Phlorotannins and Macroalgal Polyphenols: Potential As Functional Food Ingredients and Role in Health Promotion

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Abstract

Marine macroalgae are rapidly gaining recognition as a source of functional ingredients that can be used to promote health and prevent disease. There is accumulating evidence from in vitro studies, animal models, and emerging evidence in human trials that phlorotannins, a class of polyphenol that are unique to marine macroalgae, have anti-hyperglycaemic and anti-hyperlipidaemic effects. The ability of phlorotannins to mediate hyperglycaemia and hyperlipidaemia makes them attractive candidates for the development of functional food products to reduce the risk of cardiovascular diseases and type 2 diabetes. This chapter gives an overview of the sources and structure of phlorotannins, as well as how they are identified and quantified in marine algae. This chapter will discuss the dietary intake of macroalgal polyphenols and the current evidence regarding their anti-hyperglycaemic and anti-hyperlipidaemic actions in vitro and in vivo. Lastly, this chapter will examine the potential of marine algae and their polyphenols to be produced into functional food products through investigating safe levels of polyphenol consumption, processing techniques, the benefits of farming marine algae, and the commercial potential of marine functional products.

Keywords

Hyperglycaemia · Hyperlipidaemia · Macroalgae · Phlorotannin · Polyphenol

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3.1 Introduction

Polyphenols are non-nutrient compounds that are produced in both terrestrial plants [1, 2] and marine macroalgae (seaweeds) [3]. Their natural function is predominantly to act as the defence system of these organisms, protecting against infection [1, 2, 4], ultraviolet radiation [1, 2, 4, 5] and consumption by herbivores [4, 5]. They can also play a role as integral structural components of cell walls [4, 6, 7]. When consumed by humans, polyphenols demonstrate biological activity that may be beneficial to human health and disease prevention [3, 8, 10–12].

Over 8000 structurally different polyphenols have been identified in plants, from simple monomer units to complex polymerised structures [8, 9, 11, 13]. However, only several hundred of those varieties exist in edible plants [1]. Other varieties of polyphenols (e.g. phlorotannins) have been identified in both edible and nonedible forms of marine algae. There is ongoing investigation of successful extraction techniques and the potential for marine algal polyphenols to be used as supplements and functional food ingredients [14, 15–18]. This chapter discusses polyphenols from marine macroalgae, their dietary intake levels, their potential as functional food ingredients and their potential role as mediators of risk factors for cardiovascular disease and type 2 diabetes.

3.2 Macroalgal Sources of Polyphenols

There exist over 10,000 known species of macroalgae, which are classified into three categories based on their pigmentation: Chlorophyta and Charophyta (green algae), Rhodophyta (red algae) and Phaeophyta (brown algae) [19].

Green algae get their characteristic colour from a combination of chlorophyll *a* and *b*, beta-carotene and various xanthophylls. Due to the diversity of green algae, they are classified into two phyla (Chlorophyta and Charophyta) [19]. There are about 4500 species of Chlorophyta, including species found in freshwater and marine habitats, and about 3500 species of Charophyta all of which are freshwater-dwelling. Some applications of green algae include commercial use for the production of beta-carotene and use in nutritional supplements, particularly the *Chlorella* genus, to improve healing and enhance the immune system [19, 20].

Approximately 6500 species of algae are classified as red algae, due to the presence of the pigments phycoerythrin and phycocyanin, which mask the other pigments and provide the red colour [19]. Almost all red algal species are found in marine habitats and reside in the intertidal and subtidal zones. Several types of red algae are eaten as food, the most popular of which is Nori (*Porphyra*) [19, 21, 22], now the most valuable marine crop grown by aquaculture, valued at over US$1 billion [19]. Red algae are also a source of carrageenan, a commonly used food stabiliser, and agar, an ingredient used in food, pharmaceuticals, and cosmetics and as a growth medium for microorganisms [19, 23].

There are about 1800 known species of brown algae, all of which get their brown colour from the dominant pigment fucoxanthin [19]. Brown algae are larger than red and green algae, with the largest kelps growing up to 70 m in length. They are mostly found in marine habitats, commonly in cooler Northern hemisphere waters [19]. Japan, Korea and China grow brown algae for use in food and alginate production. Ireland and Scotland also use brown algae for the production of alginates and as a fertiliser for land. Other places where brown algae are harvested include Atlantic France and the coasts of California [19].

Of the three varieties of macroalgae, brown algae contain the highest levels of polyphenols, in particular phlorotannins which alone can make up 2–30% of the dry weight (DW) of the organism [4, 5], compared with approximately 0.1–4% and 0.2–20% total phenolic content in green and red algae, respectively [24, 25]. Brown algae will therefore be the focus of this chapter. A number of polyphenol classes, including catechins, flavonoids, flavonols and phlorotannins, are all found in marine macroalgae [3, 25]. However phlorotannins are the predominant class of polyphenol found in brown algae [18, 26] and are unique to marine sources [4]. Marine macroalgae thrive in harsh environments, including exposure to varying light intensity, salinity, pressure and temperatures, and therefore produce a variety of potent polyphenolic substances, which are not found in terrestrial plants [28].

3.3 Classification and Structure of Phlorotannins

Polyphenol is an umbrella term for a large group of highly heterogeneous compounds, characterised by the presence of at least one phenol structural unit (aromatic ring) [8]. Phlorotannins are a class of polyphenol that are synthesised in marine macroalgae through the acetate-malonate pathway by the polymerisation of phloroglucinol monomer units (1,3,5-tri hydroxybenzene) [3, 4, 18, 27] (Fig. 3.1). Phlorotannins are highly hydrophilic molecules that contain both phenyl (C_6H_5-) and phenoxy (C_6H_5O-) groups (Fig. 3.1) and range in size from 126 Da to 650 kDa [3], a much broader range than terrestrial polyphenols (up to 30 kDa) [28]. Phlorotannins vary in degree of polymerisation, structure and type of chemical bonds [5], resulting in a complex group of compounds with numerous isomers for any given molecular weight [4]. Phlorotannins are classified into four subclasses based on the chemical bonds they contain [3]. The subclasses are (1) fucols which have a phenyl linkage, (2) fuhalols and phlorethols which contain an ether linkage, (3) fucophloroethols which have a mixture of a phenyl and ether linkage and (4) eckols which contain a dibenzodioxin linkage (Fig. 3.1) [3]. However the literature often defines phlorotannins based on the species of their algal source or by naming the specific type of phlorotannin (e.g. phlorofucofuroeckol A, dieckol) rather than which subclass they belong to.

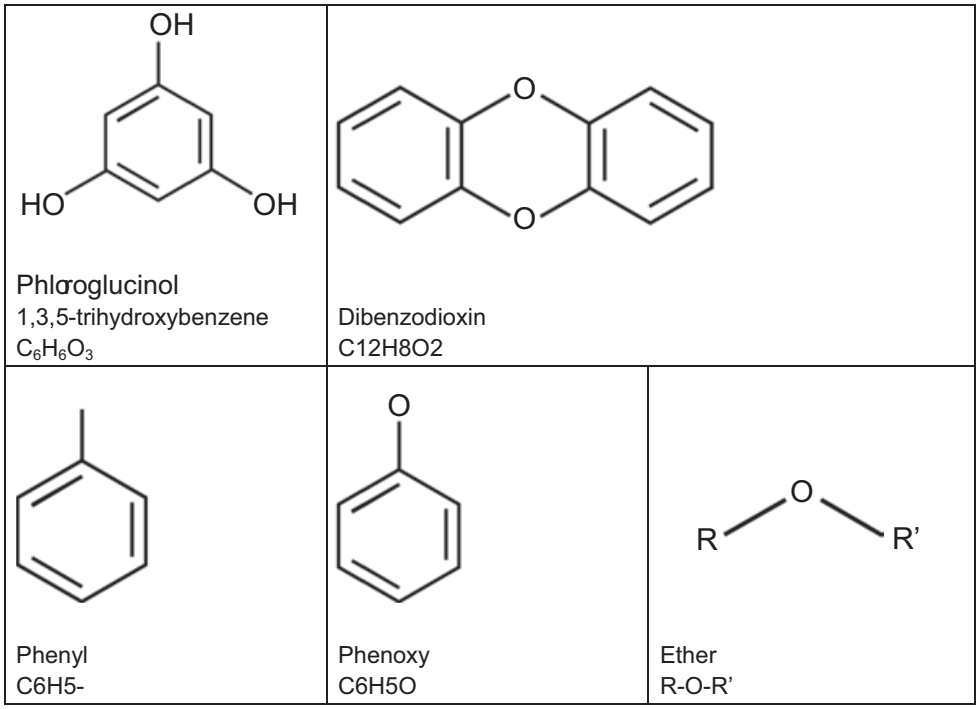

Fig. 3.1 Chemical structures of phloroglucinol, dibenzodioxin, phenyl, phenoxy and ether groups [105]

3.4 Phlorotannin Content of Macroalgae

Different algal species contain varying combinations and concentrations of phlorotannins, and a range of low- and high-molecular-weight phlorotannins can be found within a single species of marine alga [4] (Table 3.1). Within an individual algal body, phlorotannins are generally more concentrated in the outer layers of the organism, where it is exposed to the environment [27]. Phlorotannin content can fluctuate within the same population area and within a single algal body [5]. Environmental factors such as ultraviolet radiation, salinity, light and nutrient availability and herbivore grazing are likely causes for differences in phlorotannin content [5]. Furthermore, since the natural function of phlorotannins is as part of the defence system of the organism, algae that require a greater level of defence against harsh environments or attack from predators are likely to have a higher phlorotannin content [1, 4, 5]. This effect is seen in land-based fruit crops, where crops that are grown organically, without synthetic pesticides, have a higher polyphenol content than those grown the conventional way because they have had more exposure to stressful situations, resulting in an increased natural defence [29].

