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Review

Amaranth proteins as a source of bioactive peptides: a review

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Abstract

Recently, bioactive peptides have been used as an alternative to treat or prevent diseases. Although milk has been the most studied food, different crops have been investigated with the aim of diversifying peptides alternatives. Amaranth is an American crop with a high percentage of protein (> 15%), and has been used in several studies to release peptides with different bioactivities. This review presents the state of the art in peptides generation from amaranth proteins; finding that hydrolysis *in vitro* digestion is the most typical process to release peptides. This review also focuses on amaranth as a potential product for obtaining bioactive peptides.

Introduction

Amaranth is a pre-Columbian food which found in the region between Mexico and Peru, and was consumed by several ancient civilizations such as Inca, Aztec, and Maya who included it in their diet. Then, amaranth slowly spread to other geographical regions, and was found in Europe as an ornamental plant since the 18th century, and in Africa and Asia as vegetable since the 19th century, all the while not attaining similar importance in human nutrition as in its country of origin (Borneo and Aguirre, 2008). Its presence in several geographical areas is due to its adaptability to various environmental conditions, such as medium or low fertility soils and limited rain fall conditions (Bressani, 1993; Amicarelli *et al.,* 2002).

Taxonomically, this plant belongs to the order Caryophyllales, family Amaranthaceae and genus *Amaranthus*. There are over 800 amaranth species in the world, most of them weedy species, like *A. retroflexus*. Only few species are used as food, leafy vegetable, forage, and ornamental, the most common being *A. tricolor*, *A. blitum, A. caudatus, A. cruentus,* and *A. hypochondriacus* (Bressani, 1993; Aguilar *et al.,* 2011). Because of its nutritional characteristics, it is not considered a cereal; rather a "pseudo-cereal", like buckwheat and quinoa (Amicarelli and Camaggio, 2012). Amaranth is an annual dicotyledonous and herbaceous plant that can reach over 3 m in a rigid upright stems. Leaves of amaranth are greenish and reddish, which are mostly edible. Its flowers are very small in purple, dark red, or yellow green colours. Its fruits contain a tiny and lenticular seed (1.0 - 1.5 mm diameter; 0.6 - 1.2 g) which may be white, gold, red, and dark (Teutonico and Knorr, 1986; Bressani, 1993; Adhikary and Pratt, 2015).

The grain chemical composition of Amaranth is presented in Table 1. The grain of amaranth includes the coat (smooth, thin, and easy to remove), the germ (rich in fat), and the perisperm (rich in starch). Because of its quantity and quality of macronutrients (higher percentages in protein and fat), it is different from common cereals (Bressani 1993; Caselato-Sousa and Amaya-Farfán, 2012). Another important fact of amaranth is that its seed has a balanced amino acid composition, similar to the FAO/WHO guidelines for human diet (Mlakar *et al.,* 2010; Amicarelli and Camaggio, 2012; Rastogi and Shukla, 2013).

Amaranth proteins

The best source of high-quality proteins are animal proteins. However, these are usually expensive and some of them cause allergies or intolerances

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Table 1. Chemical composition and essential amino acids of amaranth grain.

Chemical composition	Per 100 g of amaranth seed	Essential amino acids	Per 100 g of protein
Energy (kcal)	365 - 370	Trp	0.98 - 1.80
Protein (g)	13.57 - 18.19	Met/Cys	4.00 - 4.90
Total lipid (g)	2.50 - 8.50	Thr	3.30 - 4.00
Carbohydrate (g)	60.54 - 66.25	Ile	2.70 - 4.00
Starch (g)	57.27 - 68.00	Val	3.90 - 4.70
Fibre (g)	2.60 - 6.70	Lys	5.00 - 6.00
		Phe/Tyr	5.00 - 8.50
		Leu	4.20 - 6.30

Sources from personal elaboration by the authors' data: Caselato-Sousa and Amaya-Farfán (2012), Becker *et al.* (1981), Mlakar *et al.* (2010), and Adhikary and Pratt (2015).

(Tavano *et al.,* 2008; Shevkani *et al.,* 2014). For this reason, many nutritional studies are focused on plant proteins like those of amaranth (Peiretti, 2018; Peiretti *et al.,* 2018; Zhang *et al.,* 2019). Amaranth grain has high digestible quality protein (13 - 19% of protein with 90% of digestibility), with good balance in amino acids, better than that in cereals and legumes; high in lysine, which is deficient in cereals, and also lacks of protein-forming gluten (gliadin) making it proper for its consumption in celiac diet (Alencar *et al.,* 2017; Kurek *et al.,* 2018).

