Recent progress in *Porphyra haitanensis* polysaccharides: Extraction, purification, structural insights, and their impact on gastrointestinal health and oxidative stress management

Kit-Leong Cheong [a,b], Keying Liu [a,b], Wenting Chen [a,b], Saiyi Zhong [a,b,*], Karsoon Tan [c,**]

[a] *College of Food Science and Technology, Guangdong Ocean University, Guangdong Provincial Key Laboratory of Aquatic Product Processing and Safety, Guangdong Province Engineering Laboratory for Marine Biological Products, Guangdong Provincial Engineering Technology Research Center of Seafood, Guangdong Provincial Engineering Technology Research Center of Prefabricated Seafood Processing and Quality Control, Zhanjiang 524088, China*
[b] *Collaborative Innovation Center of Seafood Deep Processing, Dalian Polytechnic University, Dalian 116034, China*
[c] *Guangxi Key Laboratory of Beibu Gulf Biodiversity Conservation, Beibu Gulf University, Qinzhou, Guangxi, China*

ARTICLE INFO

ABSTRACT

Porphyra haitanensis, a red seaweed species, represents a bountiful and sustainable marine resource. *P. haitanensis* polysaccharide (PHP), has garnered considerable attention for its numerous health benefits. However, the comprehensive utilization of PHP on an industrial scale has been limited by the lack of comprehensive information. In this review, we endeavor to discuss and summarize recent advancements in PHP extraction, purification, and characterization. We emphasize the multifaceted mechanisms through which PHP promotes gastrointestinal health. Furthermore, we present a summary of compelling evidence supporting PHP's protective role against oxidative stress. This includes its demonstrated potent antioxidant properties, its ability to neutralize free radicals, and its capacity to enhance the activity of antioxidant enzymes. The information presented here also lays the theoretical groundwork for future research into the structural and functional aspects of PHP, as well as its potential applications in functional foods.

1. Introduction

Marine algae, commonly known as seaweeds, abound in the world's oceans and exemplify the remarkable sustainability inherent in nature. As the world increasingly seeks environmentally conscious and sustainable solutions to meet its growing demands, marine algae provide a compelling testament to nature's capacity to supply resources that can be responsibly harnessed to promote the well-being of both humanity and the environment (Wells et al., 2017). *P. haitanensis*, a member of the Rhodophyta division, commonly referred to as red algae, predominantly thrives in the coastal waters of East Asia (Blouin, Brodie, Grossman, Xu, & Brawley, 2011). This seaweed species plays an important role in the Chinese economy, with its commercial cultivation and harvest giving rise to a robust industry that creates substantial revenue and employment opportunities in China's coastal regions (Wang et al., 2020).

Beyond its culinary uses, *P. haitanensis* holds economic significance by being integrated into various food products, including snacks, condiments, and health foods (Venkatraman & Mehta, 2019). Furthermore, the bioactive compounds derived from this seaweed, specifically *P. haitanensis* polysaccharide (PHP), have opened doors to a wide array of applications across various industrial sectors (Qiu et al., 2022).

PHP, owing to its unique chemical properties, assumes a pivotal role in unlocking the nutritional and therapeutic potential harbored by this marine resource. The pharmaceutical properties inherent in PHP encompass its antioxidant, immune-modulatory, anti-inflammatory, anti-cancer, and prebiotic properties (Shi et al., 2015; Wu et al., 2020; Xu et al., 2019; Yao, Veeraperumal, Qiu, Chen, & Cheong, 2020). Nevertheless, the comprehensive utilization of PHP in industrial applications has been limited by the lack of comprehensive information. Hence, this review embarks on a quest to summarize and discuss PHP,

initiating with an exploration of the various techniques employed for the extraction of these polysaccharides. These methodologies span from conventional water-based extraction processes to state-of-the-art approaches like ultrasonic and microwave-assisted extraction techniques, elevating extraction efficiency (Gharibzahedi, Marti-Quijal, Barba, & Altintas, 2022; Mirzadeh, Arianejad, & Khedmat, 2020). This also highlights the growing importance of eco-conscious practices in sustainable extraction methods. Transitioning our focus from extraction to purification, we venture into the methodologies implemented to characterize PHP, subsequently revealing their intricate chemical compositions. The amalgamation of advanced spectroscopic techniques, molecular weight determination, and monosaccharide composition analysis collectively furnishes a comprehensive and intricate understanding of PHP's chemical structure (Huang et al., 2022).