The location of brown algal species can also impact phlorotannin content. Brown algal species grown in the intertidal zones have the highest phlorotannin content, whereas those grown at lower and upper levels of the shore have lower phlorotannin content [30]. This is likely due to differences in exposure to environmental factors as those in the intertidal zone would experience a more rapidly changing and varied environment with the movements of the tide. The phlorotannin content of brown algae also varies according to season, and the degree of

Table 3.1 Summary of phlorotannins isolated from marine algal species [105]

Seaweed species	Reported phlorotannin content	References
Ascophyllum nodosum	Approx. 5.80% of dry weight	[30]
Bifurcaria bifurcata	3.73 (0.57)% of dry weight	[30]
Cystoseira nodicaulis	89.14 (2.57) g phloroglucinol equivalents (PGE)/mg sample	[4]
Ecklonia cava	3.3% crude phlorotannins:	[27]
	4.7% phloroglucinol	
	0.7% phloroglucinol tetramer	
	6.4% eckol	
	16.6% phlorofucofuroeckol A	
	22.2% dieckol	
	12.4% 8,8′-bieckol	
Ecklonia cava	Dieckol – 1.52 mg/g dry weight	[18]
Ecklonia cava	Phlorofucofuroeckol A – 0.93 mg/g dry weight	[18]
Ecklonia cava	Total phlorotannin content – 3.39 mg PGE/mL in crude phlorotannin extract solution	[107]
Ecklonia kurome	3.0% crude phlorotannins:	[27]
	2.6% phloroglucinol	
	0.3% phloroglucinol tetramer	
	9.2% eckol	
	28.6% phlorofucofuroeckol A	
	24.6% dieckol	
Ecklonia kurome	7.8% 8,8′-bieckol	[27]
Ecklonia stolonifera	Dieckol – 1.52 mg/g dry weight	[18]
Ecklonia stolonifera	Phlorofucofuroeckol A – approx. 1.20 mg/g dry weight	[18]
Eisenia bicyclis	3.1% crude phlorotannins:	[27]
	0.9% phloroglucinol	
	4.4% phloroglucinol tetramer	
	7.5% eckol	
	21.9% phlorofucofuroeckol A	
	23.4% dieckol	
	24.6% 8,8′-bieckol	
Eisenia bicyclis	Phlorofucofuroeckol A – 1.30 mg/g dry weight	[18]
Eisenia bicyclis	Dieckol – 1.33 mg/g dry weight	[18]
Eisenia bicyclis	Contains eckol	[106]
Eisenia bicyclis	Contains 6,6′-bieckol	[106]
Eisenia bicyclis	Contains 8,8′-nieckol	[106]
Eisenia bicyclis	Contains dieckol	[106]
Eisenia bicyclis	Contains phlorofucofuroeckol A	[106]

(continued)

Table 3.1 (continued)

Seaweed species	Reported phlorotannin content	References
Fucus serratus	4.27 (1.12)% of dry weight	[30]
Fucus serratus	180.55 (16.98) μg PGE/mg sample	[4]
Fucus spiralis	3.88 (0.65)% of dry weight	[30]
Fucus vesiculosus	Phlorotannins approx. 5.80% of dry weight	[30]
Fucus vesiculosus	231.95 (8.97) μg PGE/mg sample	[4]
Fucus vesiculosus	Total phlorotannins ranged from 12 to 23 mg/g dry weight	[6]
Himanthalia elongata	2.17 (1.40)% of dry weight	[30]
Himanthalia elongata	198.28 (9.17) μg PGE/mg sample	[4]
Laminaria digitata	0.13 (0.03)% of dry weight	[30]
Pelvetia canaliculata	3.39 (0.64)% of dry weight	[30]
Sargassum aquifolium	6.770 (0.001) mg phlorotannins/g dry weight	[32]
Sargassum denticarpum	0.978 (0.004) mg phlorotannins/g dry weight	[32]
Sargassum mcclurei	2.057 (0.003) mg phlorotannins/g dry weight	[32]
Sargassum oligocystum	2.369 (0.004) mg phlorotannins/g dry weight	[32]
Sargassum serratum	1.305 (0.008) mg phlorotannins/g dry weight	[32]
Sargassum polycystum	0.735 (0.002) mg phlorotannins/g dry weight	[32]

Abbreviations: *PGE* phloroglucinol equivalents

seasonal variation differs among species [30]. During summer the *Fucales* genus, *Pelvetia canaliculata* (3.72% dry weight (DW)) and *Ascophyllum nodosum* (7.18% DW) species exhibit their maximal phenolic content, whereas the *Laminariales* genus has its highest phenolic content in winter. During spring months the *Fucus vesiculosus* (7.84% DW) and *Ecklonia radiata* species have their highest phenolic levels [30, 31]. As well as being influenced by season, time spent in storage can impact phlorotannin content. In a study of six *Sargassum* species of macroalgae, grown in Vietnam, the amount of time spent in storage was negatively associated with phlorotannin content; however the rate at which phlorotannin content decreased varied among species [32].

3.5 Identification and Analysis of Macroalgal Phlorotannins

There is currently limited knowledge of the variety of phlorotannins in macroalgae, and the distribution of phlorotannins within specific algal species, largely due to the structural complexity and polymeric nature of phlorotannins, with variations in the number of monomer units, their positions, and chemical bonds with which they are joined [4]. Historically, only low-molecular-weight phlorotannins (2–8 phloroglucinol units) could be characterised [4]. However, recent technological advancements in chromatographic and mass spectrometric techniques allow for more thorough study of the complex structures of phlorotannins and their distribution in macroalgae, with isomers of up to 16 monomer units successfully detected [4]. One technique by which phlorotannins have been successfully separated from a crude phenolic extract is using high-performance liquid chromatography (HPLC) with UV photodiode array detection [7]. Ultrahigh performance liquid chromatography (UPLC) with mass spectrometry has also been used to profile individual phlorotannins [4, 6]. This technique has identified 61 isomers corresponding to 12 phloroglucinol units, from the brown alga *Fucus vesiculosus*, and determined the isomerisation of phlorotannins ranging from 3 to 16 monomers [4]. With improved technologies we are now able to identify and characterise a greater variety of phlorotannin molecules and accurately determine the phlorotannin content of macroalgae.

Furthermore, depending on the type of extraction solvent used, different quantities of individual phlorotannins have been extracted from the same macroalgae [18]. When phlorotannins were extracted from *Ecklonia cava*, *Ecklonia stolonifera* and *Eisenia bicyclis* using two different solvents, the dieckol yield by boiling water extraction was 86%, 93% and 98% of the organic solvent extraction, respectively. The phlorofucofuroeckol A yield from boiling water extraction was 74%, 86% and 62% of the organic solvent extraction, respectively [18]. This highlights the need for the identification of the most efficient extraction solvent.

Efficient extraction techniques result in a greater yield of phlorotannins from the same biomass, meaning greater potential for biological activity. Organic solvent, such as ethanol and methanol, extraction is the most common method used for extraction of phlorotannins [18]. Boiling water extraction produces a slightly lower yield of phlorotannins than organic solvent extraction (see figures above); however it is a safer and more cost-effective method [18]. It has been suggested that a 70% aqueous acetone solution is most efficient for extraction of phlorotannins due to its ability to inhibit interactions between tannins and proteins and to break hydrogen bonds [7]. However more recent findings indicated that this method was less effective than both boiling water and organic solvent extraction [18]. For the extraction of phlorotannins for use as a functional food ingredient, it appears that either organic solvent extraction or boiling water extraction is the most effective method, depending on the cost and safety considerations. Elucidation of the most efficient extraction technique, along with improved technologies for identifying individual phlorotannins, will enable future researchers to identify the most effective phlorotannin molecules for the promotion of health and prevention of disease in humans.

