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REVIEW

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Heart Health Peptides from Macroalgae and Their Potential Use in Functional Foods

Ciarán Fitzgerald,[†,§] Eimear Gallagher,[‡] Deniz Tasdemir,[§] and Maria Hayes*[,†]

[†]Food BioSciences Department and [‡]Department of Prepared Foods, Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland

[§]Department of Pharmaceutical and Biological Chemistry, School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX, United Kingdom

ABSTRACT: Macroalgae have for centuries been consumed whole among the East Asian populations of China, Korea, and Japan. Due to the environment in which they grow, macroalgae produce unique and interesting biologically active compounds. Protein can account for up to 47% of the dry weight of macroalgae depending on species and time of cultivation and harvest. Peptides derived from marcoalgae are proven to have hypotensive effects in the human circulatory system. Hypertension is one of the major, yet controllable, risk factors in cardiovascular disease (CVD). CVD is the main cause of death in Europe, accounting for over 4.3 million deaths each year. In the United States it affects one in three individuals. Hypotensive peptides derived from marine and other sources have already been incorporated into functional foods such as beverages and soups. The purpose of this review is to highlight the potential of heart health peptides from macroalgae and to discuss the feasibility of expanding the variety of foods these peptides may be used in.

KEYWORDS: bioactive peptides, functional foods, macroalgae, cardiovascular health, hypertension

INTRODUCTION

Cardiovascular disease (CVD) claims more lives each year than the five next leading causes of death combined, namely, cancer, influenza, chronic obstructive pulmonary disease (COPD), accidents, and diabetes mellitus.[1] Currently in the United States, an estimated 81,100,000 adults (1 in 3) have one or more symptoms of CVD, and of this figure, an estimated 74,500,000 people suffer from high blood pressure or hypertension.[2] Hypertension or high blood pressure is one of the major, yet controllable, risk factors in CVD.[3] It is defined as systolic blood pressure (SBP) above 140 mmHg and/or diastolic blood pressure (DBP) above 90 mmHg.[4] To combat hypertension, various stages of the renin−angiotensin system (RAS) can be positively affected. This system is responsible for the control of blood pressure and fluid balance in humans. The RAS system (shown in Figure 1) involves the enzymes renin and angiotensin-I-converting enzyme (ACE-I) and is capable of stimulating atherosclerosis by triggering basic reactions that ultimately lead to growth, instability, and rupture of atherosclerotic plaques and facilitation of thrombosis.[5] Two ways of inhibiting this system include (1) blocking the formation of angiotensin-II by the enzyme ACE-I from angiotensin I and (2) inhibiting the conversion of angiotensinogen into angiotensin-I by the enzyme renin.[5]

A number of other CVD risk factors have also been identified and addressed, including the development of thrombosis due to abnormalities in the coagulation of blood.[6] Thrombosis is defined as the formation of a blood clot within a blood vessel, which obstructs blood flow and may lead to embolism formation, a precursor of myocardial infarction and other vascular complications.[6] Another enzyme that is associated with the risk of cardiac events is the enzyme platelet-activating factor acetylhydrolase (PAF-AH, also known as Lp-PLA_2).[7] A proatherogenic role has been postulated for this enzyme on the basis of its ability to generate two key pro-inflammatory mediators.[7] This observation has led to suggestions of a causative role for PAF-AH in the development of atherosclerosis.[7]

With the global growth of the functional foods market, researchers have turned to sourcing natural food components to provide preventative effects against cardiovascular disease and metabolic syndromes in foods as a strategy to combat illness. Bioactive peptides are defined as food-derived peptides that exert a physiological hormone-like effect in humans beyond their basic, nutritional value.[6] These peptides are encrypted in the primary structure of dietary proteins and can be released by digestive enzymes during gastrointestinal transit or by fermentation or ripening during food processing.[8] To date, most bioactive peptides have been isolated from milk-based products.[3] ACE-I inhibitory peptides derived from milk protein precursors are split into two groups, the casokinins and the lactokinins,[9] and have known antihypertensive effects.[9] Other sources of ACE-I-peptidic inhibitors include soy,[10] meat,[11] egg,[4] cereal,[12] and marine sources such as fish muscle, shellfish, and macroalgae.[13,14]

