Comprehensive REVIEWS in Food Science and Food Safety

Identification of Bioactive Peptide Sequences from Amaranth (*Amaranthus hypochondriacus*) Seed Proteins and Their Potential Role in the Prevention of Chronic Diseases

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Abstract: Amaranth (*Amaranthus hypochondriacus*) is a pseudocereal with higher protein concentration than most cereal grains. Enzymatic hydrolysis and food processing could produce biopeptides from amaranth proteins; however, there is limited information about the bioactivity of peptides from amaranth proteins. The objective of this comprehensive review was to determine bioactive peptide sequences in amaranth proteins that may prevent cardiovascular disease, cancer, and diabetes. Amaranth proteins, reported in UniProt database, were evaluated for potential bioactive peptide using BIOPEP database. The 15 main proteins present in amaranth seed are 11S globulin, 7S globulin, α-amylase inhibitor, trypsin inhibitor, antimicrobial proteins, nonspecific lipid-transfer-protein-1, superoxide dismutase, ring-zinc finger protein, prosystemin, amaranth albumin 1, glucose-1-phosphate adenyltransferase, glucosyltransferase, polyamine oxidase, granule-bound starch synthase 1, and acetolactate synthase. All proteins showed high occurrence frequencies of angiotensin-converting enzyme-inhibitor peptides (A = 0.161 to 0.362), as well as of dipeptidyl peptidase IV inhibitor (A = 0.003 to 0.087). Other proteins showed antioxidative (A = 0.012 to 0.063) and glucose uptake-stimulating activity (A = 0.023 to 0.042), and also antithrombotic (A = 0.002 to 0.031) and anticancer sequences (A = 0.001 to 0.042). The results of this study support the concept that amaranth grain could be part of a "healthy" diet and thereby prevent chronic human diseases.

Keywords: amaranth, bioactive peptides, chronic disease

Introduction

History, classification, and botanical description of amaranth

Amaranth belongs to the order Caryophyllales, Amaranthacea family, subfamily Amaranthoideae, genus Amaranthus (Grobelnik-Mlakar and others 2009; Délano-Frier and others 2011). The *Amaranthacea* family has 70 genera and more than 80 species. The 3 principal species that produce grains are *Amaranthus hypochondriacus* (native of México), *Amaranthus caudatus* (native of Peru), and *Amaranthus cruentus* (native of México and Guatemala) (Milán-Carrillo and others 2012b; López-Mejia and others 2014). It is a herbaceous plant rising 0.3 to 5 meters in height with an erect stem and enormous inflorescence (Kigel 1994; Rastogi and Shukla 2013). The principal parts of the amaranth plant are roots, stem, leaves, inflorescences, and seeds (Rastogi and Shukla 2013). Amaranth grain was the base of the human diet in pre-Columbian civilizations (Milán-Carrillo and others 2012a). Aztec, Incan, and Mayan civilizations used amaranth in their diets (Pavlik 2012). *A. hypochondriacus* is the principal amaranth species cultivated in Mexico since pre-Columbian times, where Aztecs used amaranth as food. Also, they used amaranth in their religious practices, and for that reason, when Spaniards arrived in America, they banned amaranth, ignoring its nutritional and agricultural features. Spaniards prohibited amaranth because pre-Columbian civilizations used it during their religious events, mixing amaranth with human blood, because they believed it gave them strength (Borneo and Aguirre 2008; Rastogi and Shukla 2013).

Agronomical importance of amaranth

The amaranth grain has gained interest in the past 20 years due to its nutritional and agricultural features (Zapotoczny and others 2006; Khandaker and others 2010; Velarde-Salcedo and others 2013). Amaranth is a C4 plant, meaning it loses less water by transpiration and uses the carbon dioxide (CO_2) very efficiently

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(Zapotoczny and others 2006; Délano-Frier and others 2011). The agronomic importance of amaranth is that it is a fast-growing plant, has tolerance to drought conditions, can grow in poor soils, and can be cultivated throughout the year (Brenner and others 2000; Avanza and others 2005). These features make amaranth an important crop that can be utilized in regions where conventional crops cannot grow. Nowadays, amaranth is cultivated in many parts of the world, including South America, Africa, India, China, and the USA (Aguilar and others 2013).

Amaranth seed

The amaranth seed, a dicotyledonous product, is composed of the seed coat, which is a very thin layer of cells; the 2 cotyledons, which is the richest in protein; the perisperm, a layer rich in starch; the endosperm; the procambium; the radicle; and the root (Figure 1) (Irving and others 1981; Grobelnik-Mlakar and others 2009; Quiróga and others 2010).

The seed is very small, measuring 1 to 1.5 mm in diameter and the number of seeds per gram varies between 1000 and 3000. Seeds are circular in shape and present around 19 colors, including white, black, yellow, gold, pink, and red. All wild species are black and they have very hard covers.

Amaranth chemical composition

Amaranth grain possesses a higher protein concentration than the common cereals. This pseudocereal has a protein content of 13% to 19%, which is distributed in the endosperm, containing 35% of the total grain protein, while the remaining protein is present in the coat and in the germ (Bressani 2003). Amaranth also is a good source of lipids (5% to 13%), minerals such as Ca, Fe, Mg, Mn, K, P, S, and Na, and vitamins of B complex. The principal component of amaranth is starch (62%) (Alvarez-Jubete and others 2010b; Ferreira and Gómez-Areas 2010; Repo-Carrasco-Valencia and others 2010). Besides its nutritional features, some bioactive

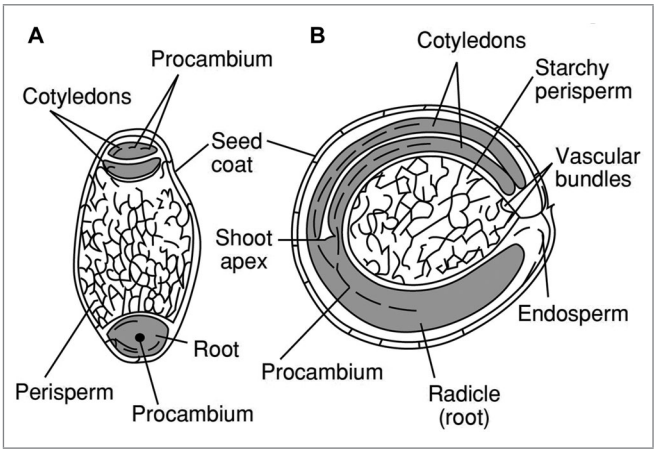

Figure 1–Amaranth seed in (A) cross- and (B) longitudinal sections as viewed in a light microscope. Source: Irving and others (1981).

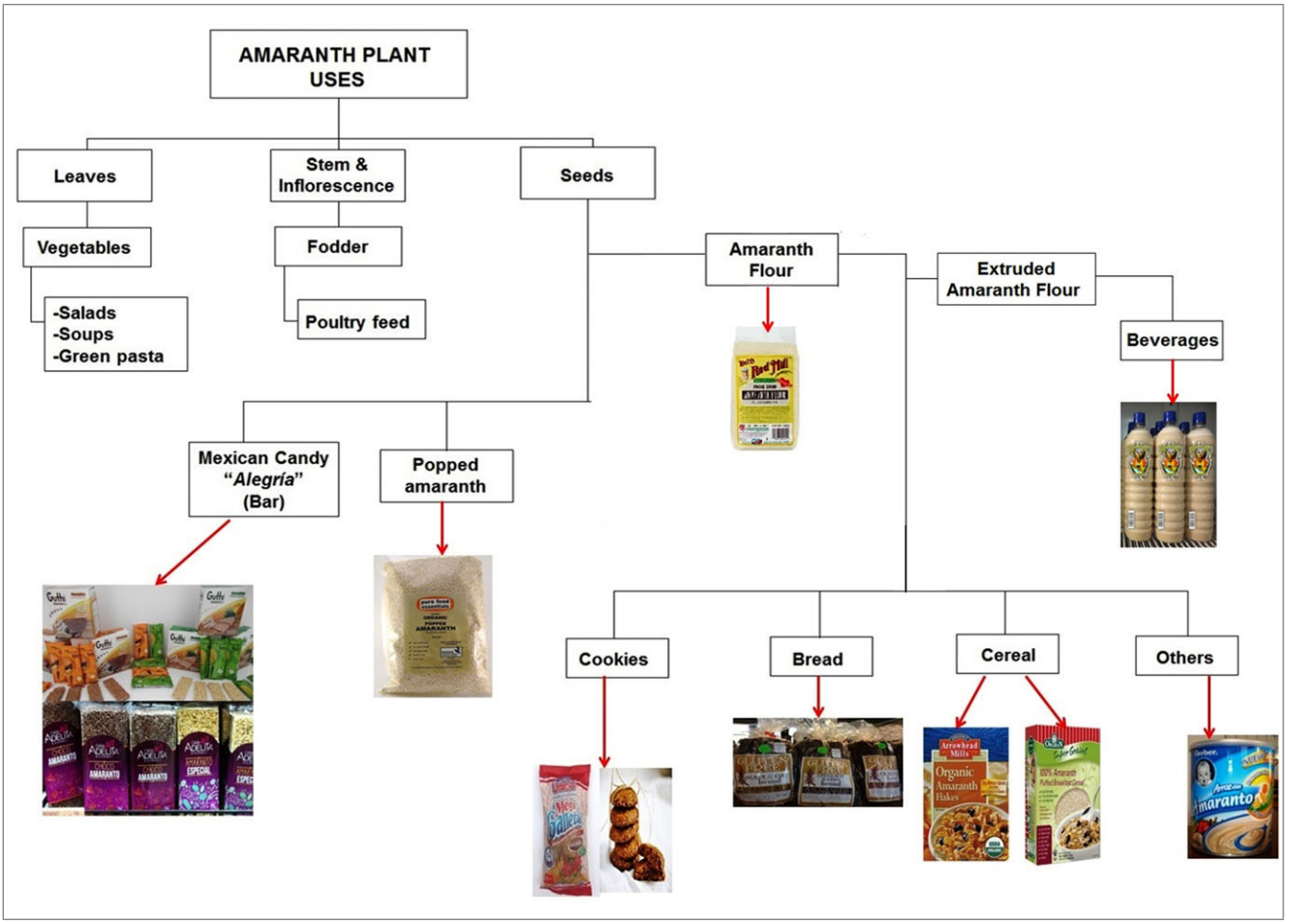

Figure 2–Uses of amaranth plant and some products obtained from amaranth grains.

Table 1–Concentrations of essential amino acids in grains of different amaranth species in comparison with some other crops.

	Amino acids (g/100 g of protein)									
Protein source	Trp	Met/Cys	Thr	Ile	Val	Lys	Phe/Tyr	Leu	LAA[A]	EAA[B]
FAO/WHO (1973)	1.0	3.5	4.0	4.0	5.0	5.5	6.0	7.0	–	–
Amaranth (average)[a]	1.3	4.4	2.9	3.0	3.6	5.0	6.4	4.7	67	87
A. cruentus[b]	–	4.1	3.4	3.6	4.2	5.1	6.0	5.1	84	89
A. cruentus[c]	0.9	4.6	3.9	4.0	4	6.0	7.9	6.2	88	95
A. cruentus[c]	–	4.6	3.9	4.0	4.5	6.1	8.5	6.1	87	96
A. caudatus[c]	1.1	4.9	4.0	4.1	4.7	5.9	8.1	6.3	90	98
A. hypochondriacus[d]	1.8	0.6	3.3	2.7	3.9	5.9	8.4	4.2	34	78
A. cruentus[e]	1.4	4.1	3.4	3.6	4.2	5.1	6.0	5.1	73	91
Amaranth (average)[a-e]	1.3	4.5	3.5	3.6	4.2	5.6	7.3	5.4	75	91
Barley[a]	1.2	3.2	3.2	4.0	4.7	3.2	8.2	6.5	83	97
Buckwheat[a]	1.4	3.7	3.9	3.8	5.2	5.9	5.8	5.8	83	97
Maize[a]	0.6	3.2	4.0	4.6	5.1	1.9	10.6	13.0	35	86
Oat[a]	1.2	3.4	3.1	4.8	5.6	3.4	8.4	7.0	62	92
Rice[a]	1.0	3.0	3.7	4.5	6.7	3.8	9.1	8.2	69	94
Soya[a]	1.4	3.1	3.9	5.4	5.3	6.3	8.1	7.7	89	98
Wheat[a]	1.2	3.5	2.7	4.1	4.3	2.6	8.1	6.3	47	86

Sources: [a]Senft (1979);
[b]Betschart and others (1981);
[c]Becker and others (1986);
[d]Dodok and others (1997);
[e]Sánchez-Marroquin and others (1986). **A** = relative value of limited amino acid according to FAO/WHO requirements. **B** = relative value of essential amino acids according to FAO/WHO requirements. Adapted from Grobelnik Mlakar and others (2009).

compounds are present in amaranth grain including flavonoids, phenolic acids, anthocyanins, tannins, and phytosterols (Alvarez-Jubete and others 2010a; Pasko and others 2011). The principal characteristic of amaranth is that it has a high concentration of proteins with excellent nutritional quality (Quiróga and others 2010). The main proteins present in amaranth grain are globulins and albumins (Tovar-Pérez and others 2009). There are some reports about its fiber content. Milán-Carrillo and others (2012a) reported total dietary fiber (soluble and insoluble) between 13.9% and 14.6% for extruded and unprocessed amaranth flour, respectively.