PHP displays a diverse array of biological activities and attributes collectively underscore its diverse and potent pharmacological potential. It's worth noting that despite the growing interest in the beneficial effects of PHP on gastrointestinal health and the management of oxidative stress, there is a noticeable shortage of recent systematic reviews that comprehensively delve into the underlying mechanisms (Cheong, Yu, Chen, & Zhong, 2022; Vaes, Idris, Boesmans, Alves, & Melotte, 2022). Hence, in this current review, we embark on a quest to unearth the evidence related to the favorable impacts of PHP on gastrointestinal health and oxidative stress. This quest involves shedding light on PHP's prebiotic capability to withstand upper gastrointestinal digestion, its role in modulating gut microbiota, its ability to generate functional metabolites such as short-chain fatty acids (SCFAs), its capacity to enhance the colonic mucus barrier, its role in reinforcing tight junctions to strengthen the intestinal barrier, and its anti-inflammatory effects (Chen, Tong, Zeng, Zheng, & Hu, 2021; Yu et al., 2023). Furthermore, in the context of preventing oxidative stress, PHP emerges as a potent antioxidant. It adeptly neutralizes free radicals, enhance the activity of crucial antioxidant enzymes, and mitigates lipid peroxidation, thereby enhancing the body's intrinsic defenses against oxidative damage (Khan, Qiu, Xu, Liu, & Cheong, 2020; Zeng et al., 2023).

As we undertake this comprehensive review, we delve into the intriguing realm of PHP, examining the extraction, purification, and characterization processes. We emphasize their impressive biological activities, particularly in promoting intestinal well-being and providing protection against oxidative stress. These insights may serve as a catalyst for breakthroughs in functional foods, healthcare, and the pharmaceutical industry, contributing to a holistic understanding of their potential health benefits.

2. Preparation and extraction of PHP

2.1. Pre-treatment of macroalgae

The importance of pretreatment before extracting marine seaweed polysaccharides cannot be overstated, as it has a profound impact on the quality, yield, and efficiency of the extraction process. Pretreatment selectively removes undesirable components like pigments and low molecular weight impurities, thus enhancing the purity of the polysaccharide extract. Additionally, it increases the bioavailability of the polysaccharides, reduces processing time, and promotes sustainable resource utilization. Fat-soluble pigments, such as chlorophyll and carotenoids, have the potential to introduce unwanted colors and off-flavors into the extracted polysaccharides (Generalić Mekinić, Šimat, Rathod, Hamed, & Čagalj, 2023), thereby affecting their suitability for various industrial applications. Furthermore, remaining pigments may influence the biological assessment of PHP due their potent antioxidant activity (Pan et al., 2021). Typically, fat-soluble pigments can be eliminated through refluxing with an organic solvent, such as methanol/dichloromethane/water (4:2:1; $v/v/v$) (Xu et al., 2019), while ethanol-soluble small molecule impurities can be removed by refluxing with 80% ethanol for 2 h at 80 °C (Cao et al., 2016). These pretreatment

procedures not only improve the purity of the polysaccharides but also make significant contributions to their overall functionality and bioactivity.

2.2. Conventional extraction method

Conventional water-based thermal extraction methods have traditionally been employed to isolate polysaccharides from various natural sources. Typically, this approach involves heating the entire apparatus or thermal system to transfer energy to the water, thereby raising its temperature (Chemat et al., 2020). In this process, red algae are immersed in hot water. The elevated temperature softens the cell walls, enhancing the permeability of the cell membranes and promoting the dissolution of PHP into the surrounding water (Padayachee, Day, Howell, & Gidley, 2017). Consequently, PHP becomes solubilized and leaches out from the algae material. Conventional extraction methods retain a prominent position due to their inherent advantages, including relative simplicity and cost-effectiveness, as demonstrated in Table 1. Consequently, they continue to be the most widely employed approach for PHP extraction. The standard procedure for obtaining a PHP solution from *P. haitanensis* typically involves using hot water (90 °C) for 2–4 h (Chen et al., 2023; Qiu et al., 2020), with the most extended extraction duration and highest temperature reaching 100 °C for 5 h (Cao et al., 2016). On occasion, it may be necessary to perform multiple cycles to fully extract all the polysaccharides from the seaweeds. For instance, the residue of *P. haitanensis* is subjected to three consecutive extractions with 20 volumes of distilled water at 95 °C, each lasting 2 h (Fu et al., 2019). (See Table 2.)