Marine organisms including seaweeds and microalgae, as a result of their exigent, competitive, and aggressive surroundings compared to terrestrial environments, produce specific and active biomolecules and secondary metabolites.[14] These secondary metabolites result as a consequence of the harsh conditions in which macroalgae exist, including extremes of salinity and temperature and UV−vis irradiation, along with nutrient deficiencies.[15] Until recently, seaweeds or macroalgae were primarily used as a source of functional, technological ingredients in the food industry for use as emulsifying agents and to enhance

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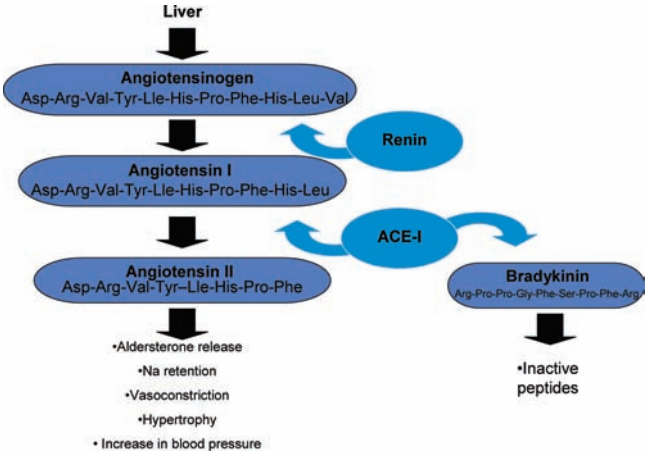

Figure 1. Renin angiotensin system. In the initial and rate-limiting reaction, renin hydrolyzes angiotensinogen, its only known substrate forming angiotensin-I. ACE-I in turn hydrolyzes angiotensin-I to form angiotensin-II, a potent vasoconstrictive molecule. ACE-I also acts on the vasodilatory peptide bradykinin, inactivating it, which in turn further raises blood pressure.

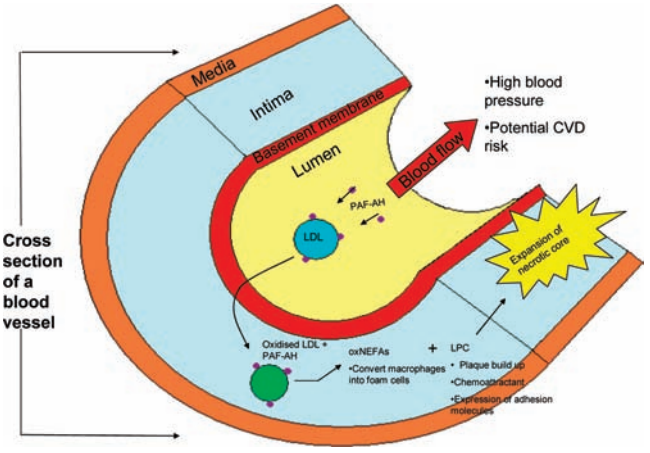

Figure 2. Expansion of necrotic core due to PAF-AH activity. In blood plasma 80% of PAF acetylhydrolase is bound to low-density lipoproteins (LDL). As the LDL is transported into the intima from the lumen, it is oxidized; PAF-acetylhydrolase then generates two pro-inflammatory mediators, lysophosphatidylcholine (LPC) and oxidized nonesterified fatty acids (oxNEFAs). LPC increases plaque buildup as it is an important chemoattractant for macrophages and increases the expression of adhesion molecules. oxNEFAs convert the attracted macrophages into foam cells, which agglutinate in the lumen and constrict blood vessels, which may lead to high blood pressure and potentially CVD.

viscosity and gelation in food formulations, pharmaceuticals, and cosmetics.[16] In addition to their technological properties, macroalgae exhibit original and interesting nutritional properties.[17] Edible marine algae are regularly consumed among the East Asian populations of China, Korea, and Japan,[18] and they are a rich source of polysaccharides, dietary fiber, minerals, and proteins.[17]

Seaweeds consumed in their whole form can have beneficial physiological effects,[19,20] and many bioactive compounds including peptides and lectins, carbohydrates, and fats including polyunsaturated fats (PUFAs) sourced from macroalgae have been identified.[21,22] Seaweeds are viewed as "natural" by consumers, and this promotes a positive response in consumers, who often regard natural entities.[23] Therefore, seaweeds may be considered a consumer friendly source of food ingredients.