Amaranth uses

Amaranth grain has been used in a wide variety of foods. From the whole grain, tasteful soups, stews, sauces, porridges, and soufflés can be prepared. Boiled grains can be used like rice and couscous, which is traditionally made with semolina of wheat. When amaranth grains are boiled, the starch is leaching out and is gelatinized. This causes the cooking water to thicken with pronounced porridge structure formation. It often occurs that the embryo-encircled gelatinous perisperm is separated during cooking (Mújica-Sánchez and others 1997; Rastogi and Shukla 2013). Also, amaranth grain can be used as an ingredient after processing by extrusion, germination, popping, or alkaline process (nixtamalization) (Milán-Carrillo and others 2012a). The entire amaranth plant could be used to prepare different foods, with the seed as the principal part used to prepare human food. Flour from the seed is used to prepare bread, cookies, amaranth candy, ready-to-eat cereals, and popped amaranth, among others. Stems and leaves normally are used for animal feed (Borneo and Aguirre 2008). Figure 2 shows different uses of the whole amaranth plant.

The objective of this review was to determine potential bioactive peptides in amaranth proteins that may prevent cardiovascular disease, cancer, and diabetes. Amaranth proteins, reported at UniProt database, have been evaluated for potential bioactive peptides using BIOPEP database. In UniProt there are 5 types of evidence for the existence of a protein: (1) evidence at a protein level, which indicates that there is clear experimental evidence for the existence of the protein. The criteria include partial or complete Edman sequencing, clear identification by mass spectrometry, X-ray, or nuclear magnetic resonance structure, good-quality protein–protein interaction, or detection of the protein by antibodies; (2) evidence at a transcript level, which indicates that the existence of a protein has not been strictly proven but that expression data (such as existence of complementary deoxyribonucleic acids, real-time polymerase chain reaction, or Northern blots) indicate the existence of a transcript; (3) evidence inferred from homology, which indicates that the existence of a protein is probable because clear orthologs exist in closely related species; (4) evidence is predicted, which is used for entries without evidence at any other level; and (5) evidence is uncertain, which indicates that the existence of the protein is unsure. In this report, only the highest or most reliable level of supporting evidence for the existence of a protein for each entry was used. For example, if the existence of a protein was supported by both the presence of expressed sequence tags and direct protein sequencing, the protein was assigned the value evidence at a protein level. The protein existence value was assigned automatically when based on the annotation elements present in the entry. In the case of the information of the sequence protein existence, it may happen that the sequence slightly differs from the genomic sequences, especially for sequences derived from gene model predictions.

Amaranth Proteins

Nutritional quality of amaranth proteins

Proteins from animal sources such as eggs, milk, and meat are the best sources of protein with high quality. However, they have a high cost and in some cases produce some allergies or intolerances. Plant proteins can be substituted for them either partially or completely (Tavano and others 2008; Shevkani and others 2014).The grain of amaranth presents a high-quality protein with an excellent amino acid balance, which is better than that of cereals and some legumes (Shevkani and others 2014). The protein in amaranth grains (13% to 19%) has high digestibility (90%) (Grobelnik-Mlakar and others 2010).

Proteins from amaranth are rich in lysine ranging from 4.9 to 6.1 g lys/100 g protein, a limiting amino acid in cereals (Grobelnik-Mlakar and others 2009). Amaranth protein is also a good source

Table 2–Proteins from amaranth (*Amaranthus hypochondriacus*) with molecular mass between 3 and 30 kDa.

Protein name	ID	Sequence[a]	AAR	MM (Da)
Alpha-amylase inhibitor1	P80403	CIPKWNRCGPKMDGVPCCEPYTCTSDYYGNCS	32	3592
Trypsin inhibitor[b]	Q7M1Q2	ARECPGKQEWPELVGEYGYKAAAIIERENPNVR DIVKHERSYGFTKDFRCDRVWVVVDYTGVVVRT YPRVT	71	8319
Antimicrobial protein[c]	Q71U16	MVNMKCVALIVIVMMAFMMVDPSMGVGECVRGR CPSGMCCSQFGYCGKGPKYCGRASTTV DHQADVAATKTAKNPTDAKLAGAGSP	86	8912
Nonspecific lipid-transfer protein 1[b]	P83167	AVTCTVVTKALGPCMTYLKGTGATPPPANCCAG VRSLKAAAQTVADRRMACNCMKSAAQKTKSLNYK VAARLASQCGVRMSYSVSPNVNCNSVQ	94	9747
Superoxide dismutase [Cu-Zn][d]	F6JRN6	MGKGVTVLNSSEGVTGTIYFTQEGDGPTTVSGN ISGLKPGLHGFHVHALGDTTNGCMSTGPHFNPAGKE HGSPEDDVRHAGDLGNITAGDDGTATFTLIDSQI PLSGANSIVGRAVVVHADPDDLGRGGHE LSKTTGNAGGRIACGIIGLQG	152	15199
RING zinc finger protein[d]	F8RNK2	MGDSHSPNYNLAPSSFNDQQISYNYNISMLY CGFFVVATAGLVLAIYHCLALNWCSDYPPVWLRT AQTGPTEQQCQARKVIEFNSIRYKYKKGEMGTNNEE CVVCLSGFEEEEDIRKLVKCKHSFHALCIDM WLFSHFDCPLCRAPVAVAVAVCS VARLDSSGSELSDSANLV	173	19355
Prosystemin[c]	Q5UAW5	MISKPKEMTMQEEPKVKLHHEKGGDEKEK IIEKETPSQDINNKDTISSYVLRDDTQEIPKMEHEEGGY VKEKTVEKETISQYIIKIEGDDDAQEKLKVEYE EEEYEKEKIVEKETPSQDINNKGDDAQEKP KVEHEEGDDKETPSQDIIKMEGEGALEITKVVCEKI IVREDLAVQSKPPSKRDPPKMQTDNNKL	195	22511
Cystatin[d]	Q0GPA4	MLIKFSFLLPHSSTILLLFSLIFFFSPSSQ GSCSDFESEPSMATLGGLRESQGAANDAEIESL ARFAVDEHNKKENALLEFARVVKAKEQVVAGTLHHFT IEAIDAGKKKLYDAKVWVKPWMNFKELQEFKHTE DSPSFTSSDLGAIREGHAPGWKEVPVHDPEVQNAAEHA VKTIQQRSNSLFPYELQEIAHAKAEVVEDTAKFNLHL KVKRGNKDEIFNVEVHKSSDGNY NLNKMGNIQPEIENQ	247	27736

[a] 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; threonine; 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; X, undetermined amino acid. Protein sequence was obtained from UniProt database (http://www.uniprot.org). **AAR** = Amino acid residues; **MM** = Molecular mass.
[b] Evidence at protein level;
[c] Predicted sequence;
[d] Evidence at transcript level;
[e] Sequence inferred from homology.

of tryptophan and sulfur-containing amino acids, which normally are limiting in other grains (Morales de Leon and others 2005; Awasthi and others 2011). Amaranth proteins are found in the embryo (65%) and only 35% in the perisperm, whereas in other grains amino acids are found in endosperm and are poorer in essential amino acids (Grobelnik-Mlakar and others 2010). Table 1 shows a comparison of the amino acid composition of amaranth grain with different species and some other crops such as maize, wheat, and oat (Senft 1979; Betschart and others 1981; Becker and others 1986; Sánchez-Marroquin and others 1986; Dodok and others 1997). The balanced amino acid composition of amaranth is close to the optimum protein reference pattern in the human diet according to FAO/WHO requirements (Grobelnik-Mlakar and others 2009; Rastogi and Shukla 2013). The combination of amaranth and maize flour in a ratio of 50:50 almost reaches the 100 score (Grobelnik-Mlakar and others 2009). The limiting amino acids in amaranth are leucine, isoleucine, and valine. However, this is not a serious problem since these are in excess in most common grains (Grobelnik-Mlakar and others 2009). The good amino acid balance of the amaranth protein, almost reaching the FAO/WHO requirements, suggests that it could be used in a mixture or combination with cereals to improve the quality of the protein and have a superior nutritive value (Gorinstein and others 2002). Also, amaranth proteins do not contribute to intolerances or allergic reactions in people with celiac disease or gluten intolerance, due to the fact that amaranth is a gluten-free grain (Alvarez-Jubete and others 2010b).

Globulins, glutelins, and albumins

One way to classify proteins is by solubility. In this category, albumins, globulins, and glutelins are found (Damodaran 2008). The main proteins in amaranth grains are globulins and albumins (Quiróga and others 2007). Silva-Sánchez and others (2008) evaluated the peptides present in amaranth grains. These authors separated the proteins and reported the presence of globulins, glutelins, and albumins. Montoya-Rodríguez and others (2014a) reported the presence of the same kind of proteins before and after the extrusion process.

Globulin 11S

Globulins constitute the principal protein fraction present in amaranth isolates, with globulin 11S, also called amarantin, the principal constituent (Quiróga and others 2009). This protein was characterized by Barba de la Rosa and others (1996). This is a protein with 501 amino acid residues and a molecular mass of 56 kDa (Barba de la Rosa and others 1996). The globulin 11S is the main grain storage protein in amaranth (Condés and others 2009).

Globulin 7S

Globulin 7S is present in amaranth protein isolates in a lower quantity than globulin 11S; it is also less studied than 11S (Tandang-Silvas and others 2010; Quiróga and other 2012). Quiróga and others (2010) described the globulin 7S as formed by 4 subunits of 66, 52, 38, and 16 kDa, with a molecular mass near 200 kDa. Garcia-Gonzalez and others (2013) reported that

Table 3–Proteins from amaranth (*Amaranthus hypochondriacus*) with molecular mass between 30 and 56 kDa.

Protein name	ID	Sequence[a]	AAR	MM (Da)
Seed protein AmA1 (Amaranth Albumin 1)[c]	Q8S390	MAGLPVIMCLKSNNNQEYLRYQSDNIQQYGL LQFSADKILDPLAQFEVEPSKTYDGLVHIKSRYTN KYLVRWSPNHYWITASANEPDENKSNWACTLF KPLYVEEGNMKKVRLLHVQLGHYTE NYTVGGSFVSYLFAESSQIDTGSKDVFHVIDWKS IFQFPKTYVTFKGNNGKYLGVITINQLPCLQ FGYDNLNDPKVAHQMFVTSNGTICIKSN YMNKFWRLSTDNWILVDGNDPRETNEAAA LFRSDVHDFNVISLLNMQKTWFIKRFTSGKP EFINCMNAATQIVDETAILEIIELGSNN	304	34959
Glucose-1-phosphate adenyltransferase[d]	J9PE35	MTVTGAITVPSSNSMTNLAFSSSSLSGDKF QSVSFLNRQNSRIFSDARRTPNVVSPKAVSDSK NSQTCLDPEASRSVLGIILGGGAGTRLYPLTKKRAKP AVPLGANYRLIDIPVSNCLNSNISKIYVLT QFNSASLNRHLSRAYASNMGGYKNEGFVEVLAAQ QSPENPNWFQGTADAVRQYLWLFEEHNVL EFLALAGDHLYRMDYERFIQAHRE TDADITVAALPMDENRATAFGLMKIDEE GRIIEFAEKPKGEQLKAMKVDTTILGLDD KRAKEMPYIASMGIYVISKDVMLNLLRDQF PGANDFGSEIIPGATSVGMRVQAYLYDGYWED IGTIEAFYNANLGITKKPVPDFSFYDRSSPIY TQPRYLPPSKMLDADITRQCYR	390	43392
Glucosyltransferase[d]	X4Y205	MDDDELQKLHVVFFPFMAYGHMIPTLDIARLFA ARGVKTTIITTPVSLPIVTQAIEKAIKHGSPAIYT EIFSFPSAENGLPDGCETVNQAIKYYMIPKFMQAVE MLNTPLEQYLEKTRPHCLVSDMFLPWTTDCAA KFNVPRLVFHGTSYFALCAEEIVRVYKPYKN VSNDEETFILPSLPHEVKMTKSQFSEDFMKEELNES KKEFELIKESEIKSYGVI VNSFYELERDYADFFSKELGRRAWHIGPVSL CNRSIEDKAKRGILEASKDEHECLKWLNSKKT NSVIYICFGSMAQINASQMLEIAMGLEASQH DFIWVVKNDRQSEEELLPQ GFEQRMEGKGLIIRGWVPQLLLLEHEAIGAL LTHCGWNSILEGISTGLPMVTWPACTEQFYN EKLVTEILKIGVPVGAKKWNVVPYNVDYLVRRNAI EKAIREVMEGDEAQERRNR AMKLKEMALKAVEVDGSSYNDLGVLI NELRHNKLKVV	487	55652
Polyamine oxidase[d]	Q8LL67	MRKINKVEAMKFLLFLVMGLLVSLISASSYPSV IVIGAGMSGISAAKTLHDNNIKDFIILEATNRI SGRIHKTEFAGYTVEKGANWLHGAEGPEKNPMYEI AEKINLKNFYSDFSNVSLNTYKQNGEKYS MEEVEAAIALADDNEEFGTKLAEQFSANTK EDDDMSLLAAQRLNKKEPKTILERMVDFYFNDGE QAEAPRVSSLKHILPRPEFSLYG DGEYFVADPRGFEGITHTIAKSFLSYTNHTVT DPRLMFNQVVTEIEYKRRSVTVKTEDGN VYKAKYVIVSPSLGVLQSDLITFTPELPLWK RRAISEFSIGIYTKIFLKFPYKFWPT GPGTEFFFYVHARRGYYAIWQQLENEYPGSNILF VTVADEESKRVEQQPDEVTKAEAMEVLRKIFGE DIPEATDIMIPRWYSDRFYRGTFTNWPVGYTNKK HKNLRAPVGRVFFTGEHTHPELFGYA DGAYFAGITTANDILARLK GGILPWHNQDMKLMKI	496	56580

