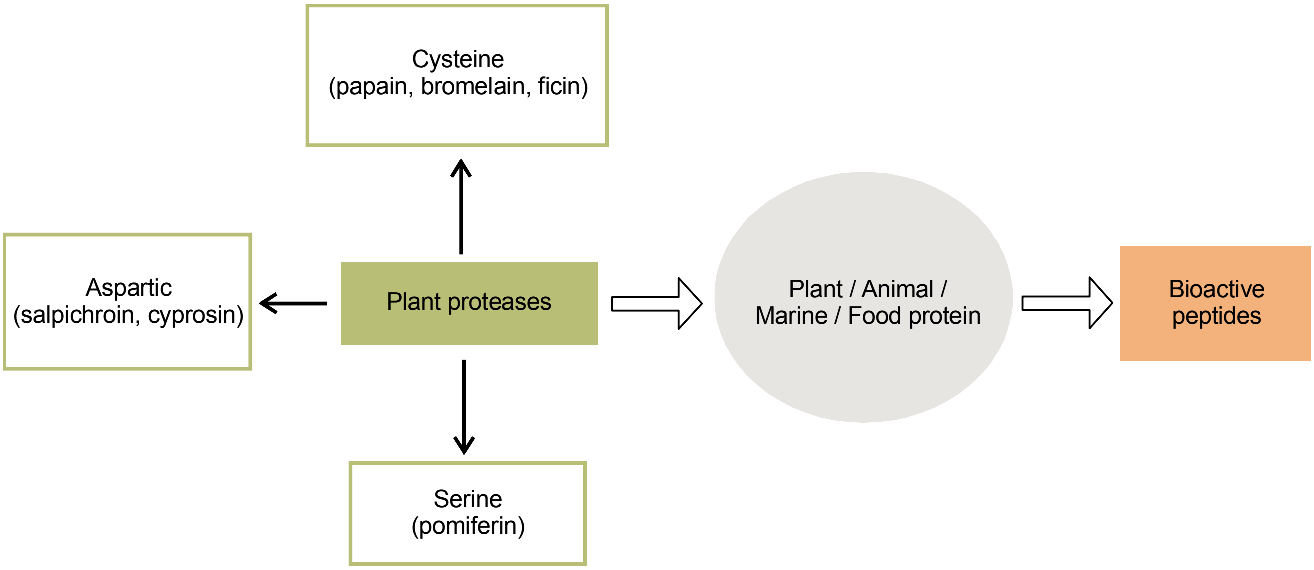

Fig. 1. An overview of the types of plant proteases in the production of bioactive peptides

exhibit broad specificity and are active over a wide range of pH and temperatures (David Troncoso et al., 2022). It is well documented that a protease with a specific mode of action leads to the release of encrypted bioactive peptides from the protein (Agyei and Danquah, 2011). Many studies have reported that bioactive peptides are produced using plant proteases with broad specificity like papain and bromelain (David Troncoso et al., 2022; Mazorra-Manzano et al., 2017). These proteases release nonpredictable products with a high degree of hydrolysis and low molecular weight, thereby releasing peptides with multifunctional properties. Some examples of plant proteases used in the production of bioactive peptides are listed in Table 1.

Cysteine proteases

Most of the plant proteases belong to the cysteine type, which predominantly take part in the mobilization and degradation of storage proteins during germination and also play an important role in protein maturation, housekeeping, and cell senescence (Martinez et al., 2019). Papain (EC 3.4.22.2), bromelain (EC 3.4.22.32), and ficin (EC 3.4.22.3) are the most used proteases in the production of bioactive peptides and are commonly utilized in the pharmaceutical and food industries in the preparation of therapeutic and nutraceutical products (Mazorra-Manzano et al., 2017). Besides these proteases, researchers have used several other plant proteases, primarily cysteine proteases such as actinidin from *Actinidia* spp. and zingibain from *Zingiber officinale* (David Troncoso et al., 2022). However, their commercial application is yet to be explored.