Amaranth grain proteins could be divided according to its solubility in albumins, globulins, and prolamins (Barba de la Rosa *et al.,* 1992). Silva-Sánchez *et al*. (2008) reported the presence of the three protein fractions and later, Montoya-Rodríguez *et al.* (2014a) observed the same proteins even after extrusion process.

Globulin 11S or amarantin

Globulin 11S or amarantin is the main fraction in amaranth protein isolates (Quiroga *et al.,* 2009). It was first characterised by Barba de la Rosa *et al.* (1996), and consists of 501 amino acids (Figure 1), and a molecular weight of 56 kDa. Globulin 11S is one of the most important storage proteins of the seed (Condés *et al.,* 2009). Globulin 11S consists of three subunits integrated with two trimmers into a homohexamer (Carrazco-Peña *et al.,* 2013). The homohexamer is made up with monomers between 52 and 59 kDa, which are linked by a SS-bond (Janssen *et al.,* 2017).

Globulin 7S

Globulin 7S is present in amaranth in fewer amounts than 11S globulin, and also less studied (Tandang-Silvas *et al.,* 2010). Quiroga *et al.* (2009) described globulin 7S as four subunits of 66, 52, 38, and 16 kDa, with a molecular weight of 200 kDa. While García-González *et al.* (2013) reported globulin 7S as three principal subunits called α (57 - 69 kDa), α´ (57 - 72 kDa) and β (42 - 52 kDa), which are linked by covalent bonds formed by a trimer with a molecular weight between 170 and 200 kDa.

STHASGFFFFHPTKMAKSTNYFLISCLLFVLFNGCMGEGRFREFQ
QGNECQIDRLTALEPTNRIQAERGLTEVWDSNEQEFRCAGVSV
IRRTIEPHGLLLPSFTSAPELIYIEQGNGITGMMIPGCPETYESGSQ
QFQGGEDERIREQGSRKFGMRGDRFQDQHQKIRHLREGDIFAM
PAGVSHWAYNNGDQPLVAVILIDTANHANQLDKNFPTRFYLA
GKPQQEHSGEHQFSRESRRGERNTGNIFRGFETRLLAESFGVSEEI
AQKLQAEQDDRGNIVRVQEGLHVIKPPSRAWEEREQGSRGSRY
LPNGVEETICSARLAVNVDDPSKADVYTPEAGRLTTVNSFNLPI
LRHLRLSAAKGVLYRNAMMAPHYNLNAHNIMYCVRGRGRIQI
VNDQGQSVFDEELSRGQLVVVPQNFAIVKQAFEDGFEWVSFKT
SENAMFQSLAGRTSAIRSLPIDVVSNIYQISREEAFGLKFNRPETT
LFRSSGQGEYRRKISIA

Figure 1. Amino acid sequence of Globulin 11S.
Amino acid nomenclature: C, cys; cysteine; H, his; histidine; I, ile; isoleucine; M, met; methionine; S, ser; serine; V, val; valine; A, ala; alanine; G, gly; glycine; L, leu; leucine; P, pro; proline; T, thr; treonine; F, phe; phenylalanine; R, arg; arginine; Y, tyr; tyrosine; W, trp; tryptophan; D, asp; aspartic acid; N, asn; asparagine; B, asx; either of D or N; E, glu; glutamic acid; Q, gin; glutamine; Z, glx; either of E or Q; K, lys; lysine. Protein sequence from database UniProt (http://www.uniprot.org)

Albumins

In amaranth, albumins are found as a group of two polypeptides called MRPs (Methionine-Rich Protein) with 16 - 18% of methionine and a molecular weight of 18 kDa (Segura-Nieto *et al.,* 1994; Silva-Sánchez *et al.,* 2004).

Prolamins

Prolamins are the less abundant protein in amaranth (Segura-Nieto *et al.,* 1992). They are formed by three fractions with apparent molecular

weight between 52 - 54, 33 - 34, and 22 - 27 kDa, linked by sulfuric bonds (Barba de la Rosa *et al.,* 1992; Janssen *et al.,* 2017).

Globulins, albumins and prolamins are the main components of amaranth protein. Protein can be hydrolysed by different methods to release peptides with different bioactivities.

