Seaweeds and Gut Health Benefits

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Macroalgae, or seaweeds, are a rich source of components which may exert beneficial effects on the mammalian gut microbiota through the enhancement of bacterial diversity and abundance. An imbalance of gut bacteria has been linked to the development of disorders such as inflammatory bowel disease, immunodeficiency, hypertension, type-2-diabetes, obesity, and cancer. This review outlines current knowledge from in vitro and in vivo studies concerning the potential therapeutic application of seaweed-derived polysaccharides, polyphenols and peptides to modulate the gut microbiota through diet. Polysaccharides such as fucoidan, laminarin, alginate, ulvan and porphyran are unique to seaweeds. Several studies have shown their potential to act as prebiotics and to positively modulate the gut microbiota. Prebiotics enhance bacterial populations and often their production of short chain fatty acids, which are the energy source for gastrointestinal epithelial cells, provide protection against pathogens, influence immunomodulation, and induce apoptosis of colon cancer cells. The oral bioaccessibility and bioavailability of seaweed components is also discussed, including the advantages and limitations of static and dynamic in vitro gastrointestinal models versus ex vivo and in vivo methods. Seaweed bioactives show potential for use in prevention and, in some instances, treatment of human disease.

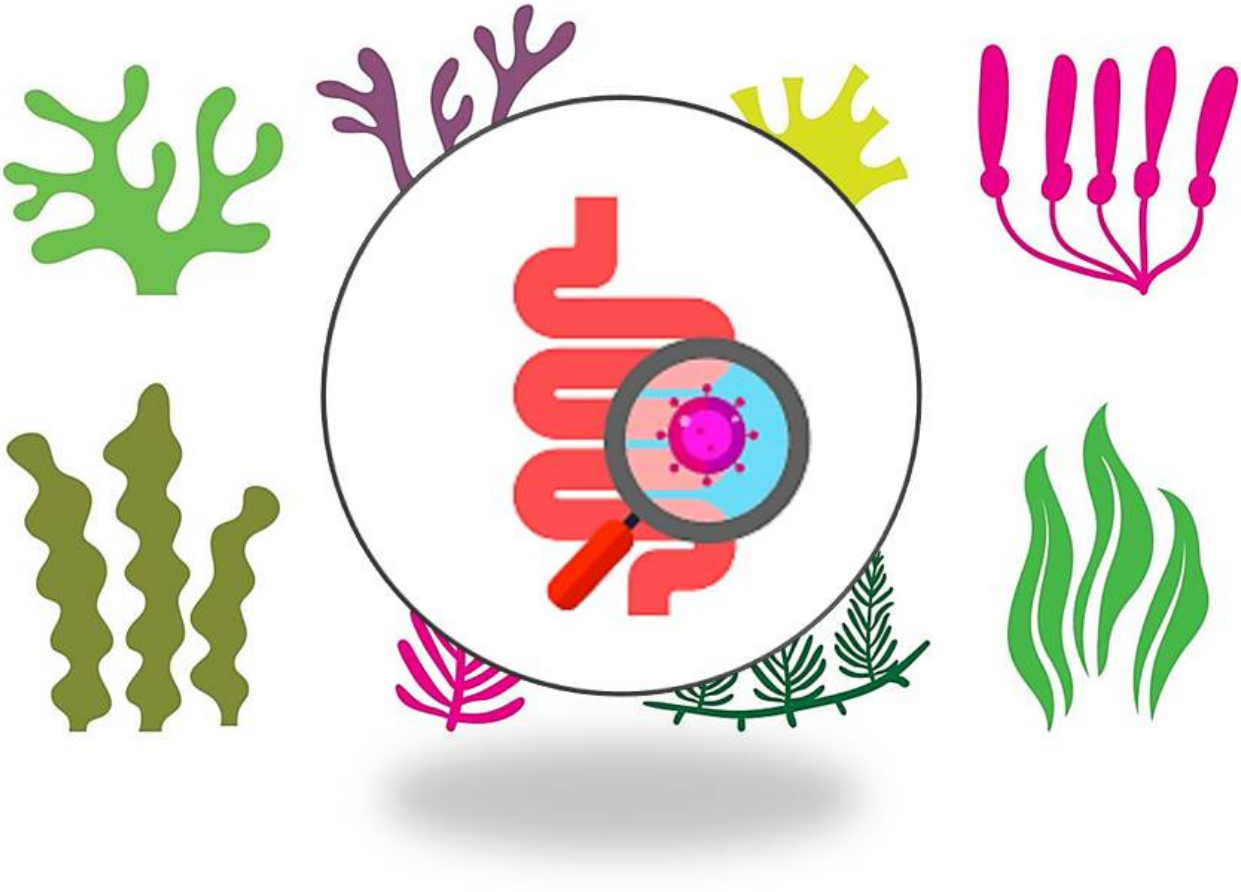
seaweed prebiotics gut microbiota polysaccharides polyphenols peptides colonic fermentation short chain fatty acids bioaccessibility simulated gastrointestinal and fermentation digestion models

1. Introduction

Seaweed-derived components with potential to impact positively on diseases of the body including hypertension [1], cancer [2], type-2-diabetes [3], obesity [4], oxidation [5], inflammation [6] and other disorders have been evaluated in a number of studies to date [7][8][9][10][11][12][13][14][15]. The pathogenesis of these disorders has been linked to the health of the gut microbiota [16]. The microorganisms that inhabit the human gastrointestinal tract—bacteria, archaea, fungi, protozoa, and viruses—are collectively termed the gut microbiota [17]. The gut microbiota is established during infancy [18]. There is a broad variance amongst individuals in microbiota composition because it is shaped by infant transitions such as the gestational period, delivery method, weaning age, breast-feeding duration, or use of formula milk [19]. The microbiota remains relatively stable throughout adulthood but is affected by factors such as enterotype, antibiotic use, diet, lifestyle, genetic traits, and body mass index [20]. Three enterotypes have been described in the human gut microbiome based on variations in levels of the bacterial genera Bacteroides, Prevotella, and Ruminococcus [21]. The gut microbiota is regarded as an endocrine organ that

co-develops with the host throughout its life. It exerts an effect on immunity, metabolism, neuroendocrine responses, and synthesises vitamins, amino acids, and enzymes [22][23].

The gut microbiota also aids in the absorption of dietary minerals and produces important short-chain fatty acids (SCFA) such as butyrate, propionate, and acetate. These SCFA are the energy source for gastrointestinal epithelial cells, provide protection against pathogens, influence intestinal mucosal immunity and barrier integrity, and induce apoptosis of colon cancer cells [24][25]. SCFA also regulate liver mitochondrial function, insulin secretion, and induce the production of gut hormones γ-aminobutyric acid and serotonin by interacting with their receptors on enteroendocrine cells [26][27]. An increase in the gut bacterial population enhances the beneficial effects of the microbiota and increases SCFA production [20]. An imbalance or decreased diversity of beneficial versus harmful bacterial species in the gut microbiota is termed dysbiosis and is linked to several diseases [28][29][30][31][32]. Therefore, maintaining the health of the microbiota through diet or supplementary means is thought beneficial to overall health [30]. Seaweed components may exert a beneficial effect on gut health by acting as prebiotics [33][34]. The potential bioactivity of seaweed components has been demonstrated previously in *in vitro* studies [35][36], however the impact of gastrointestinal enzymatic digestion and colonic bacterial fermentation *in vivo* must also be considered, since it may have an effect on the bioavailability of prebiotic and other actives [37][38][39]. As a pharmacological concept, bioavailability is a measure of drug absorption defined as the percentage of the drug that reaches blood circulation, measured by a dose-response curve [40]. However, the evaluation of bioavailability in food-derived extracts differs, since characteristic dose-response curves are not exhibited [41]. In addition, the bioaccessibility of food-derived active compounds must be taken into account, i.e., the accessible portion of the active compound released from the food or extract matrix during digestion [42][43]. Although pharmacokinetic studies are required for the development of prebiotics destined for human and animal use, such studies are not within the scope of this review. The pharmacokinetics of seaweed-derived prebiotics in terms of absorption, distribution, metabolism, and elimination has previously been documented in animal studies after oral administration [44][45][46][47][48] and topical application [49], and recently reviewed by Corino et al. [50] and Shikov et al. [51].



2. Current Insights

Seaweed components that have the potential to exert beneficial effects on the gut by modulating the abundance and diversity of bacterial populations in the gut microbiota include polysaccharides, polyphenols, and peptides. Their structure, function, and studies regarding their potential impact on the gut are considered in this review. Despite the positive results reported from cited studies concerning *in vitro* and animal work, more research is required in human dietary intervention studies, with health-related end points, to determine prebiotic potential.

2.1. Polysaccharides

Polysaccharides, or carbohydrates, are repeating units of monosaccharides linked by glycosidic bonds found in all plants, fungi, and algae. They are considered primary metabolites with structural and energy storage functions [52]. The majority of seaweed polysaccharides are composed of water-soluble and -insoluble fibre [53][54]. The total fibre content of seaweed varies between species and has been reported to range from 35–62% in brown, to 10–57% in red and 29–67% in green (DW) [55][56][57][58][59]. The principal fibres in brown seaweeds are fucoidan, laminarin, and alginate; porphyran, carrageenan, hypnean and floridean starch in red; and ulvan, sulphated-rhamnans, -

arabinogalactans and -mannans in green [60][61]. Humans do not produce the endogenous enzymes in the upper gastrointestinal tract required to degrade dietary fibre to monosaccharides. However, fibre is an excellent food substrate, or prebiotic, for human gut bacteria [62][63]. Prebiotics are food components that are indigestible in the small intestine but can be metabolised by microorganisms in the large intestine, modulating their composition and/or activity, thus conferring a beneficial physiological effect on the host [64]. Many species of gut bacteria produce endogenous carbohydrate-degrading enzymes, such as β-glucanase and β-glucosidase, capable of hydrolysing the glycosidic linkages of polysaccharides [65][66][67][68]. Several polysaccharides within seaweed that are indigestible in the upper gastrointestinal tract are thought to exert bioactive effects including glycaemic control [69] and the promotion of gut microbial- and immune-modulation by acting as prebiotics in *in vitro* and *in vivo* studies [70][71]. The bioactivity of polysaccharide fractions is influenced by a number of factors such as chemical structure, molecular weight (MW), solubility, extraction method, seaweed genus and seasonal variation [72][73]. The principal polysaccharides of brown, red, and green seaweeds are detailed below.

2.1.1. Fucoidans

Three polysaccharides—fucoidans, laminarin and alginate—occur within brown seaweeds, each of which have differing structures and functions [74]. Fucoidans comprise 5–20% (DW) of the entire seaweed thallus [75][76]. They are water-soluble sulphated-polysaccharides composed of repeating fucose and sulphate groups, and may also contain galactose, mannose, xylose, rhamnose, arabinose, glucose, acetyl groups, or glucuronic acid [77]. The molecular weight of fucoidans varies from 7 to 2300 kDa [11]. Fucoidans provide structure for the outer cell wall and a hydrophilic coating to prevent desiccation of the seaweed during low tide. They also play a role in adapting to osmotic stress caused by changes in salinity as their sulphate groups can bind to cations such as sodium, potassium, magnesium, and calcium [78][79]. Fucoidans have previously been shown in *in vitro* studies to have potential for use as anticancer [80], antiviral [81], antioxidant [77], and anti-inflammatory [82] agents; and *in vivo* as anticoagulants (human trial) [83], anticancer (human trial) [84], antitumour (mouse model) [85], antihyperglycaemic, and antihyperlipidaemic agents (mouse model) [86]. However, the oral bioavailability of fucoidan can be low due its highly polar nature and limited ability to pass through intestinal epithelial cells [68]. In recent years, the prebiotic status of fucoidan has been recognised *in vitro* [82][87] and in human [88] and animal [89][90][91][92] gastrointestinal studies.

2.1.2. Laminarin

The energy storage polysaccharide of brown seaweeds is laminarin, composed of β(1–3)-linked glucose units with β(1–6)-branches [93]. It occurs within the chloroplasts in micro-compartments called pyrenoids [94]. Laminarin is water-soluble, though increased branching of the molecule requires colder temperatures for solubility. It comprises 3–35% of brown seaweed dry mass and is most prevalent in Laminaria species [95]. It is a small polysaccharide with a molecular weight of approximately 5 kDa [96]. Laminarin has shown efficacy in *in vitro* studies carried out previously and has potential for use as an anticancer [97], antimetastatic [98], antioxidant [99] and immunostimulatory [100] agent [97][99][100]; and *in vivo* as an immunomodulatory agent [101] and prebiotic to modulate dysbiosis (animal models) [102][103][104][105].

2.1.3. Alginate

Alginate comprises up to 45% of brown seaweed dry mass [106], occurring in the cell walls as salts of alginic acid bound to sodium, calcium or magnesium ions [107]. It is a water-soluble linear polysaccharide composed of (1–4)-linked β-D-mannuronate and α-L-guluronate residues [108]. Molecular weight ranges from 20 to 350 kDa [109][110]. It is the most abundant polysaccharide in brown seaweed and imparts flexibility to the thallus to withstand the force of the ocean. Alginate is a phycocolloid that can bind up to 20 times its own mass with water, making it very useful for food and industrial applications [111]. The prebiotic effect of alginate on gut microbiota was demonstrated previously *in vitro* by Bai et al. [112] and Li et al. [113]; and in a human study by Mizuno et al. [114]. Bai et al. fermented seaweed-derived alginates *in vitro* and observed that the alginates were degraded by human-derived gut bacteria, producing a significant ($p < 0.05$) increase in SCFA compared to a starch control, and suggested that further investigations of the prebiotic effects of alginate are warranted. Li et al. also fermented seaweed-derived alginates with human faecal bacteria *in vitro* and found a significant ($p < 0.05$) increase in total SCFA in the alginate sample (78.6 ± 5.9 mM) compared to the control (62.5 ± 5.1 mM). The bacterial Richness index in the alginate ferment (15.83 ± 2.3) was also significantly greater ($p < 0.05$) than that of the control (12.67 ± 2.88). The authors propounded that alginate may be capable of sustaining the growth of human gut bacteria, and recommended further study to evaluate the potential impact that alginate food additives may exert on host health. The *in vivo* study by Mizuno et al. was an interventional study of 11 elderly patients who required enteral feeding. After 4 weeks of receiving the alginate formula (equivalent to 14.52 g fibre/day) there was a significant increase ($p = 0.039$) in Clostridium cluster XI bacteria compared with the baseline. However, there was no increase in Bifidobacterium, Lactobacillales, or Bacteroides. The patients' stool form improved ($p = 0.044$) (Bristol Stool Scale), as did mean blood concentrations of total SCFA ($p = 0.042$), acetic acid ($p = 0.042$), propionic acid ($p = 0.027$), serum albumin ($p = 0.039$), total cholesterol ($p = 0.002$), and cholinesterase ($p = 0.034$). The alginate did not induce any significant changes in stool frequency, body weight, or arm circumference. The authors suggested that the alginate-containing liquid formula may potentially exert a beneficial prebiotic effect on intestinal function through increased production of SCFA. However, the limitations of the study were noted due to the small sample size and single-center study design. In order to validate the findings, the authors recommend a larger, multicenter study.

Alginate may also be useful in the prevention of metabolic syndrome syndrome [115]. It can increase the viscosity of gastric contents, reducing postprandial glucose absorption and insulin response [116], and may thereby impact on hyperlipidaemia and hypertension [1][117].

2.1.4. Carrageenans

Within red seaweeds, carrageenans and porphyran are the prevalent polysaccharides. The family of linear, sulphated polysaccharides, carrageenans, occur as a structural component of the extracellular matrix [118]. Of the 15 different carrageenan forms, iota (ι), kappa (κ) and lambda (λ) are the most widely used as phycocolloids in the food industry [119] and as a vegan alternative to beef gelatin in pharmaceutical capsules [120]. κ and ι-carrageenan are composed of alternating d-galactose and 3,6-anhydro-galactose units with varying numbers of sulphate groups, while λ-carrageenan lacks 3,6-anhydro-galactose and has alternating α-1,3 and β-1,4 inter-galactose bonds [121].

Average molecular weight ranges from 453 to 652 kDa [122]. All forms of carrageenan are soluble in water above their gel-melting temperatures (40–70° C). In cold water, only λ-carrageenan and the sodium salts of κ and ι-carrageenan are soluble [93]. ι-carrageenan was shown to reverse the symptoms of metabolic syndrome in a rat model by significantly decreasing systolic blood pressure, body mass (BM), abdominal and liver fat, and total cholesterol, while also beneficially modulating the gut microbiota [123]. As potential antitumour agents, κ/ι hybrid carrageenans have shown activity *in vitro* against colorectal cancer stem cell-enriched tumourspheres [2]. However, simulated gastrointestinal studies have found that κ-carrageenan can be both beneficial and harmful by increasing or decreasing markers of inflammation and the growth of beneficial gut bacteria and SCFA. This is dependent on the degree of polymerisation of the carrageenan [124].

2.1.5. Porphyran

Porphyran is a sulphated polysaccharide that occurs in red seaweed, within the genus Porphyra, and comprises approximately 11–21% of the seaweed dry mass [125]. It is composed of repeating units of galactose and 3,6-anhydrogalactose, with alternating units of galactose-6-sulphate and 6-*O*-methyl-galactose [126]. Average molecular weight ranges from 14 to 201 kDa [127][128]. Porphyran is soluble in hot water and has similar structural functions to carrageenan, though its higher viscosity limits its pharmaceutical applications [128][129]. Porphyran has shown potential antioxidant and anti-inflammatory effects in cell studies using RAW264.7 cell line [125] and was found to promote cell migration and proliferation in intestinal epithelial cells [127]. It also has antitumor activity against HeLa cells [130], HT-29 colon cancer cells and AGS gastric cancer cells [131]. As a prebiotic, porphyran was previously found to increase beneficial gut bacteria and SCFA production *in vitro* in simulated digestion studies [126][132][133] and in animal studies as whole red seaweed [134][135][136].

2.1.6. Ulvans

Green seaweeds are dominated by the ulvans, which account for 38–54% of the thallus dry mass [137]. Ulvans are water-soluble, gelling polysaccharides composed of repeating units of sulphated l-rhamnose, d-xylose, d-glucuronic acid and its epimer L-iduronic acid [138]. Molecular weights range widely from 1 to 2000 kDa depending upon the degree of sulphation [139]. Ulvans have demonstrated potential anticoagulant [140], antibacterial [141], antiviral [142], and immunoregulatory (porcine intestinal epithelial cells) [143] activities *in vitro*. They have also shown potential for the use as prebiotics in animal studies [144] and *in vitro* [132][145][146].

2.2. Gastrointestinal Digestion Studies with Seaweed Polysaccharides

A number of recent studies have used simulated *in vitro* gastrointestinal digestion or *in vivo* clinical trials to investigate the effect of polysaccharides on beneficial bacterial populations and their metabolites. **Table 1** summarises the polysaccharide fraction used in each study and its impact on gut bacteria. Further characterisation and *in vivo* animal and human dietary intervention studies are required to confirm any potential therapeutic benefits.

Table 1. The impact of polysaccharides on gut bacteria.