Papain

Papain, a cysteine endopeptidase, is one of the most commercially used plant proteases as it exhibits a strong proteolytic activity toward proteins and short-chain peptides. It preferentially cleaves the peptide bonds between Arg-Phe, Arg-Lys, and Phe-Lys (Amri and Mamboya, 2012). The relatively broad specificity of papain makes it a suitable candidate for the production of bioactive peptides as it hydrolyzes peptide bonds within the peptide chain and thus produces peptides but not free amino acids (Amri and Mamboya, 2012). In fact, using relatively low concentrations of papain can result in a higher yield of low molecular weight peptides. Thus, the specific catalytic action of papain makes it a good choice for hydrolyzing food proteins. Papain is stable over a wide range of pH (3.0–9.0) and temperatures (37–70 °C) and shows activity even in the presence of organic solvents and denaturing agents (Shouket et al., 2020). It can be obtained from the latex of the papaya plant (*Carica papaya*). This tropical fruit is easily available all year round, and protein precipitation techniques can yield up to 53 g of crude enzyme per kg of latex, which can be produced in 11–12 months (Fernández-

Table 1. Plant proteases used in the production of bioactive peptides

Protease	Protein source	Time [min]	Degree of hydrolysis [%]	Bioactive peptide sequences	Biological properties	Reference
Papain	porcine liver	420	–	APAAIGPYSQAVLVDR, GLNQALVDLHALGSAR	antioxidant	López-Pedrouso et al. (2020)
	buffalo skim milk retentate	240	23	FPGPIPK, IPPK, IVPN, YPSG, HPFA, KFQ	antihepatotoxic and antioxidant	Abdel-Hamid et al. (2019)
	camel milk protein	540	35	MPSKPPLL	antidiabetic and antiobesity	Mudgil et al. (2019a)
	rice bran	150	33	–	antioxidant and anticarcinogenic	Singh et al. (2018)
	chia expeller	120	15	–	antioxidant	Cotabarren et al. (2018)
Bromelain	brown rice	180	–	FGGSGGPGG, ESDVVSDL, GSGVGGAK	ACE inhibitory, antioxidant, and flavor characteristics	Selamassakul et al. (2020)
	porcine liver	240	–	–	antimicrobial	Borrajo et al. (2020)
	stone fish	240	44.59	–	ACE inhibitory	Auwal et al. (2017)
Ficin	goat milk casein	180	–	–	antimicrobial	Esmaeilpour et al. (2016)
Salpichroin	whey	1200	8	–	antioxidant	Rocha et al. (2017)
Pomiferin	bovine casein	180	17.1	YQEPVLGPVRGPFPIIV, RFFVAPFPE	ACE inhibitory	Corrons et al. (2017)
	soy protein isolate	90	36.2	LQSGDALRVPSGTTYY, LNSGDALRVPSGTTYY	antioxidant	Reyes Jara et al. (2018)
	whey	180	31.3	KGYGGVSL	ACE inhibitory and antioxidant	Bertucci et al. (2015)
Asian pumpkin	whey	300	30.5	–	dipeptidyl peptidase-IV, alpha-glucosidase, and ACE inhibitory	Babij et al. (2014)
	egg yolk	240	46	LAPSLPGKPKPD	ACE inhibitory	Eckert et al. (2014)

Lucas et al., 2017). Further purification can be achieved using column chromatography and aqueous two-phase extraction system. After purification, the crude papain is treated with reducing agents to protect its protease activity from oxidation (Fernández-Lucas et al., 2017). Werner et al. (2015) expressed recombinant papain in *Pichia pastoris* and reported a much higher yield of papain (463 g in 3 days) compared with the conventional process of papain extraction from papaya. Monari et al. (2019) proposed the digestion of scotta, a by-product of the cheese industry, using various commercial protease preparations. Among the nine different microbial, animal, and plant proteases, peptides generated using papain showed the highest antioxidant and antityrosinase activities. Moreover, papain is preferred in the large-scale production of bioactive peptides due to its maximum yield, improved bioactivity, and lower scale-up cost. Wan Mohtar et al. (2014) hydrolyzed winged bean (*Psophocarpus tetragonolobus* (L.) DC.) seeds using four proteolytic preparations and found that papain showed the highest yield of peptides with ACE inhibitory activity. Papain generated hydrolyzates with a higher degree of hydrolysis in a shorter time compared with hydrolyzates produced from bromelain, flavourzyme, and alcalase (Wan Mohtar et al., 2014). Besides plant proteins, papain has also been proven to be successful in hydrolyzing animal proteins for the production of antioxidant and anti-inflammatory biopeptides (O'Sullivan et al., 2017).