Bioactive peptides

Bioactive peptides are defined as specific protein fractions with positive physiological functions such as diminishing arterial pressure, and having antagonist or agonist opioid effects. Additionally, bioactive peptides can also be antithrombotic, antimicrobial, anticholesterol, antioxidant, and others (Lorenzo *et al.,* 2018; Onuh and Aluko, 2019). In some cases, peptides encrypted in protein sequences have multifunctional activities. However, while they are still linked in the protein, their activity is null. Bioactive sequences can be released by three different methods: digestion, enzymatic activity, and microbiological hydrolysis (Kitts and Weiler, 2003; Hartmann and Meisel, 2007).

Digestion

In vivo or *in vitro* enzymatic process is the most common way to produce bioactive peptides. During this process, the protein is completely hydrolysed using the combination of pepsin-pancreatin and pepsin-trypsin enzymes. With this enzymatic process, peptides with different activities have been released in some food proteins, such as milk, egg and soybean (Capriotti *et al.,* 2015; Grootaert *et al.,* 2017; Su *et al.,* 2017). Peptides with antithrombotic activity in infants´ blood after the consumption of breast milk (Chabance *et al.,* 1995) and antihypertensive peptides after the consumption of sardine and yogurt in adults´ plasma (Matsui *et al.,* 2002; Foltz *et al.,* 2007) have been identified. Even though *in vitro* studies are cheap and can give a good approach to *in vivo* studies, studies where the comparison of both methods have been realised in milk, showing the generation of a great amount of peptides and free amino acids in both experiments (Sanchón *et al.,* 2018; Egger *et al.,* 2019).

Enzymatic hydrolysis

Proteinases and peptidases extracted from plants, bacteria, fungi, and animals are used to release peptides through enzymatic hydrolysis. For example, Suh *et al.* (1999) found peptides with Angiotensin Converting Enzyme inhibition (ACE-i) with the use of Pescalase (serine protease from *Bacillus licheniformis*) in maize proteins, and Zarei *et al.* (2014) released antioxidant peptides from palm wastes using papain. Also, antioxidant peptides have been identified after the use of Alcalase (*Bacillus subtilis*) and Flavourzyme (*Aspergillus oryzae*) in maize and flaxseed, respectively (de Silva *et al.,* 2017). By the use of both enzymes in a continuous process, antibacterial and cholesterol-lowering peptides from chia have been released (Coelho *et al.,* 2018).

Fermentation

Bioactive peptides can be produced using fermentation starter bacteria from dairy products. Proteolytic system of this kind of microorganism is the way to release encrypted peptides from proteins sequences. This system is divided into three principal steps, the first one involves proteinase being bonded to the cell wall, next oligo-, tri-, and di- peptides formed by the proteinase are transported into the bacteria, where finally they are newly divided by a countless amount of endo-, amino-, tri- and di-peptidases (Savijoki *et al.,* 2006). This method has been used in different proteins, such as milk, meat, soybean, tomato, pea, and others, releasing peptides with multiple activities like ACE-I, antioxidant and antimicrobial, with the use of monocultures or a combination of different bacteria (Vermeirssen et al., 2003; Aguilar-Toalá *et al.,* 2017; Daliri *et al.,* 2018; Gallego *et al.,* 2018; Mechmeche *et al.,* 2019).

The effectiveness of bioactive peptides depends on two factors- their resistance to gastroin testinal degradation by peptidases and their absorption into blood stream, which depends on the peptide transporters (Peptide Transporter 1 PEPT1, for tripeptides; pinocytosis, for soluble peptides; paracellular, aqueous transport; transcellular routes). Based on these two factors, and the amino acidic sequence, the clinical results would be different (Aluko, 2015).

Bioactivity from amaranth protein

Amaranth is a rich protein food (> 15%), which makes it a good source for the release of bioactive peptides with different activities. Herein, different studies are described. Table 2 lists the methods through which bioactive peptides have been released from amaranth.

Antihypertensive peptides

Hypertension is a public health concern worldwide, with a prevalence of 30%. The use of conventional drugs has typical side effects, which is why the use of nutraceutical in the treatment and/or prevention of cardiovascular diseases, could

Table 2. Methods used to release peptides from amaranth.