3.6 Dietary Intake of Macroalgal Phlorotannins

The difficulties associated with quantifying macroalgal phlorotannin content have likely contributed to the lack of information on population intake of macroalgal phlorotannins. However, macroalgae consumption is documented in Asian countries, such as Japan, where it is a traditional part of the diet [21, 33]. With this information it is possible to retrospectively extrapolate phlorotannin intake using existing macroalgal phlorotannin content data (Table 3.1). In 2006, Japanese households consumed 450 g per year of kombu (*Laminaria japonica* – a brown macroalgae), although generally consumption was four times higher in elders than in young adults (<29 years) [22]. Despite a decrease in kombu consumption in Japan, daily macroalgae intake remained relatively stable, 4.3 g/day in 1955 and 5.3 g/day in 1995, with an increase in wakame (*Undaria pinnatifida* – a brown macroalgae) and nori (*Porphyra* genus – a red macroalgae) varieties making up for the decline in kombu [22]. Based on an average dietary intake of 5.3 g of macroalgae per day, the Japanese population would be consuming approximately 160 mg of phlorotannins per day [27, 30]. However this value would vary depending on individual intake, macroalgal variety and other factors that affect phlorotannin content as previously described, such as season and processing. From the red alga family, *Porphyra* is most frequently consumed (nori). From the brown algae, the *Laminaria japonica* (kombu), *Undaria pinnatifida* (wakame) and *Hizikia fusiforme* (hijiki) species are the most commonly consumed [21, 22].

In most western cultures, macroalgae is relatively new to the diet, but consumption has been steadily increasing since the early 1980s [21, 33] due to consumer demand for interesting, natural and sustainable food products and due to globalisation of the food system [33, 34]. There is, however, limited literature available regarding daily intakes of macroalgae among western cultures; the red alga *Palmaria palmata*, common in Atlantic waters, is one of the few algal species that is documented to have been used for human consumption in Europe [33]. However, there are now polyphenol-rich macroalgal extracts that are commercially available as health food products in the United States of America, Canada and Korea. These supplements may significantly increase the average population intake of macroalgal polyphenols in these countries, especially given the health claims these products carry (including antioxidant and anti-inflammatory activity, improvement of lipid balance, weight loss and protection against cardiovascular disease and diabetes) and the likelihood of health claims to positively influence consumer purchase intentions [35]. However, even though these health claims are as yet unsubstantiated in humans, there is some support for their role in these health outcomes based on evidence from in vitro and animal studies.

Accurate estimation of phlorotannin intake based on dietary intake data, like any other dietary component, is difficult [36]. Collection of dietary data is predominantly self-reported and therefore is likely to be inexact and carry bias. Perceived ‘unhealthy’ foods are often under-reported, while perceived ‘healthy’ foods are typically over-reported [37], resulting in an overestimation of polyphenol intake. The difficulty of estimating polyphenol intake is further exacerbated as the

polyphenol content of foods is not included in most food composition databases. An online European database provides some information on the polyphenol content of 459 common foods (including 500 different polyphenols), but as yet this is limited to polyphenols from terrestrial food sources and does not include phlorotannins [38]. Furthermore, the variation in the phlorotannin content of algal species increases the difficulty of accurate intake estimation, as the recorded phlorotannin content of macroalgae in food databases may not be an accurate representation of all individuals in that species. However, with ongoing improvement (e.g. information on effects of cooking/processing on polyphenol content, polyphenol metabolites formed in the body and additional polyphenol sources), food databases such as Phenol-Explorer may be a useful tool to improve understanding of the impact of polyphenols on health [38]. The use of biomarkers, such as urinary excretion or plasma levels of polyphenols, is becoming more widely used to determine phlorotannin intake and can be used to make conclusions about the potential health effects of phlorotannins [39]. However only 5–10% of dietary polyphenol intake is actually absorbed into the plasma and excreted in urine [40], thus while these are more technically accurate methods of measurement than dietary intake, they may not be better tools for determining total polyphenolic intake and resulting biological activity.

3.6.1 Bioavailability of Polyphenols

Bioavailability is defined as the proportion of a compound that is digested, absorbed and metabolised in the body through normal pathways [9]. In order to properly assess the biological functioning of macroalgal polyphenols, it is important to measure their bioavailability in humans [9]. Most evidence for the biological activity of macroalgal polyphenols to date has been derived from cultured cells or animal models, which do not account for the effects of other dietary components, or human digestion and absorption, and therefore may not represent the biological actions of polyphenols in people [1, 10]. Thus polyphenols that have exhibited strong biological activity in vitro may not have the same effect in the human body [8, 41].

The bioavailability of any compound is affected by its ability to cross biological membranes, withstand pH changes in the gastrointestinal tract and maintain its structural integrity [41, 42]. Factors that affect the absorption and metabolism of polyphenols from food are presented in Table 3.2. During digestion, polyphenols are metabolised in the small intestine, in the large intestine by colonic microflora as well as in the liver and other organs whereby they go through numerous structural modifications [1, 9, 14, 43]. This means that human body tissues are not exposed to polyphenols in their original form [8, 41]. Thus, in vitro studies that examine polyphenol extracts (which have not undergone digestion) are not a true representation of the activity or concentration of the metabolites present in the human body [8, 14, 41]. Studies of polyphenols need to take into account the changes in structure and concentration that occur when the compounds enter the human body [8, 41]. To further complicate the issue, personal variations in intestinal microflora may also impact an individuals' metabolism and absorption of polyphenols; however this

Table 3.2 Factors that affect the absorption and metabolism of food-derived polyphenols

Factors	References
Chemical structure of polyphenols (degree of glycosylation/acylation, basic structure, conjugation with other phenolics, molecular size, degree of polymerisation and solubility)	[1, 9, 13, 28]
Dietary factors (interactions with proteins and polysaccharides, pH of the gut, transit time, intestinal fermentations, biliary excretion, the influence of certain food components on enzymes and carriers)	[1]
Human behavioural factors (such as smoking)	[10]
Person-to-person variation in enzyme activity	[10]
Whether absorption takes place in the small intestine or colon	[1, 41]

area is not yet well understood [41]. These issues highlight the need for more studies investigating the healthful effects of macroalgal polyphenols to be performed in humans.

3.7 Role of Macroalgal Polyphenols in Human Health

As already alluded to in this chapter, polyphenols from terrestrial sources have been linked to positive health effects regarding several risk factors for chronic conditions including obesity, diabetes and cardiovascular diseases [8, 11, 43–47]. Recent research has extended to polyphenols from marine macroalgae, possibly because of epidemiological data from Asia which indicate that a diet rich in macroalgae or seaweed is associated with longevity and a decreased risk for cardiovascular diseases, some cancers and other chronic diseases [48–50]. Another factor that has contributed to increased interest in macroalgae for health promotion is the observation of the lack of photodynamic damages that macroalgae experience during metabolism. Macroalgae grow in harsh environments where they are exposed to light and high oxygen concentrations, factors which lead to the formation of free radicals and other strong oxidising agents [51]. Despite these damaging molecules, the lack of damage that macroalgae experience implies that they have some protective antioxidant mechanisms and/or compounds [51], which may be beneficial to humans as well. The accumulating evidences do indicate that polyphenols from macroalgae can influence glycaemic control, blood cholesterol and lipid levels (Fig. 3.2), which may potentially reduce metabolic abnormalities associated with cardiovascular diseases (CVDs) and diabetic complications [15–17, 43, 52–68].