Polysaccharide	Seaweed	Extraction Method	Study Type	Statistically Significant Effects	Ref.
* (i) Crude polysaccharide-rich extract (>1 kDa) (CE) (ii) Depolymerised crude extract (>1 kDa) (DE)	*L. digitata*	(i) (CE) Hot acid and ethanol precipitation (0.1 M HCl) (ii) (DE) Fenton's reaction with iron and hydrogen peroxide	Simulated *in vitro* colonic digestion	After 24 h fermentation, compared to cellulose control: • CE increased relative abundance of *Porphyromonadaceae* (p = 0.043), *Lachnospiraceae* (p = 0.015) and *Dialister* (p = 0.005); and reduced *Fibrobacteraceae* (p = 0.026) *Streptococcaceae* (p = 0.025), *Ruminococcus*, (p = 0.027) *Streptococcus* (p = 0.022) and *Fibrobacter* (p = 0.026). • DE increased *Parabacteroides* (p = 0.017) *Lachnospiraceae* (p = 0.039), *Dialister* (p = 0.008) and reduced *Alcaligenaceae* (a Proteobacterium) (p = 0.030) and *Peptostreptococcaceae Incertae Sedis* (p = 0.027). CE and DE increased total SCFA, acetic, propionic, and butyric acid (all p < 0.05) after 10, 24, 36, and 48 h.	[147]

Polysaccharide	Seaweed	Extraction Method	Study Type	Statistically Significant Effects	Ref.
				Ratio of propionate to acetate beneficially reduced by CE and DE (both $p < 0.05$) after 24, 36, and 48 h.	
* Porphyran, ulvan and laminarin	Pyropia, Ulva and Laminaria	Ethanol (80%)	Simulated *in vitro* colonic digestion	After 24 h fermentation, growth of bacterial genera compared to fructooligosaccharide (FOS) control: Porphyran increased Lactobacilli (10.7%, $p < 0.05$). Ulvan increased Bacteroides (6.7%, $p < 0.05$). Laminarin increased Bifidobacteria (8.3%, $p < 0.05$) and Bacteroides (13.8%, $p < 0.05$). Negative results: no significant increase at 24 h in total SCFA, butyrate, lactate or acetate by laminarin, ulvan or porpyran compared to FOS.	[132]
* (i) Crude extract fraction (CF)	*E. radiata*	(i) Enzymatic (Viscozyme-β-glucanase,	Simulated *in vitro* colonic digestion	Increases (log_{10} cells/mL) after 24 h fermentation (all	[10]

Polysaccharide	Seaweed	Extraction Method	Study Type	Statistically Significant Effects	Ref.
(ii) Low MW fraction (LPF) (iii) High MW fraction (HPF)		hemicellulase, arabanase, xylanase) (ii and iii) Viscozyme and ethanol precipitation		*p* < 0.05 compared to controls): ▪ Bacteroidetes (CF 7.36 ± 0.03, LPF 7.21 ± 0.05 and HPF 7.28 ± 0.04) greater than cellulose (6.40 ± 0.05). ▪ *Faecalibacterium prausnitzii* (CF 6.34 ± 0.05, LPF 6.42 ± 0.08) greater than inulin (6.17 ± 0.04) and cellulose (6.07 ± 0.06). ▪ *Clostridium coccoides* (CF 8.29 ± 0.03, LPF 8.56 ± 0.06) greater than inulin (7.57 ± 0.06) and cellulose (7.40 ± 0.05) ▪ *Escherichia coli* (CF 7.16 ± 0.04, LPF 7.31 ± 0.05 and HPF 6.96 ± 0.04) greater than cellulose (6.81± 0.03) ▪ Bifidobacteria (LPF 7.11 ± 0.12) greater than cellulose (6.34 ± 0.06) ▪ Lactobacilli (LPF 6.56 ± 0.05) greater than inulin (6.07 ± 0.05) and cellulose (5.11 ± 0.06) SCFA production after 24 h (all *p* < 0.05):	

Polysaccharide	Seaweed	Extraction Method	Study Type	Statistically Significant Effects	Ref.
				▪ Total SCFA in CF (97.3 µmol/mL), LPF (89.0 µmol/mL) greater than inulin positive control. HPF (68.9 µmol/mL) greater than cellulose (39.7 µmol/mL) but ~20% lower than inulin. ▪ Acetic acid HPF (40.8 µmol/mL) > cellulose ▪ Propionic acid CF (54.6 µmol/mL) > inulin and cellulose ▪ Butyric acid LPF (17.3 µmol/mL) > inulin and cellulose Ratio of Firmicutes to Bacteroidetes beneficially lowered: HPF (1.08 ± 0.008), CF (1.14 ± 0.001) and LPF (1.18 ± 0.006) compared to cellulose (1.22 ± 0.004). Ratio of propionic acid to acetic acid beneficially reduced: 0.47 ± 0.04 (CF), 0.62 ± 0.06 (LPF) and 2.15 ± 0.06 (HPF) compared to 4.08 ± 0.18 (inulin) and 5.73 ± 0.13 (cellulose).	
* (i) Low MW polysaccharide (LMW) (primarily laminarin)	*E. radiata*	(i) Enzymatic (cellulase)	Simulated *in vitro* colonic digestion	24 h post fermentation (all differences $p < 0.05$):	[148]

Polysaccharide	Seaweed	Extraction Method	Study Type	Statistically Significant Effects	Ref.
(ii) High MW polysaccharide acidic water extract (HMW) (primarily fucoidan and alginate) (iii) High MW polysaccharide water and ethanol precipitate (HMWW) (primarily fucoidan and alginate)		(ii) Acidic water (pH 4.5) (iii) Water and ethanol precipitation		(i) LMW increased Bifidobacteria from 5.51 ± 0.15 log_{10} cells/mL (in cellulose fermented control) to 6.55 ± 0.08 log_{10} cells/mL; Lactobacillus from 4.73 ± 0.13 (cellulose) to 5.28 ± 0.19 log_{10} cells/mL and Bacteroidetes from 5.09 ± 0.06 (cellulose) to 6.02 ± 0.09 log_{10} cells/mL. Negative results: no significant increase by LMW on populations of *F. prausnitzii, Clostridium leptum*, *Ruminococcus bromii*, *E. coli* or *Enterococcus*. (ii) HMW increased *C. coccoides* from 5.74 ± 0.75 (cellulose) to 7.07 ± 0.04 log_{10} cells/mL, *E. coli* from 6.09 ± 0.41 (cellulose) to 7.52 ± 0.07 log_{10} cells/mL and *Enterococcus* from 5.02 ± 0.31 (cellulose) to 6.63 ± 0.11 log_{10} cells/mL. Negative results: no significant increase by HMW in any other bacterial populations. (iii) HMWW increased *E. coli* from 6.09 ± 0.41 (cellulose) to 7.01 ± 0.17	

Polysaccharide	Seaweed	Extraction Method	Study Type	Statistically Significant Effects	Ref.
				log_{10} cells/mL and *Enterococcus* from 5.02 ± 0.31 (cellulose) to 5.80 ± 0.33 log_{10} cells/mL. HMWW also had a negative effect on several bacterial populations—Bifidobacteria reduced from 5.51 ± 0.15 (cellulose) to 3.21 ± 0.61 log_{10} cells/mL, Bacteroidetes from 5.09 ± 0.06 (cellulose) to 4.08 ± 0.12 log_{10} cells/mL, Lactobacillus 4.73 ± 0.13 log_{10} cells/mL (cellulose) to not detected (ND), *C. coccoides* from 5.74 ± 0.75 log_{10} cells/mL (cellulose) to ND, *C. leptum* from 6.23 ± 0.28 log_{10} cells/mL (cellulose) to ND and *R. bromii* from 6.20 ± 0.06 (cellulose) to 4.87 ± 0.29 log_{10} cells/mL. SCFA increases in seaweed ferments vs. cellulose control after 24 h (all $p < 0.05$): • LMW ▪ Total SCFA 63.42 ± 1.76 vs. 18.59 ± 0.14 µmol/mL	

Polysaccharide	Seaweed	Extraction Method	Study Type	Statistically Significant Effects	Ref.
				▪ Acetic acid 22.81 ± 0.91 vs. 9.09 ± 0.07 µmol/mL ▪ Propionic acid 29.61 ± 2.60 vs. 3.24 ± 0.04 µmol/mL ▪ Butyric acid 9.22 ± 1.38 vs. 2.02 ± 0.03 µmol/mL 2. HMW ▪ Total SCFA 62.86 ± 0.20 vs. 18.59 ± 0.14 µmol/mL ▪ Acetic acid 20.59 ± 0.21 vs. 9.09 ± 0.07 µmol/mL ▪ Propionic acid 36.79 ± 0.57 vs. 36.79 ± 0.57 µmol/mL ▪ Butyric acid 4.27 ± 0.48 vs. 2.02 ± 0.03 µmol/mL 3. HMWW ▪ Total SCFA 50.70 ± 1.10 vs. 18.59 ± 0.14 µmol/mL ▪ Acetic acid 27.05 ± 0.58 vs. 9.09 ± 0.07 µmol/mL ▪ Propionic acid 18.20 ± 0.38 vs. 3.24 ± 0.04 µmol/mL ▪ Butyric acid—no significant increase	

Polysaccharide	Seaweed	Extraction Method	Study Type	Statistically Significant Effects	Ref.
** (i) Polysaccharide fraction (PF) (primarily fucoidan and alginate) (ii) Whole seaweed (WS)	*E. radiata*	(i) Enzymatic (Viscozyme) (ii) Whole dried *E. radiata*	*In vivo* trial with healthy Sprague-Dawley rats (7 d, 5% PF or 5% WS added to feed)	After 7 days supplementation (all differences $p < 0.05$): Reduction in potentially pathogenic Enterococci in WS group (6.04 ± 0.09 log_{10} cells/mL) vs. control (5.59 ± 0.08 log_{10} cells/mL) Increase in butyrate-producing *F. prausnitzii* in PF group (5.32 ± 0.11 log_{10} cells/mL) vs. control (4.87 ± 0.11 log_{10} cells/mL) 2-fold increase in caecal digesta mass 1.36 ± 0.17 (PF) vs. 0.60 ± 0.06 g/100 g BM (control) Putrefactive microbial products reduced (all values µg/g caecal digesta): • phenol in WS (0.36 ± 0.03) and PF (0.49 ± 0.02) vs. control (2.91 ± 0.70) • *p*-cresol in WS (0.47 ± 0.05) SCFA increase in WS (213.25 ± 14.40 µmol) and	[149]

Polysaccharide	Seaweed	Extraction Method	Study Type	Statistically Significant Effects	Ref.
				PF (208.59 ± 23.32 µmol) vs. control (159.96 ± 13.10 µmol)Negative results: – No significant *p*-cresol decrease in PF fed rats (19.34 ± 5.14) vs. control (25.18 ± 6.18 µg/g caecal digesta)	
* (i) conventional chemical extraction (CCE) (11.9% fucoidan) (ii) microwave-assisted extraction (MAE) (5.71% fucoidan) (iii) ultrasound-assisted extraction (UAE) (4.56% fucoidan) (iv) enzyme-assisted extraction (EAE) (3.89% fucoidan)	*A. nodosum*	(i, ii, and iii) Ethanol followed by acidic water (0.01 M HCl) (iv) Cellulase, acetate buffer (pH 4.5)	*L. casei* and *L. delbrueckii* ssp. *bulgaricus* broth cultures, 3.75% (*v*/*v*). *A. nodosum* extracts added at 0.1%, 0.3% and 0.5% (*w*/*v*)	All differences $p < 0.05$ compared to non-supplemented control medium: Increase in *L. delbrueckii* ssp. *bulgaricus* by CCE, MAE, UAE and EAE at 0.1%, 0.3% and 0.5%. Increase (24.5%) in *L. casei* only by MAE at 0.5% inclusion. Negative results: – No significant increase in *L. casei* by CCE, UAE or EAE vs. non-supplemented media.	[87]
* Crude sulphated polysaccharide (716 kDa) (90% galactose, 9.07% sulphate)	*C. pilulifera*	Acidic extraction (0.0.1 M HCl) and ethanol precipitation	Simulated *in vitro* saliva, gastric, small intestinal and colonic digestion	After 24 h, all differences $p < 0.05$ compared to inulin control: Increase in Bacteroides, Parabacteroides,	[150]

Polysaccharide	Seaweed	Extraction Method	Study Type	Statistically Significant Effects	Ref.
				Megamonas and Veillonella. Increase in total SCFA (22.17 ± 0.82 mmol/L) vs. control (16.17 mmol/L ± 0.39). Negative results: – No significant increase in butyrate, lactate, iso-butyrate, valerate or iso-valerate in seaweed polysaccharide supplemented ferments.	
* (i) Polysaccharides (SJP) (138 kDa) (Fucose:galactose:glucuronic acid:mannose, molar ratio of 4.1:3.6:1.2: 1.0). (ii) Oligosaccharides (SJO)	*S. japonica*	(i) Methanol, dichloromethane, water and ethanol (ii) Methanol, dichloromethane, water and ethanol, followed by 0.6 M HCl	Simulated *in vitro* colonic digestion	After 24 h, all differences $p < 0.05$ compared to FOS control • Increase in beneficial Bacteroidetes and decrease in Proteobacteria (SJP and SJO). • Increased ratio of Bacteroidetes to Firmicutes (SJP and SJO).	[91]
** Crude sulphated polysaccharide (SP) (28.807 kDa) (Galactose (59.7%), galacturonic acid (19.8%),	*G. pacificum*	Ultrasound-assisted water extraction followed by ethanol, acetone	*In vivo* trial with lincomycin hydrochloride induced diarrhoeal	After 9 d, seaweed polysaccharide group vs. non-supplemented normal recovery group (all differences $p < 0.05$):	[151]

Polysaccharide	Seaweed	Extraction Method	Study Type	Statistically Significant Effects	Ref.
xylose (7.1%) and sulphate (8.8%))		and petroleum precipitation	mice (9 days, 75 mg SP/kg BM)	Increase in beneficial Bacteroides, Oscillospira and Bifidobacterium. Decrease in Parabacteroides, Sutterella and AF12. Reduction in inflammatory cytokines, TNF-α, IL-1β and IL-2. Improved (lower) diarrhoea status scores, water intake, and less weight loss. Increase in total SCFA, acetate and propionate.	
** Fucoidan (300 kDa) (60% fucose, 14.3% sulphate)	*C. okamuranus*	Method not specified	*In vivo* trial with Traf3 ip2-mutant psoriasis mice (fucoidan diet group n = 14, normal diet group n = 9, 63 days, 1% fucoidan added to feed)	Fucoidan group vs. cellulose control group (all differences $p < 0.05$). After 56 days: ▪ Increase (% relative abundance) in Bacteroidetes (78.2 ± 6.42 vs. 59.4 ± 9.69%), Proteobacteria (3.05 ± 0.62 vs. 1.73 ± 0.53%), and Paraprevotellaceae. ▪ Decrease in Firmicutes (16.3 ± 4.98 vs. 34.3 ± 9.05%) and TM7	[89]

[152] [153] [154][155][156] [157][158] [159] [160] [161] [162] [163] [164][165] [166] [167]

germfree animals have shown that bioactive phenolic metabolites—normally found after oral administration of polyphenols—are absent in their gut [168]. This shows the importance of the gut microbiota in polyphenol metabolism.

In terrestrial plants, the predominant polyphenols are flavonoids, stilbenes, lignans, and phenolic acids [169]. Seaweeds also produce flavonoids, coumarins, phenolic terpenoids, phenolic acids, luteolin, regiolone, and

Polysaccharide	Seaweed	Extraction Method	Study Type	Statistically Significant Effects	Ref.
				Saccharibacteria (3.80 ± 0.24 vs. 1.23 ± 0.11%). ▪ After 21 days increase in mucin production in ileum and faeces ▪ After 63 days increase in IgA production in cecum+ ▪ Reduction in psoriasis area and severity index (PASI) and ethological scratch-test Negative results: – Decreases in Deferribacteres and Actinobacteria after 56 days were not significant	[170][171][172][173]
** Laminarin and fucoidan (10% laminarin,8% fucoidan and 82% ash)	*Laminaria hyperborea*	Method not specified	*In vivo* trial (10 pregnant sows/treatment) (10 g/days seaweed extract from day 107 of gestation until weaning (day 26)) and *ex vivo* lipopolysaccharide (LPS) immunological challenge	Compared with non-supplemented group, seaweed extract supplemented (SWE) sows had: ▪ Greater colostrum IgA ($p < 0.01$) and IgG ($p = 0.062$) ▪ Decreased faecal Enterobacteriaceae populations at parturition ($p < 0.05$)	[101][174][175][176][177][178][179][180][181][182][183][184][185][186][187][188][189][190][191][192][193][194][195][196][197][198][199][200][201][202][203][204][205][206][207]

2.4. In Vitro and In Vivo Gastrointestinal Digestion Studies with Seaweed Polyphenols

The effect of polyphenols, particularly phlorotannins, on the gut, metabolic syndrome, and DNA damage has been reported in some *in vitro* and *in vivo* studies which are discussed below.

2.4.1. Prebiotic Function and Attenuation of Metabolic Syndrome by Phlorotannins

Polysaccharide	[10]	Seaweed	Extraction Method	Study Type	Statistically Significant Effects	Ref.
	[10]			10	▪ Reduced faecal *Enterobacteriaceae* on expected farrowing date (7.26 vs. 8.60 log_{10} CFU/g, pooled SEM 0.463, $p < 0.05$)	10
	10			10		
			10			
	10					
	10				LPS challenge increased pro-inflammatory cytokines IL-1α and IL-6 ($p < 0.01$) in ileal tissue and tumor necrosis factor (TNF)-α in colonic ($p < 0.01$) tissue	
	[170]				Piglets suckling SWE sows had: ▪ Greater TNF-α after *ex vivo* LPS challenge ($p < 0.05$) ▪ Increased serum IgG ($p < 0.05$) on day 14 ▪ Decreased colonic *E. coli* population ($p < 0.01$) at weaning ▪ Greater Lactobacilli: *E.coli* ratio ($p < 0.05$)	
					Negative results: – No increase in faecal [208] [209] volatile fatty concentrations in SWE sows	

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downregulated by polyphenol supplementation and was lower ($p < 0.05$) than the model group.

Yuan et al. [210] investigated the ability of polyphenol extracts from the brown seaweed, *Lessonia trabeculata*, harvested in China, to alter the gut microbiota of rats in response to type-2-diabetes. Microwave-assisted methanol extraction was followed by solvent fractionation and macroporous resin adsorption separation. The polyphenol-rich fractions produced were composed primarily of phlorotannins, followed by phenolic acids and gallocatechin derivatives. Diabetes was induced in C57BL/6J rats using streptozotocin (STZ). STZ damages the insulin-

Polysaccharide	Seaweed	Extraction Method	Study Type	Statistically Significant Effects	Ref.
				– SWE diet had no effect on TNF-α mRNA expression in unchallenged sow ileal tissue – Piglet birth and weaning weight, and small intestinal morphology unaffected by SWE sow diet	

* = *in vitro* studies; ** = *in vivo* animal studies.

and non-esterified fatty acids (1.86 ± 0.05 vs. 2.02 ± 0.11 mmol/L ($p < 0.05$)). The dyslipidaemia observed in the DC group who did not receive polyphenol supplementation was most likely due to the deficiency of circulating insulin, which increases lipase activity and fatty acid mobilisation from adipose tissue [211]. 16S rRNA gene sequencing of faecal samples from the diabetic rats revealed that there was a significant ($p < 0.01$) increase in gut bacterial diversity within the polyphenol-fed PE group compared to the DC and NC groups. The PE group had a significantly greater abundance of Bacteroidetes, less Proteobacteria, and an improved (lower) ratio of Firmicutes to Bacteroidetes compared to DC ($p < 0.01$). An overabundance of Proteobacteria has been reported as a pro-inflammatory phylum and linked with the imbalance of glucose homeostasis in type-2-diabetes [170]. At the genus level, the PE group had approximately 10 times more Odoribacter ($p < 0.008$) and Muribaculum ($p < 0.005$), and twice the population of Alistipes ($p < 0.006$), Lachnospiraceae ($p < 0.015$) and Parabacteroides ($p < 0.022$) compared to the DC group. Lachnospiraceae and Alistipes are butyric acid producing bacteria that contribute to the maintenance of colonic epithelial tissue [212]. The Odoribacter genus, part of the Bacteroidetes phylum, is an acetic, propionic and butyric acid producer. Its abundance ameliorates inflammation by increasing SCFA availability [213]. An increase in Muribaculum and Parabacteroides numbers has been reported to combat dyslipidaemia, weight gain, inflammation, and insulin resistance resistance [214][215].

Quantification of gut SCFA showed a 61.1% increase in total SCFA production (from 491.31 ± 10.39 to 1276.34 ± 16.86 µg/g ($p < 0.01$)) by the rats after 4 weeks of polyphenol supplementation. The PE group also produced 68.6% more acetic acid (1202.49 ± 11.55 compared to 377.77 ± 3.46 µg/g ($p < 0.01$)) and 74.4% more butyric acid (39.77 ± 1.85 compared to 10.18 ± 0.58 µg/g ($p < 0.01$)) than the DC group. The authors of the study concluded that seaweed polyphenols may have regulated dysbiosis of the gut microbiota in diabetic rats.

2.4.2. Impact of Digestion on Phlorotannin Bioactivity, Attenuation of DNA Damage, and Cancer Cell Proliferation In Vitro

Corona et al. [216] studied the effect of *in vitro* gastrointestinal digestion and colonic fermentation on the polyphenolic content and bioactivity of high molecular weight (HMW > 10 KDa) and low molecular weight (LMW 1–10 KDa) ethanol-extracted phlorotannins from *A. nodosum* harvested in Scotland. To assess changes in

phlorotannin bioactivity post-gastric digestion and -fermentation, the ability of the extracts to prevent H_2O_2 induced DNA damage in HT-29 colon cancer cells and inhibit cell proliferation was also measured. The HMW extract had the greatest total polyphenol and total phlorotannin contents before and after digestion. The HMW extract also had the highest Trolox equivalent antioxidant capacity. The molecular weight of total phlorotannins before and after gastric digestion and colonic fermentation was evaluated by normal phase HPLC. Gastric digestion reduced the level of very high molecular weight components present in the HMW fraction by only 5.4%, while colonic fermentation caused an 89.9% reduction. In the LMW extracts, gastric digestion reduced the level of very high molecular weight components by 52.8% and colonic fermentation by 62.0%. In both cases, colonic fermentation had a far greater impact on the breakdown of phlorotannins compared to enzymatic gastric digestion, suggesting that phlorotannins have the potential to be metabolised by human gut bacteria.

A sulforhodamine B assay was used to measure changes in HT-29 colon cancer cell biomass. The addition of post-gastric digested HMW and LMW at a concentration of 500 µg/mL significantly inhibited ($p < 0.01$) HT-29 cell proliferation (number of cells by division), with HMW being the most effective. Post-gastric digested LMW did not inhibit cell growth (mass accumulation) at any concentration, but HMW did ($p < 0.05$) at concentrations of 250 and 500 µg/mL. High molecular weight phlorotannins may therefore have a potential protective effect on colonocytes against cancer. H_2O_2 induced DNA damage in HT-29 cells was evaluated by single cell gel electrophoresis (Comet) assay. Three of the four phlorotannin extracts (at 100 µg/mL) were successful in reducing DNA damage. Post-gastric digested HMW significantly ($p < 0.01$) reduced DNA damage compared to the control, while post-gastric digested LMW had no effect. However, both the HMW and LMW post-colonic fermented extracts significantly ($p < 0.001$) reduced DNA damage, suggesting that colonic bacteria may potentially metabolise phlorotannins into molecules with different bioactivity than their parent structures.

Although *in vitro* studies and animal trials do not replicate the human gut environment identically, these results show that the abundance of bacteria which normally colonise the mammalian gut may potentially be enhanced by the inclusion of dietary polyphenols. The findings are an indication of prebiotic potential, which may be used to inform the design of future human clinical studies. **Table 2** summarises the polyphenol used in each study and its potential impact on the gut microbiota *in vitro* and *in vivo*, the modulation of hyperglycaemia in animal models, and attenuation of DNA damage *in vitro*.

Table 2. The potential impact of polyphenols on the gut microbiota *in vitro* and *in vivo*, modulation of hyperglycaemia in animal models and DNA damage *in vitro*.