[a] 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; threonine; 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; X, undetermined amino acid. Protein sequence was obtained from UniProt database (http://www.uniprot.org). **AAR** = Amino acid residues; **MM** = Molecular mass.
[b] Evidence at protein level;
[c] Predicted sequence;
[d] Evidence at transcript level;
[e] Sequence inferred from homology.

the globulin 7S is composed of 3 main subunits called α (57−68 kDa), α' (57−72 kDa), and β (42−52 kDa). These are bound by noncovalent bounds to form a trimer with a molecular mass of approximately 170 to 200 kDa. Each subunit has one or two N-linked glycosyl groups. The trimmer structure is stabilized in high-ionic-strength solutions.

Other important amaranth grain proteins

Besides the previously mentioned proteins, there are some other important proteins present in amaranth that are involved in important pathways for the seed to exist (http://www.uniprot.org/) (Table 2 to 4). Some of these proteins reported in UniProt have inhibitory activities such as α-amylase inhibitor, trypsin inhibitor, cystatin, and polyamine oxidase (Valdes-Rodríguez and others 1993; Chagolla-López and others 1994; Wang and others 2002; Valdes-Rodríguez and others 2007). Nonspecific lipid-transfer protein 1 and glucosyltransferase are involved in phospholipids transfer and glycosyl group transfers across membranes, respectively (Ramírez-Medeles and others 2003; Casique and others 2014). Nonspecific lipid-transfer protein 1 regulates the cutin or wax deposition in the cell walls of expanding epidermal cells and certain secretory tissues (Ramírez-Medeles and others 2003). Superoxide dismutase protein plays a critical role in destroying radicals which are normally produced within the cells and which are toxic to biological systems in the seed (León-Galván and others 2009). Proteins such as granule-bound starch synthase 1

Table 4–Proteins from amaranth (*Amaranthus hypochondriacus*) with molecular mass between 56 and 73 kDa.

Protein name	ID	Sequence[a]	AAR	MM (Da)
11S globulin seed storage protein[b]	Q38712	STHASGFFFFHPTKMAKSTNYFLISCLLFVLFNGCMGEGRFREFQ QGNECQIDRLTALEPTNRIQAERGLTEVWDSNEQEFRCAGVSV IRRTIEPHGLLLPSFTSAPELIYIEQGNGITGMMIPGCPETYESGSQ QFQGGEDERIREQGSRKFGMRGDRFQDQHQKIRHLREGDIFAM PAGVSHWAYNNGDQPLVAVILIDTANHANQLDKNFPTRFYLA GKPQQEHSGEHQFSRESRRGERNTGNIFRGFETRLLAESFGVSEEI AQKLQAEQDDRGNIVRVQEGLHVIKPPSRAWEEREQGSRGSRY LPNGVEETICSARLAVNVDDPSKADVYTPEAGRLTTVNSFNLPI LRHLRLSAAKGVLYRNAMMAPHYNLNAHNIMYCVRGRGRIQI VNDQGQSVFDEELSRGQLVVVPQNFAIVKQAFEDGFEWVSFKT SENAMFQSLAGRTSAIRSLPIDVVSNIYQISREEAFGLKFNRPETT LFRSSGQGEYRRKISIA	501	56672
Granule-bound starch synthase I[e]	D6RSA4	METVTSSHFVSNFANTAMGSSDPKLTLANNALKSNQMSTHNGLR PLMSNIDMLRLSNNPKSTTVELRKERFHAPFIRSGMNVVFVGAE VAPWSKTGGLGDVLGGLPPALAARGHRVMTVSPRYDQYRDG WDTSVTVEFQVGNRTETVRYFHTYKRGVDRIFVDHPLFLARV WGITGSKLYGPKAGADYEDNQLRFSLLCQAALEAPRVLNLNNN PNFSGPYGENVVFIANDWHTALLPAYLKAIYQPKGIYNNAKVA FCIHNIVYQGRFALADYPRLHLPEELRPVFEFMDGYDRPIKGRK INWMKAGILQSDRVVTVSPYYAQELISGVERGVELDDVVRQTG VTGIVNGMDVQEWNPITDKYIGINFNITTVMTAKPLIKEALQAE VGLPVDRNIPLIGFIGRLEEQKGSDILAEAIPRFIKENVQIVVLGTG KEVMEKQIEQLEILYPEKARGVTKFNSPLAHMIVAGADFMLIPSR FEPCGLIQLYSMRYGTVPVVASTGGLVDTVKEGYTGFHMGRFS ANCDMVDPADISAVETTVHRALTTYNSPAMREMVINCMTQD FSWKEPARKWEELLLSLGVAGSRPGFEGTESIPLATENIATP	606	67320
Acetolactate synthase[e]	A7LIU5	MASNSSNPPFFYFTKPYKIPNLQSSIYAIPFSNSLKPTSSSSIPRRPLQ ISSSSSQSPKPKPPSATITQSPSSLTDDKPSSFVSRFSPEEPRKGCD VLVEALEREGVTDVFAYPGGASMEIHQALTRSNIIRNVLPRHEQ GGVFAAEGYARATGRVGVCIATSGPGATNLVSGLADALLDSVP LVAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLVLDVEDIPR IVKEAFFLANSGRPGPVLIDIPKDIQQQLVVPNWEQPIKLGGYLSR LPKPTYSANEEGLLDQIVRLVGESKRPVLYTGGGCLNSSEELRKFV ELTGIPVASTLMGLGAFPCTDDLSLHMLGMHGTVYANYAVDKA DLLLAFGVRFDDRVTGKLEAFASRAKIVHIDIDSAEIGKNKQPHVS ICGDVKVALQGLNKILESRKGKVKLDFSNWREELNEQKKKFPLSFK TFGDAIPPQYAIQVLDELTKGDAVVSTGVGQHQMWAAQFYKY RNPRQWLTSGGLGAMGFGLPAAIGAAVARPDAVVVDIDGDGS FIMNVQELATIRVENLPVKIMLLNNQHLGMVVQWEDRFYKAN RAHTYLGNPSNSSEIFPDMLKFAEACDIPAARVTKVSDLRAAIQ TMLDTPGPYLLDVIVPHQEHVLPMIPSGAAFKDTITEGDGRRAY	669	72858

[a] 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; threonine; 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; X, undetermined amino acid. Protein sequence was obtained from UniProt database (http://www.uniprot.org). **AAR** = Amino acid residues; **MM** = Molecular mass.
[b] Evidence at protein level;
[c] Predicted sequence;
[d] Evidence at transcript level;
[e] Sequence inferred from homology.

and glucose-1-phosphate adenylyltransferase are involved in the starch synthesis of the amaranth seed (Park and others 2010; Castrillon-Arbelaez and others 2012). Acetolactate synthase is involved in the synthesis of essential amino acids present in amaranth seed such as valine and isoleucine (Maughan and others 2007). Also, the globulin 11S seed storage protein in amaranth is reported as a nutrient reservoir protein (Barba de la Rosa and others 1996). This protein from amaranth has been widely studied, including its molecular mass, amino acid residues, and crystal structure (Tandang-Silvas and others 2012). Other proteins reported at UniProt database, such as adenosine triphosphate synthase, nicotinamide adenine dinucleotide-dependent malic enzyme, and chlorophyll a/b-binding protein are present in amaranth leaves where they are involved in photosynthesis and CO_2 fixation (Long and others 1994; Villegas-Sepulveda and others 1994; Savolainen and others 2000). Amaranth protein is known to have a good balance of essential amino acids (Tiengo and other 2009).

Analysis of Bioactive Peptides from Amaranth Proteins

For this study and review all protein sequences were reported and then evaluated for the profile of active peptides using the database BIOPEP (http://www.uwm.edu.pl/biochemia). The amaranth protein globulin 11S contained many potential bioactive peptides. As an example, Figure 3 shows the amino acid sequences of globulin 11S in amaranth and the different biological activities are mapped onto the sequence. Table 5 to 7 show the potential biological sequences for each amaranth seed protein reported in UniProt. α-Amylase inhibitor and trypsin inhibitor were high in angiotensin I-converting enzyme-inhibitory activity (ACE) activity (A = 0.188 and 0.240, respectively), dipeptidyl peptidase IV (DPP-IV) inhibitors (A = 0.063 and 0.042, respectively), and antioxidative peptides (A = 0.063 and 0.056, respectively). α-Amylase inhibitor also contained a small amount of antiamnestic and antithrombotic peptides (A = 0.031). Likewise, the trypsin inhibitor showed an anticancer activity peptide (A = 0.042). Antimicrobial protein and nonspecific lipid-transfer protein also were high in peptides with ACE (0.279 and .160, respectively) and DPP-IV (A = 0.047 and 0.021, respectively) inhibitory activities. Nonspecific lipid-transfer protein also showed antithrombotic (A = 0.0106) and antioxidative (A = 0.0319) peptides. Superoxide dismutase was high in peptides with ACE inhibitor (A = 0.3618), antioxidative (A = 0.059), and DPP-IV inhibitory (A = 0.053) activities. Also, it showed an anticancer peptide (A = 0.0065). Ring-zinc finger protein was high in ACE inhibitors and DPP-IV inhibitors (A = 0.173 and 0.087, respectively). Ring-zinc finger protein showed to have peptides with hypotensive activity (A = 0.0173) and antithrombotic activity (A = 0.0057). Prosystemin was high in ACE inhibitors and glucose uptake-stimulating

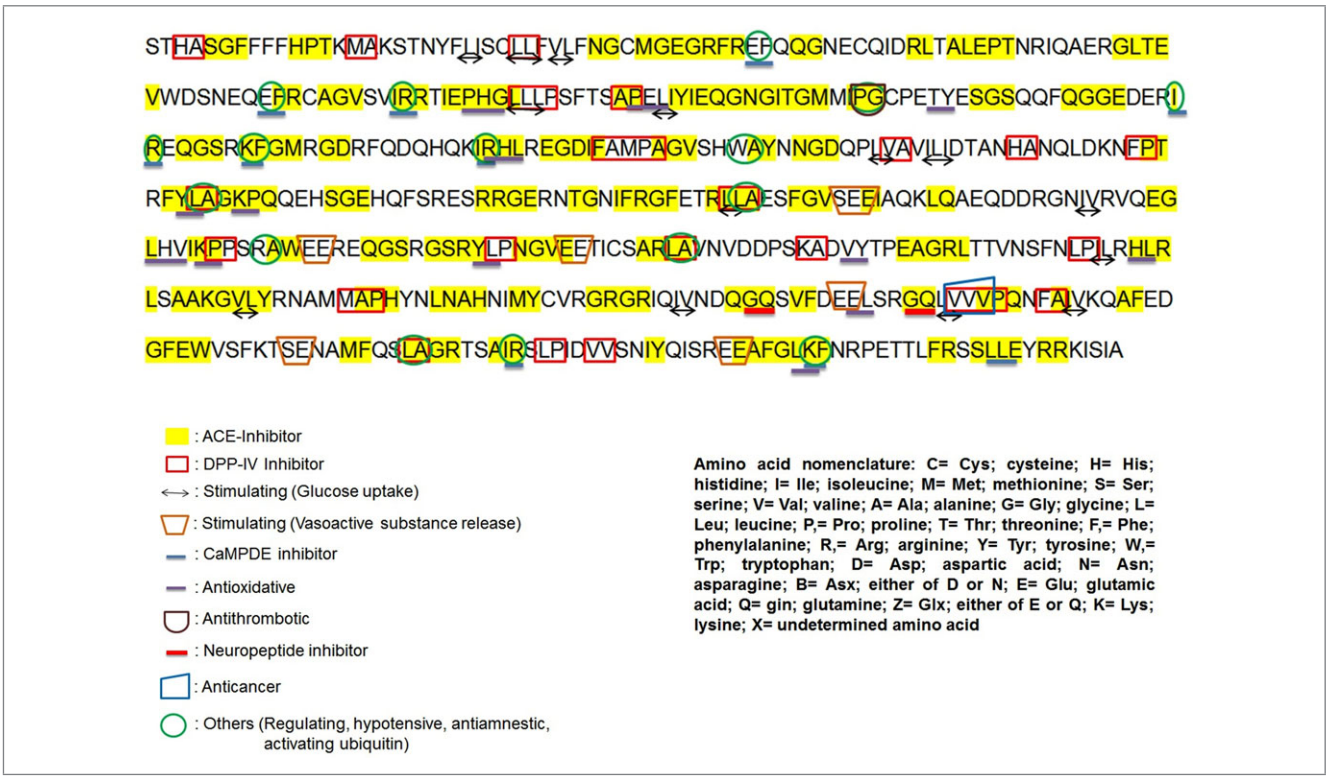

Figure 3–Illustration of bioactive peptide sequences found in amaranth globulin 11S protein.

peptides (A = 0.282 and 0.036, respectively). Also, it showed have an antioxidative peptide (A = .0307). ACE inhibitor (A = 0.259), DPP-IV inhibitor (A = 0.081), and antioxidant activity (A = 0.056) were the principal activities present in cystatin protein. Cystatin also showed peptides with antithrombotic (A = 0.004) and anticancer activity (A = 0.004). All of these proteins, except α-amylase inhibitor, showed peptides related to glucose uptake-stimulating activity.