3.7.1 Anti–hyperglycaemic Effects

Hyperglycaemia is a key characteristic and cause of type 2 diabetes [43]. Both acute and chronic hyperglycaemia cause overloading of the metabolic pathways with glucose, resulting in oxidative stress and free radical formation which lead to diabetic complications such as cardiovascular disorders, nephropathy, retinopathy, neuropathy, foot and leg ulcers and limb amputation [3, 69]. Alpha-amylase,

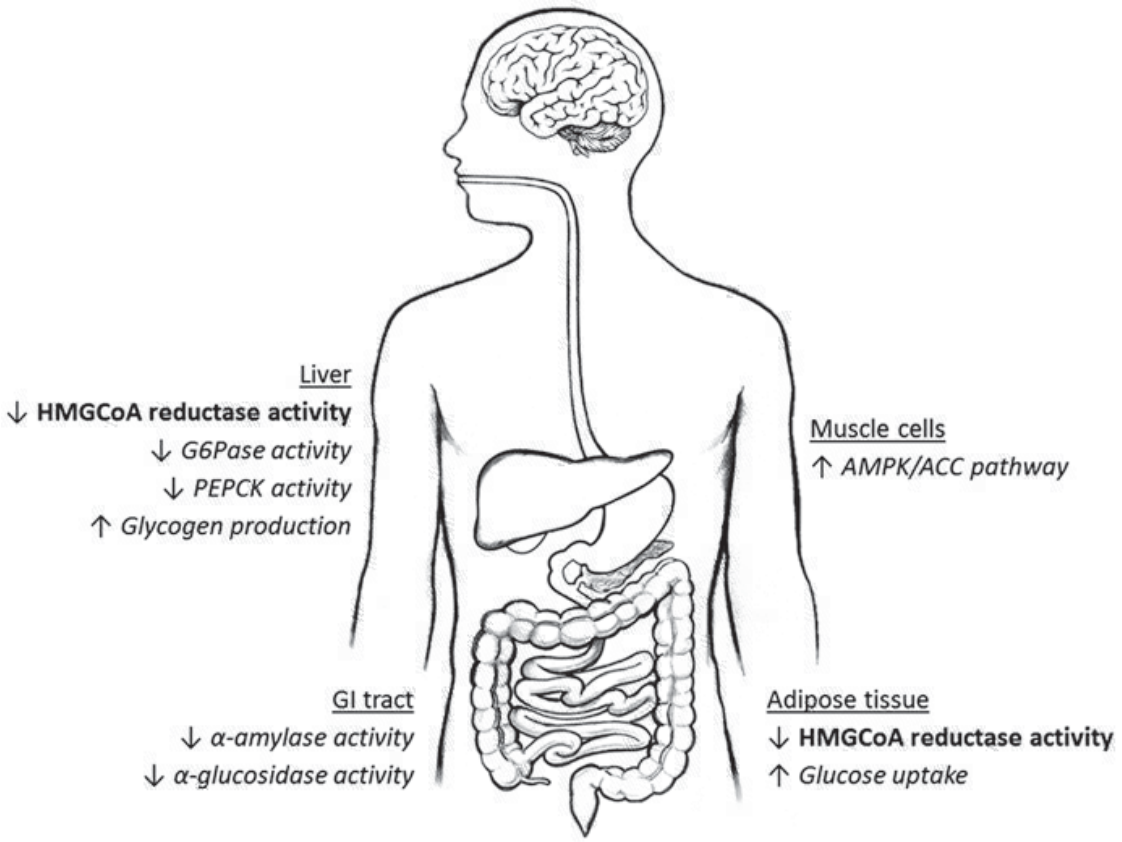

Fig. 3.2 The proposed anti-hyperglycaemic (in *italics*) and anti-hyperlipidaemic (in **bold**) effects of marine polyphenols. (Adapted from [105])
↑, increased, ↓, decreased; AMP-activated protein kinase/acetyl-CoA carboxylase (AMPK/ACC) increases GLUT4 transporters to transport glucose out of the blood and into the tissue. Inhibition of glucose-6-phosphatase (G6Pase) reduces the amount of glucose released from the liver. Inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMGCoA) reductase reduces plasma cholesterol levels. Inhibition of phosphoenolpyruvate carboxykinase (PEPCK), an enzyme involved in gluconeogenesis, reduces the amount of glucose released from the liver. Proliferator activated receptor γ (PPARγ) and CCAAT/enhancer-binding protein α (C/EBPα) are genes involved in adipogenesis and adipocyte differentiation

located in the pancreas, and α-glucosidase, at the brush border of intestinal cells, are key enzymes that break down carbohydrates into monosaccharides to be absorbed into the bloodstream, resulting in increased blood glucose levels following a meal [3, 70, 71]. Inhibition of these enzymes reduces the rate at which glucose is released from carbohydrate foods following a meal and can be an effective strategy for managing postprandial blood glucose [3, 70, 71]. Oral glucosidase inhibitor drugs are the common clinical treatment for type 2 diabetes; however long-term use can result in side effects such as renal tumours, acute hepatitis and serious hepatic injury [3]. A number of studies suggest that marine polyphenols may be safer alternatives [3, 43, 54, 63].

The inhibition of α-amylase and α-glucosidase results in short-term management of blood glucose levels; however there are a number of other ways that marine polyphenols may also contribute to longer-term glycaemic control (Fig. 3.2). Marine

polyphenols upregulate the AMP-activated protein kinase/acetyl-CoA carboxylase (AMPK/ACC) signal transduction pathways in muscle cells, which increases the number of GLUT4 glucose transporters at the cell membranes to increase the uptake of glucose into the tissues [58, 72]. They also downregulate glucose-6-phosphatase and phosphoenolpyruvate carboxykinase (PEPCK) enzymes in the liver, which reduces the formation of new glucose through gluconeogenesis. In turn, the rate of glycogen production is increased in the liver, which ultimately promotes the uptake of glucose by the liver and reduces blood glucose levels [61].

3.7.1.1 In Vitro Studies

Polyphenolic-rich extracts from the marine macroalgae *Alaria*, *Pulmaria* and *Ascophyllum* exhibited some α-amylase inhibitory activity in cultured colon cancer cells [62]. The extract from *Ascophyllum* demonstrated strongest α-amylase inhibition (IC_{50} approximately 0.1 μg/mL gallic acid equivalents (GAE)) and was the only extract that also inhibited α-glucosidase activity (IC_{50} approximately 20 μg/mL GAE) [62]. An *Ascophyllum nodosum* extract has also been shown to induce dose-dependent α-amylase and α-glucosidase inhibition [52]. Furthermore, the α-glucosidase inhibition observed from the phlorotannin-rich *Ascophyllum nodosum* extract (0.24 μg phenolics) was greater than that of acarbose (0.37 μg), a current antidiabetic drug [52]. Diphlorethohydroxycarmalol (DPHC), a phlorotannin extracted from *Ishige okamurae*, dose-dependently inhibited α-amylase and α-glucosidase activity (IC_{50} values of 0.53 and 0.16 mM, respectively) in a chemical assay and was more effective than acarbose [54]. Strong α-glucosidase inhibitory effects have also been demonstrated from phlorotannin-rich *Ecklonia stolonifera* extracts [56].

Marine polyphenols act on other enzymes involved in carbohydrate metabolism to reduce hyperglycaemia. Phloroglucinol, eckol, phlorofucofuroeckol A, dieckol, 8,8′-bieckol and an unidentified tetramer phlorotannin were extracted from *Eisenia bicyclis* and examined for enzyme inhibitory action in vitro [65]. Phlorofucofuroeckol A, dieckol and 8,8′-bieckol inhibited α-fucosidase, β-galactosidase and β-mannosidase (extracted from the turban shell of *Turbo cornutus*), whereas phloroglucinol, eckol and the unidentified tetramer were only weakly active against the enzymes [65]. An additional way in which phlorotannins from *Ascophyllum nodosum* (400 μg/mL extract) have been demonstrated to reduce hyperglycaemia is to increase basal glucose uptake in 3 T3-L1 adipocytes; during a 20-min incubation period, glucose uptake increased by approximately threefold [68]. This action is possibly due to an increase in GLUT transporters; however this detail was not reported. Additionally, marine polyphenols from *Ecklonia cava* have activated AMPK/ACC signal transduction pathways in C_2C_{12} myoblasts [58], which resulted in increased glucose uptake into the cells and is another potential mechanism for a reduction in blood glucose levels [72] (Fig. 3.2).

3.7.1.2 Animal Studies

A diet supplemented with 0.5% w/w of a polyphenol-rich extract from *Ishige okamurae* for 6 weeks reduced hepatic G6Pase, PEPCK activity and increased hepatic glycogen production in C57BL/KsJ-db/db mice, which resulted in reduced fasting blood glucose levels [61]. The treated mice also presented with reduced hyperinsulinemia and HbA1c, compared with control [61]. Similarly, diabetic KK-A^{γ} mice were administered 16.42 or 81.20 mg/day (0.2% or 1% of diet, respectively) of a phlorotannin extract from *Ecklonia stolonifera* for 4 weeks. The treated mice maintained blood glucose and insulin levels at a close-to-normal level in a dose-dependent manner, compared with untreated diabetic mice whose blood glucose and insulin levels increased over time [58]. Kang et al. [59] also identified that supplementation with dieckol (20 mg/kg body weight/day for 14 days), from *Ecklonia cava*, reduced blood glucose and insulin levels in C57BL/KsJ-*db/db* diabetic mice. Interestingly, a dose-dependent treatment effect on insulin levels but not blood glucose levels was observed [59]. Park et al. [64] reported that supplementation with 200 mg/kg body weight/day of an *Ecklonia cava* polyphenol extract for 7 weeks reduced fasting blood glucose in obese C57BL/6 mice compared with placebo. They also found that an extract taken from the geographical area of Gijang, Korea (79.70 mg polyphenols/g extract), improved glucose tolerance compared with an extract from the area of Jeju, Korea (68.78 mg/g polyphenols), and placebo [64].