Method	Bioactivity evaluated	Amaranth used	Reference
Simulated gastrointestinal digestion	Antioxidant	*Amaranthus mentagazzianus*	Delgado *et al.* (2011; 2015; 2016)
	Antihypertensive	*Amaranthus cruentus*	Tiengo *et al.* (2009)
	Antihypertensive	*Amaranthus hypochondriacus*	Quiroga *et al.* (2011); Barba de la Rosa *et al.* (2010)
	Anti-inflammatory	*Amaranthus hypochondriacus*	Montoya-Rodríguez *et al.* (2014a); Montoya-Rodríguez and González-Mejía (2015); Moronta *et al.* (2016a)
	Antithrombotic	*Amaranthus hypochondriacus*	Sabbione *et al.* (2016)
	Dipeptidyl peptidase IV inhibition	*Amaranthus hypochondriacus*	Velarde-Salacedo *et al.* (2012)
In vivo digestion	Cholesterol lowering	*Amaranthus cruentus*	Mendonça *et al.* (2009); Soares *et al.* (2015)
Enzymatic hydrolysis	Antioxidant	*Amaranthus mentagazzianus*	Tironi and Añón (2010)
	Antihypertensive	*Amaranthus mentagazzianus*	Fritz *et al.* (2011)
	Antihypertensive	*Amaranthus hypochondriacus*	Tovar-Pérez *et al.* (2009)
	Anti-inflammatory	*Amaranthus hypochondriacus*	Moronta *et al.* (2016b)
	Antithrombotic	*Amaranthus mentagazzianus*	Sabbione *et al.* (2015)
	Antioxidant, Antithrombotic, antihypertensive	*Amaranthus hypochondriacus*	Ayala-Niño *et al.* (2019a)
	Dipeptidyl peptidase IV inhibition	*Amaranthus hypochondriacus*	Soriano-Santos *et al.* (2015)
	Antitumor	*Amaranthus mentagazzianus*	Barrio and Añón (2010); Quiroga *et al.* (2015)
	Antitumor	*Amaranthus gangeticus*	Sani *et al.* (2004)
Protein isolation	Antitumor	*Amaranthus hypochondriacus*	Maldonado-Cervantes *et al.* (2010)
	Antihypertensive	*Amaranthus hypochondriacus*	Luna-Suárez *et al.* (2010)
	Antimicrobial	*Amaranthus caudatus* *Amaranthus retroflexus*	Broekaert *et al.* (1992); Lipkin *et al.* (2005)
	Insecticide	*Amaranthus hypochondriacus*	Valdes-Rodríguez *et al.* (1993); Chagolla-López *et al.* (1994)
In silico	Antihypertensive	Globulin 11S	Vecchi and Añón (2009)

hypothetically have economic saving in health expenditure (Borgui and Cicero, 2017). The present review also highlights the release of peptides with antihypertensive activity from different food matrixes.

Antihypertensive activity of peptides is one of the most studied bioactivities, and researchers have investigated them from different sources such as fish, milk, meat, and plant-derived proteins (Simonetti *et al.,* 2017; Bhat *et al.,* 2017; Ciau-Solís *et al.,* 2018; Yathisha *et al.,* 2019). Milk has been the most studied protein source of antihypertensive peptides, yielding the tripeptides Val-Pro-Pro and Ile-Pro-Pro, with high antihypertensive activity with dosages between 5 and 100 mg/day (Cicero *et al.,* 2016).

The first evidence of antihypertensive peptides from amaranth protein is from the study conducted by Vecchi and Añón (2009). In this *in silico* study, peptides from globulin 11S were screened in a peptide library, mapped via database-driven antihypertensive peptides, thus

yielding two potent and exposed tripeptides (Isoleucine-Lysine-Proline, IKP; Leucine-Glutamic Acid-Proline, LEP) showing an ACE inhibition of IC_{50} of 6.32 mM and 175 μM, respectively. This shows for the first time that amaranth protein was an antihypertensive peptides source. Once it was proven that antihypertensive peptides could be released from amaranth globulin, hydrolysates from amaranth whole proteic isolates, and their isolated proteins, such as albumin, glutelin, and globulin 11S and 7S have been realized. Additionally, fermentation of amaranth proteins has been realised with lactic acid bacteria in mono and combined culture, where greater ACE-i was obtained with the use of combined strains reaching inhibition percentage of 45.22 ± 0.28 (Ayala-Niño *et al.,* 2019a).