Polyphenol	Seaweed	Extraction Method	Study Type	Statistically Significant Effects	Ref.
* Phlorotannin enriched fraction	*E. radiata*	Ethanol (90%)	Simulated *in vitro* colonic digestion	Increases (all $p < 0.05$) in Bacteroidetes (6.52 ± 0.04 $\log_{10}$ cells/mL) compared to the cellulose control (6.40 ± 0.05 $\log_{10}$ cells/mL); *F. prausnitzii* (6.57 ± 0.05 $\log_{10}$ cells/mL) compared to	[10]

Polyphenol	Seaweed	Extraction Method	Study Type	Statistically Significant Effects	Ref.
				cellulose and inulin controls (6.17 ± 0.04 and 6.07 ± 0.06 log_{10} cells/mL, respectively); *C. coccoides* (7.97 ± 0.05 log_{10} cells/mL) compared to inulin and cellulose controls (7.57 ± 0.06 and 7.40 ± 0.05 log_{10} cells/mL, respectively); and *E. coli* (8.09 ± 0.02 log_{10} cells/mL) compared to inulin and cellulose controls (6.81 ± 0.03 and 6.94 ± 0.03 log_{10} cells/mL, respectively).	
** Polyphenols (3 kDa) (luteolin-6-c-glucoside, regiolone, neoeriocitrin and estr-5(10)-ene-3,17-diol)	*E. prolifera*	Ultrasound assisted ethanol extraction (55%) and ultrafiltration (3 kDa)	*In vivo* trial with diabetic mice (4 weeks, 300 mg polyphenol extract/kg BM/day)	Reduction after 14 days ($p < 0.05$) in mean BM of *E. prolifera*-fed diabetic group compared to model diabetic group. Reduction after 28 days ($p < 0.05$) in mean fasting blood glucose levels of *E. prolifera*-fed diabetic group and glucose tolerance increased ($p < 0.05$) compared to the model diabetic group. Increase in Alistipes ($p < 0.05$) in *E. prolifera*-fed diabetic group compared to model diabetic group. Hypoglycaemic effect via increase ($p < 0.01$) in phosphatidylinositol 3-kinase and suppression ($p < 0.05$) of c-Jun N-terminal kinase in *E. prolifera*-fed diabetic group livers compared to model diabetic group.	[170]
** Polyphenol-rich fraction (primarily phlorotannins, phenolic acids and gallocatechin derivatives)	*L. trabeculata*	Microwave assisted methanol extraction, solvent fractionation and macroporous resin adsorption separation	*In vivo* trial with diabetic rats (4 weeks, 200 mg/day phlorotannin extract/kg BM)	Increase in genera of the phylum Bacteroidetes in the PE group compared to the DC group: Odoribacter ($p < 0.008$), Muribaculum ($p < 0.005$), Alistipes ($p < 0.006$), Lachnospiraceae ($p < 0.015$) and Parabacteroides ($p < 0.022$). Decrease in Proteobacteria, and ratio of Firmicutes to	[210]

Polyphenol	Seaweed	Extraction Method	Study Type	Statistically Significant Effects	Ref.
				Bacteroidetes ($p < 0.05$ PE vs. DC group). Increase in total SCFA (491.31 ± 10.39 (DC), 1276.34 ± 16.86 µg/g (PE) ($p < 0.01$)), acetic acid (377.77 ± 3.46 (DC), 1202.49 ± 11.55 µg/g (PE) ($p < 0.01$)) and butyric acid (10.18 ± 0.58 (DC), 39.77 ± 1.85 µg/g (PE) ($p < 0.01$)). Reduction in the PE group versus the DC group in: fasting blood glucose (10.55 ± 0.94 vs. 13.99 ± 0.87 mmol/L ($p < 0.05$)), serum insulin (14.69 ± 0.11 vs. 17.70 ± 0.22 mU/L ($p < 0.01$)), HOMA-IR insulin resistance value (6.89 ± 0.42 vs. 11.01 ± 0.98 ($p < 0.01$)), total cholesterol (4.92 ± 0.14 vs. 5.64 ± 0.16 mmol/L ($p < 0.01$)), triglycerides (0.99 ± 0.04 vs. 1.43 ± 0.10 mmol/L ($p < 0.01$)), LDL cholesterol (0.68 ± 0.03 vs. 1.06 ± 0.06 ($p < 0.01$)), glycated serum protein (2.15 ± 0.16 vs. 2.74 ± 0.15 ($p < 0.01$)) and non-esterified fatty acids (1.86 ± 0.05 vs. 2.02 ± 0.11 mmol/L ($p < 0.05$)).	
(i) * Phlorotannin (HMW > 10 kDa) (ii) Phlorotannin (LMW 1–10 kDa)	*A. nodosum*	Ethanol	(a) *In vitro* gastrointestinal digestion and colonic fermentation (b) H_2O_2 induced DNA damage in HT-29 colon cancer cells	(a) Reduction in MW of phlorotannins (89.9% HMW, 62.0% LMW) by colonic fermentation, compared to enzymatic gastric digestion (5.4% HMW, 52.8% LMW), suggesting phlorotannins may potentially be metabolised by human gut bacteria. (b) Compared to the control, HMW and LMW phlorotannin extracts at a concentration of 500 µg/mL inhibited ($p < 0.01$) HT-29 colon cancer cell proliferation (number of cells by division), HMW inhibited ($p < 0.05$) HT-29 cell growth (mass accumulation) at concentrations	[216]

References

1. Strain, C.R.; Collins, K.C.; Naughton, V.; McSorley, E.M.; Stanton, C.; Smyth, T.J.; Soler-Vila, A.; Rea, M.C.; Ross, P.R.; Cherry, P.; et al. Effects of a polysaccharide-rich extract derived from Irish-

Polyphenol	Seaweed	Extraction Method	Study Type	Statistically Significant Effects	Ref.
				of 250 and 500 µg/mL. H_2O_2 induced DNA damage in HT-29 cells reduced by post-gastric digested HMW extract ($p < 0.01$) and HMW and LMW post-colonic fermented extracts (both $p < 0.001$).	

* = *in vitro* studies; ** = *in vivo* animal studies.

2.5. Seaweed-Derived Peptides

Seaweed-derived peptides have reported bioactivity as inhibitors of renin [217], angiotensin converting enzyme-I (ACE-I) [9], dipeptidyl peptidase (DPP-IV) [218], platelet activating factor acetylhydrolase (PAF-AH) [219] and α-amylase [220]. They also have reported immunostimulatory [221], antitumor [222], anti-coagulant [223], antioxidative [5], and anti-hyperglycaemic [224] activity. There is recent evidence that some peptides found *in vitro* correlate with animal studies [225]. A study conducted by Fitzgerald et al. [217] previously identified the potential heart health beneficial effects of peptides included in a bread product with peptides derived from the red seaweed *Palmaria palmata* using both *in vitro* and animal models [226]. Peptides were isolated from the seaweed and characterised to completion. However, another study carried out by Allsopp et al. [227] found that the same seaweed had a pro-inflammatory effect when consumed as a whole seaweed in a bread product. This highlights the importance of extraction and characterisation of seaweed bioactives for potential use as therapeutic agents. Allsopp et al. suggested that the iodine content of the seaweed may have been responsible for the observed pro-inflammatory effect in a human dietary intervention study. **Table 3** details the amino acid sequences of recently elucidated seaweed peptides and their bioactivities *in vitro*, *in silico*, or *in vivo*.

Table 3. Amino acid sequences of recently elucidated seaweed-derived peptides and their bioactivities *in vitro*, *in silico* or *in vivo*.

Seaweed	Extraction Method	Amino Acid Sequence	Bioactivity	Ref.
* † U. lactuca	Enzymatic (Papain), MWCO filtration, preparative RP-HPLC and *in silico* enzyme cleavage simulation	(i) Ala-Thr-Lys-Pro-Ala-Asn (ii) Ser-Gly-Ala-Ala-Ser-Ala-Ser-Gly-Ala-Ala (iii) Ala-Gly-Gly-Pro-Asn-Gln-Pro-Pro-Asn (iv) Ala-Ala-Asn-Ile-Thr-Val-Pro-Ala-Ala-Asn (v) Glu-Ala-Glu-Pro-Ala-Glu-Ala-Ala (vi) Gly-Ala-Ala-Pro-Thr-Pro-Pro-Ser-Pro-Pro-Pro-Ala-Thr-Lys-Pro-Ser-	Peptides (i) to (vii) ACE-I, DPP-IV, and enzyme 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibition (*in silico* predictive activity) *In vitro* ACE-I inhibitory activity (%) (all assayed at conc. of 1mg/mL): (a) crude seaweed protein 79.87 ± 0.18%	[9]

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le and phlorotannin-enriched extracts of the brown seaweed Ecklonia radiata influence human gut microbiota and fermentation in vitro. J. Appl. Phycol. 2017, 29, 2407–2416.
4. Charoensiddhi, S.; Conlon, M.A.; Vuaran, M.S.; Franco, C.M.M.; Zhang, W. Impact of extraction processes on prebiotic potential of the brown seaweed Ecklonia radiata by in vitro human gut bacteria fermentation. J. Funct. Foods 2016, 24, 221–230.
5. Charoensiddhi, S.; Conlon, M.A.; Methacanon, P.; Franco, C.M.M.; Su, P.; Zhang, W. Gut health benefits of brown seaweed Ecklonia radiata and its polysaccharides demonstrated in vivo in a rat model. J. Funct. Foods 2017, 37, 676–684.
6. Okolie, C.L.; Mason, B.; Mohan, A.; Pitts, N.; Udenigwe, C.C. The comparative influence of novel extraction technologies on in vitro prebiotic-inducing chemical properties of fucoidan extracts from Ascophyllum nodosum. Food Hydrocoll. 2019, 90, 462–471.
7. Wang, Y.; Chen, G.; Peng, Y.; Rui, Y.; Zeng, X.; Ye, H. Simulated digestion and fermentation in vitro with human gut microbiota of polysaccharides from Coralline pilulifera. LWT 2019, 100, 167–174.
8. Zhang, X.; Liu, Y.; Chen, X.-Q.; Aweya, J.J.; Cheong, K.-L. Catabolism of Saccharina japonica polysaccharides and oligosaccharides by human fecal microbiota. LWT 2020, 130, 109635.
9. Cui, M.; Zhou, R.; Wang, Y.; Zhang, M.; Liu, K.; Ma, C. Beneficial effects of sulfated polysaccharides from the red seaweed Gelidium pacificum Okamura on mice with antibiotic-associated diarrhea. Food Funct. 2020, 11, 4625–4637.
10. Takahashi, M.; Takahashi, K.; Abe, S.; Yamada, K.; Suzuki, M.; Masahisa, M.; Endo, M.; Abe, K.;
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Seaweed	Extraction Method	Amino Acid Sequence	Bioactivity	Ref.
		Thr-Pro-Pro-Lys-Pro-Pro-Thr (vii) Pro-Pro-Asn-Pro-Pro-Asn-Pro-Pro-Asn Amino acid sequences not defined: (a) crude seaweed protein (b) full peptide hydrolysate (c) 1 kDa-UFH (ultra-filtered hydrolysate) (d) 3 kDa-UFH (e) 10 kDa-UFH	(b) full peptide hydrolysate 82.37 ± 0.05% (c) 1 kDa-UFH (ultra-filtered hysrolysate) 93.03 ± 0.87% (d) 3 kDa-UFH 86.64 ± 2.17% (e) 10 kDa-UFH 88.12 ± 0.02%	
* *P. palmata*	Enzymatic (Papain)	Ile-Arg-Leu-Ile-Ile-Val-Leu-Met-Pro-Ile-Leu-Met-Ala	Renin inhibition (58.97 ± 1.26% inhibition *in vitro* at 1 mg/mL)	[217]
* *P. palmata*	Enzymatic (Protease)	(i) Ile-Leu-Ala-Pro (ii) Leu-Leu-Ala-Pro (iii) Met-Ala-Gly-Val-Asp-His-Ile	DPP-IV inhibition IC_{50} values *in vitro*: (i) 43.40 ± 1.40 µM (ii) 53.67 ± 0.82 µM (iii) 159.37 ± 13.67 µM	[218]
* *P. palmata*	Enzymatic (Papain)	Asn-Ile-Gly-Lys	PAF-AH inhibition IC_{50} value *in vitro* 2.32 ± 2.12 mM	[219]
* Porphyra (Laver—species not specified)	Enzymatic (Viscozyme, Alcalase, Neutrase, Pepsin and Trypsin)	(i) Gly-Gly-Ser-Lys (ii) Glu-Leu-Ser	α-amylase inhibition IC_{50} values *in vitro*: (i) 2.58 ± 0.08 mM (ii) 2.62 ± 0.05 mM	[220]
* *P. palmata*	Thermolysin hydrolysis	(i) Leu-Arg-Tyr (ii) Val-Tyr-Arg-Thr	ACE-I inhibition IC_{50} values *in vitro*: (i) 0.044 µM (ii) 0.14 µM	[228]
*,** *U. pinnatifida*	Enzymatic (Protease)	(i) Val-Tyr (ii) Ile-Tyr (iii) Phe-Tyr (iv) Ile-Trp (v) Ala-Trpvi) Val-Trp (vii) Leu-Trp	ACE-I inhibition IC_{50} values *in vitro*: (i) 35.2 µM (ii) 6.1 µM (iii) 42.3 µM (iv) 1.5 µM (v) 18.8 µM(vi) 3.3 µM (vii) 23.6 µM *In vivo* antihypertensive effect in spontaneously hypertensive rats (single oral dose, 1 mg/kg of BW). Blood pressure decreases (pre-administration vs. 9 h post):	[229]

25. Parada Venegas, D.; De la Fuente, M.K.; Landskron, G.; González, M.J.; Quera, R.; Dijkstra, G.; Harmsen, H.J.; Faber, K.N.; Hermoso, M.A. Short chain fatty acids (SCFAs)-mediated gut

Seaweed	Extraction Method	Amino Acid Sequence	Bioactivity	Ref.
			(i) Val-Tyr (228.2 ± 3.4 vs. 206.7 ± 9.5 mmHg) ($p < 0.05$) (ii) Ile-Tyr (205.6 ± 5.2 vs. 184.3 ± 4.5 mmHg) ($p < 0.05$) (iii) Phe-Tyr (208.7 ± 4.4 vs. 193.0 ± 5.1 ($p < 0.01$) (iv) Ile-Trp (213.3 ± 3.4 vs. 199.5 ± 5.9) ($p < 0.05$)	
* *U. pinnatifida*	Enzymatic (Pepsin)	(i) Ala-Ile-Tyr-Lys (ii) Tyr-Lys-Tyr-Tyr (iii) Lys-Phe-Tyr-Gly (iv) Tyr-Asn-Lys-Leu	ACE-I inhibition IC_{50} values *in vitro*:((i) 213 μM (ii) 64.2 μM (iii) 90.5 μM (iv) 21.0 μM	[230]
* *P. palmata*	Enzymatic (Protease)	Ser-Asp-Ile-Thr-Arg-Pro-Gly-Gly-Asn-Met	Antioxidant activity after simulated gastrointestinal digestion: Oxygen radical absorbance capacity 152.43 ± 2.73 nM Trolox equivalents (TE)/μmol peptide and ferric reducing antioxidant power activity 21.23 ± 0.90 nM TE/μmol peptide,	[231]

* = *in vitro* studies; ** = *in vivo* animal studies; † = *in silico* studies.

Proteins and peptides can be used as a food substrate by some families of anaerobic bacteria, including Enterobacteriaceae, Burkholderiaceae, and Desulfovibrionaceae [232] and the genera Peptostreptococcus and Clostridium [233]. Most dietary proteins are broken down by gastric enzymes in the upper gastrointestinal tract and absorbed by the host. The remaining proteins and peptides that reach the colon are metabolised by microbial proteases and peptidases via deamination or decarboxylation reactions to generate amino acids or SCFA, which are used in proteolytic fermentation, or to build microbial cell components [234][235]. The majority of microbial protein fermentation occurs in the distal colon, after passing through the proximal colon, where carbohydrate fermentation is dominant. Amino acids cannot be absorbed through the intestinal epithelium in the colon, therefore protein fermentation end-products can accumulate. The majority of protein fermentation end-products are branched-chain amino acids, while some bacteria such as Clostridia and Fusobacteria metabolise peptides into beneficial SCFA [236][237][238][239]. Other protein metabolites include hydrogen sulphide, phenylacetate, indoles, ammonia, and *p*-cresol, an excess of which can impair epithelial barrier function [240]. However, this has only been reported to occur in individuals with low fibre and high protein diets [241], as the availability of complex polysaccharides reduces protein fermentation by the gut microbiota [242][243].

2.6. Gastrointestinal Digestion Studies with Seaweed Peptides

32. Zhao, L., Zhang, F., Ding, X., Wu, G., Lam, Y.Y., Wang, X., Fu, H., Xue, X., Lu, C., Ma, J., et al. Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes. Science 2018, 359, 1151–1156.

33. Charoensiddhi, S.; Abraham, R.E.; Su, P.; Zhang, W. Chapter Four-Seaweed and seaweed-derived metabolites as prebiotics. In Advances in Food and Nutrition Research; Toldrá, F., Ed.; Academic Press: Cambridge, MA, USA, 2020; Volume 91, pp. 97–156.

34. Hu, B.; Gong, Q.; Wang, Y.; Ma, Y.; Li, J.; Yu, W. Prebiotic effects of neoagaro-oligosaccharides prepared by enzymatic hydrolysis of agarose. Anaerobe 2006, 12, 260–266.

35. Alam, M.A.; Parra-Saldivar, R.; Bilal, M.; Afroze, C.A.; Ahmed, M.N.; Iqbal, H.M.N.; Xu, J. Algae-derived bioactive molecules for the potential treatment of SARS-CoV-2. Molecules 2021, 26, 2134.

36. Ajanth Praveen, M., Karthika Parvathy, K.R., Jayabalan, R., Balasubramanian, P. Dietary fiber from Indian edible seaweeds and its in-vitro prebiotic effect on the gut microbiota. Food Hydrocoll. 2019, 96, 343–353.

37. Kawabata, K.; Yoshioka, Y.; Terao, J. Role of intestinal microbiota in the bioavailability and physiological functions of dietary polyphenols. Molecules 2019, 24, 370.

38. Quigley, E.M.M. Prebiotics and probiotics in digestive health. Clin. Gastroenterol. Hepatol. 2019, 17, 333–344.

39. Coelho, M.C.; Ribeiro, T.B.; Oliveira, C.; Batista, P.; Castro, R.; Monteiro, A.R.; Rodrigues, A.S.; Teixeira, J.; Pintado, M. In vitro gastrointestinal digestion impact on the bioaccessibility and antioxidant capacity of bioactive compounds from tomato flours obtained after conventional and ohmic heating extraction. Foods 2021, 10, 954.
40. Neal, M.J. Chap 3 Drug absorption, distribution and excretion. In Medical Pharmacology at a Glance, 9th ed.; Neal, M.J., Ed.; John Wiley & Sons: Oxford, UK, 2020; Available online: (accessed on 15 May 2020).
41. Srinivasan, V.S. Bioavailability of nutrients: A practical approach to in vitro demonstration of the availability of nutrients in multivitamin-mineral combination products. J. Nutr. 2001, 131, 1349S–1350S.
42. Fernández-García, E.; Carvajal-Lérida, I.; Pérez-Gálvez, A. In vitro bioaccessibility assessment as a prediction tool of nutritional efficiency. Nutr. Res. 2009, 29, 751–760.
43. Santos, D.I.; Saraiva, J.M.A.; Vicente, A.A.; Moldão-Martins, M. Chapter 2-Methods for determining bioavailability and bioaccessibility of bioactive compounds and nutrients. In Innovative Thermal and Non-Thermal Processing, Bioaccessibility and Bioavailability of Nutrients and Bioactive Compounds; Barba, F.J., Saraiva, J.M.A., Cravotto, G., Lorenzo, J.M., Eds.; Woodhead Publishing: Cambridge, UK, 2019; pp. 23–54.
44. Pozharitskaya, O.N.; Shikov, A.N.; Faustova, N.M.; Obluchinskaya, E.D.; Kosman, V.M.; Vuorela, H.; Makarov, V.G. Pharmacokinetic and tissue distribution of fucoidan from Fucus vesiculosus after oral administration to rats. Mar. Drugs 2018, 16, 132.
45. Nishikawa, T.; Yokose, T.; Yamamoto, T.; Yamaguchi, K.; Oda, T. Detection and pharmacokinetics of alginate oligosaccharides in mouse plasma and urine after oral administration by a liquid chromatography/tandem mass spectrometry (LC-MS/MS) method. Biosci. Biotechnol. Biochem. 2008, 72, 2184–2190.
46. Ventura, S.; Rodrigues, M.; Falcão, A.; Alves, G. Safety evidence on the administration of Fucus vesiculosus L. (bladderwrack) extract and lamotrigine: Data from pharmacokinetic studies in the rat. Drug Chem. Toxicol. 2020, 43, 560–566.
47. Zhang, L.; Chu, F.; Zhao, T.; Chai, Y.; Liang, H.; Song, S.; Ji, A. Determination of fucoidan in rat plasma by HPLC and its application in pharmacokinetics. Pak. J. Pharm. Sci. 2020, 33.
48. Lu, J.; Pan, Q.; Zhou, J.; Weng, Y.; Chen, K.; Shi, L.; Zhu, G.; Chen, C.; Li, L.; Geng, M.; et al. Pharmacokinetics, distribution, and excretion of sodium oligomannate, a recently approved anti-Alzheimer's disease drug in China. J. Pharm. Anal. 2021.
49. Pozharitskaya, O.N.; Shikov, A.N.; Obluchinskaya, E.D.; Vuorela, H. The pharmacokinetics of fucoidan after topical application to rats. Mar. Drugs 2019, 17, 687.