Zhang et al. [68] reported the effects of an *Ascophyllum nodosum* extract on diabetic markers in streptozotocin-induced diabetic mice. The mice were administered 200 mg/kg body weight of a crude extract or enriched extract (purified polyphenolic fraction) orally by daily gavage for up to 4 weeks. Both extracts reduced fasting blood glucose in the diabetic mice compared with placebo. However, only the enriched extract dampened the postprandial rise in blood glucose following an oral sucrose tolerance test [68]. Similarly, a dieckol-rich extract from *Ecklonia cava* (0.5 g/100 g diet) reduced fasting blood glucose levels, HbA1c levels and plasma insulin levels in C57Bl/6/KsJ-*db/db(db/db)* mice after 6 weeks, compared with a control diet [60]. The effect observed from the dieckol-rich extract was comparable to that of rosiglitazone (0.005 g/100 g diet), a known antidiabetic drug. Glucose tolerance also improved in the mice as a result of phlorotannin supplementation; the blood glucose area under the curve (AUC) was significantly reduced following phlorotannin treatment compared with control [60]. An *Ecklonia cava* polyphenol extract (28.2 ± 0.58% polyphenols) containing dieckol, 2,7″-phloroglucinol-6,6′-bieckol, pyrygallol-phloroglucinol-6,6′-bieckol and phlorofucofuroeckol A was administered to C57BL/6 obese male mice five times a week for 12 weeks [53]. Mice received either 100 mg/kg body weight/day or 500 mg/kg body weight/day or water. Postprandial blood glucose AUC reduced following both the low-dose and high-dose treatments compared to placebo. Additionally, the high-dose group had significantly lower plasma insulin and HOMA-IR after 12 weeks compared to placebo. However polyphenol supplementation had no effect on fasting blood glucose [53]. Furthermore, when fed to streptozotocin-induced diabetic mice, a 100 mg/kg body weight single dose of DPHC diminished postprandial blood glucose AUC to 2022 (113.0) mmol/min, compared with 2210 (125.2) mmol/min in the control mice [54].

3.7.1.3 Human Studies

In a randomised controlled trial, Shin et al. [17] gave 97 overweight adults a daily dose of either 72 mg or 144 mg of a polyphenol-rich extract (polyphenol content 98.5%) from *Ecklonia cava*, or a placebo, for 12 weeks. A reduction in fasting blood glucose was observed, but only in the high-dose group [17]. Conversely, a similar length randomised controlled trial showed that 3 months of an oral supplement (500 mg/day) containing 5% marine polyphenols increased plasma insulin levels, HOMA β-cell and HOMA-IR, compared with placebo, in overweight and obese adults. However, no change was observed in fasting blood glucose levels or blood glucose levels following an oral glucose tolerance test (OGTT) [55]. Lee and Jeon [16] administered 690 mg polyphenols or a placebo to 73 adults with high fasting blood glucose (100–180 mg/dL or 5.6–10.0 mmol/L) for 12 weeks. While an improvement in postprandial blood glucose control and a significant reduction in fasting blood insulin levels were observed following supplementation, there was no change in fasting blood glucose level. Paradis et al. [63] examined the anti-hyperglycaemic effects of a phlorotannin-rich, commercially available blend of the brown algae *Ascophyllum nodosum* and *Fucus vesiculosus* in a randomised, double-blind, placebo-controlled, cross-over postprandial study in 23 adults. Participants consumed either 500 mg seaweed capsules (containing at least 10% polyphenols) or placebo capsules 30 min prior to consumption of 50 g of available carbohydrates from bread. Plasma glucose and insulin levels were measured over a 3 h period following carbohydrate ingestion. Consumption of the capsule was associated with a 12.1% reduction in insulin incremental area under the curve (iAUC) and a 7.9% increase in the Cederholm index of insulin sensitivity, compared with placebo. There was no significant effect on postprandial blood glucose iAUC [63].

3.7.1.4 Summary

Marine polyphenols inhibit the action of α-amylase and α-glucosidase in vitro and reduce the postprandial rise in blood glucose and insulin levels in animals. However, reductions in postprandial blood glucose and insulin have not been consistently demonstrated in humans (Table 3.3). This may be due to differences in the way the molecules behave, or interact with other substances, in the human gut compared with the rodent gut; however this is as yet unknown. Marine polyphenols also reduce fasting blood glucose in animals, as also shown by one study in humans [17]. Some evidence suggests that a dose-dependent relationship exists between phlorotannin intake and the anti-hyperglycaemic effects, yet the variation in dosages, timeframes and species examined between studies make interpretation difficult.

3.7.2 Anti-hyperlipidaemic Effects

Dyslipidaemia occurs during diabetes and is a well-known risk factor for CVDs [43, 73–75]. Dyslipidaemia refers to elevated levels of low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG) in the blood and reduced high-density lipoprotein cholesterol (HDL-C) [76, 77]. A key preventative measure for reducing the

Table 3.3 Anti-hyperglycaemic effects of marine polyphenols [105]

Seaweed species	Polyphenol	Dosage and duration	Subject/medium	Antidiabetic effect	References
Alaria, *Palmaria*, *Ascophyllum*	Polyphenolic-rich extracts	NA	Chemical assay	α-Amylase inhibition	[62]
				α-Glucosidase inhibition	
Ascophyllum nodosum	Does not name specific polyphenols	NA	Chemical assay	Dose dependent	[52]
				Strong α-glucosidase inhibition	
				Dose-dependent α-amylase inhibition	
Eisenia bicyclis	Phloroglucinol	NA	Chemical assay	Glycosidase enzyme inhibition	[65]
	Eckol				
	Phlorofucofuroeckol A				
	Dieckol				
	8,8′-bieckol				
	An unidentified tetramer				
Ascophyllum nodosum	Does not name specific polyphenols	NA	Chemical assay	Dose-dependent α-glucosidase inhibition	[68]
		400 μg/mL extract, 20 min incubation	3 T3-L1 adipocytes	Glucose uptake stimulated	
				Fasting blood glucose reduced	
		200 mg/kg body weight for 4 weeks	Streptozotocin-diabetic mice	Postprandial blood glucose rise blunted	
Ecklonia stolonifera	Does not name specific polyphenols	NA	Chemical assay	Strong α-glucosidase inhibition in vitro	[56]
		16.42 or 81.20 mg for 4 weeks	Genetically non-insulin-dependent diabetic KK-A^y male mice	Suppressed postprandial blood glucose and insulin	

(continued)

Table 3.3 (continued)

Seaweed species	Polyphenol	Dosage and duration	Subject/medium	Antidiabetic effect	References
Ishige okamurae	Diphlorethohydroxycarmalol (DPHC)	NA	Chemical assay	Strong α-glucosidase and α-amylase inhibition	[54]
		100 mg/kg body weight (single dose)	Streptozotocin-induced diabetic mice	Supressed postprandial blood glucose	
Ecklonia cava	Does not name specific polyphenols	50–300 μg/ml, 1 h incubation	C_2C_{12} myoblasts	Activated AMPK/ACC and P13/Akt signal transduction pathways	[58]
		300 mg/kg body weight for 3 weeks	Streptozotocin-induced type 1 diabetes mellitus rats	Fasting blood glucose reduced	
				Insulin concentration increased	
Ecklonia cava	Polyphenol extract (28.2 ± 0.58% polyphenols):	100 or 500 mg/kg body weight for 12 weeks	C57BL/6 male mice	Reduced blood glucose 1 h after injection	[53]
	Dieckol			Reduced postprandial glucose AUC	
	2,7″-phloroglucinol-6,6′-bieckol,			High-dose reduced plasma insulin and HOMA-IR	
	Pyrygallol-phloroglucinol-6,6′-bieckol				
	Phlorofucofuroeckol A				
Ecklonia cava	CA extract – 68.78 mg/g polyphenols	200 mg/kg body weight for 7 weeks	C57BL/6 mice	Reduced fasting blood glucose	[64]
	G-CA extract – 79.70 mg/g polyphenols			Improved glucose tolerance	
Ecklonia cava	Dieckol-rich extract	0.5 g/100 g diet for 6 weeks	C57BL/KsJ-*db/db (db/db)* male mice	Reduced fasting blood glucose	[60]
				Reduced plasma insulin	
				Reduced HbA1c	