However, a significant limitation arises from the considerable energy consumption associated with heating processes (Ameer, Shahbaz, & Kwon, 2017), especially when applied to red seaweed, which is characterized by robust cell walls. This aspect can compromise the environmental sustainability and economic viability of these techniques. Thus, given the growing demand for polysaccharides across various industries, it becomes imperative to explore and adopt extraction technologies that prioritize environmental friendliness and operational efficiency, effectively overcoming these conventional limitations.

2.3. Microwave-assisted extraction

Microwave-assisted Extraction (MAE) marks a groundbreaking advancement in polysaccharide extraction, presenting an exceptionally efficient and expeditious approach to extract these valuable compounds from natural sources. MAE operates through the application of microwave radiation during the extraction process (Soni, Smith, Thompson, & Brightwell, 2020). When algae material submerged in water is exposed to microwave energy, this radiation prompts the rapid oscillation of water molecules within the sample due to their dipolar properties (Flórez, Conde, & Domínguez, 2015). This molecular movement generates heat, leading to localized temperature increases and the build-up of internal pressure within the algae (Qiu et al., 2022). As a result, the cell walls of the algae are disrupted, facilitating the release of PHP molecules into the surrounding solvent (Fig. 1A).

MAE method significantly shortens extraction times and enhances the efficiency of PHP extraction. As depicted in Table 1, the MAE method exhibits the shortest extraction duration. MAE resulted in a PHP yield of approximately 3.6% under optimized conditions determined through single-factor experiments: a microwave power of 300 W, an extraction time of 8 min, and a water-to-powder ratio of 50:1 (Yu & Zhang, 2017). Additionally, Chen and Xue further refined the MAE process utilizing a response surface methodology, achieving a PHP yield of 5.01% with a water-to-raw material ratio of 28.98 (mL/g), a microwave power of 77.84 W, and an extraction time of 14.14 min. Their findings revealed that MAE outperforms hot water extraction, notably in terms of extraction time, which requires 300 min for hot water extraction, resulting in an approximate 191% increase in yield when employing the

Table 1
The preparation, purification and structural information of *P. haitanensis* polysaccharide.