Modulation of Intestinal Epithelial Cell Differentiation

Lee et al. [244] evaluated the ability of a 25 amino acid peptide extracted from the red seaweed *Porphyra yezoensis* to modulate cell differentiation in rat intestinal epithelial (IEC-6) cells. Cells were treated with the *P. yezoensis* peptide (PY-PE) at concentrations of 125, 250, 500, and 1000 ng/mL for 24 h. An MTS tetrazolium assay showed that the PY-PE peptide significantly ($p < 0.05$) induced cell proliferation in a dose-dependent manner. Cells treated with 1000 ng/mL PY-PE experienced the greatest increase in numbers (65%). In order to decipher the mechanism by which the peptide exerted this effect, proteins related to the insulin-like growth factor-I receptor (IGF-IR) signalling pathway were measured in the cells. Four main insulin receptor substrate (IRS) proteins are involved in the pathway: IGF-IR, IRS-1, sarcoma homology collagen (Shc), and phosphotyrosine (PY-99). These substrates are adaptor proteins that send signals to the cell nucleus [245]. Protein and mRNA expression of these substrates by the intestinal cells after treatment with PY-PE was evaluated by western blotting, and reverse transcription-polymerase chain reaction (RT-PCR) of complementary (c)DNA. After 24 h, PY-PE successfully upregulated protein and mRNA expression of the four substrates with the 1000 ng/mL PY-PE treatment having the most significant ($p < 0.05$) effect.

The IGF-IR pathway in turn activates the mitogen-activated protein kinase (MAPK) signalling pathway. MAPK is a kinase (phosphate transfer enzyme) that binds with threonine and serine and directs cellular responses [246]. Expression levels of three MAPK proteins were measured: extracellular signal-regulated kinase 1/2 (ERK1/2), anti-phospho-c-Jun N-terminal kinase (JNK), and anti-phospho-p38 (P38). Treatment with PY-PE did increase ($p < 0.05$) the expression of ERK1/2 in the intestinal cells in a dose-dependent manner; however, the peptide had no effect on JNK or p38. The authors surmised that the peptide only affected ERK1/2 expression because it regulates cell growth and proliferation, while JNK and p38 are activated by cellular stress and inflammation.

The effect of PY-PE on the PI3K-Akt signalling pathway was also examined by measuring the intermediates p85, p110, PDK1, and p-Akt. This pathway is involved in cell proliferation and angiogenesis through serine and threonine phosphorylation [243]. Compared to the controls, protein and mRNA expression of p85, p110, PDK1 and p-Akt was increased ($p < 0.05$) in intestinal cells treated with PY-PE, dose dependently. Lastly, the p42/p44 mitogen-activated protein kinase (MAPK1) pathway was investigated. This pathway regulates the activation of transcription factors, such as activator protein-1 and its sub-proteins, c-Jun and c-Fos, which modulate cell proliferation and differentiation. Again, PY-PE treatment successfully upregulated protein and mRNA expression of c-Jun and c-Fos in a dose dependent manner.

Due to these positive results, the authors of the study conducted further analysis with the *P. yezoensis* derived peptide [247]. The proliferative effect of the peptide on the epidermal growth factor receptor (EGFR) signalling pathway was investigated in IEC-6 rat intestinal epithelial cells. The EGFR signalling pathway influences cell functions such as proliferation and involves several proteins including phosphorylated (p-)EGFR, Shc, growth

50. Corino, C.; Di Giancamillo, A.; Modina, S.C.; Rossi, R. Bioactive Effects of Seaweed Polysaccharides in Pigs. Animals 2021, 11, 1573.

51. Shikov, A.N.; Flisyuk, E.V.; Obluchinskaya, E.D.; Pozharitskaya, O.N. Pharmacokinetics of marine-derived drugs. Mar. Drugs 2020, 18, 557.

52. Mohammed, A.S.A.; Naveed, M.; Jost, N. Polysaccharides; classification, chemical properties, and future perspective applications in fields of pharmacology and biological medicine (A review of current applications and upcoming potentialities). J. Polym. Environ. 2021.

53. Rasmussen, R.S.; Morrissey, M.T. Marine Biotechnology for Production of Food Ingredients. In Advances in Food and Nutrition Research; Academic Press: Cambridge, MA, USA, 2007; Volume 52, pp. 237–292.

54. Peñalver, R.; Lorenzo, J.M.; Ros, G.; Amarowicz, R.; Pateiro, M.; Nieto, G. Seaweeds as a functional ingredient for a healthy diet. Mar. Drugs 2020, 18, 301.

55. Wong, K.H.; Cheung, P.C.K. Nutritional evaluation of some subtropical red and green seaweeds: Part I—Proximate composition, amino acid profiles and some physico-chemical properties. Food Chem. 2000, 71, 475–482.

56. Cherry, P.; O'Hara, C.; Magee, P.J.; McSorley, E.M.; Allsopp, P.J. Risks and benefits of consuming edible seaweeds. Nutr. Rev. 2019, 77, 307–329.

57. Kraan, S. Chapter 22-Algal polysaccharides, novel applications and outlook. In Carbohydrates-Comprehensive Studies on Glycobiology and Glycotechnology; Chang, C.F., Ed.; IntechOpen: Rijeka, Croatia, 2012.

58. de Jesus Raposo, M.F.; de Morais, A.M.; de Morais, R.M. Emergent Sources of Prebiotics: Seaweeds and Microalgae. Mar. Drugs 2016, 14, 27.

59. Sanz-Pintos, N.; Pérez-Jiménez, J.; Buschmann, A.H.; Vergara-Salinas, J.R.; Pérez-Correa, J.R.; Saura-Calixto, F. Macromolecular antioxidants and dietary fiber in edible seaweeds. J. Food Sci. 2017, 82, 289–295.

60. Fernando, I.P.S.; Kim, K.-N.; Kim, D.; Jeon, Y.-J. Algal polysaccharides: Potential bioactive substances for cosmeceutical applications. Crit. Rev. Biotechnol. 2019, 39, 99–113.

61. Olsson, J.; Toth, G.B.; Albers, E. Biochemical composition of red, green and brown seaweeds on the Swedish west coast. J. Appl. Phycol. 2020.

62. Tanneck, G.W.; Liu, Y. Guided dietary fibre intake as a means of directing short-chain fatty acid production by the gut microbiota. J. R. Soc. N. Z. 2020, 50, 434–455.

63. Hjorth, M.F.; Astrup, A. The role of viscous fiber for weight loss: Food for thought and gut bacteria. Am. J. Clin. Nutr. 2020.

Peptide	Seaweed	Study Type	Statistically Significant Effects	Ref.
* Ala-Leu-Glu-Gly-Gly-Lys-Ser-Ser-Gly-Gly-Gly-Glu-Ala-Thr-Arg-Asp-Pro-Glu-Pro-Thr	*P. yezoensis*	*In vitro* rat intestinal epithelial cells—investigating the modulation of cell differentiation.	At concentrations of 125–1000 ng/mL, the peptide, dose-depenently ($p < 0.05$): ▪ Induced intestinal epithelial cell proliferation ▪ Upregulated insulin receptor substrates IGF-IR, IRS-1, Shc and PY-99 ▪ Increased mRNA expression of p110, PDK1, p-Akt, c-Jun, c-Fos, and MAPK protein ERK1/2	[244]
* Ala-Leu-Glu-Gly-Gly-Lys-Ser-Ser-Gly-Gly-Gly-Glu-Ala-Thr-Arg-Asp-Pro-Glu-Pro-Thr	*P. yezoensis*	*In vitro* rat intestinal epithelial cells—investigating the epidermal growth factor receptor signalling pathway and Ras/Raf-p42/p44 MAPK signalling pathway, mediating signal transduction from cell surface to nucleus.	At concentrations of 125–1000 ng/mL, the peptide dose-dependently($p < 0.05$): – Increased mRNA expression of p-EGFR, Shc, Grb2, SOS, Ras, Raf, mitogen activated extracellular kinase, and p-extracellular signal-regulated kinase. – Increased mRNA expression of p-EGFR, Shc, Grb2, SOS Ras, Raf, mitogen activated extracellular kinase, and p-extracellular signal-regulated kinase. – Increased mRNA expression of proteins required for cell proliferation: cyclin D1, cyclin E, Cdk2, Cdk4, Cdk6, and pRb – Increased cell growth during Gap 1 phase (47.6, 50.6, 56.8, 62.8 and	[247]

75. Usov, A.; Zelinsky, N. Chapter 2-Chemical structures of algal polysaccharides. In Functional Ingredients from Algae for Foods and Nutraceuticals; Domínguez, H., Ed.; Woodhead Publishing: Cambridge, UK, 2013; pp. 23–86.

Peptide	Seaweed	Study Type	Statistically Significant Effects	Ref.
			64.4% following treatment with 0, 125, 250, 500, and 1000 ng/mL of peptide, respectively) – Decreased mRNA expression of p21 and p27 associated with mucosal damage and ulcerative colitis.	

* = *in vitro* studies.

2.7. Bioaccessibility and Bioavailability

Bioavailability may be defined as the fraction of ingested nutrient or bioactive compound that reaches the systemic circulation and is utilised by the body [257]. Numerous factors influence the bioavailability of compounds in food including the health status of the individual, age, diet, interactions with other dietary components during digestion, and intestinal and hepatic metabolism [257][258]. Bioavailability involves two different phases—bioaccessibility and bioactivity. Bioaccessibility is the quantity of the ingested compound that is released from its food matrix and is available for absorption in the intestine [259]. Bioactivity is the biological activity of a drug or food component and involves transport of the component to the target tissue, interaction with other biomolecules, biotransformation and/or metabolism, and the induction of a physiological response [260].

The oral bioaccessibility of food can be measured *in vitro* using static or dynamic digestive methods, or *ex vivo* using organ/tissue culture models. Bioavailability can be measured using an animal-free method such as the protein digestibility-corrected amino acid score for estimating *in vivo* protein digestibility [261], but is usually measured *in vivo* using animal or human models [262]. The advantages and limitations of *in vitro* versus *ex vivo* and *in vivo* methods are here outlined.

2.7.1. In Vitro Bioaccessibility Methods

In vitro simulated digestion methods are generally used as a preliminary test to determine the oral bioaccessibility of a food-derived component as they can be conducted in a laboratory using chemicals and enzymes that mimic the environment of the stomach and intestine without the need for live animals or human participants [263]. Experimental processes for *in vitro* simulated digestion involve several incubation steps (1–3 h) of the sample at physiological temperatures (37 °C) and conditions that simulate the mammalian digestive tract [264]. Oral digestion of the homogenised food sample begins with lingual α-amylase at pH 5–7, followed by adjustment of pH to 1–3 to

80. Usoltseva, R.V.; Anastyuk, S.D.; Surits, V.V.; Shevchenko, N.M.; Thinh, P.D.; Zadorozhny, P.A.; Ermakova, S.P. Comparison of structure and in vitro anticancer activity of native and modified fucoidans from Sargassum feldmannii and S. duplicatum. Int. J. Biol. Macromol. 2019, 124, 220–228.
82. Hwang, P.A.; Phan, N.N.; Lu, W.J.; Ngoc Hieu, B.T.; Lin, Y.C. Low-molecular-weight fucoidan and high-stability fucoxanthin from brown seaweed exert prebiotics and anti-inflammatory activities in Caco-2 cells. Food Nutr. Res. 2016, 60.
83. Irhimeh, M.R.; Fitton, J.H.; Lowenthal, R.M. Pilot clinical study to evaluate the anticoagulant activity of fucoidan. Blood Coagul. Fibrinolysis 2009, 20, 607–610.
84. Tsai, H.-L.; Tai, C.-J.; Huang, C.-W.; Chang, F.-R.; Wang, J.-Y. Efficacy of low-molecular-weight fucoidan as a supplemental therapy in metastatic colorectal cancer patients: A double-blind randomized controlled trial. Mar. Drugs 2017, 15, 122.
85. Maruyama, H.; Yamauchi, H.; Hashimoto, M.; Nakano, T. Antitumor activity and immune response of Mekabu fucoidan extracted from sporophyll of Undaria pinnatifida. In Vivo 2003, 17, 245–249.
86. Lin, H.-T.V.; Tsou, Y.-C.; Chen, Y.-T.; Lu, W.-J.; Hwang, P.-A. Effects of low-molecular-weight fucoidan and high stability fucoxanthin on glucose homeostasis, lipid metabolism, and liver function in a mouse model of type II diabetes. Mar. Drugs 2017, 15, 113.
87. Okolie, C.L.; Mason, B.; Mohan, A.; Pitts, N.; Udenigwe, C.C. The comparative influence of novel extraction technologies on in vitro prebiotic-inducing chemical properties of fucoidan extracts from Ascophyllum nodosum. Food Hydrocoll. 2019, 90, 462–471.

mimic the stomach environment, and the addition of the endopeptidase, pepsin [26]. Finally, the pH is adjusted to 6–8 to mimic the small intestine and pancreatin (a combination of amylase, protease, and lipase) is added with or without bile [265].

In vitro methods are divided into four categories: these are solubility, dialysability, gastrointestinal models, and cell models [245].

Solubility and Dialysability

Solubility involves centrifugation of the digested sample and quantification of the nutrient of interest in the supernatant by various techniques such as atomic absorption spectrophotometry (AA), mass spectrometry and HPLC [266]. Laparra et al. [267] estimated the bioaccessibility of arsenic in *Hizikia fusiforme*, *Porphyra* and *Enteromorpha* species using an *in vitro* solubility method followed by AA.

Dialysability was first described by Miller et al. [268] in 1981 to measure the bioaccessibility of iron by equilibrium dialysis, and has been modified to quantify the bioaccessibility of other micronutrients. After acidic pepsin digestion of the food sample, dialysis tubing of the required MW is filled with a basic buffer such as sodium bicarbonate and added to a vessel containing the sample in its acidic environment. The sodium bicarbonate diffuses out of the dialysis tubing and neutralises the acidity. Pancreatin is added to the sample and incubated. The dialysate that diffuses in through the tubing is the bioaccessible portion of the food sample, which is then removed from the vessel and quantified [268].

Static and Dynamic Gastrointestinal Models

Gastrointestinal models can be static or dynamic. Static models are the simpler of the two methods and involve the oral, gastric, and small intestinal stages described above. The reactions are carried out in a single bioreactor or flask with stirring and pH adjustments made at each step by addition of an acid or base, usually hydrochloric acid and sodium hydroxide [260]. One of the limitations of static methods is the broad variance in results due to the diversity of reagents used worldwide; particularly digestive enzymes, that differ in activity depending upon their source, which can be human, porcine, rabbit, bacterial, or fungal [269]. Other parameters such as incubation time, pH, ionic strength, the use of phospholipid surfactants, bile salts, and sample-to-liquid ratio also vary from one method to another [270]. In order to address this lack of cohesion in simulated digestive methods, the European Cooperation in Science and Technology (COST) began an EU-funded Action in 2011 called INFOGEST involving scientists from 45 countries [271]. In 2014, an international consensus was reached and a standardised static *in vitro* digestion method suitable for food was published by Minekus et al. [269] based on physiologically relevant conditions that can be applied to various endpoints. The method recommends specific concentrations and conditions for each step of static *in vitro* digestion. Pepsin was determined to be the factor causing most variation, the activity determination of which was found to be improved by pH stabilisation [269]. Subsequent inter-laboratory validation studies in 2016 by Egger et al. [265] using skim milk powder as a model food found that the harmonised INFOGEST method delivered increased consistency for the comparability of *in vitro* digestion studies. Recent studies have used the INFOGEST method to evaluate the potential bioaccessibility of seaweed components such

88. Kan, J.; Cheng, J.; Xu, L.; Hood, M.; Zhong, D.; Cheng, M.; Liu, Y.; Chen, L.; Du, J. The combination of wheat peptides and fucoidan protects against chronic superficial gastritis and alters gut microbiota: A double-blinded, placebo-controlled study. Eur. J. Nutr. 2020, 59, 1655–1666.
89. Takahashi, M.; Takahashi, K.; Abe, S.; Yamada, K.; Suzuki, M.; Masahisa, M.; Endo, M.; Abe, K.; Inoue, R.; Hoshi, H. Improvement of psoriasis by alteration of the gut environment by oral administration of fucoidan from Cladosiphon okamuranus. Mar. Drugs 2020, 18, 154.
90. Parnell, J.A.; Reimer, R.A. Prebiotic fiber modulation of the gut microbiota improves risk factors for obesity and the metabolic syndrome. Gut Microbes 2012, 3, 29–34.
91. Zhang, X.; Liu, Y.; Chen, X.-Q.; Aweya, J.J.; Cheong, K.-L. Catabolism of Saccharina japonica polysaccharides and oligosaccharides by human fecal microbiota. LWT 2020, 130, 109635.
92. Shang, Q.; Shan, X.; Cai, C.; Hao, J.; Li, G.; Yu, G. Dietary fucoidan modulates the gut microbiota in mice by increasing the abundance of Lactobacillus and Ruminococcaceae. Food Funct. 2016, 7, 3224–3232.
93. Olatunji, O. Aquatic Biopolymers: Understanding Their Industrial Significance and Environmental Implications; Springer Nature: Cham, Switzerland, 2020.
94. Baweja, P.; Sahoo, D. Chapter 2-Classification of algae. In The Algae World; Sahoo, D., Seckbach, J., Eds.; Springer: Dordrecht, The Netherlands, 2015; pp. 31–55.
95. Usman, A.; Khalid, S.; Usman, A.; Hussain, Z.; Wang, Y. Chapter 5-Algal polysaccharides, novel application, and outlook. In Algae Based Polymers, Blends, and Composites; Zia, K.M., Zuber, M., Ali, M., Eds.; Elsevier: Amsterdam, The Netherlands, 2017; pp. 115–153.
96. Kadam, S.U.; O'Donnell, C.P.; Rai, D.K.; Hossain, M.B.; Burgess, C.M.; Walsh, D.; Tiwari, B.K. Laminarin from Irish brown seaweeds Ascophyllum nodosum and Laminaria hyperborea: Ultrasound assisted extraction, characterization and bioactivity. Mar. Drugs 2015, 13, 4270–4280.
97. Song, K.; Xu, L.; Zhang, W.; Cai, Y.; Jang, B.; Oh, J.; Jin, J.O. Laminarin promotes anti-cancer immunity by the maturation of dendritic cells. Oncotarget 2017, 8, 38554–38567.
98. Miao, H.-Q.; Elkin, M.; Aingorn, E.; Ishai-Michaeli, R.; Stein, C.A.; Vlodavsky, I. Inhibition of heparanase activity and tumor metastasis by laminarin sulfate and synthetic phosphorothioate oligodeoxynucleotides. Int. J. Cancer 1999, 83, 424–431.
99. Cuong, D.X. Laminarin (beta-glucan) of brown algae Sargassum mcclurei: Extraction, antioxidant activity, lipoxygenase inhibition activity, and physicochemistry properties. World J. Food Sci. Technol. 2020, 4, 31.
100. Lee, J.; Kim, Y.-J.; Kim, H.; Kim, Y.-S.; Park, W. Immunostimulatory effect of laminarin on RAW 264.7 mouse macrophages. Molecules 2012, 17, 5404–5411.

as essential minerals, carrageenan [272], alginate [273] and to assess protein digestibility [274]. Static models have the advantage of being inexpensive, easy to use, and do not require specific equipment. However, continuous mechanical agitation is not representative of complex peristaltic movements and does not replicate the dynamic processes that occur during digestion, such as continuous changes in pH and secretions or gastric emptying [275].

Dynamic gastrointestinal models differ from static models in that a series of chambers are used to digest the food sample connected by peristaltic pumps [276]. The temperature, pH, enzyme concentration, incubation time and agitation are, for each chamber, controlled by a computer [264]. The first commercially dynamic gastrointestinal model was developed in 1995 by Minekus et al. [277] at the Netherlands Organisation for Applied Scientific Research (Toegepast Natuurwetenschappelijk Onderzoek (TNO)) called the TNO Gastrointestinal Model (TIM). The TIM-1 model has four compartments, representing the stomach, duodenum, jejunum, and ileum connected by peristaltic valve pumps. Bioaccessible fractions are collected by dialysis after the fourth compartment [277]. The non-bioaccessible fraction is transferred to the TIM-2 model, which has one compartment representing the large intestine. Human faecal inocula is added to study the effect of colonic fermentation on the food sample and nutrient absorption [277]. The main advantage of the TIM system is that it is a holistic *in vitro* gastrointestinal model which incorporates the large as well as the small intestine. In addition, samples can be taken at any stage of the digestive process without pausing the experiment [278]. Several studies have found that bioaccessibility results using the TIM system correlate with bioavailability of the same nutrient *in vivo*. The TIM system was used to measure the bioaccessibility of iron and phosphorus from wheat [279], folate in folate-fortified milk products [280], and the bran, flour, and protein aleurone layer of wheat [281] and were found to be comparable to *in vivo* data. The TIM system has been used to assess the bioaccessibility of heavy metals [282] and essential minerals [283] in seaweed. Drug bioaccessibility was assessed in a study by Blanquet et al. [284] comparing the ability of TIM-1 to measure the bioaccessibility of paracetamol and a lyophilised Lactobacillus strain with *in vivo* data. The TIM1 results were consistent with *in vivo* data, showing the value of TIM-1 as a predictive tool on biopharmaceutical behaviour. However, as with all *in vitro* methods, *in vivo* factors such as first pass effect, renal clearance, and metabolisation by intestinal epithelia are not represented [284].