PREVENTION OF CARDIOVASCULAR DISEASE (CVD)

One of the risk factors associated with the development of CVD includes high blood pressure, and this may be controlled by inhibition of a number of enzymes in the RAS and the human body that are known to increase blood pressure and atherosclerosis. Angiotensin converting enzyme I (ACE-I; EC 3.4.15.1) is one such enzyme. ACE-I is a zinc metalloprotease that plays an important role in RAS and the control of blood pressure and fluid regulation.[24] Inhibition of ACE-I is a well-established approach in the treatment of hypertension. ACE-I removes a dipeptide from the C-terminus of angiotensin I, converting it to angiotensin II, a potent vasoconstrictor. Chemically synthesized ACE-I inhibitors including captopril (Capoten), enalopril, alcacepril, and lisinopril are ordinarily prescribed for the treatment of high blood pressure.[25] ACE-I is also involved in the degradation of bradykinin, which is a vasodilator.[26] ACE-I also acts as an endopeptidase, shown by cleavage of peptides with amidated C-termini, as seen in the cleavage of histidine and leucine in the RAS.[24] Bradykinin-potentiating peptides prevent the hypertensive effect of angiotensin II and potentiate the hypotensive effect of the circulating vasodilatory peptide bradykinin by also inhibiting ACE-I[26] as shown in Figure 1. ACE-I inhibitors have been identified from multiple natural marine sources such as yellow fin sole, shrimp, clam, and sea cucumber as outlined in a recent review by Wijesekara and Kim.[25]

The enzyme renin (EC 3.4.23.15) belongs to a family of enzymes referred to as aspartic proteases, which also includes the enzymes pepsin, cathepsin, and chymosin.[27] Renin is a monospecific enzyme that displays remarkable specificity for its only known substrate, angiotensinogen.[27] As early as the 1950s, renin was recognized as the initial and rate-limiting substance involved in the RAS, and the approach of inhibiting circulating renin was suggested as the most likely approach to succeed in pharmacological inhibition of the whole system.[28] However, only in recent years has the first direct renin inhibitor, known as aliskiren, progressed to phase III trials and extensive clinical use as an antihypertensive drug.[29] Renin inhibitory peptides have previously been isolated from plant sources. For example, Li and Aluko used the enzyme alcalase to hydrolyze pea protein and produced three dipeptides: Ile-Arg, Lys-Phe, and Glu-Phe, each with potent renin inhibitory activity.[30]

Antithrombotic drugs have existed for many years in the form of aspirin and heparin.[31,32] Due to the dominant role of platelets in thrombosis, current strategies to inhibit thrombogenesis focus mainly on drugs that block platelet function, but also include anticoagulants for the prevention of cardioembolic events.[32] Fibrinolytic drugs are used for rapid restoration of forward-moving blood flow in patients with acute myocardial infarction and for treatment of acute ischemic stroke.[32] Previously, a protein with anticoagulant and antiplatelet activities was discovered in a yellow fin sole hydrolysate generated from fish muscle using seven different proteases.[33] Peptides isolated from this hydrolysate were found to inhibit coagulation in vitro.[33]

PAF-acetylhydrolase is a circulating enzyme produced and secreted by inflammatory cells centrally involved in atherosclerosis.[34]

It is bound predominately to apolipoprotein B-containing lipoproteins and is highly expressed in the necrotic core of atherosclerotic lesions.[34] It generates two key pro-inflammatory mediators, lysophosphatidylcholine (LPC) and oxidized nonesterified fatty acids (oxNEFAs). Evidence exists for a regulatory role of these lipids in promoting atherosclerotic plaque development that can ultimately lead to the formation of a necrotic core, a key determinant in atherosclerotic plaque vulnerability, as illustrated in Figure 2.[7] Darapladib, a PAF-acetylhydrolase inhibitory drug in development by GlaxoSmithKline (GSK), was shown previously to prevent necrotic core expansion, a key determinant in atherosclerotic plaque vulnerability.[34] Mayer et al. discovered a variety of compounds from Chlorophyta, Phaeophyta, and Rhodophyta that have inhibitory properties against bee-derived phospholipase A_2 (the same family of enzymes that PAF-AH belongs to).[35] The discovery of natural PAF-AH inhibitors and their inclusion in the treatment of CVD and incorporation into functional foods have high potential and are yet to be fully exploited.