Fraction	Extraction method	Purification process	Yield of polysaccharides	Monosaccharide composition	Molecular weight	Backbone or linkages	Ref.
PHP0.5–1-UF, PHP1.0–1-UF	Thermal extraction, 80 °C hot water at ratio of 20:1 (w/v) for 2 h	DEAE-Sepharose FF Sephacryl S-400 HR	37.1%, 48.6%	–	6.68×10^6 Da 1.14×10^6 Da	–	(Chen, Xu, et al., 2021)
PHPs	Thermal extraction, 80 °C hot water material to water ratio of 0.04 , for 3 h	–	20.48%	galactose, glucose, and fucose (76.2:2.1:1)	6.3×10^5 Da	–	(Dong et al., 2020)
PHPs	Thermal extraction, hot water	DEAE-cellulose 52	–	3,6-anhydrogalactose	–	–	(Liu et al., 2017)
PHP1 PHP2 PHP3	Thermal extraction, 90 °C hot water for 4 h	DEAE-cellulose 52	–	galactose, glucose, xylose, mannose, fructose, and glucuronic acid (98.10:0.54:0.19:0.36:0.15:0.66 94.27:3.95:0.28:0.46:0.26:0.78 96.91:1.66:0.19:0.54:0.17:0.53)	5.67×10^5 Da 4.14×10^5 Da 3.23×10^5 Da	–	(Wang, Ye, Wang, & Fu, 2022)
PHPs	Thermal extraction, 80 °C hot water at ratio of 20:1 (*w/v*) for 2 h	–	–	–	–	–	(Wang, Zhong, et al., 2023)
PHP	Thermal extraction, 90 °C hot water at ratio of 30:1 (w/v) for 2 h	–	4.10%	galactose (94.85%), glucose (3.18%), mannose (1.97%)	from 2.623×10^5 to 2.308×10^4 Da	$1 \to 3$ and $1 \to 4$ linked Galp	(Xu et al., 2019)
LP-G2	Thermal extraction, 110 °C hot water for 2 h	DEAE Sephadex A-50 Sephadex G-100	–	galactose, galacturonic acid, glucose, arabinose (14.10:0.33:1.52:0.04)	8381 Da	→4)-β-D-galactose→4)-α-L-galactose-6-sulfate segments	(Zhang et al., 2020)
PHPD-IV-4	Thermal extraction, at 80 °C hot water for 1.5 h at a ratio of 1:20 (w/v)	DEAE-cellulose Sephadex-G-50	0.24%	galactose	–	→3)-β-D-galactose $(1 \to 4)$ 3, 6-anhydro-α-L-galactose $(1\to$, and →3) β-D-galactose $(1 \to 4)$ α-L-galactose-6-S $(1\to$	(Gong et al., 2020)
PHP	Thermal extraction, at 90 °C hot water for 2 h at a ratio of 1:30 (w/v)	–	–	galactose, 3,6-anhydrogalactose (1.2:1.0)	2.5×10^5 Da.	→4–3,6-anhydro-α-L-galactopyranose-$(1 \to 3)$-β-D-galactopyranose	(Khan et al., 2020)
PHP	Thermal extraction, hot water for 2 h	DEAE-cellulose-52	–	galactose, 3,6-anhydrogalactose (1.2:1.0)	2.01×10^5 Da	$(1 \to 4)$-linked 3,6-anhydro-α-L-galactopyranose units or $(1 \to 4)$-linked α-L-galactose 6 sulphate units	(Qiu et al., 2020)
PH PY	Thermal extraction, extracted with 20 volumes of distilled water at 95 °C for 2 h	dialyze	–	–	–	–	(Fu et al., 2019)
PHPS	Thermal extraction, dried material (25 g) was crushed and boiled in distilled water (1 L) for 4 h	–	–	galactose (60.09%)	–	–	(Shi et al., 2015)
APHP	Thermal extraction, with 50 volumes of distilled water for 5 h at 100 °C	dialyze	–	–	–	–	(Cao et al., 2016)
PHP2	Thermal extraction, at a ratio of 1:20 (m/V), at 80 °C for 2 h.	DEAE-Sepharose FF Sephacryl S-400 HR	–	galactose (69.27%), mannose (21.32%), glucose (9.41%)	–	a hypothetical backbone structure of →4) Gα $(1 \to 6)$ G4Sβ $(1 \to 4)$ Glc $(1 \to$ and a side chain of Man $(1 \to 6)$ Glc	(Chen et al., 2023)
CPP PP1 PP2 PP3(PP3–1, PP3–2, PP3–3, PP3–4, PP3–4a)	Thermal extraction, hot water (80 °C) in a ratio of 1:20 (w/v) for 1.5 h	DEAE-cellulose Sephadex G-100	3.8% (CPP) 0.41% (PP1) 0.76% (PP2) 0.65% (PP3) 0.68% (PP4) 0.35% (PP5)	PP1 (galactose and 3,6-anhydrogalactose) PP2-PP4 (galactose, 3,6-anhydrogalactose, and 6-*O*-methyl-galactose) PP5 (galactose, 3,6-anhydrogalactose, mannose, and glucose)	PP3–1 $(1.02 \times 10^5$ Da) PP3–2 $(7.2 \times 10^4$ Da) PP3–3 $(3.9 \times 10^4$ Da) PP3–4 $(2.0 \times 10^4$ Da)	→3) β-D-galactose $(1 \to 4)$ 3,6-anhydro-α-L-galactose $(1\to$, and →3) β-D-galactose $(1 \to 4)$ α-L-galactose-6-S $(1\to$	(Gong et al., 2018)

Table 1 (*continued*)