Proteins reported in UniProt with a molecular mass between 30 and 56 kDa are listed in Table 3, and the biological activities of the peptide sequences are shown in Table 6. Seed protein AmA1 (amaranth albumin 1) and glucose-1-phosphate adenyltransferase were high in ACE inhibitors (A = 0.161 and A = 0.267, respectively), DPP-IV inhibitors (A = 0.003 and A = 0.0.043, respectively), stimulating glucose uptake (A = 0.036 and A = 0.028, respectively), and antioxidative peptides (A = 0.0328 and A = 0.020, respectively). Both seed proteins showed rennin inhibitor peptides (hypotensive peptides) (A = 0.007 and A = 0.008, respectively). Glucosyltransferase and polyamide oxidase also showed high frequency of ACE inhibitors (A = 0.259 and A = 0.208, respectively) and DPP-IV inhibitors (A = 0.082 and A = 0.050, respectively). Likewise, these proteins showed peptide sequences related to antioxidative (A = 0.041 and A = 0.020, respectively), glucose uptake-simulating (A = 0.037 and A = 0.030, respectively), hypotensive (A = 0.010 and A = 0.014, respectively), and antithrombotic (A = 0.004 and A = 0.008, respectively) activity. Proteins reported at UniProt with a molecular mass between 56 and 73 kDa are listed in Table 4, and their biological activities are shown in Table 7. The 11s globulin seed storage protein was high in ACE inhibitors (A = 0.264), DPP-IV inhibitors (A = 0.580), and antioxidative peptides (A = 0.040). Also, this protein showed peptides with antithrombotic activity (A = 0.002) and anticancer activity (A = 0.002). The granule-bound starch synthase I protein also showed to have high occurrence frequencies in ACE inhibitors (A = 0.276), DPP-IV inhibitors (A = 0.080), and glucose-uptake simulating peptides (A = 0.031). Likewise, this protein showed peptides with antithrombotic activity (A = 0.003) and hypotensive activity (A = 0.005). The acetolactate synthase protein was high in ACE inhibitors (A = 0.300), DPP-IV inhibitors (A = 0.066), glucose uptake-stimulating peptides (A = 0.037), and antioxidative peptides (A = 0.033). Likewise, this protein showed peptides with antithrombotic activity (A = 0.010), hypotensive activity (A = 0.007), and anticancer activity (A = 0.001). Figure 4 shows the different proteins of amaranth reported at UniProt database and their potential biological activity, likewise the peptide occurrence frequency for each biological activity. ACE-inhibitor activity and DPP-IV inhibitor activity were the most recurrent activities present in amaranth proteins.

Protein Hydrolysates from Amaranth

Amaranth as a source of bioactive peptides

Bioactive peptides are inactive within the parent protein. However with enzymatic digestion or food processing they can act as physiological modulators of metabolism (Pihlanto-Leppala and others 2000). Some studies with amaranth have reported the presence of peptides with biological activities such as antihypertensive, antioxidative, and antithrombotic among others (Silva-Sánchez and others 2008). Tovar-Perez and others (2009) reported peptides from albumins and globulins from amaranth seed with ACE-inhibitory activity. Vecchi and Añon (2009) reported tetrapeptides (ALEP and VIKP) with ACE-inhibitory activity from *Amaranthus hypochondriacus* 11S globulin protein. Most of the peptides from amaranth reported in the literature have ACE-inhibitory activity (Huerta-Ocampo and Barba de la Rosa 2011). To obtain these

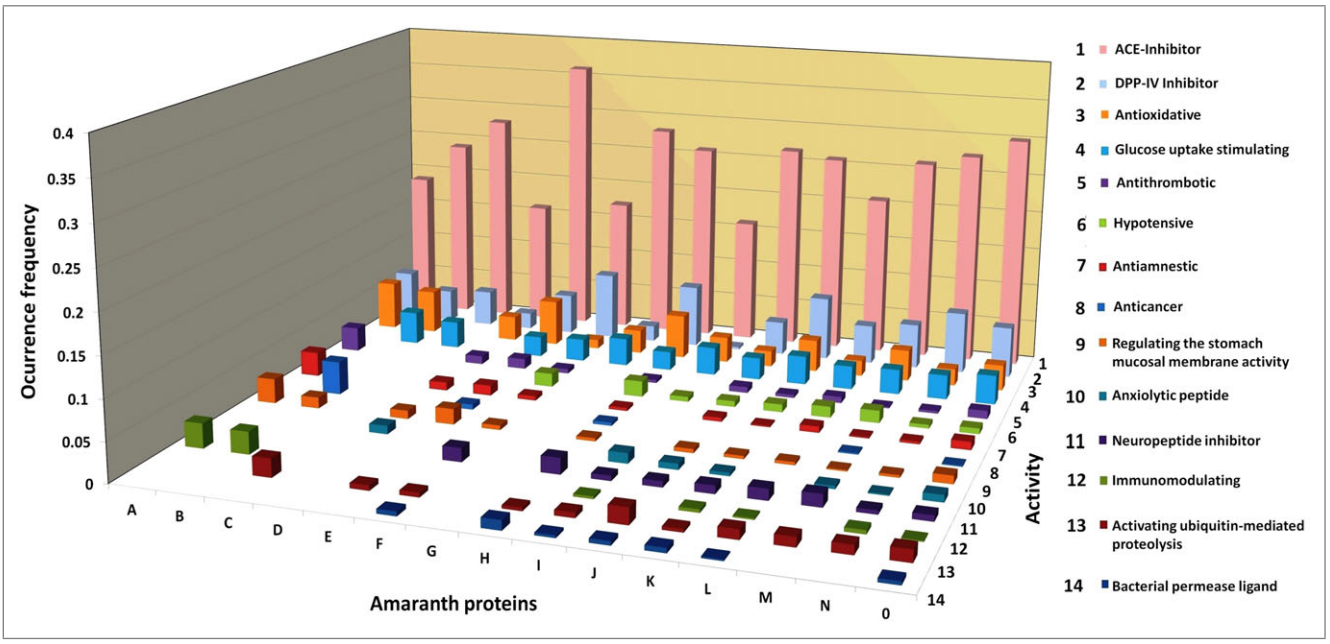

Figure 4–Potential bioactive sequences in amaranth proteins found after performing a scientific prediction of bioactive peptides present in amaranth proteins. First of all, the protein sequences were identified, and then the profiles of active peptides were evaluated using the database http://www.uwm.edu.pl/biochemia. The occurrence frequency (A) of bioactive fragments with a particular activity was calculated by the equation: A = a/N, where "a" is the number of amino acid residue-forming fragments with given activity in protein, and "N" is the number of amino acid residues of the protein. Protein legend: A = Alpha-amylase inhibitor; B = Trypsin inhibitor; C = Antimicrobial protein; D = Nonspecific lipid-transfer protein 1; E = Superoxide dismutase [Cu-Zn]; F = RING zinc finger protein; G = Prosystemin; H; Cystatin; I = Seed protein; J = Glucose-1-phosphate adenylyltransferase; K = Glucosyltransferase; L = Polyamine oxidase; M = 11S globulin seed storage protein; N = Granule-bound starch synthase I; O = Acetolactate synthase.

kinds of peptides an enzymatic hydrolysis is needed during food processing or during the digestion of the food in the human body.

Enzymes used to produce peptides

The human body has different gastrointestinal enzymes, with the principal digesting enzymes being trypsin, chymotrypsin, and pepsin. Trypsin is produced in the pancreas as the inactive proenzyme trypsinogen. Trypsin cleaves peptide chains mainly at the carboxyl side of the amino acids lysine and arginine, except when either is followed by proline (Rawlings and Barret 1994). Likewise, chymotrypsin is also a digestive enzyme found in the pancreatic juice acting in the duodenum where it performs proteolysis, the breakdown of proteins and polypeptides (Wilcox 1970). Pepsin, as well as trypsin and chymotrypsin, is one of the principal protein-degrading, or proteolytic, enzymes in the digestive system. During the process of digestion, these enzymes, each of which is specialized in cutting links between particular types of amino acids, collaborate to break down dietary proteins into their components (peptides and free amino acids) which can be readily absorbed by the intestinal lining and passed into the circulatory system. Pepsin is most efficient in cleaving peptide bonds between hydrophobic, and preferably, aromatic amino acids such as phenylalanine, tryptophan, and tyrosine (Fruton 2002).

On the other hand, there are commercial enzymes such as alcalase. Alcalase belongs to a group of serine proteases that initiate the nucleophilic attack on the peptide (amide) bond through a serine residue at the active site. This enzyme can be obtained from certain types of soil bacteria, for example, *Bacillus amyloliquefaciens,* which produce it in large amounts (Ottesen and Svendsen 1970). Table 8, 9, 10, 11, and 12 show the potential biological peptides formed from globulin 11S using enzymes such as pepsin, trypsin, chymotrypsin, alcalase, and the combination of pepsin/chymotrypsin, respectively. Most of the peptides showed ACE-inhibitory activity, followed by DPP-IV inhibitory activity and antioxidative activity. Other activities present in this protein are anticancer, antithrombotic, and glucose uptake-stimulating activities.

The technology used to release bioactive peptides from food depends on several factors, including the method used, the bioactive peptides of interest to be released, and the intended use of the peptide(s). Some methods include chemical hydrolysis, which uses acid to break down larger proteins or fermentation, which releases bioactive peptides after the application of cultured microorganisms to food products (Wang and De Mejia 2005). Another alternative technology is extrusion, a high-temperature short-time process, with partial denaturation of the proteins; it is used to make precooked flours (Milán-Carrillo and others 2006). Montoya-Rodríguez and others (2014a) reported that the extrusion process caused the formation of free amino acids and small peptides with biological activity. Although the complete mechanisms of absorption and bioavailability of specific peptides are still under investigation, there is sufficient evidence to conclude that food bioactive peptides are bioavailable and can be absorbed into the body (González de Mejia and others 2012).

Amaranth Health Benefits

Antioxidative capacity

The antioxidant capacity of amaranth, as well of other pseudocereals, is comparable to that of soybean and rice. The principal compounds that provide the antioxidant activity in amaranth grain are polyphenols. Also, proteins play an important role as radical scavengers (Gorinstein and others 2007). Barba de la Rosa and others (2009) evaluated different amaranth cultivars and identified some polyphenols such as isoquercetin and rutin; likewise,

Table 5–Predicted biological activity of peptide sequences from amaranth proteins with molecular mass between 3 and 30 kDa.