Ecklonia cava	Dieckol	20 mg/kg body weight for 14 days	C57BL/KsJ-*db/db*, a type II diabetes mice	Reduced blood glucose	[59]
				Dose-dependently reduced plasma insulin	
Ishige okamurae	Does not mention specific polyphenols	0.5% w/w for 6 weeks	C57BL/KsJ-*db/db* mice	Reduced G6Pase and PEPCK activity	[61]
				Increased hepatic glycogen production	
				Reduced fasting blood glucose	
				Reduced HbA1c	
Ascophyllum nodosum, Fucus vesiculosus	Commercially available blend of brown seaweeds containing a minimum of 10% polyphenols	50 mg polyphenols, single dose prior to postprandial testing	23 non-diabetic adults	Reduced postprandial insulin iAUC	[63]
				Increased insulin sensitivity	
Ecklonia cava	Polyphenol extract	72 or 144 mg/day for 12 weeks	97 non-diabetic overweight adults	Reduced fasting blood glucose (high dose only)	[17]
Ecklonia cava	Dieckol-rich extract	690 mg/day for 12 weeks	73 adults with high fasting blood glucose	Improved postprandial blood glucose control	[16]
				Reduced fasting blood insulin level	
				No change in fasting blood glucose level	
Not specified	Polyphenol containing (5%) oral supplement	25 mg polyphenols for 3 months	25 non-diabetic overweight or obese volunteers	Increased plasma insulin, HOMA β-cell and HOMA -IR	[55]

Abbreviations: *AMPK/ACC* AMP–activated protein kinase/acetyl–CoA carboxylase, *AUC* area under the curve, *G6Pase* glucose–6–phosphatase, *HbA1c* glycated haemoglobin, *HOMA–IR* homeostatic model assessment – insulin resistance, *HOMA β–cell* homeostatic model assessment – beta-cell function, *iAUC* incremental area under the curve, *NA* not available/not applicable, *P13/Akt* phosphatidylinositol 3-kinase/Akt (a serine/threonine protein kinase), *PEPCK* phosphoenolpyruvate carboxykinase

risk of CVDs is to lower total cholesterol (TC), LDL-C and TG levels in the blood [78]. Polyphenols from terrestrial sources have been shown to reduce digestion and absorption of dietary lipids, decrease synthesis and secretion of apolipoprotein B, inhibit cholesterol esterification and intestinal lipoprotein production and inhibit key enzymes in lipid biosynthesis pathways [79–81] resulting in an improved lipid profile and lowered cardiovascular risk. Recent studies indicate that macroalgal polyphenols may have similar lipid-lowering actions. One of the proposed lipid-lowering actions of macroalgal polyphenols is the inhibition of the enzyme HMGCoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase in the liver and adipose tissue [66]. This is a key enzyme involved in the cholesterol production pathway and also the target of successful TC- and LDL-C-lowering drugs [82].

3.7.2.1 In Vitro Studies

Seapolynol™, a polyphenol extract containing 98.5% unspecified polyphenols, and dieckol, an isolated phlorotannin from *Ecklonia cava*, both dose-dependently (0–200 μg/mL) inhibited the activity of HMGCoA reductase in vitro [66].

Macroalgal phlorotannins have also been shown to inhibit lipid accumulation and adipocyte differentiation in 3 T3-L1 adipocytes through reducing the expression levels of adipocyte marker genes PPARγ and C/EBPα [57, 66]. This mechanism has been suggested as a way of managing obesity via reduction of the formation of mature adipocytes and adipose tissue [57, 83–85] and has been associated with reduced body weight gain in high-fat diet-fed mice [83]. This mechanism has also been associated with a reduction in the activity of TG synthetic hormones, such as diacylglycerol acyltransferase 1 (DGAT1) and glycerol-3-phosphate acyltransferase 3 (GPAT3), resulting in reduced triglyceride accumulation within adipocytes [86].

Interestingly, a reduction in serum TG levels in high-fat diet-fed mice was also observed [66]; however it was not clear how inhibition of intracellular lipid/TG accumulation and adipocyte differentiation would result in changes to serum TG levels. While the inhibition of lipid accumulation and adipocyte differentiation may contribute to a reduction in body fat, it is unlikely that this mechanism would directly reduce blood lipid levels. A combination of reduced body weight and the impact on liver enzymes may have effect on blood lipids; however this is yet to be shown.

3.7.2.2 Animal Studies

Yeo et al. [66] investigated the reduction of hyperlipidaemia in imprinting control region (ICR) mice using a generic polyphenol extract (Seapolynol – containing 98.5% polyphenols) and dieckol, from *Ecklonia cava.* Mice were fed a high-fat diet (20% fat) for 5 weeks and supplemented with Seapolynol™ (1.25, 2.5 or 5 mg/day) or dieckol (0.5, 1 or 2 mg/day) for 4 weeks, starting 1 week after high-fat diet feeding. Both treatments reduced serum TC, TG and LDL-C levels compared with mice fed a high-fat diet only [66]. Similarly, in C57BL/KsJ-*db/db (db/db)* male mice, a dieckol-rich extract from *Ecklonia cava* (0.5 g dieckol/100 g diet) reduced TC levels and free fatty acids (FFAs) after 6 weeks, compared with placebo. The same treatment reduced TG levels to an extent similar to treatment with rosiglitazone (0.005 g/100 g diet) and increased HDL-C to a greater extent than rosiglitazone

treatment [60]. Eo et al. [53] examined a polyphenol extract from *Ecklonia cava* (28.2 ± 0.58% polyphenols) containing dieckol, 2,7″-phloroglucinol-6,6′-bieckol, pyrygallol-phloroglucinol-6,6′-bieckol and phlorofucofuroeckol A in high-fat diet-fed obese C57BL/6 male mice. Mice were administered 100 mg/kg body weight/day or 500 mg/kg body weight/day or water, five times per week for 12 weeks. Both doses of polyphenol extract reduced TG and TC levels compared with mice fed the high-fat diet alone. No changes were reported in HDL-C [53]. A phlorotannin-rich extract (100–250 mg/kg body weight/day) and isolations of the phlorotannins eckol and dieckol (10 or 20 mg/kg body weight/day) from *Ecklonia stolonifera* were investigated for their anti-hyperlipidaemic effects in high-cholesterol diet- or poloxamer 407-induced hyperlipidaemic rats [67]. The phlorotannin-rich ethyl acetate and *n*-butanol fractions, extracted using ethanol, reduced TG, TC and LDL-C levels and increased HDL-C levels in a dose-dependent manner after 3 days of treatment. Both eckol and dieckol isolations reduced TG, TC and LDL-C levels after 3 days. Dieckol treatment alone produced a greater hypolipidaemic effect than lovastatin (50 mg/kg) and increased HDL-C levels in the hyperlipidaemic rats after 3 days [67]. Conversely, Park et al. [64] administered polyphenol-rich extracts from *Ecklonia cava* (grown in two different geographical areas in Korea: Jeju and Gijang) to high-fat diet-induced obese C57BL/6 mice at 200 mg/kg body weight/day for 8 weeks. Treatment with the extract from Jeju had no effect on TC, TG, LDL-C or HDL-C levels; however, the extract from Gijang reduced TC level compared with placebo [64], highlighting the potential for differences in polyphenol content based on geographical location of cultivation, even within the same species of algae [5, 30].

3.7.2.3 Human Studies

In a randomised, double-blind, placebo-controlled parallel-design trial in 97 overweight adults, consumption of a phlorotannin-rich extract from *Ecklonia cava*, at doses of 72 or 144 mg polyphenols per day, reduced TC levels, LDL-C levels and TC to HDL-C ratio, in a dose-dependent manner following 12 weeks of treatment, compared with placebo. An increase in HDL-C levels was only observed following the highest dose [17]. A comparable trial in 80 adults with raised cholesterol (>200 mg/dL TC or > 110 mg/dL LDL-C) demonstrated that consumption of a dieckol-rich *Ecklonia cava* extract (400 mg/day, 8.2% dieckol) for 12 weeks resulted in reduced TC and LDL-C, compared with placebo, without change in TG or HDL-C levels [15]. Conversely, in a randomised, double-blind, placebo-controlled clinical trial of 25 overweight or obese volunteers, no changes were reported in TC, TG or HDL-C levels following 500 mg of a polyphenol-containing oral supplement (5% polyphenols) daily for 3 months. However, LDL-C levels were reduced following the supplementation treatment compared with no change in placebo group [55].