Bromelain

Bromelain, a thiol endopeptidase, is obtained from the fruit or stem of pineapple (*Ananas comosus*) and has been used as a medicinal compound since 1875 (Pavan et al., 2012). Commercially, it is extracted from the pineapple stem using a three-step process, namely centrifugation, ultrafiltration, and lyophilization. Being an inexpensive waste product, the stem has a higher concentration of proteases as than fruits (Rathnavelu et al., 2016). Nowadays, chromatographic techniques and aqueous two-phase extraction process are used in the purification of bromelain. These techniques simplify the recovery of the enzyme and enhance the overall yield of the product (Ramli et al., 2016). Several studies have also reported the production of recombinant stem bromelain in *Escherichia coli* to increase the activity of the enzyme and enhance the yield (George et al., 2014; Muntari et al., 2011). Bromelain is active over a wide range of pH range – 3.0–9.0 – and has the optimum temperature between 37 and 70°C. It catalyzes the hydrolysis of peptide bonds within the polypeptide chain, exhibiting specificity for hydrophobic and nonpolar amino acid residues (Pavan et al., 2012). However, the exact mechanism of action of bromelain is still unknown, which limits its use in the hydrolysis of food proteins. Selamassakul et al. (2020) hydrolyzed brown rice protein using bromelain and reported the formation of low molecular weight peptides (<1 kDa) with bioactive properties. Similarly, Hu et al. (2020) synthesized bioactive peptides from corn gluten meal using papain, bromelain, and ficin and found bromelain and ficin to be more effective in the production of antioxidant peptides than papain. However, Bah et al. (2015) reported the use of fungal proteases over bromelain and papain in the production of bioactive peptides using animal plasma.

Ficin

The term "ficin" refers to the endoproteolytic activity present in the latex of fig (*Ficus carica*) (Zare et al., 2013). Various active forms or isoforms of ficin are expressed in plants depending on the health of the tree, fruit ripening, watering, and environmental conditions during growth (Morellon-Sterling et al., 2020). Several isoforms of ficin have been purified using ammonium sulfate precipitation followed by gel filtration chromatography (Haesaerts et al., 2015). Ficin isoforms have a slightly varying specificity but prefer arginine at the substrate residue N terminal to the cleavage site (P1) position and a hydrophobic amino acid at the substrate residue N terminal to the P1 (P2) position in the polypeptide. The optimum temperature and pH range for ficin activity is 50–60°C and 6–7 pH, respectively (Morellon-Sterling et al., 2020). Till now, only a few reports exist on protein hydrolysis by ficin in the production of biopeptides due to its specific mode of action. Shahidi et al. (2018) hydrolyzed gelatin from *Uroteuthis duvauceli* (quid) using ficin. *In vitro* studies proved that these hydrolyzates can inhibit the growth of cancer cells. Esmaeilpour et al. (2016) digested goat milk with ficin and trypsin and reported that ficin was more efficient in hydrolyzing casein in the synthesis of bioactive peptides than trypsin. Similarly, Pierro et al. (2014) hydrolyzed bovine casein using ficin and obtained hydrolyzates with antioxidant properties. Moreover, Hernandez and de Mejia (2019) theoretically analyzed the potential of ficin in hydrolyzing chickpea protein, which could generate bioactive peptides with antioxidant, hypolipidemic, antihypertensive, anticarcinogenic, and antidiabetic properties. They suggested that plant proteases can be used in combination with other commercially available proteases for the production of bioactive peptides from chickpea with decreased bitterness. Ficin offers great diversity due to the change in its enzyme composition with varying environmental conditions such as the health of the fig tree, ambient conditions, watering of plant, and fruit ripening (Morellon-Sterling et al., 2020). Thus, it shows great potential for generating bioactive peptides by differentially hydrolyzing food proteins. However, the cost of commercial production of ficin is much higher than those of other proteases available on the market.