Activity	Occurrence frequency	Potential bioactive peptide	References
ALPHA-AMYLASE INHIBITOR (molecular mass = 3592 Da)			
ACE-inhibitor	0.1875	IP, KW, GP, DG, GV, YG	Cheung and others (1980); Byun and Kim (2002)
Dipeptidyl peptidase IV inhibitor	0.0625	GP, VP	Bella and others (1982)
Antioxidative peptide	0.0625	DYY, YYG	Saito and others (2003)
Antithrombotic peptide	0.0312	GP	Ashmarin and others (1998)
Ion flow regulating peptide	0.0312	DY	Ziganshin and others (1994)
Immunostimulating peptide	0.0312	YG	Kayser and Meisel (1996)
Prolyl endopeptidase inhibitor (antiamnestic)	0.0312	GP	Ashmarin and others (1998)
Peptide regulating the stomach mucosal membrane activity	0.0312	GP	Ashmarin and others (1998)
TRYPSIN INHIBITOR (molecular mass = 8319 Da)			
ACE-Inhibitor	0.2394	AR, GK, EW, VG,GE, GY, AA, IE, GF, FR, TG, GV, YP, PR, YG, YPR,	Cheung and others (1980); Bella and others (1982); Sentandreu and Toldra (2007)
Antioxidative peptide	0.0563	EL,TY, YGY, PEL	Suetsuna and others (2000)
Dipeptidyl-aminopeptidase IV inhibitor	0.0422	KA, VV,	Bella and others (1982)
Glucose uptake-stimulating peptide	0.0422	LV, II,IV,	Morifuji and others (2009)
Dvl protein binding (Anticancer)	0.0422	VWV, VVV,	Lee and others (2009)
Immunostimulating peptide	0.0281	YG	Kayser and Meisel (1996)
Peptide regulating the stomach mucosal membrane activity	0.0140	PG	Ashmarin and others (1998)
Ion flow regulating peptide	0.0140	DY	Ziganshin and others (1994)
ANTIMICROBIAL PROTEIN (molecular mass = 8912 Da)			
ACE inhibitor	0.2790	AF, MG, GV, VG, GE, GR, SG, GM, FG, GY, GK, KG, GP, AA, PT, DA, LA, AG, GA, GS, VAA, AGSP	Cheungand and others (1980); van Platerink and others (2008); Balti and others (2010)
Dipeptidyl-aminopeptidase IV inhibitor	0.0465	VA, MA, LA,	Bella and others (1982)
Glucose uptake-stimulating peptide	0.0348	LI, IV,	Morifuji and others (2009)
Ubiqitin-mediated proteolysis activating peptide	0.0232	LA, RA	Turner and others (2000)
NONSPECIFIC LIPID-TRANSFER PROTEIN 1 (molecular mass = 9747 Da)			
ACE inhibitor	0.1595	LG, KG, GT, TG, GA, PP, AG, GV, AA, AR, VAA, VSP, RL	Cheung and others (1980); van Platerink and others (2008)
Antioxidative peptide	0.0319	LK, TY	Huang and others (2010)
Dipeptidyl-aminopeptidase IV inhibitor	0.0212	VV, KA, PA, RR, MA, LN, VA, LA, GP, PP, PPPA	Bella and others (1982); Maruyama and others (1993)
Antithrombotic peptide	0.0106	GP	Ashmarin and others (1998)
Prolyl endopeptidase inhibitor (Antiamnestic)	0.0106	GP	Ashmarin and others (1998)
Peptide regulating the stomach mucosal membrane activity	0.0106	GP	Ashmarin and others (1998)
Anxiolytic peptide (Neuropeptide)	0.0106	YL	Kanegawa and others (2010)
SUPEROXIDE DISMUTASE [CU-ZN] (molecular mass = 15199 Da)			
ACE inhibitor	0.3618	MG, GK, KG, GV, EG, TG, GT, IY, TQ, GD, PT, SG, GL, HG, GF, LG, NG, PH, AG, KE, GS, TF, IP, GA, VG, GR, GG, GI, IG, GH, LQ, QG, LKP	Cheung and others (1980); Bella and others (1982); Sentandreu and Toldra (2007); van Platerink and others (2008)
Dipeptidyl-aminopeptidase IV inhibitor	0.0526	LN, HA, PA, VV, GP	Bella and pothers (1982)
Antioxidative peptide	0.0592	LK, KP, LH, EL, LKP, LHG, HVH, PHF, RHA	Huang and others (2010)
Glucose uptake-stimulating peptide	0.0263	VL, LI, IV, II	Morifuji and others (2009)
Peptide regulating the stomach mucosal membrane activity	0.0197	GP, PG	Ashmarin and others (1998)
Antithrombotic peptide	0.0131	GP	Ashmarin and others (1998)
Prolyl endopeptidase inhibitor (Antiamnestic)	0.0131	GP	Ashmarin and others (1998)
Stimulating vasoactive substance release	0.0065	SE	Ringseis and others (2005)
Ubiqitin-mediated proteolysis activating peptide	0.0065	RA	Turner and others (2000)
Dvl protein binding (Anticancer)	0.0065	VVV	Lee and others (2009)
RING ZINC FINGER PROTEIN (molecular mass = 19355 Da)			
ACE inhibitor	0.1734	MG, GD, AP, LY, GF, AG, GL, AI, IY, LN, YP, PP, TG, PT, TE, AR, IE, RY, KG, GE, GT,SG,RA, GS, RL, IR, LVL	Cheung and others (1980); Bella and others (1982); Sentandreu and Toldra (2007); van Platerink and others (2008)
Dipeptidyl-aminopeptidase IV inhibitor	0.0867	LA, AP, VV, VA,PP, HA, GP	Bella and others (1982)
Stimulating vasoactive substance release	0.0289	EE, SE, EEE	Ringseis and others (2005)

(*Continued*)

Table 5–Continued.

Activity	Occurrence frequency	Potential bioactive peptide	References
Glucose uptake-stimulating peptide	0.0231	LV, VL	Morifuji and others (2009)
CaMPDE inhibitor	0.0173	EF, IR	Li and Aluko (2010)
Renin inhibitor(Hypotensive)	0.0173	EF, IR	Li and Aluko (2010)
Antioxidative peptide	0.0115	EL, YKY	Suetsuna and others (2000)
Prolyl endopeptidase inhibitor (Antiamnestic)	0.0057	GP	Ashmarin and others (1998)
Peptide regulating the stomach mucosal membrane activity	0.0057	GP	Ashmarin and others (1998)
Antithrombotic peptide	0.0057	GP	Ashmarin and others (1998)
Ion flow regulating peptide	0.0057	DY	Ziganshin and others (1994)
Bacterial permease ligand	0.0057	KK	Sleigh and others (1997)
Activating ubiquitin-mediated proteolysis	0.0057	RA	Turner and others (2000)
PROSYSTEMIN (molecular mass = 22511 Da)			
ACE Inhibitor	0.2820	KE, EK, KG, GG, GD, IE, TQ, EI, IP, ME, EG, GY, VE, DA, KG, GE, GA, PP, KR,	Cheung and others (1980); van Platerink and others (2008)
Stimulating vasoactive substance release	0.0358	EE, EEE	Ringseis and others (2005)
Glucose uptake-stimulating peptide	0.0358	II, VL, IV	Morifuji and others (2009)
Antioxidative peptide	0.0307	KP, LH, HH, LK	Huang and others (2010)
Dipeptidyl-aminopeptidase IV inhibitor	0.0205	VV, LA, PP	Bella and others (1982)
CYSTATIN (molecular mass = 27736 Da)			
ACE Inhibitor	0.2591	KF, IF, QG, GS, LG, GG, GL, GA, AA, DA, EI, IE, LA, AR, KE, KA, AG, GT, EA, AI, GK, LY, LQ, TE, EG, GH, AP, PG, GW, EV, VP, AH, VE, HL, KR, HK, LN, MG, RL, IQP	Cheung and others (1980); Bella and others (1982); Sentandreu and Toldra (2007); van Platerink and others (2008)
Dipeptidyl-aminopeptidase IV inhibitor	0.0809	LL, LP, MA, LA, FA, VV, KA, VA, HA, AP, VP, FP,	Bella and others (1982)
Antioxidative peptide	0.0566	LH, HH,KP, EL, IR, HL, LK, LHH, PHS, HHF, LHL, LLPH	Chen and others (1996)
Glucose uptake-stimulating peptide	0.0242	LI, LL, IL	Morifuji and others (2009)
Renin inhibitor (Hypotensive)	0.0202	KF, EF, IR	Li and Aluko (2010)
CaMPDE inhibitor	0.0202	KF, EF, IR	Li and Aluko (2010)
Bacterial permease ligand	0.0121	KK, KKK	Sleigh and others (1997)
Stimulating vasoactive substance release	0.0080	SE, LLL	Ringseis and others (2005)
Peptide regulating the stomach mucosal membrane activity	0.0040	PG	Ashmarin and others (1998)
Ubiqitin-mediated proteolysis activating peptide	0.0040	LA	Turner and others (2000)
Dvl protein binding (Anticancer)	0.0040	VWV,	Lee and others (2009)
Antithrombotic peptide	0.0040	PG	Ashmarin and others (1998)
Prolyl endopeptidase inhibitor (Antiamnestic)	0.0040	PG	Ashmarin and others (1998)

Bioactive peptide sequence was obtained from BIOPEP database.

phenolic acids such as syringic and vanillic acids. These compounds showed antioxidant activity. Lopez-Mejía and others (2014) evaluated the antioxidant capacity from leaf and seed extracts, concluding that both tissues have antioxidant capacity attributed not only to phenolic compounds. Tiengo and others (2009) also reported that amaranth is rich in amino acids such as cysteine, methionine, tyrosine, tryptophan, lysine, histidine, proline, glycine, alanine, and threonine, which are well known to possess antioxidant capacity. Peptides found in the extruded and unprocessed amaranth hydrolysates contain amino acids reported to possess antioxidant activity (sulfur and aromatic ones, as well as lysine, proline, histidine, glycine, alanine, and threonine) (Montoya-Rodriguez and others 2014a).

Cholesterol-lowering effect

Plate and Areas (2002) demonstrated that the consumption of extruded amaranth reduced low-density lipoprotein (LDL) and total cholesterol levels in hypercholesterolemic rabbits and suggested that extruded amaranth may be another option to prevent coronary heart disease.

Mendonca and others (2009) reported that amaranth protein isolates, fed to hamsters, showed a reduction of the total plasma cholesterol concentration at the end of the experimental period. Amaranth protein isolates intake led to a significant reduction in non-HDL-cholesterol. There are different hypotheses to explain the hypocholesterolemic effect of amaranth; one of them refers to the fiber content and possibly to the amino acid profile of the proteins (Berger and others 2003; Mendonca and others 2009). The fiber apparently decreased cholesterol absorption from the intestine and thus the blood cholesterol level (Pavlik 2012). Milán-Carrillo and others (2012a) and Ferreira and Gómez-Areas (2010) reported that amaranth represents an excellent source of total dietary fiber before and after extrusion.

Antidiabetic activity

The effect of amaranth on the reduction of blood glucose is not well known yet. However, there are studies where amaranth showed antidiabetic properties. Conforti and others (2005) reported antidiabetic activity with 2 amaranth varieties via inhibition of α-amylase. Velarde-Salcedo and others (2013)

Table 6–Predicted biological activity of peptide sequences in amaranth protein with molecular mass between 30 and 56 kDa.