3.7.2.4 Summary

Similar to the anti-hyperglycaemic evidence, phlorotannins improved dyslipidaemia in animal models and in vitro via a number of mechanisms, although results in humans are few and inconsistent (Table 3.4). Phlorotannins have potential as an

Table 3.4 Anti-hyperlipidaemic effects of marine polyphenols

Seaweed species	Polyphenol	Dosage and duration	Subjects/medium	Anti-hyperlipidaemic effect	References
Ecklonia cava	Generic polyphenol extract (Seapolynol™ – 98.5% polyphenols) and dieckol	50 μg/mL concentration	Chemical assay	Inhibited HMGCoA reductase	[66]
		1.25, 2.5 or 5.0 mg Seapolynol™, or 0.5, 1.0 or 2.0 mg dieckol for 4 weeks	ICR mice	Reduced TC, TG and LDL-C	
Ecklonia cava	Dieckol-rich extract	0.5 g/100 g diet for 6 weeks	C57BL/KsJ-*db/db* *(db/db)* male mice	Reduced TC, TG and FFA	[60]
				Increased HDL-C	
Ecklonia cava	CA extract – 68.78 mg/g polyphenols	200 mg/kg body weight for 8 weeks	Obese C57BL/6 mice	G-CA extract reduced TC	[64]
	G-CA extract – 79.70 mg/g polyphenols				
Ecklonia cava	Polyphenol extract (28.2 ± 0.58% polyphenols):	100 or 500 mg/kg body weight for 12 weeks	Obese C57BL/6 male mice	Reduced TG and TC levels	[53]
	Dieckol			No change in HDL-C	
	2,7″-phloroglucinol-6,6′-bieckol,				
	Pyrygallol-phloroglucinol-6,6′-bieckol,				
	Phlorofucofuroeckol A				
Ecklonia stolonifera	Polyphenol extract	100–250 mg/kg body weight (polyphenol extract) or 10 or 20 mg/kg body weight (eckol and dieckol) for 3 days	Hyperlipidaemic rats	Both treatments dose-dependently reduced TC, TG and LDL-C and increased HDL-C.	[67]
	Eckol			Different extraction techniques yielded different actions	
	Dieckol				

Ecklonia cava	Polyphenol extract	72 or 144 mg for 12 weeks	97 overweight adults	Dose-dependently reduced TC, LDL-C and TC/HDL-C ratio	[17]
				High-dose increased HDL-C	
Ecklonia cava	Dieckol-rich extract (8.2% dieckol)	400 mg/day (32.8 mg dieckol) for 12 weeks	80 adults with raised cholesterol	Reduced TC and LDL-C levels	[15]
				Intervention had no effect on HDL-C or TG levels	
Not specified	Polyphenol containing (5%) oral supplement	25 mg polyphenols for 3 months	25 overweight or obese volunteers	Reduced LDL-C only	[55]

Adapted from Murray et al. [105]

Abbreviations: *TC* total cholesterol, *LDL-C* low density lipoprotein cholesterol, *HDL-C* high density lipoprotein cholesterol, *TG* triglyceride, *HMGCoA* 3-hydroxy-3-methylglutaryl-coenzyme A, *CA* Jeju geographical area, Korea, *G-CA* Gijang geographical area, Korea

Table 3.5 Health-promoting effects of marine polyphenols according to algal species

Species	Effect	Subject/medium	References
Ascophyllum nodosum	Anti-hyperglycaemic	Chemical assay	[52, 62, 68]
		3 T3-L1 adipocytes	[68]
		Diabetic mice	[68]
		Non-diabetic adults	[63]
Ecklonia cava	Anti-hyperglycaemic	C_2C_{12} myoblasts	[58]
		Diabetic rats	[58]
		Mice	[53, 64]
		Diabetic mice	[59, 60]
		Non-diabetic overweight adults	[17]
		Pre-diabetic adults	[16]
	Anti-hyperlipidaemic	Chemical assay	[66]
		3 T3-L1 preadipocytes	[66]
		Mice	[66]
		Diabetic mice	[60]
		Obese mice	[53, 64]
		Overweight adults	[17]
		Adults with raised cholesterol	[15]
Ecklonia stolonifera	Anti-hyperglycaemic	Chemical assay	[56]
		Diabetic mice	[56]
	Anti-hyperlipidaemic	3 T3-L1 preadipocytes	[57]
		Hyperlipidaemic rats	[67]
Fucus vesiculosus	Anti-hyperglycaemic	Non-diabetic adults	[63]
Ishige okamurae	Anti-hyperglycaemic	Chemical assay	[54]
		Diabetic mice	[54, 61]

Adapted from [105]

anti-hyperlipidaemic agent in humans, but due to factors such as bioavailability and dosing, which differ considerably between humans and animals, further research is required to determine a consistent effect and appropriate dosage and treatment schedule in humans.

3.7.3 Health Effects According to Algal Species

Phlorotannin-rich extracts from the *Ascophyllum nodosum*, *Ecklonia stolonifera*, *Ishige okamurae* and *Ecklonia cava* macroalgae varieties are the most predominantly tested with relation to their potential health-promoting effects (Table 3.5).

Macroalgae from the *Ascophyllum* species have been investigated in cell culture, animal models and humans and have been shown to improve diabetic risk factors. Phlorotannin-rich extracts from this species have inhibited α-amylase and α-glucosidase activity [52, 62, 68], stimulated glucose uptake [68], reduced fasting and postprandial blood glucose in diabetic mice [68] and reduced postprandial insulin and improved insulin sensitivity in non-diabetic adults [63].

Phlorotannins from the *Ecklonia stolonifera* species have exhibited both anti-hyperglycaemic and anti-hyperlipidaemic effects. These include the inhibition of α-glucosidase activity in vitro [56] and reductions in postprandial blood glucose and insulin in diabetic mice [56] and dose-dependent reductions in TC, TG and LDL-C and increasing HDL-C levels in hyperlipidaemic rats [67].

Ishige okamurae phlorotannins have demonstrated anti-hyperglycaemic effects; α-amylase and α-glucosidase inhibition has been demonstrated in vitro [54], along with reductions in postprandial blood glucose, fasting blood glucose and HbA1c levels in diabetic mice [54, 61].

Phlorotannin-rich extracts from *Ecklonia cava* are by far the most extensively tested and have demonstrated both anti-hyperglycaemic and anti-hyperlipidaemic effects. Anti-hyperglycaemic effects, including reduced fasting blood glucose and insulin levels, reduced postprandial blood glucose and improved insulin sensitivity, have been shown in animal models [53, 58–60, 64] and non-diabetic [17] and pre-diabetic adults [16]. The anti-hyperlipidaemic effects of *Ecklonia cava* phlorotannins include the reduction of TC, LDL-C, TG and FFA levels and increase of HDL-C, shown in animal models [53, 60, 64, 66] and humans [15, 17]. The inhibition of HMGCoA reductase by *Ecklonia cava* phlorotannins has also been shown in 3 T3-L1 preadipocytes [66].

While there are numerous different algal species, the research to date has tended to focus on the aforementioned species with a particular emphasis on *Ecklonia cava*, despite the fact that it is not a commonly consumed alga. Phlorotannins from *Ecklonia cava* have demonstrated all of the health effects examined in this chapter and are beginning to be tested in different human populations [15–17]. Future research is warranted to investigate the health effects of *Ecklonia cava* phlorotannins particularly in human populations, as they show potential to be used as a functional food ingredient. However, the potential of other species of macroalgae that are not yet as well investigated should not be neglected.

3.8 Macroalgae As a Functional Food Ingredient

A functional food is defined as a "natural or processed food that contains known or unknown biologically-active compounds, which in defined, effective, non-toxic amounts, provide a clinically proven and documented health benefit for the prevention, management, or treatment of chronic disease" [87]. The potential of macroalgae derived polyphenols as a functional food component and its limitation will be discussed below.

3.8.1 Safe Levels of Consumption

Healthy diet and exercise are the best methods for prevention of chronic lifestyle diseases. However in the absence of a healthy diet and exercise, drugs are the current accepted treatment for blood sugar and cholesterol control. Long-term use of

oral antidiabetic and anti-hyperlipidaemic drugs can cause unpleasant side effects, including muscle cramping, fatigue, muscle breakdown, vomiting and diarrhoea [3, 43, 88, 89]. Macroalgal polyphenols are thought to be relatively safe for consumption [54, 66, 90–93] as they lack unpleasant side effects [3, 43, 63], and therefore they may be safer alternatives as functional food ingredients if efficacy can be proven. The safety of a polyphenol-rich supplement from *Fucus vesiculosus* has been demonstrated at up to 750 mg/kg/day in rats over 4 weeks [93]. Extrapolating these data to an equivalent dose in humans, based on an average body weight of 65 kg, suggests up to 48.75 g/day would be safe for consumption. This amount is far greater than what would realistically be consumed. Macroalgae-derived DPHC has also shown no cytotoxicity in human umbilical vein epithelial cells (HUVECs) at concentrations up to 3.91 mM after 20 h incubation [54]. It should be noted, however, that green tea polyphenols have been shown to cause hepatotoxicity and other adverse effects at higher doses. A dose of 500 mg/kg/day pure epigallocatechin gallate (EGCG) for 13 weeks increased bilirubin and decreased fibrinogen in rats [94]. This equates to a dose of 32.5 g/day for a 65 kg human. The risk of toxicity increased when ingested in the fasting state or over long periods of time, or when the polyphenols were administered intraperitoneally [94]. If the safety of marine polyphenols and efficacy for blood glucose or cholesterol control can be shown in a human population, then marine polyphenols would have great potential for commercialisation as a functional food ingredient. It should be a research priority to assess the safety and efficacy of marine polyphenol consumption for health promotion and disease prevention in humans.