| Fraction	Extraction method	Purification process	Yield of polysaccharides	Monosaccharide composition	Molecular weight	Backbone or linkages	Ref.
PP4 PP5]							
PH	Thermal extraction, with 20 volumes of distilled water at 95 °C for 2 h	DEAE-cellulose 52 Sephadex G-100	–	–	–	–	(Wang, Lin, et al., 2023)
PHP1	Thermal extraction, hot water (80 °C) in a ratio of 1:20 (w/v) for 2 h	–	–	galactose	–	→3) G4Sβ (1 → 3) G (1 → 6) G4Sα (1 → 4) LA (1 → 6) G4Sα (1→	(Chen, Tong, et al., 2021)
PHPs	Thermal and ultrasonicCytotoxicity of-assisted extraction, sonicate at 450 W for 30 min, at 100 °C hot water for 4 h at the ratio of 1:40 (w/v)	dialyze	–	galactose (more than 75%)	–	β-type glycosidic bond	(Ji et al., 2022)
PHPs	Thermal and ultrasonic-assisted/microwave-assisted extraction, at the ratio of 1:42 (w/v), in an ultrasonic/microwave instrument (CW-2000) and kept at 80 °C for 30 min	DEAE-52	20.53%		2.01×10^5 Da	(1 → 4)-linked 3,6-anhydro-α-lgalactopyranose units or (1 → 4)-linked α-l-galactose 6 sulfate units	(Yao et al., 2020)
PHP	Thermal, ultrasonic-assisted and microwave-assisted extraction, microwave power of 500 W, ultrasonic power of 50 W, at 79.94 °C hot water for 29.64 min at a ratio of 1:41.79 (g/mL)	–	20.98%	–	–	–	(Xu et al., 2020)
PHP UHP-PHP US-PHP M-PHP	Microwave-assisted extraction, microwave treatment at 440 W for 2 min	dialyze	–	galactose, glucose, xylose, mannose, galacturonic acid (83.26:5.94:0.56:2.97:7.27 79.41:7.07:0.74:2.31:10.53 85.57:2.79:0.28:3.24:8.12 73.09:10.03:1.08:6.11:9.70)	2.176×10^6 Da 1.080×10^6 Da 1.0×10^6 Da 9.46×10^5 Da	-β-D-galactose and 3,6-anhydro-α-L-galactose units	(Zheng et al., 2023)
PHP	Microwave-assisted extraction, ratio of water to raw material 28.98 (mL/g), microwave power 77.84 W, extraction time 14.14 min	–	4.90%	rhamnose, arabinose, xylose, mannose, glucose, galactose (10.25:9.38:1:12.45:9.9:11.55)	–	–	(Chen & Xue, 2019)
PY1 PY2 PY3	Microwave-assisted extraction, the microwave power 300 W, at the ratio of 1:50 (w/v) for 8 min	DEAE-52 Sephadex G-100	3.6%	galactose and 3,6-anhydrogalactose (PY1 34% and 4.6% PY2 31.3% and 7.5% PY3 26.9% and 3.4%)	–	PY1 had α-glycosidic bonds, PY2 and PY3 had the β-amide pyranose.	(Yu & Zhang, 2017)
PHP	–	–	–	galactose, 3,6-anhydrogalactose (1.2:1.0)	2.01×10^5 Da	(1 → 4)-linked 3,6-anhydro-α-L-galactopyranose residues or (1 → 4)-linked α-L-galactose 6 sulfate residues	(Malairaj et al., 2023)

Table 2
Evidence of prebiotic activities and gastrointestinal health performance of *P. haitanensis* polysaccharides.

Model	Metabolite	Changes in gut microbiota	Other performance	References
in vitro simulated fermentation	acetic acid ↑ propionic acid ↑ butyric acid ↑ SCFAs ↑	*Ruminococcaceae*_UCG-005 ↑ *Escherichia-Shigella* ↓ *Lactobacillus* ↓ *Helicobacter* ↓ *Prevotella_9* ↓ *Desulfovibrio* ↑ *Ruminococcus_2* ↑	bile acid ↓	(Chen et al., 2023)
in vitro simulated fermentation	SCFAs ↑ acetic acid ↑ butyric acid ↑	Bacteroidetes ↓ Proteobacteria ↑ Cyanobacteria ↓ *Bacteroidaceae* ↓	Mucin-2 ↑ ZO-1 ↑ occludin. ↑	(Malairaj et al., 2023)
in vitro simulated fermentation	acetate acid ↑ propionate ↑ SCFAs ↑	*Bifidobacteria* ↑ *Bacteroides* ↑ *Lactobacillus* ↑ *Lactobacilli* ↑	–	(Seong et al., 2019)
in vitro simulated fermentation	propionic acid ↑ SCFAs ↑	propionic acid-producing bacteria ↑ *Escherichia-Shigella* ↓ *Fusicatenibacter* ↑ *Ruminiclostridium_5* ↑ *Bifidobacterium* ↑ *Bacteroides* ↑ *Ruminococcaceae* ↑	cholesterol ↓	(Chen, Tong, et al., 2021)
in vitro simulated fermentation	SCFAs ↑ acetic acid ↑ propionic acid ↑ butyric acid ↑	pathogenic bacteria ↓ *Firmicutes* ↑ *Bacterioidetes* ↑ *Proteobacteria* ↑	–	(Xu et al., 2019)
in vitro simulated fermentation	–	Proteobacteria ↓ *Bacteroides* ↑ *Escherichia_Shigella* ↓	–	(Xu et al., 2020)
in vivo mouse model	–	–	TNF-α ↑ IL-6 ↑	(Liu et al., 2019)
in vivo mouse model	–	Bacteroidetes ↑ *Firmicutes* ↓ *Bacteroidales* S24–7 ↑ *Ruminococcaceae* UCG-014 ↑ *Lactobacillus* ↑	–	(Zhang, Wang, Han, Liu, & Liu, 2018)
in vivo mouse model of diabetes mellitus	hypotaurine ↑ pyruvate ↑	*Helicobacter* ↓ *Desulfovibrio* ↓ *Mucispirillum* ↓ *Enterorhabdus* ↓ *Blautia* ↑ *Muribaculaceae* ↑ *Lactobacillus* ↑	the enrichment factors of the taurine and hypotaurine pathways ↑	(Ou et al., 2023)
in vivo high-fat mouse model	–	Bacteroidetes ↑ Proteobacteria ↑ Verrucomicrobia ↑ Firmicutes ↓ *Roseburia* ↑ *Eubacterium* ↑ *Helicobacter* ↓ *Bacteroides* ↑ *Alistipes* ↑	lipid accumulation ↓ FFA ↓ PGC 1α↑ UCP 1 ↑	(Wang, Dong, et al., 2022)
in vivo high-fat mouse model	butyric acid ↑	*Muribaculaceae* ↑ *Faecalibaculum* ↑ *Bifidobacterium* ↓	*Fabp5* ↓ Lower blood lipids *Fasn* ↓ *Me1* ↓ *Acaca* ↓ *Elovl6* ↓ *Cd36* ↑	(Zeng et al., 2023)
in vivo mouse model of food allergy	–	Lachnospiraceae ↑ *Bacteroidia* ↓ *Clostridia* ↑ *Actinobacteria* ↑ *Coriobacteriia* ↑ *Bifidobacterium* ↑	IL-2 ↓ IL-4 ↓ IFN-γ ↓ IL-17 A ↓	(Wei et al., 2023)