The concept of functional foods as a means to protect the health of consumers was developed at the beginning of the 1980s in Japan as a way to reduce the high health costs of a population with long life expectancy projections.[36] Functional foods describe food products fortified with special constituents that possess advantageous physiological effects.[37] They have been more specifically defined as foods that beneficially affect one or more target functions in the body, beyond basic nutritional effects, in a way that is relevant to either an improved state of health and well-being and/or reduction of disease risk.[38] From 2011 onward the functional foods market currently is expected to reach U.S. $167 billion with a yearly growth potential of 10%.[39] Increasing demand for such foods can be explained by increasing healthcare costs coupled with a steady increase in life expectancy and a desire for an improved quality of life in advancing years.[37]

MACROALGAE AS A RICH RESERVOIR OF MARINE BIOACTIVE COMPONENTS

Edible macroalgae, including algae from the Protista orders, Phaeophyta (brown), Chlorophyta (green), and Rhodophyta (red), have a long history of use in the human diet.[40] The Japanese are the main consumers of macroalgae, eating 1.6 kg (dry weight) per capita per year.[40] In Japanese and Korean cuisine, red algae consumed include "nori" (or "kim") and "laver" from *Porphyra* species. Additionally, the red algae *Palmaria palmata*, known as "dulse", has a long tradition of consumption in coastal European and North American regions.[41] Brown kelp is also consumed in Japan. For example, "hijiki" (*Hijikia fusiformis*), "wakame" (*Undaria pinnatifida*), and "makonbu" (*Laminaria japonica*) and species of the brown algae *Laminaria* are also eaten in China, called "hai dai". Furthermore, green algae from *Ulva* species are consumed as part of traditional Hawaiian cuisine known as "limu palahalaha".[41] From a nutritional point of view edible macroalgae are a low-calorie food, with a high concentration of minerals, vitamins, and proteins and low lipid content. Macroalgae are an excellent source of vitamins A, C, D, and E along with the B vitamins (riboflavin, niacin, pantothenic acid, and folic acid) as well as minerals including calcium (Ca), phosphorus (P), sodium (Na), and potassium (K).[42] Macroalgae also contain large amounts of polysaccharides, notably cell wall, structural polysaccharides. Most of these polysaccharides, which include the agars, carrageenans, ulvans, and fucoidans, cannot be digested in the human gastrointestinal tract and therefore may be regarded as a good source of dietary fiber and a potential source of prebiotics.[17,43] Prebiotics are selectively fermented ingredients that allow specific changes in both the composition and/or activity of the gastrointestinal microflora that confers benefits upon the host's well-being and health.[43] Regular consumption of macroalgae in the whole form is associated with many positive health benefits.[17] For example, a recent case study of 362 women aged 30−65 years old suggested that daily consumption of "gim" (*Porphyra* species), an edible macroalga traditionally eaten in Korea, was inversely associated with the risk of breast cancer development.[19] Further research suggests that alga consumption may decrease the risk of diabetes mellitus in Korean men.[20]

BIOACTIVE PEPTIDES PREVIOUSLY ISOLATED FROM MACROALGAE

In the sourcing of bioactive peptides from macroalgae, it is important to take into account differences in protein content between species and also within species collected at different locations and during different seasons.[40] Generally, the protein fraction of brown macroalgae is low (3−15% of dry weight) compared with that of the green (10−26% of dry weight) or red macroalgae (35−47% of dry weight).[40] As mentioned, the season in which the macroalga is harvested influences the protein content. For example, the protein content of *P. palmata* can vary between 9 and 25% depending on the collection date and is generally highest during the winter season and lowest during the summer months.[40,44]