Activity	Occurrence frequency	Potential bioactive peptide	References
SEED PROTEIN AmA1 [Amaranth Albumin 1] (molecular mass = 34959 Da)			
ACE inhibitor	0.1611	AG, GL, RY, LQ, LA, EV, VE, RW, LY, EG, LG, GH, TE, VG, GG, GS, TG, VF, IF, TF, KG, NG, GK, GV, FG, GY, YG, LN, AH, MF,GT, KF, PR, EA, AA, FR, KR, SG, TQ, AI, EI, IE, RL	Cheung and others (1980); Bella and others (1982); Sentandreu and Toldra (2007); van Platerink and others (2008)
Glucose uptake-stimulating peptide	0.0361	LL, IL, LV, IV, II	Morifuji and others (2009)
Antioxidative peptide	0.0328	LK, TY, RW, LH, KP, EL, LHV, LVR	Huang and others (2010)
Anxiolytic peptide (Neuropeptide)	0.0131	YL	Kanegawa and others (2010)
Ubiqitin-mediated proteolysis activating peptide	0.0065	LA, WA	Turner and others (2000)
CaMPDE inhibitor	0.0065	KF, EF	Li and Aluko (2010)
Renin inhibitor (Hypotensive)	0.0065	KF, EF	Li and Aluko (2010)
Immuno-stimulating peptide	0.0032	YG	Kayser and Meisel (1996)
Dipeptidyl-aminopeptidase IV inhibitor	0.0032	MA, LP, LL, LA, FA, VA, FP	Bella and others (1982)
Stimulating vasoactive substance release	0.0032	EE	Ringseis and others (2005)
Bacterial permease ligand	0.0032	KK,	Sleigh and others (1997)
GLUCOSE-1-PHOSPHATE ADENYLTRANSFERASE (molecular mass = 43392 Da)			
ACE-Inhibitor	0.2667	TG, GA, AI, VP, AF, SG, GD, KF, LN, IF, DA, AR, RR, KA, EA, LG, GI, GG, AG, GT, LY, YP, KR, IP, IY, TQ, HL, AY, YA, MG, GY, EG, GF, VE, EV, AA, QG, AH, FG, GL, GR, IE, EK, KG, GE, KE, PG, GS, EI, VG, GM, IG, RL, FY, PR, RY, PP, VAA, LYP, VSP	Cheung and others (1980); Bella and others (1982); Sentandreu and Toldra (2007); van Platerink and others (2008)
Dipeptidyl-aminopeptidase IV inhibitor	0.0435	VP, LA,VV, KA, PA, VA, LP, FA, FP, PP	Bella and others (1982)
Glucose uptake-stimulating peptide	0.0282	LI, LL, VL, IL, II	Morifuji and others (2009)
Antioxidative peptide	0.0205	KP, HL, LK, YLY, RHL	Huang and others (2010)
Ubiqitin-mediated proteolysis activating peptide	0.0205	LA, RA	Turner and others (2000)
CaMPDE inhibitor	0.0076	KF, EF	Li and Aluko (2010)
Renin inhibitor (Hypotensive)	0.0076	KF, EF	Li and Aluko (2010)
Anxiolytic peptide (Neuropeptide)	0.0076	YL	Kanegawa and others (2010)
Stimulating vasoactive substance release	0.0076	EE, SE	Ringseis and others (2005)
Antithrombotic peptide	0.0076	PG, DEE	Lee and Kim (2005)
Bacterial permease ligand	0.0051	KK	Sleigh and others (1997)
Peptide regulating the stomach mucosal membrane activity	0.0051	PG	Ashmarin and others (1998)
Prolyl endopeptidase inhibitor (Antiamnestic)	0.0051	PG	Ashmarin and others (1998)
Ion flow regulating peptide	0.0025	DY	Ziganshin and others (1994)
7S GLOBULIN SEED STORAGE PROTEIN (molecular mass = 52000 to 66000 Da)[a]			
ACE-inhibitor		*Potential Bioactive Peptide*	Quiróga and others (2012)
Sub-Unit			
P66		DA, GK, QG, LQ, RL 2VF, PR, PL, AP, GF, FR, VG, AG, 2GE, MG GK, GD, EG, EA, 2LQ, LN, PQ, EW, HP	
P52		LVL, HY, FP, PR, 4LF,GPL, 2GP, PL, VK, 2AF, 2LA, KR, VP, 3FR, GL, GH, GR, 2FG, GK, GT, GG, 2EG, NG,2PG, VR, GHF, NY, NF, 2LQ, LN, EK	
P38		VLP, LNP, 2YL, GPL, PL, AF, AP, 3GA, 3AG, 2FG, GS, 2GV, 2GT,QG, 4SG, GHF, RR, AR, PH, 2VF,GY, AY, AA,VG, GE, QG, EA, NG, NF, YK, EV	
P35		2LY, LVL, RF, HY, GY, LNP, YL, 2LF, VK, 2AF, AP, 2LA, FR, GA, 3GL, GR, FG, GS, GT, 2SG, 2LG, 2EG, NY, 2SF, KL, LVE, VE, LQ, LN, TQ, EK. KE, PH	
P16′		VLP, RL, PR, LSP, LF, FFL, VP, GF, AG, SY, LN, PQ, TF, EL, FA, LP, VLP, VF, HY, GY, AF, LA, KR, FR, VG, 2NF	
P16		VF, FP, GF, GL, FG, LN, HY, PR, LF, GP, AF, LA, KR, PG, NF	
GLUCOSYLTRANSFERASE (molecular mass = 55652 Da)			
ACE-inhibitor	0.2587	LQ,VF, AY, YG, GH, IP, PT, AR, AA, GV, TQ, AI, IE, EK, KA, HG, GS, IY, TE, EI, IF, NG, GL, KF, VE, LN, RP, PH, MF, VP, PR, GT, TF, EV, KE, FY, YA, GR, RR, RA, AW, IG, GP, KR, GI, EA, KW, FG, MG, IW, PQ, QG, GF, ME, EG, GK, KG, GW, GA, TG, VG, GD,RL, LVR,	Cheung and others (1980); Bella and others (1982); Sentandreu and Toldra (2007); van Platerink and others (2008)
Dipeptidyl-aminopeptidase IV inhibitor	0.0821	VV, FP, MA, FA, LP, KA, PA, LN, VP, LL, RR, GP,	Bella and others (1982)
Antioxidative peptide	0.0410	EL, LH, VY, KP, LK, LHV, KYY, YYM, PWT, RHN,	Chen and others (1996)
Glucose uptake-stimulating peptide	0.0369	II, IV, LV, IL, LI, LL, VL	Morifuji and others (2009)

(*Continued*)

Table 6–Continued.

Activity	Occurrence frequency	Potential bioactive peptide	References
Stimulating vasoactive substance release	0.0225	EE, SE, EEE, LLL	Ringseis and others (2005)
CaMPDE inhibitor	0.0102	KF, EF, IR	Li and Aluko (2010)
Renin inhibitor (hypotensive)	0.0102	KF, EF, IR	Li and Aluko (2010)
Bacterial permease ligand	0.0061	KK,	Sleigh and others (1997)
Immunostimulating peptide	0.0041	YG	Kayser and Meisel (1996)
Anxiolytic peptide (Neuropeptide)	0.0041	YL	Kanegawa and others (2010)
Ion flow regulating peptide	0.0041	DY	Ziganshin and others (1994)
Antithrombotic peptide	0.0041	GP, DEE	Lee and Kim (2005)
Ubiqitin-mediated proteolysis activating peptide	0.0041	RA	Turner and others (2000)
Peptide regulating the stomach mucosal membrane activity	0.0020	GP	Ashmarin and others (1998)
Prolyl endopeptidase inhibitor (antiamnestic)	0.0020	GP	Ashmarin and others (1998)
POLYAMIDE OXIDASE (molecular mass = 56580 Da)			
ACE inhibitor	0.2076	ME, MG, GS, NG, RP, VE, KE, SG, GM, VF, VG, GA, EV, AP, TG, GG, GL, LG, GD, AA, AR, PR, RW, KR, GV, IF, HP, GI, YG, GP, AG, EA, GE, AY, AI, IY, KG, YP, GY, GR, LQ, YA, IG, GF, IP, EK, IE, EI, LY, KF, HK, TE, HG, EG, MY, FY, FG, GT, RR, LA, TF, PG, IW, RL, VSP	Cheung and others (1980); Bella and others (1982); Sentandreu and Toldra (2007); van Platerink and others (2008)
Dipeptidyl-aminopeptidase IV inhibitor	0.0504	FA, LA, VV, VA, AP, LP, FP, GP, KA, LL, VL, LN, HA	Bella and others (1982)
Glucose uptake-stimulating peptide	0.0302	VL, LL, IV, IL, LI, LV, II	Morifuji and others (2009)
Antioxidative peptide	0.0201	LK, EL, TY, VY, LH,	Chen and others (1996)
Renin inhibitor (hypotensive)	0.0141	EF, KF	Li and Aluko (2010)
CaMPDE inhibitor	0.0141	EF, KF	Li and Aluko (2010)
Ubiqitin-mediated proteolysis activating peptide	0.0120	LA, RA	Turner and others (2000)
Antithrombotic peptide	0.0080	GP, PG	Lee and Kim (2005)
Stimulating vasoactive substance release	0.0080	EE, SE,	Ringseis and others (2005)
Prolyl endopeptidase inhibitor (Antiamnestic)	0.0080	GP, PG	Ashmarin and others (1998)
Peptide regulating the stomach mucosal membrane activity	0.0040	GP	Ashmarin and others (1998)
Immunostimulating peptide	0.0020	YG	Kayser and Meisel (1996)
Bacterial permease ligand	0.0020	KK	Sleigh and others (1997)

Bioactive peptide sequence was obtained from BIOPEP database.
[a] The globulin 7S is composed of main subunits of 66, 52, 38, and 16 kDa. These are bound with a molecular mass near to 200 kDa. The data of globulin 7S were adapted from Quiróga and others (2012). There is no occurrence frequency due to there is no crystal structure yet.

reported an *in vitro* inhibition of DPP-IV by peptides derived from amaranth proteins. DPP-IV is an enzyme that deactivates hormones (incretins) involved in insulin secretion. They suggest that amaranth peptides could be used as functional food ingredients in the prevention of diabetes.

Antiatherosclerotic activity

The high content of fiber in amaranth could be one of the responsible compounds to reduce the risk to develop cardiovascular diseases such as atherosclerosis (Pavlik 2012). New research has highlighted the important role of squalene present in amaranth grain. The inhibition of squalene monooxygenase enzyme results in a decrease of cholesterol synthesis. This enzyme has been identified as a key regulatory site of cholesterol, which is then responsible for the development of atherosclerosis (Caselato-Sousa and Amaya-Farfán 2012).

Atherosclerosis is considered as a progressive disease derived from chronic inflammation (Xia-Hua and others 2014). Montoya-Rodriguez and others (2014a) described peptides that inhibit the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathway and thus reduce the risk to develop atherosclerosis. Kabiri and others (2010) reported an antiatherosclerotic effect for amaranth in hypercholesterolemic rabbits via reducing levels of LDL, triglycerides, and oxidized low-density lipoproteins (ox-LDL). ox-LDL is the key molecule in the development of atherosclerosis (Shin-Ichi 2007). The principal scavenger receptor for ox-LDL is lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1), which is the single class of ox-LDL receptor on human coronary artery localized in the surface of endothelial cells (Reiss and others 2009). Montoya-Rodríguez and others (2014b) also reported that hydrolysates from extruded amaranth inhibit markers of atherosclerosis by reducing the expression of proteins in the LOX-1 signaling pathway, probably attributed to peptides formed during the extrusion process. Hydrolysates from extruded amaranth flour inhibited markers such as IL-6, IL1α, and TNF-α, involved in the activation of LOX-1 signaling. The expression of LOX-1 was decreased, and also the expression of markers that start the atherosclerosis process.

Anticancer activity

Some amaranth species such as *A. caudatus* and *A. mantegazzianus* have shown antitumor effects (Yu and others 2001; Barrio and Añon 2010). Maldonado-Cervantes and others (2010) reported that amaranth lunasin-like peptide is efficient as a cancer-preventive peptide, due to its internalization into the cell nucleus, there inhibiting the transformation of NIH-3T3 cells to

Table 7–Predicted biological activity of peptide sequences in amaranth protein with molecular mass between 56 and 73 kDa.