Serine proteases

Although cysteine proteases are more abundant in plants, more than 200 different types of serine proteases are encoded by plant genomes. Serine proteases are involved in a wide range of metabolic functions such as microsporogenesis, symbiosis, signal transduction, hypersensitive response, cell differentiation, and senescence (Mazorra-Manzano et al., 2017). They are commonly found in legumes such as soybean and grains like barley and rice (Mazorra-Manzano et al., 2017). They have also been extracted from latex of *Euphorbia supina*, African milkbush (*Synadenium grantii* Hook), and Jackfruit (*Artocarpus heterophyllus*). Cucumisin from melon, pomiferin from *Maclura pomifera* latex, and hordolisin from barley are a few examples of plant serine proteases used in the production of bioactive peptides (Ha et al., 2012). Serine proteases such as wrightin are active at the pH range of 7.5–10 and a temperature of 70°C (Tomar et al., 2008); therefore, they can be used in commercial applications that require high temperatures.

Pomiferin

Maclura pomifera (Raf.) Schneid. (Moraceae), commonly known as Osage orange, is a thorny dioecious tree that can grow in most types of soils and is drought and termite resistant (Bertucci et al., 2015). The peptidases extracted from the latex of this plant are commonly termed pomiferin. However, the yield of the protease extract varies due to the difference in the size of fruits and the amount of latex recovered. Reyes Jara et al. (2020) hydrolyzed different food proteins such as egg white, soy protein isolate, and casein using pomiferin to yield antioxidant peptides with numerous applications in the food industry. Nevertheless, the simple one-step purification strategy used to isolate this protease makes it a suitable candidate in the food and pharmaceutical industries. Similarly, Corrons et al. (2017) and Bertucci et al. (2015) hydrolyzed bovine casein and whey protein, respectively, using pomiferin to obtain ACE inhibitory and antioxidant peptides that can be used in the food industry.

Proteinase from Asian pumpkin

Proteinase extracted from *Cucurbita ficifolia* (Asian pumpkin) has also been used in the hydrolysis of proteins for the production of bioactive peptides (Eckert et al., 2014). It constitutes about 15% of the total proteins extracted from the pumpkin pulp, thus requiring low production costs (Zambrowicz et al., 2015). Moreover, its stability over a wide range of temperatures can make it a good choice for the food and pharmaceutical industries. However, its commercial use has not yet been optimized. Babij et al. (2014) hydrolyzed whey protein and β-lactoglobulin using this serine protease and obtained peptides with DPP-IV, α-glucosidase, and ACE inhibition properties. These peptides can be used as ingredients in functional foods in the diet of diabetic patients. Eckert et al. (2014) hydrolyzed egg yolk protein to obtain ACE inhibitory peptides, and Dabrowska et al. (2013) hydrolyzed casein using this enzyme to obtain antimicrobial peptides.

Aspartic proteases

Aspartic proteases are active at low pH and are less abundant in plants than serine and cysteine proteases (González-Rábade et al., 2011). Eukaryotic aspartic proteases include pepsins, cathepsins, and renins. The majority of plant aspartic proteases belong to the A1 family along with pepsin-like enzymes from other origins, are specifically inhibited by pepstatin, and have two aspartic acid residues responsible for their catalytic activity (Simoes and Faro, 2004). Aspartic proteases are involved in the digestion process of carnivorous plants and protein degradation in various developmental processes, during stress responses, defense against pathogens, and cell senescence (González-Rábade et al., 2011). These proteases have been extracted from various tissues of *Arabidopsis thaliana*, barley, hempseed, tomato, potato, and cucumber (Feijoo-Siota and Villa, 2011). Bueno-Gavilá et al. (2019) hydrolyzed bovine casein using the proteolytic extract of *Cynara scolymus* and obtained bioactive peptides with enhanced ACE inhibitory and antioxidant activities compared with other animal and microbial proteases. A proteolytic mixture of aspartic proteases can be easily extracted from the artichoke flower and is found in abundance as an agricultural by-product (Bueno-Gavilá et al., 2019). In addition, aspartic proteases such as salpichroin from *Salpichroa originifolia*, cyprosin from *Cynara cardunculus*, and onopordosin from *Onopordum acanthium* have been used in the production of bioactive peptides (Shah and Mir, 2019).