3.8.2 Additional Bioactive Compounds in Macroalgae

While polyphenols are a key contributor to the biological activity and health benefits of brown macroalgae [4, 5], macroalgae also contain a variety of other biologically active compounds that may contribute to their value as functional food ingredients. They are a rich source of dietary fibre (33–50 g/100 g DW), in particular soluble fibre. Consumption of macroalgae alone, or its incorporation into other foods, can increase fibre intake in the diet which may help to reduce the risk of some chronic diseases that are associated with low-fibre intakes, such as diabetes and heart disease [51]. In addition to contributing to fibre content, macroalgal polysaccharides have also been shown to possess antioxidant properties and anti-tumour and anticoagulant bioactivity and to lower LDL-C in cholesterol-rich diet-fed rats [51, 95]. Fucoidans are sulfated polysaccharides found in the cell walls of brown macroalgae, but not in other algae or terrestrial plants. They are reported to have antioxidant, antiviral, anticoagulant and antiobesity effects and are being investigated as a functional food ingredient for disease prevention and health promotion [51, 95]. Other biologically active compounds found in macroalgae include omega-3 and omega-6 essential fatty acids, which help in the prevention of atherosclerosis, reduction of blood pressure, cancer prevention, promotion of bone health and improvement in brain function in children [51]. Macroalgae are a rich source of

alginate, or alginic acid, which is reported to reduce cholesterol concentration, have antihypertensive effects, reduce cancer risk and contribute to dietary fibre intake [95]. Macroalgae also contain chlorophyll, carotenoids, hydroquinones, flavonoids, sterols and phospholipids, which contribute to their antioxidant capacity [51, 95]. While these health benefits may sound promising, there is as yet little evidence that these effects occur in humans, since the majority of research uses only cell culture or animal models. This is an important gap that needs to be filled before macroalgae can be used as functional food ingredients for disease prevention and health promotion in humans.

3.8.3 Availability and Sustainability of Macroalgae As a Functional Food Source

There are many environmental and economic benefits associated with marine sources, as opposed to land-based sources, to obtain biologically active compounds. With the ocean making up more than 70% of the Earth's surface [26], it provides an abundant source of marine products, and algal species are easy to harvest from the wild as well as to culture in the sea and in pools on land [33]. The cultivation of marine algae has a number of advantages over terrestrial plant cultivation such as it requires less freshwater, produces a higher biomass, can be grown in lower quality agricultural environments and can be grown in seawater avoiding the need for herbicides and pesticides [96]. Furthermore, recent advances in biotechnological tools for the extraction and identification of phlorotannins and polyphenols from macroalgae have led to an upward trend in the use of these products as functional food ingredients [3]. Therefore, there is likely to be a large market for marine polyphenols as a functional food ingredient if efficacy can be demonstrated.

3.8.4 Processing of Macroalgae for Consumption

In order to be incorporated into food products, macroalgae must be processed to ensure they are palatable for human consumption. Drying methods and other processing techniques aim to ensure the acceptability of macroalgae as a food product by altering its sensory properties, e.g. minimising textural issues, while maintaining maximum levels of biologically active compounds [51]. Higher drying temperatures (40 °C) minimise the losses of phenolic content due to drying, compared with lower temperatures (25 °C). This may be because lower drying temperatures do not completely inactivate oxidative enzymes, and some oxidation of the phenolic substances takes place [51]. This is reflected in higher losses of antioxidant activity when macroalgae were dehydrated at lower temperatures (25 °C dehydration resulted in 17.3% loss in radical scavenging ability), compared with higher temperatures (40 °C dehydration resulted in 4.5% loss in radical scavenging ability) [51].

Hydrothermal processing (treatment of macroalgae in water at 80–100 °C) is another technique used to minimise the tough texture of macroalgae to improve

their sensory acceptability as a food [51]. Hydrothermal processing alone can achieve an acceptable texture within 40 min of treatment; however this results in up to 85% losses in phenolic content at both 80 and 100 °C. A combination of drying followed by hydrothermal processing can dramatically reduce these losses. When *Himanthalia elongata* was dried for 12 h at 25 °C first, an acceptable texture could be achieved with only 25 min of hydrothermal processing at 100 °C resulting in only ~9% loss in total phenolic content [51]. The pretreatment of macroalgae is essential for their successful utilisation in functional foods and to ensure the final product has acceptable sensory and textural properties. It is also vital that these treatments allow the retention of maximum levels of phenolics and other biologically active compounds [51].

3.8.5 Incorporation of Macroalgae into Foods

While maintaining high levels of bioactivity is vital in functional food development, it is also important that the resulting products are appealing to consumers and have an acceptable sensory profile [51]. Macroalgae have been successfully incorporated into a number of food products to add functional or structural properties. Macroalgae, or seaweed, as a whole component has been added to pork sausages to replace animal fat [97], beef patties to reduce salt and fat levels [98], pork patties to decrease fat content and increase fibre content [99], pasta to increase antioxidant levels [100] and noodles to increase the cooking yield [51, 101]. Products with added macroalgae have also been tested by sensory panels for their palatability and found to be acceptable, in some cases with better sensory scores than the original product [99, 102].

Meat and bread products appear to be the two main candidates for fortification using macroalgae. Macroalgae, fully dried and ground into a fine powder, has been incorporated into a wholemeal and white flour bread base mix to be made into breadsticks. It was determined that up to 10% macroalgae in the mix produced a highly acceptable product for sensory and consumer appeal [51]. When 15% or more of the mix was dried macroalgae, this resulted in a harder texture and level of aroma that were not acceptable to consumers. It is important to determine the appropriate amount of macroalgae that can be added to any particular food to get a balance between functional capacity and sensory appeal. This amount will vary depending on the acceptable sensory and texture parameters associated with individual food items [51]. The right balance will ensure a food product that is acceptable for consumption and has benefits to human health and disease prevention.

3.8.6 Commercial Potential of Functional Foods

Recent trends have shown an increase in consumer preferences for natural and sustainable health products and functional foods [33, 103]; thus there is interest in marine-based food products [3]. The global market for functional foods has

experienced rapid growth in recent years, and this growth is forecast to continue [51, 104]. In the United States of America, the expected compound annual growth rate from 2016 to 2020 for functional foods is 8.8% compared with 6.0% for the nutraceutical market as a whole [51, 104]. The functional food market is often categorised as a subsection under the umbrella of nutraceuticals and pooled with the dietary supplement (tablets, capsules and liquids) market. The following data refer to the nutraceutical market as a whole, as individual data for the functional food market was not available. In 2014 the global nutraceutical market was valued at approximately US$250 billion and is forecast to be worth around US$385 billion by 2020 [104]. While this figure is an overestimation of the actual value of the functional foods market, it indicates the commercial ability and saleability of functional food products. Key factors driving this growth of the functional food industry have been the increase in population, particularly the ageing population, an increase in diet-related diseases and our understanding of how diet affects health, in addition to consumer's demand for health and wellness products [51].

3.9 Conclusion

Macroalgae show great potential as functional food ingredients. They can be grown and harvested in a sustainable and environmentally friendly way and have already been successfully incorporated into foods. Ongoing improvements in scientific technologies will allow more thorough characterisation of the polyphenol and phlorotannin content of macroalgae and improved understanding of the health-promoting effects of individual phlorotannins and phlorotannins working in synchrony. A better understanding of what happens to macroalgal polyphenols when they enter the body and how dietary intake reflects health outcomes is required. This will get clearer as more studies investigating the health effects of marine polyphenols are conducted in humans. Under experimental conditions phlorotannins from marine macroalgae have many positive health-related effects. *Ecklonia cava* has shown great potential as a source of bioactive marine polyphenols, with evidence for both anti-hyperglycaemic and anti-hyperlipidaemic effects, in the trials already completed in human populations. However, other macroalgal species should not be ignored as potential functional food ingredients. Health benefits of macroalgal polyphenols are a relatively novel area of research, with much yet to be discovered. As research in this area continues, it will be exciting to see the true potential of macroalgal polyphenols revealed.

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Conflicts of Interest None to declare

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