MAE method (Chen & Xue, 2019).

Differing from conventional heating methods, MAE possesses the capability to target the heating of the solvent rather than the entire extraction apparatus. This characteristic results in reduced energy consumption and cost-effectiveness (Zia et al., 2022). Furthermore, MAE offers precise control over temperature and irradiation duration, a critical factor in preserving the structural integrity and bioactivity of the extracted polysaccharides (Shakoor, Hussain, Younas, & Bilal, 2023). MAE's adaptability extends across a diverse range of source materials, making it a versatile and environmentally-conscious choice for both
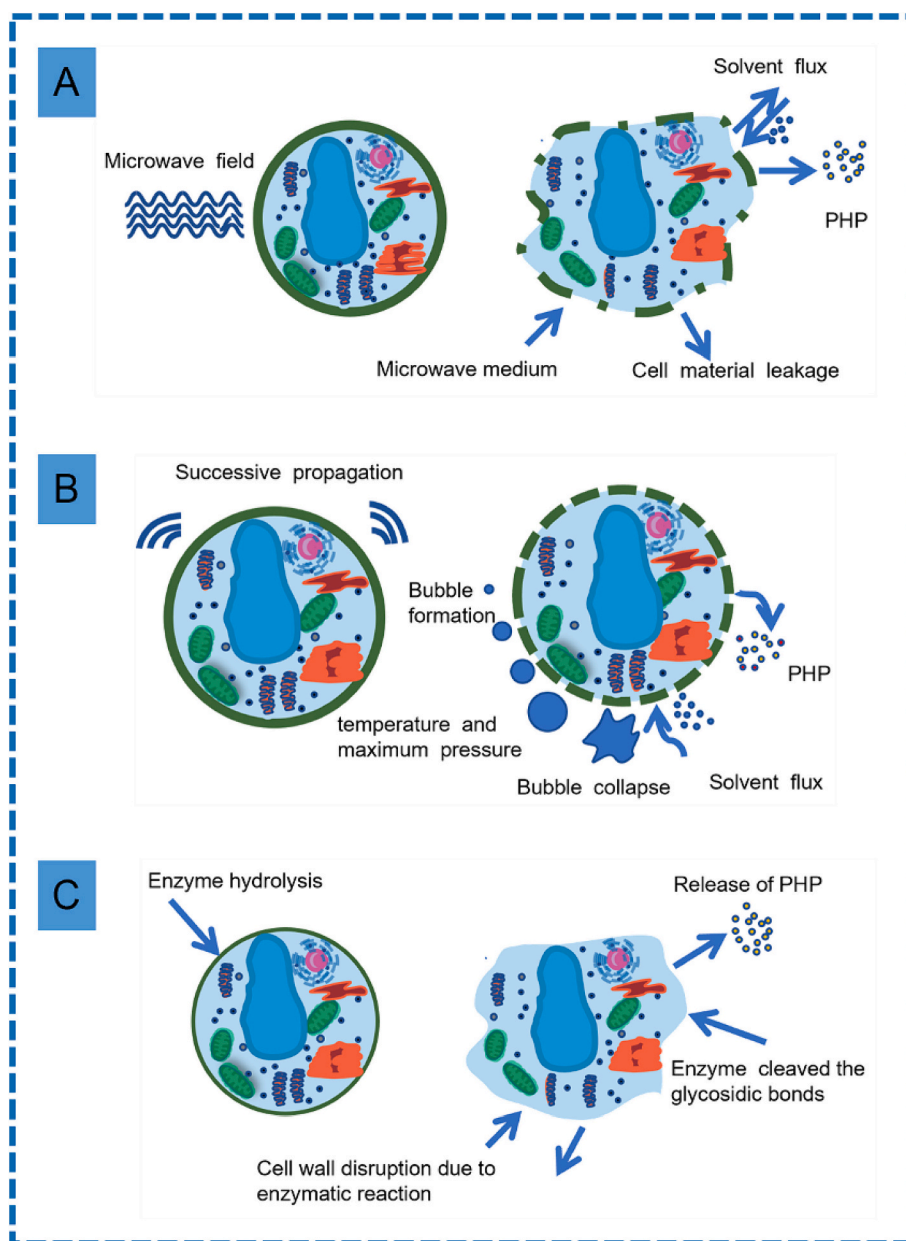
Fig. 1. The extraction mechanisms of the different extraction methods for PHP: (A) Microwave-assisted extraction, (B) Ultrasound-assisted extraction, (C) Enzyme-assisted extraction.