Most of the plants used for the extraction of proteases grow in the tropical or subtropical region. The protease enzyme can be extracted from various plant tissues such as flowers, stem, leaves, and roots by aqueous maceration, followed by purification of the crude extract (Shah and Mir, 2019). However, changing environmental conditions such as drought, excessive rains, and soil nutrition may cause internal or external stress to plants, which affects the quality and quantity of protease production. Selecting high-yielding varieties is difficult due to the long cultivation periods between planting and harvesting, which leads to increased production costs (Shah and Mir, 2019). Recent advances in tissue culture techniques have proven to be successful in producing plant proteases on a large scale, such as micropropagation, somatic embryogenesis, callus, and cell suspension culture (David Troncoso et al., 2022). Plants grown *in vitro* are independent of varying climatic conditions, economic challenges faced during cultivation, problems associated with plant diseases, and variation in product content.

Application of bioactive peptides

Plants are an important source of proteases that are commercially used in meat tenderization, dairy processing, and the baking industry for a long time (González-Rábade et al., 2011). Recently, plant proteases have gained importance in bioactive peptide production. Some of the imperative properties of these bioactive peptides that make them potential candidates for commercial use as nutraceuticals are discussed below.

Antihypertensive activity

Antihypertensive peptides, or ACE inhibitory peptides, are one of the most commonly studied bioactive peptides. ACE is the key enzyme regulating blood pressure in our body as it catalyzes the transformation of angiotensin I to potent vasoconstrictor angiotensin II, which is responsible for increasing the blood pressure (Sanchez and Vazquez, 2017). Nowadays, hypertension has become a serious health problem and may lead to cardiovascular diseases (Bertucci et al., 2015). Tripeptides like VPP and IPP generated from various protein sources such as milk and chia seeds have been proven to be potent ACE inhibitors (Daliri et al., 2017). In addition, bioactive peptides with antihypertensive properties have also been derived from several other plant sources such as potato, yam, rape seed, red seaweed, chickpea, lentil, and soy (Hayes and Bleakley, 2018). Reports suggest that besides animal and microbial proteases, plant proteases have also been proven to be efficient in producing ACE inhibitory peptides. ACE inhibitory peptides with an IC_{50} of 1.72 ± 0.25 mg/ml were produced from bovine casein using the protease extract of *M. pomifera* (Corrons et al., 2017). Bertucci et al. (2015) also used this protease extract to synthesize ACE inhibitory peptides with an IC_{50} of 0.53 ± 0.02 mg/ml from whey protein. Even though bioactive peptides have moderate ACE inhibitory potency compared with antihypertensive drugs with a low IC_{50} of 0.02 μM, nowadays these peptides are preferred due to their minimal side effects. Studies have shown that peptides can exhibit ACE inhibitory activities only if they become resistant to gastrointestinal digestion and reach the peripheral organs to show their physiological effects (Bhat et al., 2015). Such peptides can be incorporated into functional foods as a preventive measure for hypertension. Auwal et al. (2017) obtained ACE inhibitory peptides from stone fish using bromelain, which can be incorporated into food and beverages for controlling blood pressure.