The Institute of Food Research in Norwich, England also developed a dynamic method, published by Wickham et al. [285] in 2012, called the Dynamic Gastric Model (DGM). It was designed to simulate the discrete mechanical aspects of gastric digestion as well as the biochemical and is more complex than earlier dynamic models [276]. The masticated sample is added slowly over the course of several minutes to mimic the swallowing of food. The DGM system has several functionally distinct zones in which the masticated food bolus is processed to mimic the human stomach environment. A secretion distributer gradually introduces gastric acid and enzymes to the flexible main body around the food bolus, which is then gently kneaded. Contents then move to the antrum, where they are subjected to physiological shear and grinding forces [285]. The sample, or chyme, can be removed at this stage or further digested in the duodenal chamber with pancreatic enzymes, bile salts, lecithin and cholesterol, which is often used for gastro-resistant pharmaceutical formulations to monitor dispersal and dissolution in the duodenal phase [276]. A study by Vardakou et al. [286] compared the disintegration and dissolution capabilities of the DGM system with a standard Dissolution Apparatus USP II using agar gel beads, and compared the results to those previously observed when the same beads were given to human volunteers [287]. The DGM system was found to

101. Leonard, S.; Sweeney, T.; Bahar, B.; O'Doherty, J. Effect of maternal seaweed extract supplementation on suckling piglet growth, humoral immunity, selected microflora, and immune response after an ex vivo lipopolysaccharide challenge. J. Anim. Sci. 2012, 90, 505–514.
102. Vigors, S.; O'Doherty, J.V.; Rattigan, R.; McDonnell, M.J.; Rajauria, G.; Sweeney, T. Effect of a laminarin rich macroalgal extract on the caecal and colonic microbiota in the post-weaned pig. Mar. Drugs 2020, 18, 157.
103. Rattigan, R.; Sweeney, T.; Maher, S.; Thornton, K.; Rajauria, G.; O'Doherty, J.V. Laminarin-rich extract improves growth performance, small intestinal morphology, gene expression of nutrient transporters and the large intestinal microbial composition of piglets during the critical post-weaning period. Br. J. Nutr. 2019, 123, 255–263.
104. Lynch, M.B.; Sweeney, T.; Callan, J.J.; O'Sullivan, J.T.; O'Doherty, J.V. The effect of dietary Laminaria-derived laminarin and fucoidan on nutrient digestibility, nitrogen utilisation, intestinal microflora and volatile fatty acid concentration in pigs. J. Sci. Food Agric. 2010, 90, 430–437.
105. Zhang, Y.; Zhao, N.; Yang, L.; Hong, Z.; Cai, B.; Le, Q.; Yang, T.; Shi, L.; He, J.; Cui, C.-B. Insoluble dietary fiber derived from brown seaweed Laminaria japonica ameliorate obesity-related features via modulating gut microbiota dysbiosis in high-fat diet-fed mice. Food Funct. 2020.
106. O'Sullivan, L.; Murphy, B.; McLoughlin, P.; Duggan, P.; Lawlor, P.G.; Hughes, H.; Gardiner, G.E. Prebiotics from marine macroalgae for human and animal health applications. Mar. Drugs 2010, 8, 2038–2064.
107. Pereira, L.; Cotas, J. Chapter 1-Introductory chapter: Alginates-a general overview. In Alginates-Recent Uses of This Natural Polymer; Pereira, L., Ed.; IntechOpen: Rijeka, Croatia, 2020.
108. Mei, X.; Chang, Y.; Shen, J.; Zhang, Y.; Xue, C. Expression and characterization of a novel alginate-binding protein: A promising tool for investigating alginate. Carbohydr. Polym. 2020, 246, 116645.
109. Ramos, P.E.; Silva, P.; Alario, M.M.; Pastrana, L.M.; Teixeira, J.A.; Cerqueira, M.A.; Vicente, A.A. Effect of alginate molecular weight and M/G ratio in beads properties foreseeing the protection of probiotics. Food Hydrocoll. 2018, 77, 8–16.
110. Mancini, F.; Montanari, L.; Peressini, D.; Fantozzi, P. Influence of alginate concentration and molecular weight on functional properties of mayonnaise. LWT 2002, 35, 517–525.
111. Jönsson, M.; Allahgholi, L.; Sardari, R.R.; Hreggviðsson, G.O.; Nordberg Karlsson, E. Extraction and modification of macroalgal polysaccharides for current and next-generation applications. Molecules 2020, 25, 930.
112. Bai, S.; Chen, H.; Zhu, L.; Liu, W.; Yu, H.D.; Wang, X.; Yin, Y. Comparative study on the in vitro effects of Pseudomonas aeruginosa and seaweed alginates on human gut microbiota. PLoS ONE 2017, 12, e0171576.

113. Liu, M.; Liu, B.; Shang, Q.; Chen, X.; Li, W.; Pi, X.; Zhao, L.; Yin, Y.; Yu, G. Wang, X. In vitro fermentation of alginate and its derivatives by human gut microbiota. Anaerobe 2016, 39, 19–25.
114. Mizuno, H.; Bamba, S.; Abe, N.; Sasaki, M. Effects of an alginate-containing variable-viscosity enteral nutrition formula on defecation, intestinal microbiota, and short-chain fatty acid production. J. Funct. Foods 2020, 67, 103852.
115. Georg Jensen, M.; Pedersen, C.; Kristensen, M.; Frost, G.; Astrup, A. Efficacy of alginate supplementation in relation to appetite regulation and metabolic risk factors: Evidence from activated human studies. Obes. Rev. 2013, 14, 129–144.
116. Guo, L.; Goff, H.D.; Xu, F.; Liu, F.; Ma, J.; Chen, M.; Zhong, F. The effect of sodium alginate on nutrient digestion and metabolic responses during both in vitro and in vivo digestion process. Food Hydrocoll. 2020, 107, 105304.
117. Hu, Y.; Feng, Z.; Feng, W.; Hu, T.; Guan, H.; Mao, Y. AOS ameliorates monocrotaline-induced pulmonary hypertension by restraining the activation of P-selectin/p38MAPK/NF-κB pathway in rats. Biomed. Pharmacother. 2019, 109, 1319–1326.
118. Amimi, A.; Mouradi, A.; Bennasser, L.; Givernaud, T. Seasonal variations in thalli and carrageenan composition of *Gigartina pistillata* (Gmelin) Stackhouse (Rhodophyta, Gigartinales) harvested along the Atlantic coast of Morocco. Phycol. Res. 2007, 55, 143–149.
119. Jiao, G.; Yu, G.; Zhang, J.; Ewart, H.S. Chemical structures and bioactivities of sulfated polysaccharides from marine algae. Mar. Drugs 2011, 9, 196–223.
120. Oladzadabbasabadi, N.; Ebadi, S.; Nafchi, A.M.; Karim, A.; Kiahosseini, S.R. Functional properties of dually modified sago starch/κ-carrageenan films: An alternative to gelatin pharmaceutical capsules. Carbohydr. Polym. 2017, 160, 43–51.
121. McKim, J.; Willoughby, J.; Blakemore, W.; Weiner, M. Clarifying the confusion between poligeenan, degraded carrageenan, and carrageenan: A review of the chemistry, nomenclature, and in vivo toxicology by the oral route. Crit. Rev. Food Sci. Nutr. 2018, 59, 1–70.
122. Uno, Y.; Omoto, T.; Goto, Y.; Asai, I.; Nakamura, M.; Maitani, T. Molecular weight distribution of carrageenans studied by a combined gel permeation/inductively coupled plasma (GPC/ICP) method. Food Addit. Contam. 2001, 18, 763–772.
123. Du Preez, R.; Paul, N.; Mouatt, P.; Majzoub, M.E.; Thomas, T.; Panchal, S.K.; Brown, L. Carrageenans from the red seaweed Sarconema filiforme attenuate symptoms of diet-induced metabolic syndrome in rats. Mar. Drugs 2020, 18, 97.
124. Sun, Y.; Cui, X.; Duan, M.; Ai, C.; Song, S.; Chen, X. In vitro fermentation of κ-carrageenan oligosaccharides by human gut microbiota and its inflammatory effect on HT29 cells. J. Func. Foods 2019, 59, 80–91.

125. Isaka, S.; Chan, K.; Nakazono, S.; Abu, R.; Ueno, M.; Kimiya, D.; Oda, T. Antioxidant and anti-inflammatory activities of porphyran isolated from dried laver (Porphyra yezoensis). Int. J. Biol. Macromol. 2015, 74, 68–75.

126. Xu, S.-Y.; Aweya, J.J.; Li, N.; Deng, R.-Y.; Chen, W.-Y.; Tang, J.; Cheong, K.-L. Microbial catabolism of Porphyra haitanensis polysaccharides by human gut microbiota. Food Chem. 2019, 289, 177–186.

127. Qiu, H.-M.; Veeraperumal, S.; Lv, J.-H.; Wu, T.-C.; Zhang, Z.-P.; Zeng, Q.-K.; Liu, Y.; Chen, X.-Q.; Aweya, J.J.; Cheong, K.-L. Physicochemical properties and potential beneficial effects of porphyran from Porphyra haitanensis on intestinal epithelial cells. Carbohydr. Polym. 2020, 246, 116626.

128. Zhao, T.; Zhang, Q.; Qi, H.; Zhang, H.; Niu, X.; Xu, Z.; Li, Z. Degradation of porphyran from Porphyra haitanensis and the antioxidant activities of the degraded porphyrans with different molecular weight. Int. J. Biol. Macromol. 2006, 38, 45–50.

129. Bhatia, S.; Sharma, A.; Sharma, K.; Kavale, M.; Chaugule, B.; Dhalwal, K.; Namdeo, A.; Mahadik, K. Novel algal polysaccharides from marine source: Porphyran. Pharmacogn. Rev. 2008, 2, 271. Available online: (accessed on 8 June 2020).

130. He, D.; Wu, S.; Yan, L.; Zuo, J.; Cheng, Y.; Wang, H.; Liu, J.; Zhang, X.; Wu, M.; Choi, J.-I.; et al. Antitumor bioactivity of porphyran extracted from Pyropia yezoensis Chonsoo2 on human cancer cell lines. J. Sci. Food Agric. 2019, 99, 6722–6730.

131. Kwon, M.-J.; Nam, T.-J. Chromatographically purified porphyran from Porphyra yezoensis effectively inhibits proliferation of human cancer cells. Food Sci. Biotechnol. 2007, 16, 873–878. Available online: (accessed on 13 July 2020).

132. Seong, H.; Bae, J.-H.; Seo, J.S.; Kim, S.-A.; Kim, T.-J.; Han, N.S. Comparative analysis of prebiotic effects of seaweed polysaccharides laminaran, porphyran, and ulvan using in vitro human fecal fermentation. J. Funct. Foods 2019, 57, 408–416.

133. Xu, S.-Y.; Chen, X.-Q.; Liu, Y.; Cheong, K.-L. Ultrasonic/microwave-assisted extraction, simulated digestion, and fermentation in vitro by human intestinal flora of polysaccharides from Porphyra haitanensis. Int. J. Biol. Macromol. 2020, 152, 748–756.

134. Kulshreshtha, G.; Rathgeber, B.; Stratton, G.; Thomas, N.; Evans, F.; Critchley, A.; Hafting, J.; Prithiviraj, B. Feed supplementation with red seaweeds, Chondrus crispus and Sarcodiotheca gaudichaudii, affects performance, egg quality, and gut microbiota of layer hens. Poult. Sci. 2014, 93, 2991–3001.

135. Liu, S.; Kandasamy, S.; Zhang, J.; Kirby, C.W.; Karakach, T.; Hafting, J.; Critchley, A.T.; Evans, F.; Prithiviraj, B. Prebiotic effects of diet supplemented with the cultivated red seaweed Chondrus

has advantages over the Ussing-chamber model in that it is less labour intensive and has significantly higher throughput [310].

The advantage of *ex vivo* organ models, in general, over single cell lines is that they are a multi-cell system and therefore more representative of intestinal epithelial behaviour in terms of food absorption [311][312]. Compared to *in vivo* studies, *ex vivo* organ models remove the need for human participants. Limitations of *ex vivo* organ models include the lack of inclusion of gut microbial influence and time constraints. The epithelial intestinal tissue must be excised from the animal post-mortem, and the viability of the intestinal tissues once the experiment begins is only ~150 min and, therefore, it is unsuitable for any bioavailability studies that require more time [313]. The intestinal segment model has the added disadvantage of no distinction between the apical and basolateral side of the epithelium in the way that the mounted Ussing model does, as the segments are completely submerged in the same solution on both sides [314].

2.7.3. In Vitro Fermentation Models

In vitro fermentation models allow the impact of gut microbial populations on food bioaccessibility and bioactivity to be studied, without using *in vivo* human or animal methods. Batch or dynamic fermentation models can be used [315]. Batch fermentation models entail the use of a sealed vessel under anaerobic conditions containing the food sample or extract of interest in sterile media to which is added either a pure, or mixed, bacterial culture or faecal slurry, fermented for ~2 to 24 h [316]. The advantage of batch models is that they are simple to set up and inexpensive, however, since it is a static sealed model, fermentation products such as SCFA can accumulate, and there is a finite amount of substrate available for the bacteria, all of which can affect the fermentation environment [316]. Dynamic multistage models can be used to overcome this issue. In 1988, Gibson et al. [317] first described a three-stage continuous culture system with a mixed human faecal inocula fermented over 120 days that represented the environment of the proximal, transverse and distal colon. Since the 1980s, more sophisticated, computerised dynamic models have been developed including the TIM-2 (previously discussed), The Simulator of the Human Intestinal Microbial Ecosystem (SHIME®) and SIMulator of the GastroIntestinal tract (SIMGI).

The SHIME model is a 5-step multi-chamber bioreactor developed by Molly et al. [318] in 1993 that simulates the entire digestive tract from stomach to colon. The SHIME system involves allowing the microbial inoculum acclimate for 14 to 20 days to produce an environment that is representative of the *in vivo* colon in terms of bacterial populations and SCFA production [319]. Two advanced models have been developed from the original SHIME system—TWIN-SHIME and M-SHIME. Possemiers et al. [320] devised the first TWIN-SHIME model, which involves running two parallel SHIME reactors, making it possible to assess the impact of different diets or antibiotics on the same gut microbiota, as well as the metabolism and bioaccessibility of nutrients, and the pre- and probiotic effect of selected foods or microorganisms. Van den Abbeele et al. [321] incorporated mucin-covered microcosms in the M-SHIME model to create a more realistic microbial community of mucosal microbes such as *Lactobacillus mucosae* and *Pediococcus acidilactici* that are normally present on the gut epithelium. The SHIME model was used by Marzorati et al. [322] to investigate the potential of fucoidan to modulate a gut bacterial community and by Fu et

crispus or with fructo-oligo-saccharide on host immunity, colonic microbiota and gut microbial metabolites. BMC Complement. Altern. Med. 2015, 15, 279.

136. Balasubramanian, B.; Shanmugam, S.; Park, S.; Recharla, N.; Koo, J.S.; Andretta, I.; Kim, I.H. Supplemental impact of marine red seaweed (Halymenia palmata) on the growth performance, total tract nutrient digestibility, blood profiles, intestine histomorphology, meat quality, fecal gas emission, and microbial counts in broilers. Animals 2021, 11, 1244.

137. Lahaye, M. NMR spectroscopic characterisation of oligosaccharides from two Ulva rigida ulvan samples (Ulvales, Chlorophyta) degraded by a lyase. Carbohydr. Res. 1998, 314, 1–12.

138. Michel, G.; Czjzek, M. Chapter 16-Polysaccharide-degrading enzymes from marine bacteria. In Marine Enzymes for Biocatalysis: Sources, Biocatalytic Characteristics and Bioprocesses of Marine Enzymes; Trincone, A., Ed.; Woodhead Publishing: Cambridge, UK, 2013; pp. 429–464.

139. Kidgell, J.T.; Magnusson, M.; de Nys, R.; Glasson, C.R.K. Ulvan: A systematic review of extraction, composition and function. Algal Res. 2019, 39, 101422.

140. Adrien, A.; Bonnet, A.; Dufour, D.; Baudouin, S.; Maugard, T.; Bridiau, N. Anticoagulant activity of sulfated ulvan isolated from the green macroalga Ulva rigida. Mar. Drugs 2019, 17, 291.

141. Klongklaew, N.; Praiboon, J.; Tamtin, M.; Srisapoome, P. Antibacterial and antiviral activities of local Thai green macroalgae crude extracts in pacific white shrimp (Litopenaeus vannamei). Mar. Drugs 2020, 18, 140.

142. Chi, Y.; Zhang, M.; Wang, X.; Fu, X.; Guan, H.; Wang, P. Ulvan lyase assisted structural characterization of ulvan from Ulva pertusa and its antiviral activity against vesicular stomatitis virus. Int. J. Biol. Macromol. 2020, 157, 75–82.

143. Berri, M.; Olivier, M.; Holbert, S.; Dupont, J.; Demais, H.; Le Goff, M.; Collen, P.N. Ulvan from Ulva armoricana (Chlorophyta) activates the PI3K/Akt signalling pathway via TLR4 to induce intestinal cytokine production. Algal Res. 2017, 28, 39–47.

144. Cañedo-Castro, B.; Piñón-Gimate, A.; Carrillo, S.; Ramos, D.; Casas-Valdez, M. Prebiotic effect of Ulva rigida meal on the intestinal integrity and serum cholesterol and triglyceride content in broilers. J. Appl. Phycol. 2019, 31, 3265–3273.

145. Shalaby, M.; Amin, H. Potential using of ulvan polysaccharides from Ulva lactuca as a prebiotic in synbiotic yogurt production. J. Probiot. Health 2019, 7, 1–9.

146. Kong, Q.; Dong, S.; Gao, J.; Jiang, C. In vitro fermentation of sulfated polysaccharides from E. prolifera and L. japonica by human fecal microbiota. Int. J. Biol. Macromol. 2016, 91, 867–871.

147. Strain, C.R.; Collins, K.C.; Naughton, V.; McSorley, E.M.; Stanton, C.; Smyth, T.J.; Soler-Vila, A.; Rea, M.C.; Ross, P.R.; Cherry, P.; et al. Effects of a polysaccharide-rich extract derived from Irish

al. [323] used Laminaria digitata on the composition and metabolic activity of the human gut microbiota using an in vitro colonic model. Eur. J. Nutr. 2020, 59, 309–325.

148. Charoensiddhi, S.; Conlon, M.A.; Vuaran, M.S.; Franco, C.M.M.; Zhang, W. Impact of extraction processes on prebiotic potential of the brown seaweed Ecklonia radiata by in vitro human gut bacteria fermentation. J. Funct. Foods 2016, 24, 221–230.

149. Charoensiddhi, S.; Conlon, M.A.; Vuaran, M.S.; Franco, C.M.M.; Zhang, W. Gut health benefits of the brown seaweed Ecklonia radiata and its polysaccharides demonstrated in vitro using a simulated human colonic model. J. Funct. Foods 2017, 37, 676–684.

150. Wang, Y.; Chen, G.; Peng, Y.; Rui, Y.; Zeng, X.; Ye, H. Simulated digestion and fermentation in vitro with human gut microbiota of polysaccharides from Coralline pilulifera. LWT 2019, 100, 167–174.

151. Cui, M.; Zhou, R.; Wang, Y.; Zhang, M.; Liu, K.; Ma, C. Beneficial effects of sulfated polysaccharides from the red seaweed Gelidium pacificum Okamura on mice with antibiotic-associated diarrhea. Food Funct. 2020, 11, 4625–4637.

152. Sun, L.; Warren, F.J.; Gidley, M.J. Natural products for glycaemic control: Polyphenols as inhibitors of alpha-amylase. Trends Food Sci. Technol. 2019, 91, 262–273.

153. Martinez, A.M.; Michalak, I. Evaluation of phenolic compounds from Mediterranean fucoid algae and seagrasses: An overview on the genus Cystoseira sensu lato and Posidonia oceanica (L.) Delile. J. Mar. Sci. Eng. 2020, 8, 19.

154. Holdt, S.L.; Kraan, S. Bioactive compounds in seaweed: Functional food applications and legislation. J. Appl. Phycol. 2011, 23, 543–598.

155. Poole, J.; Dion, A.; Rainville, L.C.; Barnabé, S. Bioextracting polyphenols from the brown seaweed Ascophyllum nodosum from Québec's north shore coastline. Ind. Biotechnol. 2019, 15, 212–218.

156. Wekre, M.E.; Kåsin, K.; Underhaug, J.; Holmelid, B.; Jordheim, M. Quantification of polyphenols in seaweeds: A case study of Ulva intestinalis. Antioxidants 2019, 8, 612.

157. Quideau, S.; Deffieux, D.; Douat-Casassus, C.; Pouységu, L. Plant polyphenols: Chemical properties, biological activities, and synthesis. Angew. Chem. Int. Ed. 2011, 50, 586–621.

158. Freile-Pelegrín, Y.; Robledo, D. Chapter 6-Bioactive phenolic compounds from algae. In Bioactive Compounds from Marine Foods: Plant and Animal Sources; Hernández-Ledesma, B., Herrero, M., Eds.; Wiley-Blackwell: Chichester, UK, 2014; pp. 113–129.

159. Murray, M.; Dordevic, A.L.; Cox, K.; Scholey, A.; Ryan, L.; Bonham, M.P. Twelve weeks' treatment with a polyphenol-rich seaweed extract increased HDL cholesterol with no change in other

2.7.4. In Vivo Bioavailability Methods

The most accurate method for measuring the bioavailability of a food component is *in vivo* evaluation [335]. *In vitro* and *ex vivo* methods provide very useful data on bioaccessibility, and to a certain extent, bioavailability if cell models are used but they can never fully express the digestive fate of a food component in a living person or animal. This is primarily due to the complex metabolism that occurs during absorption, where food metabolites reaching the blood system may be different from the original compounds [336]. Bioavailability involves the phases of liberation, absorption, metabolism, tissue distribution, bioactivity, and elimination [257]. Balance studies can be used to measure the oral bioavailability of a nutrient by the amount that is eliminated. This entails the collection of all urine and stools after giving a known amount of the nutrient to test subjects over several days or weeks [337]. Balance studies provide accuracy, but are laborious and more suited to laboratory animal models than human subjects [338]. Tissue distribution studies also provide bioavailability data on the extent of absorption, but are almost

exclusively conducted on animals due to the invasive nature of the procedure [339][340]. In human *in vivo* studies, the oral bioavailability of a bioactive food component is most commonly measured by analysis of its metabolites in blood plasma and/or urine after a single dose, or controlled long-term consumption [336][341]. These are the methods used in the seaweed bioavailability studies discussed in the following sections.