Activity	Occurrence frequency	Potential bioactive peptide	References
11S GLOBULIN SEED STORAGE PROTEIN (molecular mass = 56672 Da)			
ACE inhibitor	0.2614	SG, GF, HP, PT, NG, MG, GE, EG, GR, FR, QG, GL, TE, EV, AG, GV, RR, IE, PH, HG, AP, IY, GI, TG, GM, IP, PG,GS, GG, KF, FG, GD, HL, IF, AY, FY, LA, GK, PQ, EI, LQ, AW, RY, VE, AR, EA, AA, KG, LY, LN, AH, MY, GQ, VF, VP, AI, AF, EW, MF,RL, IR, IKP, GKP, IEP, ALEP,	Cheung and others (1980); Bella and others (1982); Sentandreu and Toldra (2007); van Platerink and others (2008)
Dipeptidyl-aminopeptidase IV inhibitor	0.0578	HA, MA, LL, LP, AP, FA, MP, PA, VA, FP, LA, PP, KA, VV, VP,	Bella and others (1982)
Antioxidative peptide	0.0399	IR, EL, TY, HL, YL, LH, KP, VY, LK, PHG, PEL, RHL, LHV	Huang and others (2010)
Glucose uptake-stimulating peptide	0.0319	LI, LL, VL, LV, IL, IV	Morifuji and others (2009)
CaMPDE inhibitor	0.0159	EF, IR, KF	Li and Aluko (2010)
Renin inhibitor (Hypotensive)	0.0159	EF, IR, KF	Li and Aluko (2010)
Stimulating vasoactive substance	0.0139	SE, EE	Ringseis and others (2005)
Activating ubiquitin-mediated proteolysis	0.0119	WA, LA, RA	Turner and others (2000)
Neuropeptide inhibitor	0.0059	GQ	Parish and others (1983)
Anxiolytic peptide (Neuropeptide)	0.0039	YL	Kanegawa and others (2010)
Peptide regulating the stomach mucosal membrane activity	0.0019	PG	Ashmarin and others (1998)
Antithrombotic	0.0019	PG	Ashmarin and others (1998)
Prolyl endopeptidase inhibitor (Antiamnestic)	0.0019	PG	Ashmarin and others (1998)
Dvl protein binding (Anticancer)	0.0019	VVV	Lee and others (2009)
GRANULE-BOUND STARCH SYNTHASE I (molecular mass = 67320 Da)			
ACE inhibitor	0.2755	ME, MG, GS, NG, GL, RP, VE, KE, SG, GM, VF, VG, GA, EV, AP, TG, GG, GL, LG, GD, PP, AA, AR, GH, PR, RY, DG, GW, TE, KR, GV, IF, HP, GI, YG, GP, AG, EA, GE, AY, AI, IY, KG, AF, QG, GR, YP, GY, LQ, YA, EW, IG, GF, IP, GK, EK, IE, EI, LY, KF, AH, EG, RL, TQ, LYP	Cheung and others (1980); Bella and others (1982); Sentandreu and Toldra (2007); van Platerink and others (2008)
Dipeptidyl-aminopeptidase IV inhibitor	0.0792	FA, LA, VV, VA, AP, LP, PP, PA, GP, KA, LL, VL, LN,	Bella and others (1982)
Glucose uptake-stimulating peptide	0.0313	VL, LL, IV, IL, LI,	Morifuji and others (2009)
Antioxidative peptide	0.0214	LK, EL, TY, VY, LH, HL, LHL,	Chen and others (1996)
Ubiqitin-mediated proteolysis activating peptide	0.0115	LA	Turner and others (2000)
Stimulating vasoactive substance release	0.0066	EE, LLL	Ringseis and others (2005)
CaMPDE inhibitor	0.0049	EF, KF	Li and Aluko (2010)
Renin inhibitor (Hypotensive)	0.0049	EF, KF	Li and Aluko (2010)
Immunostimulating peptide	0.0049	YG	Kayser and Meisel (1996)
Ion flow regulating peptide	0.0033	DY	Ziganshin and others (1994)
Antithrombotic peptide	0.0033	GP	Lee and Kim (2005)
Prolyl endopeptidase inhibitor (Antiamnestic)	0.0033	GP	Ashmarin and others (1998)
Peptide regulating the stomach mucosal membrane activity	0.0033	GP	Ashmarin and others (1998)
Anxiolytic peptide (Neuropeptide)	0.0016	YL,	Kanegawa and others (2010)
ACETOLACTATE SYNTHASE (molecular mass = 72858 Da)			
ACE inhibitor	0.3004	PP, FY, IP, LQ, IY, YA, AI, PT, PR, RR, RP, TQ, KG, VE, EA, EG, GV, VF, AY, PG, GG, GA, ME, EI, QG, AA, GY, AR, TG, GR, VG, SG, GP, GL, DA, VP, VA, GQ, IG, GT, AF, EV, KE, LG, GE, KR, LY, KF, GI, MG, GM, HG, KA, FG, GK, PH, GD, TF, PQ, GF, GS, HL, AH, RL, IF, TE, GRP	Cheung and others (1980); Bella and others (1982); Sentandreu and Toldra (2007); van Platerink and others (2008)
Dipeptidyl-aminopeptidase IV inhibitor	0.0657	MA, PP, RR, FA, LP, GP, LL, VP, VA, FP, KA, PA, VV	Bella and others (1982)
Glucose uptake-stimulating peptide	0.0373	VL, LV, II, LL, IV, LI	Morifuji and others (2009)
Antioxidative peptide	0.0328	KP, LK, TY, EL, LH, VY, HL, LHM, RHE, YKY, PHQ	Chen and others (1996)
Ubiqitin-mediated proteolysis activating peptide	0.0149	RA, LA, WA	Turner and others (2000)
Stimulating vasoactive substance	0.0134	EE, SSS	Ringseis and others (2005)
Antithrombotic peptide	0.0104	PG, GP	Ashmarin and others (1998)
Prolyl endopeptidase inhibitor (Antiamnestic)	0.0104	PG, GP	Ashmarin and others (1998)
Peptide regulating the stomach mucosal membrane activity	0.0104	PG, GP	Ashmarin and others (1998)
Anxiolytic peptide (Neuropeptide)	0.0089	YL, KPT	Kanegawa and others (2010)
CaMPDE inhibitor	0.0074	IR, KF	Li and Aluko (2010)
Renin inhibitor (Hypotensive)	0.0074	IR, KF	Li and Aluko (2010)
Bacterial permease ligand	0.0044	KK, KKK	Sleigh and others (1997)
Neuropeptide inhibitor	0.0029	GQ	
Dvl protein binding (Anticancer)	0.0014	VVV	Lee and others (2009)
Immunostimulating peptide	0.0014	KRP	Kayser and Meisel (1996)

Bioactive peptide sequence was obtained from BIOPEP database.

Table 8–Potential peptide sequences found *in silico* in the globulin 11S amaranth protein using pepsin (EC 3.4.23.1) as a cutting enzyme (pH 2.0).

Cleavage site	Resulting peptide sequence	Biological activity	Cleavage site	Resulting peptide sequence	Biological activity
6	ST***HASG***	ACE-Inhibitor; DPP-IV inhibitor	286	QAEQDDRGN***IV***RVQ***EG***	ACE-Inhibitor; Glucose Uptake
20	F***HPT***KMAKSTN	ACE-inhibitor	296	HV***IKPPSRA***	ACE-Inhibitor; Antioxidative; Activating ubiquitin-mediated proteolysis
41	***NGCMGEGR***F	ACE-inhibitor			
55	FQ***QG***NECQID***RL***	ACE-inhibitor	323	W***EE***RE***QGS***R***GSRYLPNGVEE***TICS***ARL***	ACE-Inhibitor; Stim Vasoactive; DPP-IV inhibitor
69	***LEPT***NRIQAERG	ACE-inhibitor	335	AVNVDDPS***KA***DV	DPP-IV inhibitor
73	L***TEV***	ACE-inhibitor	343	YTP***EAGRL***	ACE-Inhibitor
97	RC***AGV***SV***IR***RTIEPHG	ACE-inhibitor; Hypotensive	359	L***RL***	ACE-Inhibitor
100	***LLL***	DPP-IV inhibitor; Glucose Uptake	366	S***AAKGVL***	ACE-Inhibitor; Glucose Uptake
109	TS***APEL***	ACE-inhibitor; Antioxidative	376	RNAM***MAPH***Y	ACE-Inhibitor; DPP-IV inhibitor
129	***IEQGNGITGM***MI***PG***CPET	ACE-inhibitor; Antithrombotic	384	N***AH***NIM	ACE-Inhibitor
136	E***SGS***QQ	ACE-inhibitor	403	CVR***GRGR***IQ***IV***ND***QGQ***SV	ACE-Inhibitor; Glucose Uptake
152	***QGG***EDER***IR***E***QG***SRK	ACE-inhibitor; CaMPDE inhibitor	407	D***EE***	Stim Vasoactive
160	***FGM***R***GD***RF	ACE-inhibitor	413	SR***GQ***L	ACE-Inhibitor; Neuropeptide Inhibitor
175	LR***EGD***I	ACE-inhibitor	419	***VVV***PQN	Anticancer
184	***AMPAG***VSH	ACE-Inhibitor; DPP-IV inhibitor	427	***AIV***KQAF	ACE-Inhibitor; Glucose Uptake
187	***AY***	ACE-inhibitor	443	KT***SE***NAM	Stimulate Vasoactive
193	N***NGD***QP	ACE-inhibitor	465	***AGR***TS***AIRSLP***ID***VV***SNI	ACE-Inhibitor; Hypotensive; DPP-IV
198	***LVA***VI	DPP-IV inhibitor; Glucose Uptake	473	QISR***EEA***	ACE-Inhibitor; Stim Vasoactive
208	IDTAN***HA***NQ	DPP-IV inhibitor	493	RS***SGQGE***	ACE-Inhibitor; Neuropeptide Inhibitor
217	DKN***FPT***RF	ACE-Inhibitor; DPP-IV inhibitor	501	***RR***KISIA	ACE-Inhibitor
219	***YL***	Neuropeptide			
232	***AGKPQ***QEH***SGE***HQ	ACE-Inhibitor; Antioxidative			
247	FSRES***RRGE***RN***TG***NI	ACE-Inhibitor			
255	FET***RL***	ACE-Inhibitor			
259	***LA***ES	ACE-Inhibitor; DPP-IV inhibitor			
269	***GVSEE***IAQK	ACE-Inhibitor; Stim Vasoactive			

[a] 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; threonine; 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; X, undetermined amino acid. Peptides sequences were obtained using ExPASy database (http://www.expasy.org). **Da** = Daltons. *Italic/bold letter = sequence with potential biological activity reported at BIOPEP database.

cancerous foci. Inflammation, in some cases, produces a critical situation resulting in a chronic disease such as cancer (Oseguera-Toledo and others 2011). Extruded amaranth hydrolysates have shown anti-inflammatory effects by reducing the activation of the NF-κB pathway (Montoya-Rodríguez and others 2014a).

Antihypertensive activity

ACE inhibitory activity is the main biological activity studied with amaranth (Caselato-Sousa and Amaya-Farfán 2012). Fritz and others (2011) reported that amaranth seed protein hydrolysates have *in vitro* and *in vivo* antihypertensive activities via inhibition of ACE enzyme. Barba de la Rosa and others (2010) reported that tryptic digests of amaranth glutelins can induce the production of endothelial nitric oxide through inhibition of ACE. Endothelial nitric oxide is involved in the regulation of vascular tone by inhibiting smooth muscle contraction and platelet aggregation. Quiróga and others (2012) reported that the globulin fraction 7S has ACE-inhibitory activity similar to the 11S globulin faction. Tovar-Perez and others (2009) and Tiengo and others (2009) also reported ACE-inhibitory activity from amaranth proteins; they found that hydrolysates produced using enzymes such as pepsin, pancreatin, and alcalase, produced peptides with ACE-inhibitor activity.

Concluding Remarks

Amaranth grain is an alternative crop that possesses excellent nutritional and nutraceutical properties. The proteins from amaranth have excellent quality with a good balance of amino acids. The *in silico* results have demonstrated that the use of commercial enzymes can produce peptides with a high occurrence of potential ACE-inhibitory activity, likewise can produce peptides associated to blood glucose control (DPP-IV inhibitory activity). In the same way, *in silico* results have demonstrated that digestive enzymes found in the human body produced peptides with high occurrence frequency of potential ACE-inhibitory activity. Also, peptides with antidiabetic potential were present. The principal biological activity for amaranth peptides was ACE-inhibitory, followed by DPP-IV inhibitory activity, likewise some antithrombotic activity. So, amaranth grain could be used as a functional food; or peptides derived from amaranth could be used as ingredients in functional foods to help in the prevention and reduction of chronic diseases. The study of a C4 plant of agronomic and nutritional relevance makes this alternative crop a subject of more research in the formulation of functional foods to improve and motivate the general use of the bioactive principles from the amaranth proteins reported in this study.

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Authors' Contributions

JM-C and CR-M proposed the initial project. AM-R developed and wrote the manuscript. MAG-F and AM-R completed the tables. EG de M provided the original idea for this manuscript, gave scientific advice throughout the research, revised, and edited the manuscript. All authors read and approved the manuscript.

Table 9–Potential peptide sequences found *in silico* in the globulin 11S amaranth protein using trypsin (EC 3.4.21.4) as cutting enzyme.

Cleavage site	Resulting peptide sequence	Biological activity	Cleavage site	Resulting peptide sequence	Biological activity
14	ST***HASGF***FFF***HPT***K	ACE-Inhibitor; DPP-IV inhibitor	308	***GS***R	ACE-Inhibitor
17	***MA***K	DPP-IV inhibitor	322	***YLPNGVEE***TICS***AR***	ACE-Inhibitor; DPP-IV inhibitor; Antioxidative ; Stim Vasoactive
40	STNYF***LI***SC***LL***F***VL***F***NG***C***MGEGR***	ACE-Inhibitor; DPP-IV inhibitor; Glucose uptake stimulating			
			332	***LA***VNVDDPSK	ACE-Inhibitor; DPP-IV inhibitor
54	***FREF***Q***QG***NECQIDR	ACE-Inhibitor; Hypotensive	342	AD***VY***TP***EAGR***	ACE-Inhibitor; Antioxidative
63	LT***ALEPT***NR	ACE-Inhibitor	355	LTTVNSFN***LPIL***R	DPP-IV inhibitor; Glucose uptake
82	***GLTEV***WDSNEQ***EFR***	ACE-Inhibitor; Hypotensive; CaMPDE inhibitor	358		
	HLR	ACE-Inhibitor; Antioxidative			
90	C***AGV***SV***IR***	ACE-Inhibitor; Hypotensive	363	LS***AA***K	ACE-Inhibitor
146	***IR***	ACE-Inhibitor; Hypotensive	368	***GVLY***R	ACE-Inhibitor; Glucose uptake
151	E***QGS***R	ACE-Inhibitor	388	NAM***MAPH***YN***LNAH***NI***MY***CVR	ACE-Inhibitor; DPP-IV inhibitor
156	***FGM***R	ACE-Inhibitor	390	***GR***	ACE-Inhibitor
159	***GD***R	ACE-Inhibitor	392	***GR***	ACE-Inhibitor
168	***IR***	ACE-Inhibitor; Hypotensive	410	IQIVNDQGQSVFDEELSR	ACE-Inhibitor
171	***HL***R	ACE-Inhibitor; Antioxidative	424	***GQLVVVPQ***N***FAIV***K	ACE-Inhibitor; DPP-IV inhibitor; Glucose uptake ; Anticancer; Neuropeptide
216	***EGDIFAMPAGV***SH***WAY***N***NGD***QP ***LVA***V***ILI***DTAN***HA***NQLDKN***FPT***R	ACE-Inhibitor; DPP-IV inhibitor; Glucose uptake ; Activ Ubiquitin			
235	***FYLAGKPQ***QEH***SGE***HQFSR	ACE-Inhibitor; DPP-IV inhibitor; Antioxidative ; Neuropeptide	437	Q***AF***ED***GFEW***VSFK	ACE-Inhibitor
242	***GE***R	ACE-Inhibitor	450	T***SE***NA***MF***QS***LAGR***	ACE-Inhibitor; DPP-IV inhibitor
249	N***TG***N***IFR***	ACE-Inhibitor	455	TS***AIR***	ACE-Inhibitor; Hypotensive
254	***GF***ETR	ACE-Inhibitor	470	S***LP***ID***VV***SN***IY***QISR	ACE-Inhibitor; DPP-IV inhibitor
269	***LLA***ES***FGVSEEI***AQK	ACE-Inhibitor; DPP-IV inhibitor; Glucose uptake ; Stim Vasoactive	477	***EEAFGLK***	ACE-Inhibitor; DPP-IV inhibitor; Antioxidative ; Stim Vasoactive
277	***LQ***AEQDDR	ACE-Inhibitor	487	FNRPETTL***FR***	ACE-Inhibitor
282	GN***IV***R	Antioxidative	495	S***SGQGE***YR	ACE-Inhibitor: Neuropeptide inhibitor
295	VQ***EGLH***V***IKPP***SR	ACE-Inhibitor; DPP-IV inhibitor			
300	***AWEE***R	ACE-Inhibitor; Stim Vasoactive			
305	E***QGS***R	ACE-Inhibitor			

[a] 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; threonine; 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; X, undetermined amino acid. Peptides sequences were obtained using ExPASy database (http://www.expasy.org). **Da =** Daltons. *Italic/bold letter = sequence with potential biological activity reported at BIOPEP database.