Bioactive peptides and proteins have been isolated from a variety of species of macroalgae as discussed in a review by Aneiros and Garateix.[13] These peptides include the lectins and phycobiliproteins. Lectins, sometimes referred to as hemagglutinins or agglutinins, are glycoproteins with an ability to agglutinate red blood cells.[45] Lectins may be divided into four main subgroups, namely, legume lectins, chitin binding lectins, monocot mannose binding lectins, and type-2 ribosome inactivating proteins (RIP).[46] The main characteristic of this class of protein is their ability to interact specifically with carbohydrates and to combine with the glycocomponents of the cell surface.[47] Macroalgal lectins have been detected and isolated from the Rhodophyta, including *Solieria filiformis*, *Enantiocladia duperreyi*, *Pterocladiella capillacea*, *Vidalia obtusiloba*, *Gracilaria cornea*, and *Gracilaria ornate*.[47] Lectins have biotechnological applications in several scientific and medicinal fields of research including biology, cytology, biochemistry, and medicine.[45] Macrolagal and many other plant-derived lectins have been used for clinical blood typing in medicinal biology. For example, lectins derived from the green alga *Codium fragile* have been shown to recognize GalNAc, an antigen for blood group A.[48] They also have a number of valuable bioactive properties. For example, lectins from *Perocladiella capillacea* demonstrated analgesic and anti-inflammatory properties in rodent models.[47] Holanda et al. showed that a lectin extracted from the red algae *Solieria filiformis* had antibacterial activity against six pathogenic Gram-negative species including *Serratia marcescens*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Proteus* sp., and *Pseudomonas aeruginosa*.[49] The lectin known as amansin isolated from the red alga *Amansia multifida* is used as a mitogen, which is a substance that encourages cell division, for human lymphocytes, and it therefore has potential for use in anticancer therapies.[50] Phycobiliproteins found in macroalgae are usually divided into three

separate groups on the basis of their color and absorption properties, namely, phycoerythrin, phycocyanin, and allophycocyanin.[51] They are components of the macromolecular light harvesting complex of macroalgae called the phycobilisome.[52] In many red algae, phycoerythrin, the most abundant phycobiliprotein found in the Rhodophyta, is the major soluble protein of the cell.[52] The primary potential of these molecules seems to be as natural dyes, but an increasing number of investigations have shown their health-promoting properties and broad range of pharmaceutical applications.[53] Phycoerythrin is a powerful and highly sensitive fluorescent reagent, which can serve as a label for antibodies, receptors, and other biological molecules in a fluorescence-activated cell sorter, and phycobiliproteins in general are used in immunolabeling experiments and fluorescence microscopy and diagnostics.[53] These macromolecules also have potent bioactivities such as antioxidant, antidiabetic, and anticancer properties. Bermejo et al. demonstrated the antioxidant capabilities of the phycobiliprotein phycocyanin isolated from a protein extract of the green alga *Spirulina platensis* and suggested these bioactive capabilities are attributed to the protein's ability to chelate metal and to scavenge free radicals.[54] Furthermore, purified C-phycoerythrin was shown to relieve the symptoms of diabetic complications in rats through significant reductions in oxidative stress and oxidized LDL-triggered atherogenesis.[55] Following administration of 25 and 50 mg/kg per body weight per day over 28 days, C-phycoerythrin decreased food intake, organ weight, serum concentration of glucose, cholesterol, TAG, VLDL-cholesterol, creatinine, uric acid, and thiobarbituric acid-reactive substances in the rats, suggesting the possible therapeutic role in human diabetes of C-phycoerythrin.[55] Purified recombinant allophycocyanin was found to have a significant inhibition effect on S-180 carcinoma in mice with inhibition rates ranging from 7.9 to 61.9% with doses ranging from 4.65 to 18.6 mg/(kg day).[56] Other peptides include hexapeptide from *Ulva* species, which displays mitogenic properties, and agglutinin glycoprotein from *Soleria robusta* and *Eucheuma serra*, which displays mitogenic, cytotoxic, and anticancer properties.[57]

Digestion of macroalga proteins with proteolytic enzymes has led to the discovery of many bioactive peptides. ACE-I inhibitory peptides were released from *Undaria pinnatifida* proteins using enzymes including pepsin[21] and from the parent proteins of *Polysiphonia urceolata* using the enzymes Protamex, Alcalase, and Flavourzyme.[58] Peptide fractions isolated from the red macroalga *Porphyra yezeonsis* ("nori") had a hypotensive effect when administered orally to SHR models.[59]