Although *in vivo* studies are considered the gold standard for assessing the oral bioavailability of food components [263], some disadvantages exist compared to *in vitro* and *ex vivo* models, including ethical approval for *in vivo* studies in animals (which is difficult due to the potential harm that may be caused to animals) or human participants, and in many cases, the necessary sacrifice of animal subjects [43]. *In vivo* studies are generally more expensive and time-consuming than other methods [342] and are not suitable for high-throughput screening of bioavailability [343]. It is more difficult to control all variables *in vivo* because of naturally occurring differences in living organisms, which can affect the reliability of results [344]. *In vivo* trials involving small cohorts may not be reflective of the bioavailability of a nutrient in the wider population [345].

However, these limitations are sufficiently outweighed by the advantages *in vivo* studies offer. *In vivo* studies consider the effect of digestion, first pass metabolism, the gut microbiota, and biotransformation of an orally consumed nutrient [43][257]. In addition, *in vivo* studies show the impact of the nutrient on the body as a whole, rather than in one localised area or on one particular biological process [342]. Data from *in vivo* studies is more clinically relevant and any side-effects induced by the consumed sample can be observed [262][346]. Although gaps exist in *in vitro* methods of measuring digestion and bioavailability compared to animal models, *in vitro* studies still provide very relevant and useful data regarding bioaccessibility. **Table 5** summarises the advantages and limitations of each gastrointestinal digestion model system.

Table 5. Advantages and limitations of gastrointestinal (GI) digestion model systems.

In Vitro Bioaccessibility Methods	Advantages	Limitations
Solubility and Dialysability	• Simple and inexpensive to conduct with enzymes and dialysis filters that chemically mimic oral, gastric and small intestinal digestion • Inexpensive • No human or animal subjects required	• Does not represent peristaltic movements, secretions, or gastric emptying of the GI tract • No gut microbial component
Static GI models	• Simple to conduct in single bioreactor or flask with stirring and pH adjustments	• Broad variance in results due to reagent diversity, particularly digestive enzymes which differ in

biomarkers of chronic disease risk in overweight adults: A placebo-controlled randomised trial. J. Nutr. Biochem. 2021, 108777.

160. Haskell-Ramsay, C.F.; Jackson, P.A.; Dodd, F.L.; Forster, J.S.; Bérubé, J.; Levinton, C.; Kennedy, D.O. Acute post-prandial cognitive effects of brown seaweed extract in humans. Nutrients 2018, 10, 85.

161. Hata, Y.; Nakajima, K.; Uchida, J.-I.; Hidaka, H.; Nakano, T. Clinical effects of brown seaweed, Undaria pinnatifida (wakame), on blood pressure in hypertensive subjects. J. Clin. Biochem. Nutr. 2001, 30, 43–53.

162. Derosa, G.; Pascuzzo, M.D.; D'Angelo, A.; Maffioli, P. Ascophyllum nodosum, Fucus vesiculosus and chromium picolinate nutraceutical composition can help to treat type 2 diabetic patients. Diabetes. Metab. Syndr. Obes. 2019, 12, 1861–1865.

163. Murray, M.; Dordevic, A.L.; Ryan, L.; Bonham, M.P. A single-dose of a polyphenol-rich Fucus vesiculosus extract is insufficient to blunt the elevated postprandial blood glucose responses exhibited by healthy adults in the evening: A randomised crossover trial. Antioxidants 2019, 8, 49.

164. Cardona, F.; Andrés-Lacueva, C.; Tulipani, S.; Tinahones, F.J.; Queipo-Ortuño, M.I. Benefits of polyphenols on gut microbiota and implications in human health. J. Nutr. Biochem. 2013, 24, 1415–1422.

165. Tomás-Barberán, F.A.; Selma, M.V.; Espín, J.C. Interactions of gut microbiota with dietary polyphenols and consequences to human health. Curr. Opin. Clin. Nutr. Metab. Care 2016, 19, 471–476.

166. Kumar Singh, A.; Cabral, C.; Kumar, R.; Ganguly, R.; Kumar Rana, H.; Gupta, A.; Rosaria Lauro, M.; Carbone, C.; Reis, F.; Pandey, A.K. Beneficial effects of dietary polyphenols on gut microbiota

seaweeds in Japan. J. Tokyo Univ. Fish. 2003, 89, 1–6. Available online: (accessed on 3 July

In Vitro Bioaccessibility Methods	Advantages	Limitations
	• Inexpensive • No human or animal subjects required	activity dependent on their source (human, porcine, rabbit, bacterial, or fungal) • Continuous mechanical agitation is not representative of complex peristaltic movements, secretions, or gastric emptying of the GI tract • No gut microbial component
INFOGEST static *in vitro* model	• Addresses worldwide lack of cohesion in simulated digestive methods • Standardised static method suitable for food based on physiologically relevant conditions which can be applied for various endpoints • Pepsin determined to be the factor causing most variation—activity determination may be improved by pH stabilisation • Inexpensive • No human or animal subjects required	• Continuous mechanical agitation is not representative of complex peristaltic movements, secretions, or gastric emptying of the GI tract • No gut microbial component
Dynamic GI models	• Holistic *in vitro* gastrointestinal model incorporating the large and small intestine • More representative of human GI digestion as changing physicochemical conditions and peristaltic forces are simulated in functionally distinct zones • Human faecal inoculum included to study the effect of colonic fermentation on the	• More costly and lower throughput than static models • Lack of *in vivo* factors such as first pass effect, renal clearance, and metabolisation by intestinal epithelial cells.

and Leathesia nana extract in vivo. Chin. J. Oceanol. Limnol. 2009, 27, 277–282.

184. Shi, D.; Li, X.; Li, J.; Guo, S.; Su, H.; Fan, X. Antithrombotic effects of bromophenol, an alga-derived thrombin inhibitor. Chin. J. Oceanol. Limnol. 2010, 28, 96–98.

In Vitro Bioaccessibility Methods	Advantages	Limitations
	food sample and nutrient absorption • Samples can be taken at any stage of the digestive process without pausing the experiment • Bioaccessibility results of dynamic models have been shown to correlate with bioavailability of the same nutrient *in vivo* • No human or animal subjects required	
Cell models	• Representative of intestinal epithelial cells • Parallels human *in vivo* absorption studies • May be used to mimic the ability of food components to be actively or passively transported and assimilated across the intestinal epithelium • Human cell lines can be used as well as animal cells • Mucus-producing cell lines can be co-cultured to more closely resemble *in vivo* conditions	• Time-consuming to culture cell lines • Costly • First pass effect, renal clearance, interaction of the food sample with other nutrients and anti-nutrients, and different absorptive capacities at each stage of the gastrointestinal tract are not represented
***Ex vivo* bioavailability methods**	• Multi-cell systems are more representative of intestinal epithelial behaviour in terms of food absorption than single cell lines • Animal organ or tissue models can measure the oral bioavailability of bioactive food components	• Extensive preparation • Lack of inclusion of gut microbial influence • Low throughput (mounted tissue models, such as Ussing chambers)

in phlorotannin levels in an assemblage of brown algae. Bot. Mar. 2004, 47, 410–416.

In Vitro Bioaccessibility Methods	Advantages	Limitations
	• Mimics arterial blood haemoglobin delivery by maintaining oxygen and carbon dioxide levels • Precise measurement of electrical and transport parameters of intact epithelium • Any type of intestinal epithelium from duodenum to colon can be studied, as well as other epithelia, such as the placental barrier • No human subjects required	• Intestinal segment models have greater throughput, but no distinction between apical and basolateral side of the epithelium as tissue segments are fully submerged • Short viability–epithelial intestinal tissue must be excised from animal within ~5 min of sacrifice • Viability of intestinal tissues once the experiment begins is only ~150 min and not suitable for many oral bioavailability studies that require more time • Limited range of measurements that do not fully describe the complex physiological system of the intestinal mucosa
***In vitro* fermentation models**	• Static batch or dynamic fermentation models can be used • Batch models are simple to set up and inexpensive • Evaluates the impact of gut microbial populations on food bioaccessibility and bioactivity without using invasive human or animal methods • Dynamic multistage models overcome the issue of fermentation product build-up in static batch models. pH and nutrient availability within each chamber are controlled throughout fermentation	• Dynamic multistage models are costly and complex to set-up • In static sealed batch models, fermentation products such as SCFA and p-cresol can accumulate and there is a finite amount of substrate available for the bacteria • Lack of realistic peristalsis; expensive set-up costs; and absence of a dialysis component and mucosal cells (in the original SHIME model)

208. Zhao, C.; Yang, C.; Chen, M.; Lv, X.; Liu, B.; Yi, L.; Cornara, L.; Wei, M.-C.; Yang, Y.-C.; Tundis, R.; et al. Regulatory efficacy of brown seaweed Lessonia nigrescens extract on the gene

In Vitro Bioaccessibility Methods	Advantages	Limitations
	• Computerised dynamic models such as TIM-2, SHIME and SIMGI create an anaerobic environment representative of the upper and lower digestive tracts rather than the colon alone in terms of bacterial populations and SCFA production • Long-term stability of the microbiome—can be assessed as it adapts • SHIME has option to set parameters found in diverse groups—humans, animals, diseased, healthy, elderly, or infants, and compare alternate treatments in parallel • Possible to create a luminal or a mucosal microbiome • Easier to obtain ethical approval compared to *in vivo* studies	• Lack of realistic peristalsis in SHIME model and absence of a dialysis component and mucosal cells (in the original model) • Lack of intestinal epithelial and immune cells in some systems. No feed-back mechanisms • Use of parameters such as pH, redox potential, and transit time based on healthy individuals may not be representative of many groups
***In vivo* bioavailability methods**	• Considered the gold standard and most accurate method for measuring bioavailability – analysis of metabolites in blood plasma and/or urine after a single dose, or controlled long-term consumption • Reflects complete effect of digestion, first pass metabolism, Phase I/II biotransformation, host microbiota and fermentation on an orally consumed nutrient • Balance studies collecting urine and stools to measure oral bioavailability are accurate	• Balance studies are laborious and more suited to laboratory animal models than human subjects • Tissue distribution studies almost exclusively conducted on animals due to invasive nature • Difficult to obtain ethical approval due to potential harm to animal or human participants and sacrifice of animals • Usually more expensive and time-consuming than other methods

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172, 400–406.

219. Fitzgerald, C.; Gallagher, E.; O’Connor, P.; Prieto, J.; Mora-Soler, L.; Grealy, M.; Hayes, M. Development of a seaweed derived platelet activating factor acetylhydrolase (PAF-AH) inhibitory

In Vitro Bioaccessibility Methods	Advantages	Limitations
	• Tissue distribution studies provide bioavailability data on the extent of absorption • Data from *in vivo* studies is more clinically relevant and any side-effects induced by the consumed sample can be observed	• Not suitable for high-throughput screening of bioavailability • More difficult to control all variables due to naturally occurring differences in living organisms • *In vivo* trials involving small cohorts may not be reflective of the bioavailability of a nutrient in the wider population

A number of recent studies have evaluated the bioaccessibility of seaweed-derived polysaccharides, polyphenols, and peptides after ingestion in human and animal subjects and are discussed below.

2.7.5. Bioaccessibility of Seaweed Polysaccharides

Gueven et al. [347] showed that a single dose of orally ingested fucoidan is sufficient to affect the expression of genes related to immunity, inflammation, cancer, and neurological function. A placebo-controlled double-blind study was performed in nine healthy, male volunteers (age 25–65 years-old). Fucoidan (85.1% pure, MW 47.7 kDa) was water-extracted from *U. pinnatifida* harvested in Tasmania. A capsule containing 1 g seaweed extract (851 mg fucoidan) or a cellulose placebo was administered. Blood was taken immediately before and 24 h after ingestion. 754 micro RNA (miRNA) strands were isolated and analysed as biomarkers of physiological function. Fucoidan ingestion was found to affect 35 miRNAs. Fifteen were upregulated and 38 downregulated. Only one upregulated and five downregulated miRNAs were common to both the placebo and fucoidan groups. The pathways and processes affected by the identified miRNAs are associated with cell surface receptor signalling, the enhancement of brain-derived neurotrophic factor, epidermal growth factor receptor, insulin receptors, and the associated MAPK downstream signalling.

Ikeda-Ohtsubo et al. [348] evaluated the *in vivo* modulatory effects of fucoidan on the gut microbiota in an animal model. Fucoidan (>95% pure, MW 43.8 kDa) was extracted from *Cladosiphon okamuranus* (Okinawa mozuku) harvested in Japan. Adult zebrafish had their food supplemented (1:1) with fucoidan for 3 weeks. The presence of pro- and anti-inflammatory cytokines was determined by quantitative (q)PCR. Then, 16S rRNA sequencing was used to analyse changes in the microbiota. There was a significant decrease in expression levels of the pro-inflammatory cytokine IL-1β in the fucoidan-fed zebrafish compared to the control. In terms of beneficial changes to

223. Venkatraman, K.L.; Syed, A.A.; Indumathi, P.; Mehta, A. VITPOR AI, a coagulation factor XIIa inhibitor from Porphyra yezoensis: In vivo mode of action and assessment of platelet function analysis. Protein Pept. Lett. 2020, 27, 243–250.
224. McLaughlin, C.M.; Sharkey, S.J.; Harnedy-Rothwell, P.; Parthsarathy, V.; Allsopp, P.J.; McSorley, E.M.; FitzGerald, R.J.; O'Harte, F.P.M. Twice daily oral administration of Palmaria palmata protein hydrolysate reduces food intake in streptozotocin induced diabetic mice, improving glycaemic control and lipid profiles. J. Funct. Foods 2020, 73, 104101.
225. Dave, L.A.; Hayes, M.; Mora, L.; Rutherfurd, S.M.; Montoya, C.A.; Moughan, P.J. Bioactive peptides originating from gastrointestinal endogenous proteins in the growing pig: In vivo identification. Curr. Pharm. Des. 2021, 27, 1382–1395.
226. Fitzgerald, C.; Aluko, R.E.; Hossain, M.; Raj, D.K.; Hayes, M. Potential of a renin inhibitory peptide from the red seaweed Palmaria palmata as a functional food ingredient following confirmation and characterization of a hypotensive effect in spontaneously hypertensive rats. J. Agric. Food. Chem. 2014, 62, 8352–8356.
227. Allsopp, P.; Crowe, W.; Bahar, B.; Harnedy, P.A.; Brown, E.S.; Taylor, S.S.; Smyth, T.J.; Soler-Vila, A.; Magee, P.J.; Gill, C.I.R.; et al. The effect of consuming Palmaria palmata-enriched bread on inflammatory markers, antioxidant status, lipid profile and thyroid function in a randomised placebo-controlled intervention trial in healthy adults. Eur. J. Nutr. 2016, 55, 1951–1962.
228. Furuta, T.; Miyabe, Y.; Yasui, H.; Kinoshita, Y.; Kishimura, H. Angiotensin I converting enzyme inhibitory peptides derived from phycobiliproteins of dulse Palmaria palmata. Mar. Drugs 2016, 14, 32.
229. Sato, M.; Hosokawa, T.; Yamaguchi, T.; Nakano, T.; Muramoto, K.; Kahara, T.; Funayama, K.; Kobayashi, A.; Nakano, T. Angiotensin I-converting enzyme inhibitory peptides derived from

the Wakame (Undaria pinnatifida) and their antihypertensive effect in spontaneously hypertensive rats. J. Agric. Food. Chem. 2002, 50, 6245–6252.

230. Suetsuna, K.; Nakano, T. Identification of an antihypertensive peptide from peptic digest of wakame (Undaria pinnatifida). J. Nutr. Biochem. 2000, 11, 450–454.

231. Harnedy, P.A.; O'Keeffe, M.B.; FitzGerald, R.J. Fractionation and identification of antioxidant peptides from an enzymatically hydrolysed Palmaria palmata protein isolate. Food Res. Int. 2017, 100, 416–422.

232. Amaretti, A.; Gozzoli, C.; Simone, M.; Raimondi, S.; Righini, L.; Pérez-Brocal, V.; García-López, R.; Moya, A.; Rossi, M. Profiling of protein degraders in cultures of human gut microbiota. Front. Microbiol. 2019, 10.

233. Neis, E.P.J.G.; Dejong, C.H.C.; Rensen, S.S. The role of microbial amino acid metabolism in host metabolism. Nutrients 2015, 7, 2930–2946.

234. Diether, N.E.; Willing, B.P. Microbial fermentation of dietary protein: An important factor in diet-microbe-host interaction. Microorganisms 2019, 7, 19.

235. Fan, P.; Li, L.; Rezaei, A.; Eslamfam, S.; Che, D.; Ma, X. Metabolites of dietary protein and peptides by intestinal microbes and their impacts on gut. Curr. Protein Pept. Sci. 2015, 16, 646–654.

236. Chiprian, K.; Allen-Vercoe, E. Macronutrient metabolism by the human gut microbiome: Major fermentation by-products and their impact on host health. Microbiome 2019, 7, 91.

237. Kim, J.; Hetzel, M.; Boiangiu, C.D.; Buckel, W. Dehydration of (R)-2-hydroxyacyl-CoA to enoyl-CoA in the fermentation of α-amino acids by anaerobic bacteria. FEMS Microbiol. Rev. 2004, 28, 455–468.

238. Blachier, F.; Mariotti, F.; Huneau, J.F.; Tomé, D. Effects of amino acid-derived luminal metabolites on the colonic epithelium and physiopathological consequences. Amino Acids 2007, 33, 547–562.

239. Feng, W.; Ao, H.; Peng, C. Gut microbiota, short-chain fatty acids, and herbal medicines. Front. Pharmacol. 2018, 9, 1354.

240. Portune, K.J.; Beaumont, M.; Davila, A.-M.; Tomé, D.; Blachier, F.; Sanz, Y. Gut microbiota role in dietary protein metabolism and health-related outcomes: The two sides of the coin. Trends Food Sci. Technol. 2016, 57 Pt B, 213–232.

241. Yao, C.K.; Muir, J.G.; Gibson, P.R. Review article: Insights into colonic protein fermentation, its modulation and potential health implications. Aliment. Pharmacol. Ther. 2016, 43, 181–196.

242. Korpela, K. Diet, microbiota, and metabolic health: Trade-off between saccharolytic and proteolytic fermentation. Annu. Rev. Food Sci. Technol. 2018, 9, 65–84.

2.7.6. Bioaccessibilty of Seaweed Polyphenols

Human clinical studies on the bioaccessibility of seaweed polyphenols are limited to brown species, and phlorotannins in particular. **Table 6** summarises the polyphenol used in each study and the impact of digestion on their bioaccessibilty.

Bacteria of the Enterobacteriaceae (genus *Escherichia*) and Comamonadaceae (genus unclassified) became dominant at the expense of *E. coli*-related Enterobacteriaceae. Intestinal Enterobacteriaceae have been reported to have pro-inflammatory effects [349]. The reduction in Enterobacteriaceae after fucoidan supplementation may have been responsible for the downregulation of the pro-inflammatory cytokine IL-1β. This illustrates the potential modulatory role of seaweed polysaccharides in the diet–microbiota–host interplay.

Fucoidan extracted from Japanese Okinawa mozuku was also shown to be bioaccessible to rats fed 2% fucoidan-supplemented food for 8 weeks [350]. Immunohistochemical staining revealed that fucoidan had been absorbed across the intestinal epithelium and taken up by intestinal macrophages and hepatic Kupffer cells. The same research group went on to investigate factors concerning the absorption of the Okinawa mozuku-derived fucoidan in a cross-sectional human study (n = 396) by Kadena et al. [351]. Okinawa mozuku is a species of brown seaweed endemic to a small group of islands south of the Japanese mainland called the Okinawa prefecture or region. Of the study population, 68% were native to Okinawa, while 32% were from other regions of Japan. Participants (227 male, 169 female, age 20 to >70 years old) were administered a drink of 3.75 g mozuku extract (containing 3 g pure fucoidan) in 200 mL purified water. The fucoidan had an average MW of 73.4 kDa and a composition of 51.2% L-fucose, 14.4% uronic acid, and 18.8% sulphate. Participants refrained from consuming seaweed or fucoidan supplements the day before and throughout the day of trial. Urine samples were collected before administration and 3, 6, and 9 h after. The presence of fucoidan was measured using a purpose-designed ELISA [352]. The assay antibody was specific to fucoidan and did not react with other sulphated polysaccharides. Fucoidan concentration was expressed as a corrected urinary creatinine value (μg/gCr) as fucoidan was calculated to be equivalent to one eighth of urinary excreted creatinine.

The results showed that intestinal absorption of Okinawa mozuku-derived fucoidan occurred in 97% of study participants (385 of 396). There was a highly significant difference ($p < 0.01$) in fucoidan absorption in native Okinawa participants compared to those from other regions. Eight of the 11 participants who did not excrete fucoidan lived Tokyo, Miyagi, Ehime, After 9 h, the total mean urinary fucoidan content of native Okinawa participants (332.3 μg/gCr) was 38.4% greater ($p < 0.01$) than those from other regions (240.1 μg/gCr). Of the 5.7–8.7% that excreted the highest levels fucoidan (>1200 μg/gCr) were native to Okinawa. By age bracket, participants in their 40′s had the greatest mean urinary fucoidan value (392.8 μg/gCr). The authors hypothesised that the gut bacteria of native Okinawa participants may have acquired genes from marine bacteria that produce the digestive enzyme fucoidanase. This horizontal transfer of genes from ocean-dwelling bacteria that normally colonise and feed on seaweed has previously been reported in populations that have consumed seaweed for thousands of years [353][354][355][356].