Table 10–Potential peptide sequences found *in silico* in the globulin 11S amaranth protein using chymotrypsin (EC 3.4.21.1) as cutting enzyme.

Cleavage site	Resulting peptide sequence	Biological activity
41	ST***HASGF***FFF***HPT***K***MA***KSTNYF***LI***SC***LL***F***VL***F***NG***C***MGEGR***F	ACE-Inhibitor; DPP-IV inhibitor; Glucose uptake stimulating
81	R***EF***Q***QG***NECQIDRLT***ALEPT***NRIQAER***GLTEV***WDSNEQ***EF***	ACE-Inhibitor; Hypotensive; CaMPDE inhibitor
103	RC***AGV***SV***IRR***T***IEPHGLLLP***SF	ACE-Inhibitor; DPP-IV inhibitor; Hypotensive; Antioxidative
111	TS***APELIY***	ACE-Inhibitor; DPP-IV inhibitor; Glucose uptake; Antioxidative
130	***IEQGNGITG***MM***IPG***CPE***TY***	ACE-Inhibitor; Antithrombotic ; Antioxidative
153	E***SGS***QQF***QGGE***DER***IREQGSRKF***	ACE-Inhibitor; Hypotensive; CaMPDE inhibitor
176	***GM***R***GD***RFQDQHQK***IRHL***R***EGDIF***	ACE-Inhibitor; DPP-IV inhibitor ; Hypotensive; CaMPDE inhibitor
185	A***MPAGV***SHW	ACE-Inhibitor; DPP-IV inhibitor
187	***AY***	ACE-Inhibitor
218	N***NGD***QP***LVA***V***ILI***DTAN***HA***NQLDKN***FPT***R***FY***	ACE-Inhibitor; DPP-IV inhibitor; Glucose uptake stimulating
248	***LAGKPQ***QEH***SGE***HQFSRES***RRGE***RNTGN***IF***	ACE-Inhibitor; DPP-IV inhibitor
251	R***GF***	ACE-Inhibitor
260	ET***RLLA***ESF	ACE-Inhibitor; DPP-IV inhibitor; Glucose uptake stimulating
309	***GVSEEI***AQK***LQ***AEQDDRGN***IV***RVQ***EGLHVIKPPSRAWEE***REQ***GS***R***GSRY***	ACE-Inhibitor; DPP-IV inhibitor; Glucose uptake; Antioxidative
336	***LPNGVEE***TICS***ARLA***VNVDDPS***KA***D***VY***	ACE-Inhibitor; DPP-IV inhibitor; Stim Vasoactive; Antioxidative
367	TP***EAGRL***TTVNSFN***LPILRHLRL***S***AAKGVLY***	ACE-Inhibitor; DPP-IV inhibitor; Glucose uptake; Antioxidative
376	RNAM***MAPH***Y	ACE-Inhibitor; DPP-IV inhibitor
385	N***LNAH***NI***MY***	ACE-Inhibitor
420	CVR***GRGR***IQ***IV***ND***QGQ***S***VF***D***EEL***SR***GQLVVVPQ***NF	ACE-Inhibitor; DPP-IV inhibitor; Anticancer; Antioxidative; Stimulate Vasoactive; Neuropeptide
427	***AIV***KQ***AF***	ACE-Inhibitor; Glucose uptake stimulating
431	ED***GF***	ACE-Inhibitor
433	***EW***	ACE-Inhibitor
466	KT***SE***NA***MF***QS***LAGR***TS***AIRSLP***ID***VV***SN***IY***	ACE-Inhibitor; DPP-IV inhibitor; Hypotensive; CaMPDE inhibitor: Antioxidative
474	QISR***EEAF***	ACE-Inhibitor; Stimulate Vasoactive substance
486	***GLKF***NRPETTLF	ACE-Inhibitor; Hypotensive; CaMPDE inhibitor
494	RS***SGQGE***Y	ACE-Inhibitor; Neuropeptide inhibitor
501	***RR***KISIA	ACE-Inhibitor

[a] 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; threonine; 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; X, undetermined amino acid. Peptides sequences were obtained using ExPASy database (http://www.expasy.org). **Da** = Daltons. *Italic/bold letter = sequence with potential biological activity reported at BIOPEP database.

Table 11–Potential peptide sequences found *in silico* in the globulin 11S amaranth protein using alcalase (Asp-N Endopeptidase, EC 3.4.24.33) as cutting enzyme.

Cleavage site	Resulting peptide sequence	Biological activity
52	ST***HASGF***FFF***HPTKMA***KSTNYF***LI***SC***LL***F***VL***F***NG***C ***MGEGR***FR***EF***Q***QG***NECQI	ACE-Inhibitor; DPP-IV inhibitor; Glucose uptake-stimulating; Hypotensive; CaMPDE inhibitor
74	D***RL***T***ALEPT***NRIQAER***GLTEV***W	ACE-Inhibitor
141	DSNEQEFRC***AGV***SV***IRR***TI***EPHGLLLP***SFTS***APELIYIE*** ***QGNG***IT***GMMIPGCPE***TYE***SG***SQQF***QGGE***	ACE-Inhibitor; DPP-IV inhibitor; Glucose uptake-stimulating; Antithrombotic activity; Hypotensive; CaMPDE inhibitor
157	DER***IR***E***QGSRKFGM***RG	ACE-Inhibitor; Hypotensive; CaMPDE inhibitor
173	DQHQK***IRHL***R***EG***	ACE-Inhibitor; Hypotensive; CaMPDE inhibitor; Antioxidative
190	DI***FAMPAG***VSH***WA***YN***NG***	ACE-Inhibitor; Activating ubiquitin mediated
200	DQP***LVA***V***ILI***	ACE-Inhibitor; Glucose uptake-stimulating
209	DTAN***HA***NQL	DPP-IV inhibitor
274	DKN***FPT***R***FYLAGKP***QQEH***SGE***HQFSRES***RRGE***RN***T*** ***G***N***IFRGF***ET***RLLA***ES***FGVSEEI***AQKLQAEQ	ACE-Inhibitor; DPP-IV inhibitor; Activating ubiquitin mediated; Stimulating vasoactive substance
327	DRGN***IV***RVQ***EGLHVIKPP***SRAW***EE***RE***QGS***R***G*** ***SRYLPNGVEE***TICS***ARLA***VNV	ACE-Inhibitor; DPP-IV inhibitor; Glucose uptake-stimulating; Antioxidative; Neuropeptide
397	D***VY***TP***EAGRL***TTVNSFN***LPIL***R***HLRL***S***AAKGVLY***RNAM ***MAPH***YNLNAHNIMYCVR***GRGR***IQ***IV***N	ACE-Inhibitor; DPP-IV inhibitor; Glucose uptake-stimulating; Antioxidative;
404	D***QGQ***SVF	ACE-Inhibitor; Neuropeptide inhibitor
428	D***EE***LSR***GQLVVVPQ***NFA***IV***KQAFE	ACE-Inhibitor; Anticancer; Glucose uptake-stimulating ; Stimulating vasoactive substance; Neuropeptide inhibitor
459	DGF***EW***VSFKT***SE***NA***MF***QS***LAGR***TS***AIRSLP***I	ACE-Inhibitor; DPP-IV inhibitor; Stimulating vasoactive substance; Hypotensive; CaMPDE inhibitor; Activating ubiquitin mediated
501	DVVSN***IY***QISR***EEAFGLKF***NRPETTLFR***SSGQGE***Y***RR***KISIA	ACE-Inhibitor; Stimulating vasoactive substance; Hypotensive; CaMPDE inhibitor

[a] 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; threonine; 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; X, undetermined amino acid. Peptides sequences were obtained using ExPASy database (http://www.expasy.org). **Da** = Daltons. *Italic/bold letter = sequence with potential biological activity reported at BIOPEP database.

Table 12–Potential peptide sequences found *in silico* in the globulin 11S amaranth protein using pepsin (EC 3.4.23.1) and chymotrypsin (EC 3.4.21.1) as cutting enzymes, simulating gastrointestinal digestion.

Cleavage site	Resulting peptide sequence	Biological activity	Cleavage site	Resulting peptide sequence	Biological activity
6	A***SG***	ACE-Inhibitor	269	***GVSEEI***AQK	ACE-Inhibitor; Stimulating vasoactive
15	***HPT***KM	ACE-Inhibitor	286	QAEQDDRGN***IV***RVQ***EG***	ACE-Inhibitor; Glucose uptake-stimulating
36	***NG***CM	ACE-Inhibitor	296	V***IKPP***SRA	ACE-Inhibitor
41	***GEGR***F	ACE-Inhibitor	309	***EE***RE***QGSRGSRY***	ACE-Inhibitor; Stimulating vasoactive
55	Q***QG***NECQID***RL***	ACE-Inhibitor	323	***LPNGVEE***TICSA***RL***	ACE-Inhibitor; DPP-IV inhibitor
69	E***PT***NRIQAERG	ACE-Inhibitor	343	TPE***AGRL***	ACE-Inhibitor
73	***TEV***	ACE-Inhibitor	354	P***IL***	Glucose uptake-stimulating
96	RC***AGV***SV***IRR***T***IEPH***	ACE-Inhibitor; Hypotensive	366	S***AAKGV***L	Glucose uptake-stimulating
109	TS***APEL***	ACE-Inhibitor; Antioxidative	375	***APH***	ACE-Inhibitor; DPP-IV inhibitor
121	***IEQGNG***IT***GM***	ACE-Inhibitor	403	CVR***GRGR***IQ***IV***ND***QGQ***SV	ACE-Inhibitor; Glucose uptake-stimulating
129	***IPG***CPET	ACE-Inhibitor; Antithrombotic	407	D***EE***	Stimulating vasoactive substance
136	E***SGS***QQ	ACE-Inhibitor	413	SR***GQ***L	Neuropeptide inhibitor
152	***QGGE***DER***IR***E***QGS***RK	ACE-Inhibitor; Hypotensive	419	***VVVP***QN	Anticancer; DPP-IV inhibitor
160	R***GD***RF	ACE-Inhibitor	427	***AIV***KQ***AF***	ACE-Inhibitor; Glucose uptake-stimulating
169	QK***IR***H	ACE-Inhibitor; Hypotensive	465	***AGR***TS***AIRSLP***ID***VV***SNI	ACE-Inhibitor; Hypotensive
175	R***EGD***I	ACE-Inhibitor	473	QISR***EEA***	ACE-Inhibitor; Stimulating vasoactive
184	A***MPAGV***SH	ACE-Inhibitor	493	RS***SGQGE***	ACE-Inhibitor; Neuropeptide inhibitor
193	N***NGD***QP	ACE-Inhibitor	501	***RR***KISIA	ACE-Inhibitor
198	***VA***VI	ACE-Inhibitor			
217	DKNF***PT***RF	ACE-Inhibitor			
227	***AGK***PQQEH	ACE-Inhibitor			
231	***SG***EH	ACE-Inhibitor			
247	SRES***RRGE***RNTGNI	ACE-Inhibitor			
255	ET***RL***	ACE-Inhibitor			

[a] 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; threonine; 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; X, undetermined amino acid. Peptides sequences were obtained using ExPASy database (http://www.expasy.org). **Da =** Daltons. *Italic/bold letter = sequence with potential biological activity reported at BIOPEP database.

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