Hydrolysis with alcalase was carried out in globulin and albumin, obtaining peptidic fractions with low molecular weight (550 Da albumin; 400 Da globulin) with an IC_{50} of 636 μM and 375 μM, respectively (Tovar-Pérez *et al.,* 2009; Soriano-Santos *et al.,* 2015). They also observed that a more extensive hydrolysis showed negative results, and diminished ACE-i activity (Tovar-Pérez et al., 2009; Soriano-Santos et al., 2015). It has been proven that simulated gastrointestinal digestion from amaranth protein hydrolysates with alcalase does not significantly alter the ACE-i activity, having more of the double on activity when it is first hydrolysed with alcalase than just by gastrointestinal digestion, thus concluding that amaranth protein hydrolysates may be a good option as hypotensive product (Tiengo *et al.,* 2009). Also with the use of alcalase combined with flavourzyme, peptide structures with possible antihypertensive activity have been found such as NIDMLRL and LVRW (Ayala-Niño *et al.,* 2019b). Nevertheless, antihypertensive activity can be evaluated by different actions; the most common is the competitive and/or non-competitive inhibition of ACE. Other mechanisms of action are related to the increase in the activity of vasodilator agents such as endothelial nitric oxide (NO), inhibition of renin, or reducing the sympathetic system, thus inducing vasodilation (Aluko, 2015).

Induction of NO production through the inhibition of ACE has been evaluated in amaranth proteins. It has been shown that glutelin's tryptic hydrolysis induces endothelial NO production and vasodilation, with an IC_{50} value of 200 μg/mL; explaining for the first time the specific association of amaranth peptides with vascular physiology (Barba de la Rosa *et al.,* 2010). On the other hand, globulin 7S is a minor globulin component in amaranth which Quiroga *et al.* (2011) compared its properties against globulin 11S. In this study, they showed that it can be denatured at lower temperature, it has higher emulsifying properties, and solubility in neutral buffer, thus making it more suitable for food requirements, and by bioinformatics analysis they found that antihypertensive peptidic sequences were released from globulin 7S after a gastrointestinal digestion with an IC_{50} of 0.17 g/L.

As shown by Tovar-Pérez *et al.* (2009), alcalase is a suitable enzyme to release antihypertensive peptides, while Fritz *et al.* (2011) compared the action of different enzymes, such as papain, trypsin, chymotrypsin, and alcalase. They showed by *in vitro* studies that the best enzyme for the release of antihypertensive peptides was alcalase, with a dose-dependent effect in spontaneously hypertensive rats with IC_{50} of 0.12 mg/mL. Meanwhile, in other study, no changes in blood pressure were shown when rats were fed with no hydrolysed protein isolates (Lado *et al.,* 2015).

A new way to obtain bioactive peptides is by the modification of known molecules, thus obtaining diversified activities. In this sense, globulin 11S or amarantin is modified to obtain a higher antihypertensive activity. This is because its physicochemical properties in its acidic subunit make it available for changes. In order to improve its antihypertensive activity, the insertion of four Val-Tyr and one Ile-Pro-Pro antihypertensive peptides in the primary structure in the third variable region of globulin 11S was performed. The experiment was carried out through a plasmid expressed in *Escherichia coli* Origami and was called AMC3. Once the protein was expressed and purified, an *in vitro* gastrointestinal process was performed to validate if the peptides inserted were released; an eightfold higher activity was found as compared to the non-modified protein (IC_{50} 0.064 mg/mL) (Luna-Suárez *et al.,* 2010; Castro-Martínez *et al.,* 2012; Morales-Camacho *et al.,* 2016). When *in vivo* studies were performed, positive effects where observed in spontaneous hypertensive rats; the group that ingested a dose of 100 mg/kg of a previously hydrolysed (*in vitro* digestion) AMC3, had similar effects than the groups that were treated with captopril (Medina-Godoy *et al.,* 2013).

Antioxidant peptides

Antioxidant capacity in peptides has been related with the enzyme used to release them, the nature of the protein, the structure of the peptide, its molecular weight, and the hydrophobicity and amino acidic composition (Pihlanto, 2006; Udenigwe and Aluko, 2012). The exact mechanisms of how

antioxidant peptides work is not totally understood. Some studies have demonstrated its capacity to inhibit lipoperoxidation, to scavenge free radicals, to chelate metal ions or by avoiding oxidative damage by inducing genes that codify the production of endogenous enzymes (Sarmadi and Ismail, 2010; Undenigwe and Aluko, 2012). For instance, Chen *et al.* (1996) postulated that histidine, because of its imidazole groups position, is identified as an important hydrogen donator, peroxyl radical scavenger, and metal chelator; and hydrophobic amino acids increase antioxidant accessibility to cellular targets like polyunsaturated chain of fatty acids. On the other hand, tryptophan, tyrosine, and phenylalanine could donate protons to free radicals and chelate metal ions while cysteine and methionine, because their SH groups, could also scavenge radicals (Liang and Kitts, 2014; David-Birman *et al.,* 2018). The importance of antioxidant capacity lies on the prevention or oxidation delay of major biomolecules, preventing cell damage and related diseases to maintain cell components in reduced state (Tohma *et al.,* 2017).