Table 3. Bioaccessibility of seaweed polyphenols.

Seaweed	Polyphenol	Extraction Method	Study Type	Observed Effects	Ref.
A. nodosum *, ***	Phlorotannins	Ethanolic crude phlorotannin extract (CE) and high-molecular-weight (HMW) fraction (>10 kDa) by tangential flow ultrafiltration. Combined as CE (58%) and HMW (42%)	(i) *In vitro* gastrointestinal enzymatic digestion, colonic fermentation, and dialysis to simulate absorption into the bloodstream. (ii) Cross-sectional human clinical trial (12 male, 12 female, healthy 18–65 years-old) (one capsule 101.89 mg phlorotannins). Blood and urine collected (0 to 24 h).	Phlorotannin metabolites detected in 15 of 24 participants after 24 h (total phlorotannins ranged from 0.011–7.76 µg/mL in blood plasma and from 0.15–33.52 µg/mL in urine).	[206]
A. nodosum ***	Phlorotannins	Ethanol CE extract and HMW fraction (>10 kDa) by tangential flow ultrafiltration. Combined as CE (57%) and HMW (43%)	24 week crossover study (8 weeks, 100 mg phlorotannin/d, or placebo capsule) (39 men, 41 women, mean BMI 30.2, mean age 42.7 years-old), 8 weeks washout phase, then repeat 8 weeks intervention or placebo treatment. Plasma and urine collected before/after each phase (0, 8, 16 and 24 weeks).	Polyphenol metabolites (0.5–11.8 mg/day total polyphenols) detected in 36 of 78 participants.	[358]

* *in vitro* studies, *** = human clinical intervention studies.

These studies, along with [illegible] have a commonality that oral bioaccessibilty of polyphenols in some individuals is poor. There are a number of reasons for this, such as host-related factors. These can be systemic factors including age, gender, genetics, and existing health disorders, or intestinal factors such as gastric enzyme activity, intestinal transit time, and gut microflora composition intestinal factors such as gastric enzyme activity, intestinal transit time, and gut microflora composition [359]. The food matrix in which polyphenols are consumed and interactions with macronutrients impact bioaccessibility. In the digestive tract, the amine group of proteins can form irreversible covalent bonds with the carboxylic group of polyphenols [360], which interferes with the ability of digestive enzymes to catabolise them. Since enzymes are proteins, polyphenols also interact with their amine groups, further inhibiting digestion. [361][362].

The capacity for polyphenols to bind with proteins increases with their molecular weight. Some seaweed polyphenols, such as phlorotannins, have a MW of up to 100 kDa [363], making them suitable candidates for multiple protein-polyphenol interactions.

Lipids have been shown to enhance polyphenol bioaccessibility. Hydrophobic interactions between lipids and polyphenols have a protective effect and increase the stability of polyphenols during digestion [364]. Complexing

248. Wang, X.; Gibson, G.R.; Costabile, A.; Sailer, M.; Theis, S.; Rastall, R.A. Prebiotic supplementation of in vitro fecal fermentations inhibits proteolysis by gut bacteria, and host diet [illegible] 2019, 85, [illegible]
24[illegible] ne [illegible] athway.
24[illegible] rane [illegible] on [illegible] athol.
24[illegible] nasov, [illegible] cancer.
24[illegible] cts of [illegible] or
24[illegible]
24[illegible] athway [illegible].
25[illegible] 53 in metastasizing canine mammary adenocarcinomas versus normal mammary glands. Res. Vet. Sci. 2009, 87, 91–96.
251. Paunovic, B.; Khomenko, T.; Deng, X.; Xiong, X.; Sandor, Z.; Szabo, S. Overexpression of cyclin-dependent kinase (CDK) inhibitors p21 and p27 is a common mechanism of experimental duodenal ulcer and ulcerative colitis. FASEB J. 2010, 24, 1027.4.
252. Abdelhedi, O.; Nasri, M. Basic and recent advances in marine antihypertensive peptides: Production, structure-activity relationship and bioavailability. Trends Food Sci. Technol. 2019, 88, 543–557.
253. Samarakoon, K.; Jeon, Y.-J. Bio-functionalities of proteins derived from marine algae: A review. Food Res. Int. 2012, 48, 948–960.
254. Vizcaíno, A.J.; Galafat, A.; Sáez, M.I.; Martínez, T.F.; Alarcón, F.J. Partial characterization of protease inhibitors of Ulva ohnoi and their effect on digestive proteases of marine fish. Mar. Drugs 2020, 18, 319.

with phlorotannins can also increase the accumulation of polyphenols in the liver, which acts as a slow-release reservoir that prolongs their residence time in the body [365]. Polysaccharides in the form of dietary fibre can bind with polyphenols. The hydroxyl groups of polyphenols form hydrogen bonds with the oxygen atoms of polysaccharide glycosidic linkages [366] or covalent bonds, such as esters [367]. While this reduces the ability of gastric enzymes to make them bioaccessible in the upper gastrointestinal tract, polyphenols can be released from their non-digestible polysaccharide complex in the colon through the action of gut microbial digestive enzymes [368]. In fact, polysaccharides such as alginate have been used to encapsulate polyphenols, delaying their release until they reach the colon [369].

Despite their low oral bioaccessibility, the biological activity of polyphenols is generally found to be high, leading to a low bioaccessibility/high bioactivity paradox. This is most likely due to the biotransformation of polyphenols in the liver and enterocytes mediated by phase I cytochrome P450 enzymes and phase II conjugation enzymes (uridine 5'-diphospho-glucuronosyltransferase and sulphotransferase) [343]. Phase I and II biotransformation is a detoxification system that modifies compounds that the body perceives as xenobiotics for easier excretion via urine, faeces, and bile [370]. This biotransformation results in conjugated compounds with different polarity, MW, ionic form, and greater intrinsic biological effects than their parent compounds [371][372]. After compounds such as polyphenols are conjugated, they re-enter the gastrointestinal tract in bile via enterohepatic recirculation [373]. Gut bacterial enzymes, particularly β-glucuronidase, can metabolise many of these polyphenol conjugates, further modifying their chemical structure, bioactivity, and bioavailability [341]. This enterohepatic recycling prolongs the presence of polyphenols within the body. Therefore, the limited oral bioaccessibilty of seaweed polyphenols does not determine their ultimate bioactivity. The biotransformation of native polyphenols through the action of digestive enzymes and microbial fermentation produces metabolites with disparate bioaccessibilty and bioactivity.

2.7.7. Bioaccessibility of Seaweed Peptides

There is a dearth of literature on the *in vivo* bioaccessibility of seaweed-derived peptides in human studies; however, some *in vivo* studies have reported the effect of digestion on their bioactivity in animal models or *in vitro*. **Table 7** summarises the peptide used in each study and the impact of digestion on their bioactivity.

Table 7. Bioactivity of seaweed peptides.

Seaweed	Peptide	Extraction Method	Study Type	Statistically Significant Effects Post-Digestion	Ref.
** *U. pinnatifida*	(i) Tyr-His (ii) Lys-Tyr (iii) Phe-Tyr (iv) Ile-Tyr	Hot water	*In vivo* study in spontaneously hypertensive rats. (a) Single oral administration of each dipeptide (50 mg/kg BM) (b) Continuous administration for	(a) All dipeptides decreased ($p < 0.05$) blood pressure after single oral dose: • Tyr-His decreased 50 mm Hg after 3 h • Lys-Tyr decreased 45 mm Hg after 6 h	[374]

255. Mahomoodally, M.F.; Bibi Sadeer, N.; Zengin, G.; Cziáky, Z.; Jekő, J.; Diuzheva, A.; Sinan, K.I.; Palaniveloo, K.; Kim, D.H.; Rengasamy, K.R.R. In vitro enzyme inhibitory properties, secondary metabolite profiles and multivariate analysis of five seaweeds. Mar. Drugs 2020, 18, 198.
256. Pan, S.; Wang, S.; Jing, L.; Yao, D. Purification and characterisation of a novel angiotensin-I converting enzyme (ACE)-inhibitory peptide derived from the enzymatic hydrolysate of Enteromorpha clathrata protein. Food Chem. 2016, 211, 423–430.
257. Rein, M.J.; Renouf, M.; Cruz-Hernandez, C.; Actis-Goretta, L.; Thakkar, S.K.; da Silva Pinto, M. Bioavailability of bioactive food compounds: A challenging journey to bioefficacy. Br. J. Clin. Pharmacol. 2013, 75, 588–602.
258. Nova, P.; Pimenta-Martins, A.; Laranjeira Silva, J.; Silva, A.M.; Gomes, A.M.; Freitas, A.C. Health benefits and bioavailability of marine resources components that contribute to health–what's new? Crit. Rev. Food Sci. Nutr. 2020, 1–13.
259. Thakur, N.; Raigond, P.; Singh, Y.; Mishra, T.; Singh, B.; Lal, M.K.; Dutt, S. Recent updates on bioaccessibility of phytonutrients. Trends Food Sci. Technol. 2020, 97, 366–380.
260. Alegría, A.; Garcia-Llatas, G.; Cilla, A. Chapter 1-Static digestion models: General introduction. In The Impact of Food Bioactives on Health: In Vitro and Ex Vivo Models; Verhoeckx, K., Cotter, P., López-Expósito, I., Kleiveland, C., Lea, T., Mackie, A., Requena, T., Swiatecka, D., Wichers, H., Eds.; Springer: Cham, Switzerland, 2015; pp. 3–12.
261. Plank, D.W. In Vitro Method for Estimating In Vivo Protein Digestibility; General Mills Inc.: Minneapolis, MN, USA, 2017; Available online: (accessed on 19 July 2020).
262. Bohn, T.; Carrière, F.; Day, L.; Deglaire, A.; Egger, L.; Freitas, D.; Golding, M.; Lefeunteun, S.; Macierzanka, A.; Ménard, O.; et al. Correlation between in vitro and in vivo data on food digestion. What can we predict with static in vitro digestion models? Crit. Rev. Food Sci. Nutr. 2017, 58, 2239–2261.
263. Dima, C.; Assadpour, E.; Dima, S.; Jafari, S.M. Bioavailability and bioaccessibility of food bioactive compounds; overview and assessment by in vitro methods. Compr. Rev. Food Sci. Food Saf. 2020, 19, 2863–2884.
26 ... z- ... plant ... -436.
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26 ... ilability methods for calcium, carotenoids, folate, iron, magnesium, polyphenols, zinc, and vitamins B6,

Seaweed	Peptide	Extraction Method	Study Type	Statistically Significant Effects Post-Digestion	Ref.
			7 days (10 mg/day/kg BM)	• Phe-Tyr decreased 46 mm Hg after 3 h • IleTyr decreased Hg 33 mm Hg after 3 h (b) After 7 days continuous oral administration blood pressure was lowered (all $p < 0.05$ compared to pre-adminstraton): • Tyr-His decreased 34 mm Hg • Lys-Tyr decreased 26 mm Hg • Phe-Tyr decreased 34 mm Hg • IleTyr decreased 25 mm Hg Hypotensive effect of all four dipeptides lasted 3–8 weeks after ceasing continuous administration.	
*, ** *U. pinnatifida*	(i) Ile-Trp (ii) Val-Trp (iii) Ile-Tyr (iv) Ala-Trp (v) Leu-Trp (vi) Val-Tyr (vii) Phe-Tyr	Enzymatic (Protease from *Bacillus stearothermophilus*) and HPLC separation to butanol-soluble fractions	(a) *In vitro* ACE-I inhibitory activity digestion stability study with pepsin, trypsin and chymotrypsin. (b) *In vivo* study in spontaneously hypertensive rats. Single oral administration of each dipeptide (1 mg/kg BM).	(a) No loss in ACE-I inhibitory activity post *in vitro* digestion.IC_{50} values: (i) Ile-Trp 1.5 µM (ii) Val-Trp 3.3 µM (iii) Ile-Tyr 6.1 µM (iv) Ala-Trp 18.8 µM (v) Leu-Trp 23.6 µM (vi) Val-Tyr 35.2 µM (vii) Phe-Tyr 42.3 µM (b) *In vivo* antihypertensive effect in spontaneously hypertensive rats (single oral dose, all 1 mg/kg of BW). Blood pressure decreases (pre-administration vs. 9h post): (i) Val-Tyr (228.2 ± 3.4 vs. 206.7 ± 9.5 mmHg) ($p < 0.05$) (ii) Ile-Tyr (205.6 ± 5.2 vs. 184.3 ± 4.5 mmHg) ($p < 0.05$) (iii) Phe-Tyr (208.7 ± 4.4 vs. 193.0 ± 5.1 ($p < 0.01$) (iv) Ile-Trp (213.3 ± 3.4 vs. 199.5	[229]

209.

Seaweed	Peptide	Extraction Method	Study Type	Statistically Significant Effects Post-Digestion	Ref.
				± 5.9) ($p < 0.05$) Captopril control (238.7 ± 6.9 vs. 224.9 ± 4.1 ($p < 0.05$)	

* = *in vitro* studies; ** = *in vivo* animal studies.

Despite the lack of human clinical data on the bioavailability of seaweed peptides, *in vivo* animal trials and *in vitro* simulated gastrointestinal digestion studies provide relevant information on the ability of bioactive peptides, sometimes termed cryptides, to retain their efficacy after transit through the mammalian gut [375][376].

3. Conclusions

The aetiology of many leading global chronic disorders, such as inflammatory diseases, immunodeficiency, metabolic syndrome and cancer has been linked to dysbiosis of the gut. *In vitro*, animal and human studies collated in this review show that food components of seaweed origin have the potential to beneficially modulate the microbiota of the mammalian gut. Seaweed polysaccharides such as fucoidan, laminarin, alginate, ulvan and porphyran have shown particular efficacy as modulators of the gut by acting as prebiotics, which increase gut bacterial numbers and the production of short chain fatty acids. There are, however, many factors that can reduce the bioaccessibility and bioavailability of seaweed components. These include antagonistic or synergistic interactions with other food components; physiochemical digestibility parameters such as solubility, polarity, molecular weight, surrounding food matrix; and the impact of first-pass metabolism. However, the low bioavailability of some seaweed components can be modified by gastric, enterohepatic, and bacterial biotransformation resulting in compounds with enhanced bioactivity. Another factor that affects bioaccessibility and bioavailability is the composition of each individual's gut microbiota, which varies broadly. This may lead to the absence of certain bacterial families required for the metabolism of seaweed components. This can be augmented by introducing bacterial strains capable of digesting them. There is a dearth of data available in the literature on human dietary intervention studies with seaweed polysaccharides, polyphenols and peptides. Although *in vitro* studies and *in vivo* animal trials are an indication of the prebiotic potential of seaweed components, they are not fully representative of how the component will be metabolised in humans. Further randomised controlled clinical trials are required in large human cohorts, with measureable endpoints, to validate any putative health effects observed in animal models, simulated digestion models, or *in vitro*. With the practice of mariculture becoming more widespread globally, seaweeds represent a sustainable source of bioactive compounds with potential to be used as modulators of the gut microbiota.

278. ... t, S. ... 0157:H7

279. Larsson, M.; Minekus, M.; Havenaar, R. Estimation of the bioavailability of iron and phosphorus in cereals using a dynamic in vitro gastrointestinal model. J. Sci. Food Agric. 1997, 74, 99–106.

280. Verwei, M.; Freidig, A.P.; Havenaar, R.; Groten, J.P. Predicted serum folate concentrations based on in vitro studies and kinetic modeling are consistent with measured folate concentrations in humans. J. Nutr. 2006, 136, 3074–3078.

281. Mateo Anson, N.; Havenaar, R.; Bast, A.; Haenen, G.R.M.M. Antioxidant and anti-inflammatory capacity of bioaccessible compounds from wheat fractions after gastrointestinal digestion. J. Cereal Sci. 2010, 51, 110–114.

282. Torres-Escribano, S.; Denis, S.; Blanquet-Diot, S.; Calatayud, M.; Barrios, L.; Vélez, D.; Alric, M.; Montoro, R. Comparison of a static and a dynamic in vitro model to estimate the bioaccessibility of As, Cd, Pb and Hg from food reference materials Fucus sp. (IAEA-140/TM) and Lobster hepatopancreas (TORT-2). Sci. Total Environ. 2011, 409, 604–611.

283. Bellmann, S.; Miyazaki, K.; Chonan, O.; Ishikawa, F.; Havenaar, R. Fucoidan from Cladosiphon okamuranus Tokida added to food has no adverse effect on availability for absorption of divalent minerals in the dynamic multicompartmental model of the upper gastrointestinal tract. Food Digestion 2014, 5, 19–25.

284. Blanquet, S.; Zeijdner, E.; Beyssac, E.; Meunier, J.-P.; Denis, S.; Havenaar, R.; Alric, M. A dynamic artificial gastrointestinal system for studying the behavior of orally administered drug dosage forms under various physiological conditions. Pharm. Res. 2004, 21, 585–591.

285. Wickham, M.; Faulks, R.; Mann, J.; Mandalari, G. The design, operation, and application of a dynamic gastric model. Dissolut. Technol. 2012, 19, 15–22.

286. Vardakou, M.; Mercuri, A.; Barker, S.; Craig, D.; Faulks, R.; Wickham, M. Achieving antral grinding forces in biorelevant in vitro models: Comparing the USP Dissolution Apparatus II and the Dynamic Gastric Model with human in vivo data. AAPS PharmSciTech. 2011, 12, 620–626.

287. Marciani, L.; Gowland, P.A.; Fillery-Travis, A.; Manoj, P.; Wright, J.; Smith, A.; Young, P.; Moore, R.; Spiller, R.C. Assessment of antral grinding of a model solid meal with echo-planar imaging. Am. J. Physiol. Gastrointest. Liver Physiol. 2001, 280, G844–G849.

288. Lea, T. Chapter 9-Epithelial cell models; general introduction. In The Impact of Food Bioactives on Health: In Vitro and Ex Vivo Models; Verhoeckx, K., Cotter, P., López-Expósito, I., Kleiveland, C., Lea, T., Mackie, A., Requena, T., Swiatecka, D., Wichers, H., Eds.; Springer: Cham, Switzerland, 2015; pp. 95–102.

289. Lv, Q.; He, Q.; Wu, Y.; Chen, X.; Ning, Y.; Chen, Y. Investigating the bioaccessibility and bioavailability of cadmium in a cooked rice food matrix by using an 11-day rapid Caco-2/HT-29 co-culture cell model combined with an in vitro digestion model. Biol. Trace Elem. Res. 2019, 190, 336–348.

290. Kuhre, R.E.; Wewer Albrechtsen, N.J.; Deacon, C.F.; Balk-Møller, E.; Rehfeld, J.F.; Reimann, F.; Gribble, F.M.; Holst, J.J. Peptide production and secretion in GLUTag, NCI-H716, and STC-1 cells: A comparison to native L-cells. J. Mol. Endocrinol. 2016, 56, 201–211.

291. Reggi, S.; Giromini, C.; Dell'Anno, M.; Baldi, A.; Rebucci, R.; Rossi, L. In vitro digestion of chestnut and quebracho tannin extracts: Antimicrobial effect, antioxidant capacity and cytomodulatory activity in swine intestinal IPEC-J2 cells. Animals 2020, 10, 195.

292. Sambuy, Y.; De Angelis, I.; Ranaldi, G.; Scarino, M.L.; Stammati, A.; Zucco, F. The Caco-2 cell line as a model of the intestinal barrier: Influence of cell and culture-related factors on Caco-2 cell functional characteristics. Cell Biol. Toxicol. 2005, 21, 1–26.

293. Glahn, R.P.; Wien, E.M.; van Campen, D.R.; Miller, D.D. Caco-2 cell iron uptake from meat and casein digests parallels in vivo studies: Use of a novel in vitro method for rapid estimation of iron bioavailability. J. Nutr. 1996, 126, 332–339.

294. Trigo, J.P.; Engström, N.; Steinhagen, S.; Juul, L.; Harrysson, H.; Toth, G.B.; Pavia, H.; Scheers, N.; Undeland, I. In vitro digestibility and Caco-2 cell bioavailability of sea lettuce (Ulva fenestrata) proteins extracted using pH-shift processing. Food Chem. 2021, 356, 129683.

295. Flores, S.R.L.; Dobbs, J.; Dunn, M.A. Mineral nutrient content and iron bioavailability in common and Hawaiian seaweeds assessed by an in vitro digestion/Caco-2 cell model. J. Food Compos. Anal. 2015, 43, 185–193.

296. Domínguez-González, M.R.; Chiocchetti, G.M.; Herbello-Hermelo, P.; Vélez, D.; Devesa, V.; Bermejo-Barrera, P. Evaluation of iodine bioavailability in seaweed using in vitro methods. J. Agric. Food. Chem. 2017, 65, 8435–8442.

297. Hur, S.J.; Lim, B.O.; Decker, E.A.; McClements, D.J. In vitro human digestion models for food applications. Food Chem. 2011, 125, 1–12.

298. Boisen, S.; Fernández, J.A. Prediction of the total tract digestibility of energy in feedstuffs and pig diets by in vitro analyses. Anim. Feed Sci. Technol. 1997, 68, 277–286.

299. Hayes, M. Food proteins and bioactive peptides: New and novel sources, characterisation strategies and applications. Foods 2018, 7, 38.

300. Popova, A.; Mihaylova, D. Antinutrients in plant-based foods: A review. Open Biotechnol. J. 2019, 13, 68–76.

301. Fabiano, A.; Brilli, E.; Mattii, L.; Testai, L.; Moscato, S.; Citi, V.; Tarantino, G.; Zambito, Y. Ex vivo and in vivo study of Sucrosomial® iron intestinal absorption and bioavailability. Int. J. Mol. Sci. 2018, 19, 2722.

302. Ussing, H.H. The active ion transport through the isolated frog skin in the light of tracer studies. Acta Physiol. Scand. 1949, 17, 1–37.