EXTRACTION TECHNIQUES USED FOR THE ISOLATION OF BIOACTIVE PEPTIDES FROM MACROALGAE

In screening for compounds of interest from plant sources, several traditional techniques have been used. Classical techniques for bioactive component extraction include the use of solvents. Bioactive component extractions from plant-based materials depend on the choice of solvent coupled with the use of heat and/or agitation.[60] Existing classical techniques used for the extraction of bioactive peptides and components from plants include Soxhlet and maceration with an alcohol−water mixture or hot fat.[61] These methods however are time and labor intensive and introduce potential quantitative errors to the process. Furthermore, the large volume of organic solvents necessary also poses human health and environmental risks.[61] More modern extraction and purification techniques use compressed fluids as the extracting agents. Examples of such techniques include supercritical fluid extraction (SFE), pressurized liquid extraction (PLE), also known as accelerated solvent extraction (ASE (R), a Dionex method), and subcritical water extraction (SWE).[61] Ultrasound-assisted extraction often enhances the effiency of these methods, along with microwave-assisted extraction, sonication, and ultrafiltration.

In SFE, a solvent, usually carbon dioxide (CO_2), is liquefied under pressure and heated to a point at which the solvent has the properties of both a liquid and a gas. This liquefied fluid then acts as the solvent in extracting the desired compounds.[62] Although SFE has been used in the past to extract compounds such as antitumor and antioxidant polysaccharides from macroalgae including *Sargassum pallidum*,[63] it is mainly used to extract lipids and essential oils.[60]

PLE is a sample preparation technique that combines elevated temperature and pressure with liquid solvents to achieve fast and efficient extraction of the analytes from the solid matrix.[64] This technique is considered to be a "green", environmentally friendly, extraction method due to its decreased solvent use, short operating time, and light- and oxygen-free environment.[64] PLE was previously used to extract fucoxanthin from kelp[64] and in the extraction of antioxidant and antimicrobial compounds from the alga *Himanthalia elongata* using the solvents hexane, ethanol, and water.[36] PLE also allows for the use of extraction solvents at temperatures greater than their normal boiling point.[61] SWE is a particular subset of PLE that uses pressurized water at high temperatures to keep it in the liquid state, achieving safe, environmentally friendly, and rapid extractions. Using this method, elevated temperatures modify the dielectric constant of the water, resulting in the possibility of tuning its polarity, thus obtaining selective extractions. Rodríguez-Meizoso et al. used this method to extract bioactive compounds with antimicrobial and antioxidant activity from the microalga *Haematococcus pluvialis*[65] previously. Plaza et al. also isolated antimicrobial and antioxidative agents from the brown alga *Himanthalia elongata* using this method.[36]

Sonicated-assisted extraction is another novel method used in the extraction of bioactive components from plant-based materials and can be coupled with SFE and PLE methods.[60] The principle of sonicated-assisted extraction is based on sound waves traveling through a medium, creating expansion and compression cycles as they do. This mechanical activity induces a greater penetration of solvent into cellular materials and improves mass transfer. It can also lead to the disruption of cell walls, facilitating release of their contents and improving extract yields.[60] Ye et al. used this technique in conjunction with SFE to extract bioactive compounds from the brown alga *Sargassum pallidum*.[63] Galland-Irmouli et al. also utilized this technique in obtaining protein extracts from the edible red macroalga *Palmaria palmata* to estimate its protein content.[66]

Ultrafiltration (UF) is often used coupled with the enzymatic digestion of marine proteins. It is used to fractionate hydrolysates, purify extracts, and also improve the organoleptic quality of hydrolysates as well as clarify and remove color from them.[67] UF membranes constitute a physical barrier, which retains all compounds bigger than the membrane molecular weight cutoff.[68] The main advantages of UF techniques are their ability to filter large quantities of extract and their moderate energy consumption (due to its one-step nature). The possibility to treat without phase change sensitive biological solutions makes this a

user-friendly extraction technique for peptides.[68] A common methodology is to pass a hydrolysate through a series of UF membranes with various molecular weights and compare the specific activity of each fraction with that of the original hydrolysate and thereby identify the most active fraction.[67] Denis et al. successfully scaled up UF methodologies to purify and concentrate the protein R-phycoerythrin from the red alga *Grateloupia turuturu*.[68] Galland-Irmouli et al. also used this method to concentrate R-phycoerythrin from another red alga, *Palmaria palmata*.[66] Pihlanto-Leppala et al. discovered ACE-I inhibitory peptides in whey protein digests by fractionating the hydrolysates with a 30 kDa membrane initially, followed by a 1 kDa membrane. They demonstrated that the 1 kDa fractions had higher ACE-I inhibitory activity, indicating that UF is capable of enriching ACE-I inhibitory peptide from crude enzymatic hydrolysates.[69]