Amaranth is a crop which contents different antioxidant compounds such as β-carotene, vitamin C, polyphenols, flavonoids, and fatty acids (Peiretti *et al.,* 2017; Sarker *et al.,* 2018). The main hydrolytic method used for the release of antioxidant peptides in amaranth has been the simulated gastrointestinal digestion (Delgado *et al.,* 2011; 2015; 2016). It has been observed that by the hydrolysis of amaranth proteins, an increase on soluble peptides was observed, which could be responsible for the antioxidant capacities. For the measurement of scavenging capacity, always a dose-dependent activity has been observed, showing higher IC_{50} values than that for Trolox (known antioxidant used as positive control) (Karamać *et al.,* 2019). The matter which was measured is a mixture of species with different antioxidant potency, including prooxidant molecules and others with high antioxidant capacity (Delgado *et al.,* 2011). Even when simulated, gastrointestinal hydrolysis had the ability to increase antioxidant capacity; a hydrolysis performed with alcalase according to Soriano-Santos and Escalona-Buendía (2015) and Tironi and Añon (2010) suggested that this enzyme has the capacity to enhance antioxidant peptides, releasing fractions with molecular size lower than 0.5 kDa with up to 66% of scavenging activity. When alcalase hydrolysis was added to an *in vitro* gastrointestinal digestion, no changes were observed in the antioxidant activity (Delgado *et al.,* 2015). Delgado *et al.* (2016) were further able to characterise four peptides: Ala-Trp-Glu-Glu-Arg-Glu-Gln-Gly-Ser-Arg (IC_{50} = 6.7 µg/mL), Tyr-Leu-Ala-Gly-Lys-Pro-Gln-Gln-Glu-His (IC_{50} = 16 µg/mL), Ile-Tyr-Ile-Glu-Gln-Gly-Asn-Gly-Ile-Thr-Gly-Met (IC_{50} = 71 µg/mL) and TEVWDSNEQ (IC_{50} = 20 µg/mL) from an *in vitro* gastrointestinal digestion.

According to studies, the presence of His and Pro residues are essential for the antioxidant effect, suggesting that specific amino acid residues in peptides chains play a significant role in antioxidant activity (Zou *et al.,* 2016).

Anti-inflammatory

Inflammation, which can be acute or chronic, is the answer of the host to invasion of foreign substances and/or inflammatory stimulus produced by different inflammatory mediators such as eicosanoids, vasoactive amines, cytokines, and chemokines (Serhan and Savilla, 2005). Even when acute inflammatory events are well described, chronic inflammation, particularly in chronic infections and autoimmune diseases, are not fully understood (Laveti *et al.,* 2013). Chronic inflammatory is related to a wide variety of diseases, such as asthma, cancer, cardiovascular diseases, Parkinson's, and others, which are associated with tissue malfunction (Scrivo *et al.,* 2011). Because of the relation between chronic inflammation and chronic diseases, recent studies are focused on the development of bioactive peptides with anti-inflammatory action based on cultured mammalian cells (especially macrophages) and chemically induced inflammation in animal models (Majumder *et al.,* 2016). Peptides derived from food sources such as milk, edible insects, eggs, and soybean have been tested for potential beneficial anti-inflammatory effects (Lin *et al.,* 2017; Meram and Wu, 2017). Figure 2 describes the anti-inflammatory effects that bioactive peptides might have.

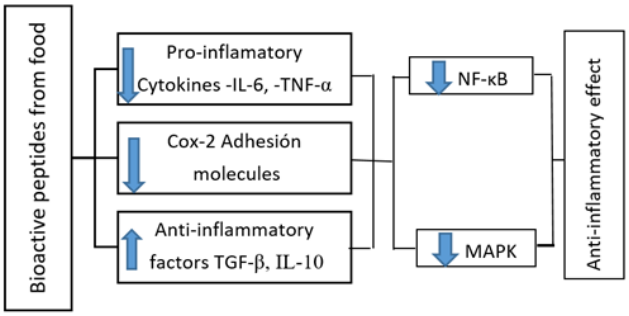

Figure 2. Anti-inflammatory peptides possible activity. Bioactive peptides from food may mediate Nuclear Factor-κB (NF-κB) and Mitogen-activated protein kinase (MAPK), by modifying cytokines -IL-6, -TNF-α, chemokines, and adhesion molecules.