303. Clarke, L.L. A guide to Ussing chamber studies of mouse intestine. Am. J. Physiol. Gastrointest. Liver Physiol. 2009, 296, G1151–G1166.

304. Awati, A.; Rutherfurd, S.M.; Plugge, W.; Reynolds, G.W.; Marrant, H.; Kies, A.K.; Moughan, P.J. Ussing chamber results for amino acid absorption of protein hydrolysates in porcine jejunum must be corrected for endogenous protein. J. Sci. Food Agric. 2009, 89, 1857–1861.

305. Westerhout, J.; Wortelboer, H.; Verhoeckx, K. Chapter 24-Ussing chamber. In The Impact of Food Bioactives on Health: In Vitro and Ex Vivo Models; Verhoeckx, K., Cotter, P., López-Expósito, I., Kleiveland, C., Lea, T., Mackie, A., Requena, T., Swiatecka, D., Wichers, H., Eds.; Springer: Cham, Switzerland, 2015; pp. 263–273.

306. Luo, Z.; Liu, Y.; Zhao, B.; Tang, M.; Dong, H.; Zhang, L.; Lv, B.; Wei, L. Ex vivo and in situ approaches used to study intestinal absorption. J. Pharmacol. Toxicol. Methods 2013, 68, 208–216.

307. Agar, W.T.; Hird, F.J.R.; Sidhu, G.S. The uptake of amino acids by the intestine. Biochim. Biophys. Acta 1954, 14, 80–84.

308. Hillgren, K.M.; Kato, A.; Borchardt, R.T. In vitro systems for studying intestinal drug absorption. Med. Res. Rev. 1995, 15, 83–109.

309. Nossol, C.; Barta-Böszörményi, A.; Kahlert, S.; Zuschratter, W.; Faber-Zuschratter, H.; Reinhardt, N.; Ponsuksili, S.; Wimmers, K.; Diesing, A.-K.; Rothkötter, H.-J. Comparing two intestinal porcine epithelial cell lines (IPECs): Morphological differentiation, function and metabolism. PLoS ONE 2015, 10, e0132323.

310. Ripken, D.; Hendriks, H. Chapter 23-Porcine ex vivo intestinal segment model. In The Impact of Food Bioactives on Health: In Vitro and Ex Vivo Models; Verhoeckx, K., Cotter, P., López-Expósito, I., Kleiveland, C., Lea, T., Mackie, A., Requena, T., Swiatecka, D., Wichers, H., Eds.; Springer: Cham, Switzerland, 2015; pp. 255–262.

311. Pearce, S.C.; Coia, H.G.; Karl, J.P.; Pantoja-Feliciano, I.G.; Zachos, N.C.; Racicot, K. Intestinal in vitro and ex vivo models to study host-microbiome interactions and acute stressors. Front. Physiol. 2018, 9.

312. Roeselers, G.; Ponomarenko, M.; Lukovac, S.; Wortelboer, H.M. Ex vivo systems to study host–microbiota interactions in the gastrointestinal tract. Best Pract. Res. Clin. Gastroenterol. 2013, 27, 101–113.

313. van de Merbel, A.F.; van der Horst, G.; van der Mark, M.H.; van Uhm, J.I.M.; van Gennep, E.J.; Kloen, P.; Beimers, L.; Pelger, R.C.M.; van der Pluijm, G. An ex vivo tissue culture model for the assessment of individualized drug responses in prostate and bladder cancer. Front. Oncol. 2018, 8.

314. Ripken, D.; van der Wielen, N.; Wortelboer, H.M.; Meijerink, J.; Witkamp, R.F.; Hendriks, H.F.J. Steviol glycoside rebaudioside a induces glucagon-like peptide-1 and peptide yy release in a porcine ex vivo intestinal model. J. Agric. Food. Chem. 2014, 62, 8365–8370.

315. Aura, A.-M.; Maukonen, J. Chapter 25-One compartment fermentation model. In The Impact of Food Bioactives on Health: In Vitro and Ex Vivo Models; Verhoeckx, K., Cotter, P., López-Expósito, I., Kleiveland, C., Lea, T., Mackie, A., Requena, T., Swiatecka, D., Wichers, H., Eds.; Springer: Cham, Switzerland, 2015; pp. 281–292.

316. Ouwehand, A.C.; Tiihonen, K.; Mäkeläinen, H.; Rautonen, N.; Hasselwander, O.; Sworn, G. Chapter 5-Non-starch polysaccharides in the gastrointestinal tract. In Designing Functional Foods; McClements, D.J., Decker, E.A., Eds.; Woodhead Publishing: Cambridge, UK, 2009; pp. 126–147.

317. Gibson, G.R.; Cummings, J.H.; Macfarlane, G.T. Use of a three-stage continuous culture system to study the effect of mucin on dissimilatory sulfate reduction and methanogenesis by mixed populations of human gut bacteria. Appl. Environ. Microbiol. 1988, 54, 2750–2755.

318. Molly, K.; Vande Woestyne, M.; Verstraete, W. Development of a 5-step multi-chamber reactor as a simulation of the human intestinal microbial ecosystem. Appl. Microbiol. Biotechnol. 1993, 39, 254–258.

319. Van den Abbeele, P.; Grootaert, C.; Marzorati, M.; Possemiers, S.; Verstraete, W.; Gérard, P.; Rabot, S.; Bruneau, A.; El Aidy, S.; Derrien, M. Microbial community development in a dynamic gut model is reproducible, colon region specific, and selective for Bacteroidetes and Clostridium cluster IX. Appl. Environ. Microbiol. 2010, 76, 5237–5246.

320. Possemiers, S.; Rabot, S.; Espín, J.C.; Bruneau, A.; Philippe, C.; González-Sarrías, A.; Heyerick, A.; Tomás-Barberán, F.A.; De Keukeleire, D.; Verstraete, W. Eubacterium limosum activates isoxanthohumol from hops (Humulus lupulus L.) into the potent phytoestrogen 8-prenylnaringenin in vitro and in rat intestine. J. Nutr. 2008, 138, 1310–1316.

321. Van den Abbeele, P.; Roos, S.; Eeckhaut, V.; MacKenzie, D.A.; Derde, M.; Verstraete, W.; Marzorati, M.; Possemiers, S.; Vanhoecke, B.; Van Immerseel, F. Incorporating a mucosal environment in a dynamic gut model results in a more representative colonization by Lactobacilli. Microb. Biotechnol. 2012, 5, 106–115.

322. Marzorati, M.; Verhelst, A.; Luta, G.; Sinnott, R.; Verstraete, W.; de Wiele, T.V.; Possemiers, S. In vitro modulation of the human gastrointestinal microbial community by plant-derived polysaccharide-rich dietary supplements. Int. J. Food Microbiol. 2010, 139, 168–176.

323. Fu, Y.; Yin, N.; Cai, X.; Du, H.; Wang, P.; Sultana, M.S.; Sun, G.; Cui, Y. Arsenic speciation and bioaccessibility in raw and cooked seafood: Influence of seafood species and gut microbiota. Environ. Pollut. 2021, 280, 116958.

324. Calatayud, M.; Xiong, C.; Du Laing, G.; Raber, G.; Francesconi, K.; van de Wiele, T. Salivary and gut microbiomes play a significant role in in vitro oral bioaccessibility, biotransformation, and intestinal absorption of arsenic from food. Environ. Sci. Technol. 2018, 52, 14422–14435.

325. Boever, P.D.; Wouters, R.; Vermeirssen, V.; Boon, N.; Verstraete, W. Development of a six-stage culture system for simulating the gastrointestinal microbiota of weaned infants. Microb. Ecol. Health Dis. 2001, 13, 111–123.

326. Van de Wiele, T.; Van den Abbeele, P.; Ossieur, W.; Possemiers, S.; Marzorati, M. Chapter 27-The simulator of the human intestinal microbial ecosystem (SHIME®). In The Impact of Food Bioactives on Health: In Vitro and Ex Vivo Models; Verhoeckx, K., Cotter, P., López-Expósito, I., Kleiveland, C., Lea, T., Mackie, A., Requena, T., Swiatecka, D., Wichers, H., Eds.; Springer: Cham, Switzerland, 2015; pp. 305–317.

327. Williams, C.; Walton, G.; Jiang, L.; Plummer, S.; Garaiova, I.; Gibson, G. Comparative analysis of intestinal tract models. Annu. Rev. Food Sci. Technol. 2015, 6.

328. Barroso, E.; Cueva, C.; Peláez, C.; Martínez-Cuesta, M.C.; Requena, T. Development of human colonic microbiota in the computer-controlled dynamic SIMulator of the GastroIntestinal tract SIMGI. LWT 2015, 61, 283–289.

329. Barroso, E.; Cueva, C.; Peláez, C.; Martínez-Cuesta, M.C.; Requena, T. Chapter 28-The computer-controlled multicompartmental dynamic model of the gastrointestinal system SIMGI. In The Impact of Food Bioactives on Health: In Vitro and Ex Vivo Models; Verhoeckx, K., Cotter, P., López-Expósito, I., Kleiveland, C., Lea, T., Mackie, A., Requena, T., Swiatecka, D., Wichers, H., Eds.; Springer: Cham, Switzerland, 2015; pp. 319–327.

330. Payne, A.; Zihler, A.; Chassard, C.; Lacroix, C. Advances and perspectives in in vitro human gut fermentation modeling. Trends Biotechnol. 2011, 30, 17–25.

331. Nissen, L.; Casciano, F.; Gianotti, A. Intestinal fermentation in vitro models to study food-induced gut microbiota shift: An updated review. FEMS Microbiol. Lett. 2020, 367.

332. Wissenbach, D.K.; Oliphant, K.; Rolle-Kampczyk, U.; Yen, S.; Höke, H.; Baumann, S.; Haange, S.B.; Verdu, E.F.; Allen-Vercoe, E.; von Bergen, M. Optimization of metabolomics of defined in vitro gut microbial ecosystems. Int. J. Med. Microbiol. 2016, 306, 280–289.

333. Marzorati, M.; Vanhoecke, B.; De Ryck, T.; Sadaghian Sadabad, M.; Pinheiro, I.; Possemiers, S.; Van den Abbeele, P.; Derycke, L.; Bracke, M.; Pieters, J.; et al. The HMI™ module: A new tool to study the Host-Microbiota Interaction in the human gastrointestinal tract in vitro. BMC Microbiol. 2014, 14, 133.

334. Venema, K.; van den Abbeele, P. Experimental models of the gut microbiome. Best Pract. Res. Clin. Gastroenterol. 2013, 27, 115–126.

335. Wood, R.J.; Tamura, T. Methodological issues in assessing bioavailability of nutrients and other bioactive substances in dietary supplements: Summary of workshop discussion. J. Nutr. 2001, 131, 1396S–1398S.

336. Carbonell-Capella, J.M.; Buniowska, M.; Barba, F.J.; Esteve, M.J.; Frígola, A. Analytical methods for determining bioavailability and bioaccessibility of bioactive compounds from fruits and vegetables: A review. Compr. Rev. Food Sci. Food 2014, 13, 155–171.

337. Jahreis, G.; Hausmann, W.; Kiessling, G.; Franke, K.; Leiterer, M. Bioavailability of iodine from normal diets rich in dairy products-results of balance studies in women. Exp. Clin. Endocrinol. Diabetes 2001, 109, 163–167.

338. Lu, Y.L.; Li, S.J.; Liu, G.Y.; Li, X.C.; Yang, D.; Jia, J.Y.; Zhang, M.Q.; Zheng, H.C.; Yu, C.; Zhu, F.; et al. Oral bioavailability and mass balance studies of a novel anti-arrhythmic agent sulcardine sulfate in Sprague-Dawley rats and beagle dogs. Eur. J. Drug Metab. Pharmacokinet. 2017, 42, 453–459.

339. Shin, B.S.; Hong, S.H.; Bulitta, J.B.; Hwang, S.W.; Kim, H.J.; Lee, J.B.; Yang, S.D.; Kim, J.E.; Yoon, H.S.; Kim, D.J.; et al. Disposition, oral bioavailability, and tissue distribution of zearalenone in rats at various dose levels. J. Toxicol. Environ. Health A 2009, 72, 1406–1411.

340. Bohn, T.; Desmarchelier, C.; Dragsted, L.O.; Nielsen, C.S.; Stahl, W.; Rühl, R.; Keijer, J.; Borel, P. Host-related factors explaining interindividual variability of carotenoid bioavailability and tissue concentrations in humans. Mol. Nutr. Food Res. 2017, 61, 1600685.

341. Manach, C.; Scalbert, A.; Morand, C.; Rémésy, C.; Jiménez, L. Polyphenols: Food sources and bioavailability. Am. J. Clin. Nutr. 2004, 79, 727–747.

342. Dias, D.M.; Costa, N.M.B.; Nutti, M.R.; Tako, E.; Martino, H.S.D. Advantages and limitations of in vitro and in vivo methods of iron and zinc bioavailability evaluation in the assessment of biofortification program effectiveness. Crit. Rev. Food Sci. Nutr. 2018, 58, 2136–2146.

343. García, Y.; Díaz-Castro, J. Advantages and disadvantages of the animal models v. in vitro studies in iron metabolism: A review. Animal 2013, 7, 1651–1658.

344. Martinez, M.N.; Rathbone, M.J.; Burgess, D.; Huynh, M. Breakout session summary from AAPS/CRS joint workshop on critical variables in the in vitro and in vivo performance of parenteral sustained release products. J. Control. Release 2010, 142, 2–7.

345. Gibson, T.M.; Ferrucci, L.M.; Tangrea, J.A.; Schatzkin, A. Epidemiological and clinical studies of nutrition. Semin. Oncol. 2010, 37, 282–296.

346. Huang, J.; Liu, C.; Wang, Y.; Wang, C.; Xie, M.; Qian, Y.; Fu, L. Application of in vitro and in vivo models in the study of food allergy. Food Sci. Hum. Wellness 2018, 7, 235–243.

347. Gueven, N.; Spring, K.J.; Holmes, S.; Ahuja, K.; Eri, R.; Park, A.Y.; Fitton, J.H. Micro RNA expression after ingestion of fucoidan; a clinical study. Mar. Drugs 2020, 18, 143.

348. Ikeda-Ohtsubo, W.; López Nadal, A.; Zaccaria, E.; Iha, M.; Kitazawa, H.; Kleerebezem, M.; Brugman, S. Intestinal microbiota and immune modulation in Zebrafish by fucoidan from Okinawa mozuku (Cladosiphon okamuranus). Front. Nutr. 2020, 7.

349. Roche-Lima, A.; Carrasquillo-Carrión, K.; Gómez-Moreno, R.; Cruz, J.M.; Velázquez-Morales, D.M.; Rogozin, I.B.; Baerga-Ortiz, A. The presence of genotoxic and/or pro-inflammatory bacterial genes in gut metagenomic databases and their possible link with inflammatory bowel diseases. Front. Genet. 2018, 9.

350. Nagamine, T.; Nakazato, K.; Tomioka, S.; Iha, M.; Nakajima, K. Intestinal absorption of fucoidan extracted from the brown seaweed, Cladosiphon okamuranus. Mar. Drugs 2015, 13, 48–64.

351. Kadena, K.; Tomori, M.; Iha, M.; Nagamine, T. Absorption study of mozuku fucoidan in Japanese volunteers. Mar. Drugs 2018, 16, 254.

352. Tokita, Y.; Nakajima, K.; Mochida, H.; Iha, M.; Nagamine, T. Development of a fucoidan-specific antibody and measurement of fucoidan in serum and urine by sandwich ELISA. Biosci. Biotechnol. Biochem. 2010, 74, 350.

353. Mathieu, S.; Touvrey-Loiodice, M.; Poulet, L.; Drouillard, S.; Vincentelli, R.; Henrissat, B.; Skjåk-Bræk, G.; Helbert, W. Ancient acquisition of “alginate utilization loci” by human gut microbiota. Sci. Rep. 2018, 8, 8075.

354. Song, T.; Xu, H.; Wei, C.; Jiang, T.; Qin, S.; Zhang, W.; Cao, Y.; Hu, C.; Zhang, F.; Qiao, D.; et al. Horizontal transfer of a novel soil agarase gene from marine bacteria to soil bacteria via human microbiota. Sci. Rep. 2016, 6, 34103.

355. Pudlo, N.A.; Pereira, G.V.; Parnami, J.; Cid, M.; Markert, S.; Tingley, J.P.; Unfried, F.; Ali, A.; Campbell, A.; Urs, K.; et al. Extensive transfer of genes for edible seaweed digestion from marine to human gut bacteria. bioRxiv 2020, 2020.06.10.142968.

356. Hehemann, J.-H.; Correc, G.; Barbeyron, T.; Helbert, W.; Czjzek, M.; Michel, G. Transfer of carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota. Nature 2010, 464, 908–912.

357. Thomas, F.; Barbeyron, T.; Tonon, T.; Génicot, S.; Czjzek, M.; Michel, G. Characterization of the first alginolytic operons in a marine bacterium: From their emergence in marine Flavobacteriia to their independent transfers to marine Proteobacteria and human gut Bacteroides. Environ. Microbiol. 2012, 14, 2379–2394.

358. Baldrick, F.R.; McFadden, K.; Ibars, M.; Sung, C.; Moffatt, T.; Megarry, K.; Thomas, K.; Mitchell, P.; Wallace, J.M.; Pourshahidi, L.K.; et al. Impact of a (poly) phenol-rich extract from the brown algae Ascophyllum nodosum on DNA damage and antioxidant activity in an overweight or obese population: A randomized controlled trial. Am. J. Clin. Nutr. 2018, 108, 688–700.

359. D'Archivio, M.; Filesi, C.; Varì, R.; Scazzocchio, B.; Masella, R. Bioavailability of the polyphenols: Status and controversies. Int. J. Mol. Sci. 2010, 11, 1321–1342.

360. Sęczyk, Ł.; Świeca, M.; Kapusta, I.; Gawlik-Dziki, U. Protein-phenolic interactions as a factor affecting the physicochemical properties of white bean proteins. Molecules 2019, 24, 408.

361. Buitimea-Cantúa, N.E.; Gutiérrez-Uribe, J.A.; Serna-Saldívar, S.O. Phenolic–protein interactions: Effects on food properties and health benefits. J. Med. Food 2018, 21, 188–198.

362. Zhang, Q.; Cheng, Z.; Wang, Y.; Fu, L. Dietary protein-phenolic interactions: Characterization, biochemical-physiological consequences, and potential food applications. Crit. Rev. Food Sci. Nutr. 2020, 1–27.

363. Imbs, T.; Zvyagintseva, T. Phlorotannins are polyphenolic metabolites of brown algae. Russ. J. Mar. Biol. 2018, 44, 263–273.

364. Zhang, H.; Yu, D.; Sun, J.; Liu, X.; Jiang, L.; Guo, H.; Ren, F. Interaction of plant phenols with food macronutrients: Characterisation and nutritional–physiological consequences. Nutr. Res. Rev. 2013, 27, 1–15.

365. Mignet, N.; Seguin, J.; Chabot, G.G. Bioavailability of polyphenol liposomes: A challenge ahead. Pharmaceutics 2013, 5, 457–471.

366. Jakobek, L.; Matić, P. Non-covalent dietary fiber-polyphenol interactions and their influence on polyphenol bioaccessibility. Trends Food Sci. Technol. 2019, 83, 235–247.

367. Bohn, T. Dietary factors affecting polyphenol bioavailability. Nutr. Rev. 2014, 72, 429–452.

368. Wojtunik-Kulesza, K.; Oniszczuk, A.; Oniszczuk, T.; Combrzyński, M.; Nowakowska, D.; Matwijczuk, A. Influence of in vitro digestion on composition, bioaccessibility and antioxidant activity of food polyphenols-a non-systematic review. Nutrients 2020, 12, 1401.

369. Sookkasem, A.; Chatpun, S.; Yuenyongsawad, S.; Wiwattanapatapee, R. Alginate beads for colon specific delivery of self-emulsifying curcumin. J. Drug Deliv. Sci. Technol. 2015, 29, 159–166.

370. Hussain, M.B.; Hassan, S.; Waheed, M.; Javed, A.; Farooq, M.A.; Tahir, A. Chapter 5-Bioavailability and metabolic pathway of phenolic compounds. In Plant Physiological Aspects of Phenolic Compounds; Soto-Hernández, M., Palma-Tenango, M., García-Mateos, R., Eds.; IntechOpen: Rijeka, Croatia, 2019.

371. Luca, S.V.; Macovei, I.; Bujor, A.; Miron, A.; Skalicka-Woźniak, K.; Aprotosoaie, A.C.; Trifan, A. Bioactivity of dietary polyphenols: The role of metabolites. Crit. Rev. Food Sci. Nutr. 2020, 60,

626–659.

372. Carregosa, D.; Carecho, R.; Figueira, I.; Santos, C.N. Low-molecular weight metabolites from polyphenols as effectors for attenuating neuroinflammation. J. Agric. Food Chem. 2020, 68, 1790–1807.
373. Liu, Z.; Hu, M. Natural polyphenol disposition via coupled metabolic pathways. Expert Opin. Drug Metab. Toxicol. 2007, 3, 389–406.
374. Suetsuna, K.; Maekawa, K.; Chen, J.-R. Antihypertensive effects of Undaria pinnatifida (wakame) peptide on blood pressure in spontaneously hypertensive rats. J. Nutr. Biochem. 2004, 15, 267–272.
375. Pimenta, D.C.; Lebrun, I. Cryptides: Buried secrets in proteins. Peptides 2007, 28, 2403–2410.
376. Hayes, M.; García-García, M.; Fitzgerald, C.; Lafarga, T. Chapter 27-Seaweed and milk derived bioactive peptides and small molecules in functional foods and cosmeceuticals. In Biotechnology of Bioactive Compounds: Sources and Applications; Gupta, V.K., Tuohy, M.G., Eds.; John Wiley & Sons: Oxford, UK, 2015; pp. 669–691.