Antioxidant activity

Oxidation plays a significant role in cellular metabolism; however, excessive amounts of free radicals may lead to cell destruction by inducing apoptosis and oxidizing vital enzymes and proteins required for the proper functioning of the cell. Liu et al. (2016) reported that bioactive peptides ranging between 4 and 16 amino acids with a molecular weight of 400–2000 Da are considered antioxidative peptides as they can reach the target site easily without undergoing gastrointestinal digestion. The amino acid composition of the peptides also plays an important role in determining their antioxidative properties (Mora et al., 2018). Moreover, the presence of these antioxidative peptides in food products enhances their quality by reducing the deterioration of food components such as lipids and proteins (Hu et al., 2020). Reports suggested that many bioactive peptides with antioxidative properties are generated by the hydrolysis of proteins obtained from plants, animals, and marine sources (Bhat et al., 2015). Such peptides have been utilized in commercial food preparations (Toldrá Vilardell et al., 2017). López-Pedrouso et al. (2020) synthesized antioxidant peptides from porcine liver using a mixture of papain, bromelain, alcalase, and flavourzyme. Abdel-Hamid et al. (2019) used papain for producing antioxidant peptides from buffalo milk retentate. Huang et al. (2017) proposed bromelain to be efficient in synthesizing antioxidant peptides from *Scomberomorus niphonius* protein. Rocha et al. (2017) used aspartic protease from *S. origanifolia* to produce antioxidant peptides from whey proteins. Among plant proteases, papain and bromelain are found to be efficient in the synthesis of antioxidant peptides (Mazorra-Manzano et al., 2017). The antioxidant activity of the bioactive peptides can be detected by measuring their ability to reduce free radicals via hydrogen donation or via transfer of electrons to reduce an oxidant.

Antimicrobial activity

Peptides that are 20–46 amino acid residues long, less than 10 kDa in molecular weight, basic (lysine- or arginine-rich), and amphipathic are regarded as antimicrobial peptides (Sanchez and Vázquez, 2017). They can inhibit a large number of food spoilage microorganisms as well as pathogens and thus serve as a promising alternative to therapeutic agents (Hayes and Bleakley, 2018). Additionally, they are safer and ecofriendlier than synthetic antimicrobial agents. Ghanbari and Ebrahimpour (2018) obtained antibacterial peptides against *Pseudomonas* sp., *E. coli*, and *Staphylococcus aureus* by hydrolysis of *Actinopyga lecanora* protein with bromelain. Mudgil et al. (2019b) hydrolyzed quinoa and amaranth proteins using three proteases, namely chymotrypsin, bromelain, and a microbial protease, and reported that bromelain was more efficient in producing antimicrobial peptides than the other proteases. Similarly, Borrajo et al. (2020) also found bromelain to be efficient in hydrolyzing porcine liver for the production of antimicrobial peptides. Antimicrobial peptides produced by the enzymatic hydrolysis of milk, marine, and plant sources have been reported in the literature (Bhandari et al., 2020). Currently, more than 80 peptide-based drugs are available on the market for a wide range of diseases, including diabetes, cancer, osteoporosis, multiple sclerosis, HIV infection, and chronic pain, and approximately 150 peptides are undergoing clinical trials for the treatment of cancer, type 2 diabetes, autoimmune diseases, etc. (Muttenthaler et al., 2021).

Opioid activity

Several bioactive peptides derived from food and other proteins have been characterized as opioid drugs and are termed exorphins. These peptides can bind to their respective opioid receptors present in the peripheral tissues and exhibit a range of physiological functions such as immunomodulation, gastrointestinal function control, and reproductive mechanism control. Besides, they can regulate central nervous system functions such as stress handling, depression, and other emotional behaviors (Mora et al., 2018). Bioactive peptides with opioid activity are mostly 5–31 residues in length and share a common N-terminal sequence of Tyr-Gly-Gly-Phe-(Met or Leu), which is known as the opioid motif (Kaur et al., 2020). Reports suggest that exorphins are a thousand times more potent than endogenous opioid peptides; however, they are unable to resist gastrointestinal digestion when administered orally (Toldrá Vilardell et al., 2017). Such peptides have been derived from milk and wheat and are comparable in structure and function to endogenous opioids (Mora et al., 2018). Bueno-Gavilá et al. (2019) produced opioid peptides by hydrolyzing bovine casein protein using aspartic proteases from the artichoke flower (*C. scolymus* L.).