In recent studies, amaranth has been investigated for peptides with anti-inflammatory effects, submitting it to different processes and evaluating diverse anti-inflammatory answers. The first evidence of anti-inflammatory peptides was observed after the extrusion of amaranth flour (Montoya-Rodríguez *et al.,* 2014a). After the flour was processed and passed through an *in vitro* digestion, anti-inflammatory activity in different inflammatory biomarkers increased, and yielded three peptides: His-Gly-Ser-Glu-Pro-Phe-Gly-Pro-Arg, Arg-Pro-Arg-Pro-Trp-Arg-Tyr-Thr, and Arg-Asp-Gly-Pro-Phe-Pro-Trp-Tyr-Ser-His. The first peptide showed a higher reduction in oxidised low-density lipoprotein receptor 1 (83%) and matrix metalloproteinase-9 (52%); and the second peptide had higher decrease in intracellular adhesion molecules-1 (39%). As a result of these studies, it was concludedd that extrusion is a technology that releases peptides with anti-inflammatory effects (Montoya-Rodríguez *et al.,* 2014a; 2014b; Montoya-Rodríguez and de Mejía, 2015).

Amaranth protein hydrolysed with alcalase has also shown anti-inflammatory activities. With this hydrolytic method, it was observed that a hydrolysis degree of 23 - 30% was ideal for the release of peptides with this bioactivity, thus reducing the expression of Chemokine Ligand 20, better known as CCL20, through the activation of Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway in activated colonic epithelial cells. The peptides responsible of such activity was identified as SSEDIKE, which also was proven not to be toxic, and to inhibit allergy reactions in mouse model, with suppression of IgE secretion and control of intestinal inflammation (Moronta *et al.,* 2016a; 2016b). These studies concludes that different methods could release different peptides from the same protein matrix.

Antitumor

Protein and peptide studies with anticancer potentials are an innovative strategy for cancer prevention and cure (Gaspar *et al.,* 2013; Chalamaiah *et al.,* 2018; Freitas *et al.,* 2019). This is because they possess advantages like low cost, high affinity, and strong specificity to target tissues, low toxicity, and less adverse side effects (Bhutia and Maiti, 2008; Silva-Sánchez *et al.,* 2008). Antitumor peptides act on different stages of cancer such as initiation, promotion, and progression (de Mejia and Dia, 2010), thus reducing tumour progression through multiple mechanisms including apoptotic, function-blocking, antiangiogenic, and immunomodulatory activities (Bhutia and Maiti, 2008; Hernández-Ledesma and Hsieh, 2017). Even though high anticancer activity has been evaluated in protein hydrolysates (Ayyash *et al.,* 2018; González-Montoya *et al.,* 2018), plants lectins and lunasins, which are glycoproteins of nonimmune origin distributed in seeds, roots, stems and leaves, have also shown high antitumor activity, as in the case of soy and amaranth (Moreira *et al.,* 1991; de Mejía *et al.,* 2003).

In amaranth, lunasin is present in all protein fractions (albumin, globulins, and prolamins), having higher concentration in amaranth glutein with 2.71 to 3.01 μg lunasin equivalent/g of protein (Silva-Sánchez *et al.,* 2008; Maldonado-Cervantes *et al.*, 2010). Lunasin is a peptide with 43 amino acids; its carboxyl-end contains nine aspartic acids residues, an Arg-Gly-Asp (RGD) cell adhesion motif, and a helix with structural homology to chromatin-binding proteins (De Lumen, 2005). It has demonstrated cancer preventive properties against mammalian cell culture models and in skin cancer mouse model against chemical carcinogens, oncogenes and inactivators of tumour suppressor proteins (De Lumen, 2005). It has been found in soy, barley and wheat (Jeong *et al.,* 2002; 2007; González de Mejía *et al.,* 2004).