EFFECTS OF FOOD PROCESSING ON BIOACTIVE PEPTIDES

Food processing provides an additional value to foods by improving food safety, shelf life, palatability, nutritive, and functional values.[8] However, depending on the processing technique employed, food processing may be detrimental to the survival of bioactive peptides.[8] Changes in the molecular structure of an amino acid may lead to changes in the bioactivity and absorption of the peptide of interest.[8]

Heat, the most common and oldest form of food processing, modifies the food proteins to make them more edible in terms of texture and flavor.[70] Heat can be used to enhance functional properties of proteins. By denaturing proteins, for example, the water binding and emulsification properties improve; heat also decreases protein solubility due to aggregation and coagulation.[70] During heating, the lysine residues of proteins may react with reducing carbohydrates in the same food system, resulting in what is known as the Maillard or nonenzymatic browning reaction.[70] This can reduce the nutritional value of proteins as the bioavailability of lysine is reduced.[70] Heat may also destroy the bioactivity of peptides. However, it has been shown that ACE-I inhibitory peptides can retain their activity when heated at temperatures of 70 and 100 °C for 20 min.[71]

Other food production processes may facilitate the release of bioactive peptides from their parent proteins.[8] For example, during milk fermentation lactic acid bacteria (LAB) hydrolyze caseins into peptides and amino acids.[8] High hydrostatic pressure also promotes the proteolysis and release of bioactive peptides. Quiros et al. showed that proteolysis of ovalbumin with pepsin for the production of ACE-I inhibitory peptides was accelerated under pressures of 200−400 MPa.[72]

Another factor to consider when bioactive peptides are incorporated into a food matrix is the often bitter taste of hydrolysates, which is attributed to the formation of low molecular weight peptides composed of mainly hydrophobic amino acids.[8] This can limit the use of some bioactive peptides that possess proven bioactivities.[8] Strategies to debitter protein hydrolysates include treatment with activated carbon, extraction with alcohol, isoelectric precipitation, chromatographic separation, masking the taste by using ingredients such as monosodium glutamate, and enzymatic hydrolysis.[73] The latter method has limited capability due to the enzymatic hydrolysis of the active peptides themselves, rendering them inactive.[8]

Microencapsulation is a technology that has solved many problems that limit the use of bioactive peptides and additives in foods, as it may mask undesirable flavors and reduce volatility, hygroscopicity, and reactivity.[74] Furthermore, microencapsulation improves the stability of the products under adverse environmental conditions and provides controlled liberation of the encapsulated material under pre-established conditions.[74] For example, the delivery of tuna-derived antihypertensive OA3 oligopeptides composed of hydrophilic peptides was achieved by encapsulating them in liposomes.[75] Moreover, antimicrobial lysozymes were previously encapsulated using zein micromolecules[76] to minimize the binding between antimicrobials and the food matrix.[76] In addition, casein hydrolysates were previously encapsulated in maltodextrin to mask the off-flavor of the hydrolysate, which facilitated its inclusion in a protein bar.[74]

SUITABLE FOOD VEHICLES

Beverage and Hydrolysate Formulations. There are many products already on the market, which utilize bioactive peptides as functional components. For example, there are two products available that contain the bioactive peptides Ile-Pro-Pro and Val-Pro-Pro, namely, Calpis Ameal-S drink in Japan and Evolus developed by Valio Ltd.; both products have ACE-I inhibitory effects and are generated from fermented milk protein and are proven to lower blood pressure in SHRs and in human clinical trials.[77] Furthermore, a thermolysin digest of dried bonito containing antihypertensive peptides has also been used in a soup product in Japan with antihypertensive effects.[77,87] Table 1 displays more products available currently containing bioactive peptides with various health-promoting properties.