industries and researchers. This aligns perfectly with the growing demand for sustainable and efficient techniques for extracting PHP.

2.4. Ultrasound-assisted extraction

Ultrasound-assisted extraction (UAE) has emerged as a robust and eco-friendly method for extracting polysaccharides from a diverse range of natural sources. UAE's underlying mechanism harnesses the energy of high-frequency sound waves to facilitate the extraction process (Yan, Mei, Li, & Xie, 2022). When ultrasonic waves are applied to seaweed suspended in a solvent, they generate intense pressure fluctuations within the solvent, a phenomenon known as cavitation (Holkar, Jadhav, Pinjari, & Pandit, 2019). During cavitation, the rapid formation and collapse of minute bubbles within the solvent produce localized heat, shockwaves, and microjets (Udepurkar, Clasen, & Kuhn, 2023). These dynamic forces disrupt the cell walls of the seaweed, leading to their rupture. As a result, PHP is liberated from their cellular matrix and become dispersed in the solvent (Picó, 2013) (Fig. 1B). This method offers several salient advantages, including its energy efficiency and minimal environmental footprint. UAE diminishes the need for elevated temperatures and protracted extraction durations (More, Jambrak, & Arya, 2022). For instance, comparing UAE to hot water extraction, PHP extracted via UAE under ultrasonic treatment at 100 W and 20 °C for 8 min exhibited higher total sugar content (67.28%), sulfated content (25.22%), and uronic acid content (12.38%) (Zheng et al., 2023).

On the contrary, ultrasonic/microwave-assisted extraction (UMAE) represents an innovative hybrid extraction technique that leverages the advantages of both ultrasound and microwave technologies to extract polysaccharides from seaweed (Xu et al., 2018). In UMAE, the sample is subjected to a dual exposure of ultrasonic waves and microwave irradiation, either concurrently or consecutively. This synergistic use of energy sources leads to an exceptionally effective extraction process (Sun et al., 2019). In our previous study, we employed a response surface methodology to optimize the parameters for extracting polysaccharides

from *P. haitanensis* using the UMAE technique. The most favorable extraction conditions involved a microwave power of 500 W, ultrasonic power of 50 W, an extraction duration of 30 min, an extraction temperature of 80 °C, and a liquid–solid ratio of 42 mL/g. These conditions yielded a PHP extraction rate of 20.53%. Notably, this yield surpassed that typically obtained through the conventional thermal method, which usually resulted in a PHP extraction rate lower by approximately 5% (Xu, Chen, Liu, & Cheong, 2020). UAE and UMAE have gained recognition for their remarkable efficiency and sustainability, positioning them as promising advancements in the field of polysaccharide extraction. These techniques address the growing demand for environmentally friendly and high-yield extraction methods.