Antiinflammatory activity

Inflammation is a natural defensive mechanism of our body against various infections. Regulation of inflammatory molecules is essential for restoring homeostasis in damaged tissues (Mudgil et al., 2019a). Pharmacological drugs used in the treatment of inflammation disorders often have high toxicity and undesirable side effects (La Manna et al., 2018). Therefore, bioactive peptides with antiinflammatory activity can serve as potential therapeutic agents for the treatment of inflammation. Mudgil et al. (2019a) obtained peptides with antiinflammatory activity from camel milk using papain. Although peptides obtained from the hydrolysis of milk, plant, and marine proteins (Sanchez and Vázquez, 2017) have shown anti-inflammatory effects *in vitro*, further research on the biological effects of these peptides is required to use them as nutraceuticals.

Antidiabetic activity

Type II diabetes is one of the major epidemic diseases that affect around 422 million people worldwide (WHO Report). Currently, synthetic drugs and several natural compounds have been used to regulate the blood glucose level, increase glucose uptake, stimulate insulin secretion, and inhibit enzymes involved in glucose regulation (Daliri et al., 2017). Bioactive peptides inhibit alpha-glucosidase and DPP-IV – key enzymes regulating blood glucose levels – and thus can be used as an alternative to pharmacological drugs (Wang et al., 2019). The hydrolyzates produced from fish, meat, and milk by proteolytic enzymes have been reported to have antidiabetic properties (Mora et al., 2018). Guo et al. (2020) suggested the release of peptides with DPP-IV and ACE inhibitory activities from quinoa protein using papain, bromelain, and ficin via in silico approach. Similarly, Gomez et al. (2019) reported the release of DDP-IV and ACE inhibitory peptides from Portuguese oyster (*Crassostrea angulata*) using papain and bromelain. However, these studies lack *in vitro* and *in vivo* experimental analysis. Novo Nordisk developed a peptide-based drug named Semaglutide, which is now available on the market as an oral formulation used in the treatment of diabetes (Patil et al., 2020).

Conclusions and future perspectives

Plants produce a wide range of proteases, which have diverse substrate specificities and are active over a wide range of temperatures and pH. Plant proteases can be easily extracted from various tissues or produced using *in vitro* culture techniques. Plant proteases such as papain, bromelain, ficin, actinidin, and cardosin have already been used for commercial purposes. According to the Code of Federal Regulations of the US Food and Drug Administration, papain, bromelain, and ficin are approved as generally recognized as safe; hence, they are now being largely used in the food industry, mainly in the hydrolysis of proteins. However, plant proteases face major competition from animal and microbial proteases due to their high production cost, inconsistency in purification techniques, and lack of availability of plant material. However, recent advances in tissue culture techniques, extensive research on immobilization of proteases, and new purification strategies promise a prominent place for plant proteases in the future. Consequently, the production of bioactive peptides using plant proteases is one of the emerging areas of research. So far, researchers have proven the ACE inhibitory, antioxidative, antimicrobial, antidiabetic, and antiinflammatory properties of plant protease-derived bioactive peptides. However, commercial use of these peptides is dependent on a number of factors. The specific target bioactive peptide must be isolated from the mixture of peptides. The peptide should confer superior stability and shelf life along with fine taste, if it is used as a functional food. While using bioactive peptides as a therapeutic drug, the route of delivery is a matter of concern as they must be delivered at a high concentration to exert a physiological effect in the host's body. Several bioactive peptides are resistant to digestion and thus can be delivered orally. However, alternative routes of administration are also being explored to secure the activity of peptides. The major production cost associated with bioactive peptides can be reduced by using abundantly available proteins or waste protein sources for hydrolysis. Multifunctional bioactive peptides can be explored to expand their biological activity and usability. These peptides have a bright future as they are considered natural, with minimal toxicity and side effects to the host. The expanding knowledge about plant proteases makes them a suitable candidate for hydrolyzing the vast repository of proteins for the development of bioactive peptides.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors declare that they have no conflict of interest.

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