Bakery Products. *Porphyra* species are already traditionally used in Wales to make a bread known as laver bread.[78] Bakery and pasta products, being the most widely consumed food products in the world,[79] are some of the best potential food vehicles for delivery of bioactive compounds.[79] Prabhasankar et al. incorporated wakame (*Undaria pinnatifida*) into pasta to improve its biofunctional properties and found that incorporation of up to 20% wakame enhanced interactions between starch granules and the protein matrix. This resulted in an improved pasta quality.[80]

In an attempt to increase the amount of fiber in the form of alginate, Hall et al. previously demonstrated that the macroalga *Ascophyllum nodosum*, when incorporated in amounts of between 20 and 400 g per loaf of bread, had no significant difference in acceptability under the terms of appearance, aroma, flavor, aftertaste, and texture when compared to a non-macroalga-enriched control bread.[81] This was assessed using a taste panel of 79 untrained sensory panelists using visual analogue scales (1−9; extremely unacceptable to extremely acceptable).[81] The introduction of alginate into a diet is thought to slow gastric emptying and create a prolonged sense of satiety.[82] By the incorporation of brown macroalgae (10−40% of which is alginate) into food products, researchers have postulated that it may be possible to increase the antiobesity and antidiabetic properties of the food.[82] Microencapsulated polyunsaturated fatty acids (PUFA) derived from tuna have been incorporated into bread and are proven to increase PUFA levels in human blood plasma.[83] PUFAs are known to lower blood pressure, reduce serum triglyceride levels, have anti-inflammatory activities, and decrease the risk of cardiac events.[84] Macroalgae and microalgae are the nutritional source of PUFAs for many fish;

Table 1. Food Products Containing Bioactive Peptides

effect	product	peptide(s)	company	source	ref
heart healthy					
hypotensive	Ameal S	Val-Pro-Pro Ile-Pro-Pro	Calpis	milk	77
hypotensive	Evolus	Val-Pro-Pro Ile-Pro-Pro	Valio Ltd.	milk	77
hypotensive	peptide soup	Leu-Lys-Pro-Asn-Met (katsuobushi oligopeptide)	Nippon Supplement, Inc.	katsuobushi (dried bonito)	88
hypotensive	Casein DP	FFVAPFPEVFGK	Kanebo Ltd.	milk	6
hypotensive	C12 peptide	FFVAPFPEVFGK	DMV (De Melkindustrie Veghel) International	milk	6
hypotensive	Valtyron	Val-Tyr	Senmi Ekisu Co. Ltd.	sardine	89
hypotensive	BioZate	whey-derived peptides	Davisco	milk	6
cholesterol lowering	CSPHP	enzymatically decomposed lecithin bound to hydrolyzed isolated soy protein (ratio of 80:20)	Kyowa Hakko	soy	90
other					
energy boost	cysteine peptide food supplement	mixture of whey protein derived peptides comprising at least 6.5 wt % cysteine	DMV International	milk	91
satiety tooth	glycomacropeptide (GMP)	casein-derived whey peptide	Davisco	milk	92
remineralization					
dental plaque reduction					

hence, macroalgae are also a viable source for these bioactive molecules.[84] Moreover, certain components of marine algae appear to improve the rheological properties of flour dough and the quality of the finished products made from it.[85] Oxidoreductase derived from the algae *Chondrus crispus* when added to flour dough increased resistance to extension and extensibility by at least 10% compared to those of a similar dough that did not contain oxidoreductase. It also increased the final volume of a finished baked product by 20%, compared to that of a similarly baked product not containing oxidoreductase.[85]

FUTURE WORK

In the past, the primary source of bioactive peptides was dairy products.[3] The variety of macroalga species and the environments in which they are found and their ease of cultivation make macroalgae a relatively untapped source of new bioactive compounds,[36] and more efforts are needed to fully exploit their potential for use and delivery to consumers in food products. Furthermore, the majority of research published to date concerned with heart health peptides details the extraction and use of ACE-I-inhibitory peptides.[86] Future work should aim to widen the scope of heart health peptide research and should include renin and PAF-AH inhibitory peptides.

More research is needed to fully ascertain the effect that food-processing methods such as heating have on the bioactivity of peptides. Although it has been shown that peptides can retain bioactivity after being heated up to 100 °C,[71] many processes such as baking reach temperatures as high as 230 °C. If these high temperatures become a barrier to the survival of the bioactivities of peptides, methods of protecting these bioactivities in these environments, such as microencapsulation methods, need to be investigated.[8] Microencapsulation is also seen as a method for creating a barrier against bioactive peptides interacting with the ingredients in the surrounding environment.[76]

AUTHOR INFORMATION

Corresponding Author

*Phone: +353 (0) 18059500. Fax: +353 (0) 1 8059500. E-mail: maria.hayes@teagasc.ie.

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