Amaranth lunasin administration in mammalian cells showed a faster nucleus penetration as compared to the one reported by soy lunasin, and it has shown 38.8% of apoptosis in HeLa cells, 5.0% in fibroblast cells with a glutelin concentration of 5 μ g/mL, and inhibition of histone acetylation thus inhibiting the transformation of mouse embryo fibroblast cells (NIH-3T3 cells) to cancerous foci (Silva-Sánchez *et al.,* 2008; Maldonado-Cervantes *et al.,* 2010). Amaranth protein hydrolysates with alcalase and trypsin have also shown antitumor activity, where specific structured peptides different to lunasin have this bioactivity and they also have antiproliferative activity against mouse osteoblast precursor cell MC3T3E1, rat bone with osteosarcoma UMR106, human heterogeneous epithelial colorectal adenocarcinoma Caco-2, and human homogeneous epithelial colorectal adenocarcinoma TC7 cells (Silva-Sánchez *et al.,* 2008; Barrio and Añón, 2010; Quiroga *et al.,* 2015).

Other bioactivities

In amaranth, antithrombotic peptides have been found in its hydrolysates using alcalase and trypsin, or *in vitro* digestion using trypsin and pancreatin. In both studies, an increase in clotting inhibition was observed, having higher bioactivity in

glutalin hydrolysates, and in fractions with molecular weight lower than 4 kDa, having the ability to be absorbed through the intestinal epithelium (Sabbione *et al.,* 2015; 2016). Cholesterol lowering activity by different methods has also been proven. By *in vitro* digestion, the release of three peptides with HMG-CoA reductase (3-hidroxi-3-metilglutaril-coenzime A reductase) inhibitory activity was characterised: Gly-Gly-Val, Ile-Val-Gly or Leu-Val-Gly, and Val-Gly-Val-Ile (Soares *et al.,* 2015). When amaranth proteins were administrated to hamster with hypercholesterolemia, they showed 27% in the reduction of plasma cholesterol, while the group fed with milk casein showed a reduction of 48% (Mendonça *et al.,* 2009), showing similar results with Wistar rats (Lado *et al.,* 2015). In both *in vivo* studies, the possible mechanism of cholesterol reduction in plasma was the increase in the faecal cholesterol excretion.

Diabetes is a metabolic disorder characterised by high levels of glucose in plasma. It affects over 422 million people around the world, and its prevalence is rapidly rising in middle- and low-income countries (WHO, 2016). Some of the possible treatments is the use of incretin-based therapy which is peptidic hormones released by intestinal enteroendocrine cells to the bloodstream in response to nutrient intake where they stimulate insulin secretion. However, these have short life because of their inactivation by dipeptidyl peptidase IV (DPPIV). New interest in DPPIV inhibitors arises which have shown promising results as antidiabetic agents (Ojeda-Montes *et al.,* 2018; Liu *et al.,* 2019). Amaranth protein hydrolysates from an *in vitro* digestion were proven for this bioactivity, yielding inhibition of DPPIV with IC_{50} of 1.1 mg/Ml in a dose-dependent manner. *In silico* analysis identified the tripeptide Iso-Pro-Glu as the inhibitor (Velarde-Salcedo *et al.,* 2013). When amaranth proteins hydrolysed with alcalase are used in diabetic mice, it improved their glucose tolerance, with remarkable increments in plasma insulin was observed (Soriano-Santos *et al.,* 2015).

Not only health promoting peptides have been proven from amaranth proteins, insecticide against insect larvae (*Tribilium castaneum* and *Prostephanus trancatus*), and antimicrobial, against different fungi (*Fusarium culmorum* (Smith) Sacc., *Helminthosporium sativum* Pammel., King et Bakke, *Alternaria consortiale* Fr., and *Botrytis cinerea* Pers.,) have been released and tested from different amaranth plants such as *Amaranthus retroflexus, A. caudatus,* and *A. hypochondriacus*. These peptides are able to inhibit larvae trypsin or α amylase from insects; or the growth of the tested fungi, being more effective than other chemical insecticides or antimicrobial products (Broekaert *et al.,* 1992; Valdes-Rodríguez *et al.,* 1993; Chagolla-López *et al.,* 1994; Lipkin *et al.,* 2005).

Conclusion

Research towards novel bioactive peptides' discovery is currently under way and will be helpful to discover functional and benefits to human health in order to improve the value of amaranth. Although this kind of grain is an attractive source of bioactive peptides, continuous isolation has limited their application in new functional foods. In order to resolve the current problems and commercial applications, more attention should be provided. Furthermore, it is necessary to establish relationship between concentration, activity and chemical structure to explore further the mechanism of their *in vivo* biological functions. Food science and technology have various new challenges and opportunities alike. An example includes new optimised process to obtain new peptide sequences. These looming challenges will be addressed and opportunities captured in sciences and technology options to strengthen the industry and increase the value of traditional food.

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