2.5. Enzyme-assisted extraction

Enzyme-assisted extraction (EAE) presents a revolutionary approach to extracting polysaccharides, particularly from plant-based materials. This technique relies on specific enzymes that have an affinity for the structural components found in cell walls, including cellulose and hemicellulose (Nadar, Rao, & Rathod, 2018). These enzymes work by breaking down the intricate polysaccharide matrices, essentially cleaving the glycosidic bonds that hold them together (Fig. 1C). This enzymatic process effectively weakens the structural integrity of the seaweed material, making it more permeable and amenable to extraction (Charoensiddhi et al., 2016). One of the primary merits of EAE lies in its selectivity (Rhein-Knudsen, Ale, & Meyer, 2015). Researchers can precisely target specific polysaccharides by carefully choosing the appropriate enzymes, thus enabling a customized extraction process. Commonly used enzymes in EAE for the selective extraction of particular polysaccharides include cellulase, hemicellulase, and pectinase. For instance, Li et al. utilized pectinase at a concentration of 50 U/mL, conducted the extraction at a pH of 5.0 and a temperature of 47.5 °C, for a duration of 2 h, resulting in the production of PHP with a molecular weight of 217.4 kDa (Li et al., 2020). Another noteworthy advantage of EAE is its operation under milder conditions in comparison to certain other extraction methods, such as those involving high temperatures (Puri, Sharma, & Barrow, 2012). This gentle approach contributes significantly to preserving the bioactivity and structural integrity of the extracted polysaccharides.

The infusion of eco-friendly and cutting-edge technologies into the extraction of PHP has undeniably marked the onset of a transformative era within this field. These technologies have paved the path toward more sustainable and environmentally conscious practices, resulting in reduced energy consumption and a diminished overall environmental footprint associated with the extraction process. Furthermore, the maintenance of polysaccharide bioactivity and structural integrity, alongside heightened extraction efficiency, has unlocked fresh possibilities for their utilization across diverse industries, encompassing food, pharmaceuticals, and cosmetics. The horizon of polysaccharide extraction promises substantial potential, with ongoing innovations and refinements by researchers and industries alike. One notable emerging trend involves the incorporation of artificial intelligence and computation learning algorithms into the extraction procedures (Rathore, Nikita, Thakur, & Mishra, 2023). These technological advancements possess the capacity to fine-tune extraction parameters, predict optimal conditions, and enhance yields, ultimately streamlining the entire process. Additionally, the quest for sustainability will steer the development of more environmentally benign solvents and greener extraction methodologies.

3. Purification techniques

3.1. Ethanol precipitation

The process of ethanol precipitation is a pivotal step in the isolation of PHP from the initial crude polysaccharide extract. When ethanol is introduced to the polysaccharide solution, it serves to diminish the solubility of the polysaccharides in the solvent, owing to the addition of an appropriate precipitant. This precipitant disrupts the hydrogen bonding and electrostatic interactions occurring among the polysaccharide molecules, resulting in their aggregation and subsequent insolubility, ultimately forming a precipitate. In this approach, the extract polysaccharide solution is gradually mixed with chilled 95% ethanol, typically at a ratio of 1:2 or 1:3 (polysaccharide solution to ethanol) (Zhang et al., 2003). Following a suitable incubation period, which often extends for several hours or overnight, it is suggest to uphold the mixture at low temperatures (Khan et al., 2020), either by employing an ice bath or a refrigerated centrifuge, to facilitate effective precipitation. Ethanol precipitation is highly esteemed for its simplicity, cost-effectiveness, and its capability to yield relatively pure polysaccharide fractions, establishing it as a cornerstone in the methodologies employed for PHP purification.

3.2. Protein removal from PHP

During the ethanol precipitation step, it's possible for proteins to co-precipitate with PHP, potentially causing interference with PHP's intended applications or analysis. Some techniques are employed to mitigate this issue, and one commonly used method is the Sevag method, also known as the chloroform-butanol method (Wu, Hu, Huang, & Jiang, 2013). The Sevag method capitalizes on the varying solubility of proteins and polysaccharides in a biphasic solvent system consisting of chloroform and *n*-butanol (in a 4:1 ratio, *v/v*), separated by the addition of water to facilitate polysaccharide extraction (Wang, Zhong, Zheng, Zhang, & Zeng, 2023). Proteins, being insoluble in the chloroform-butanol phase, precipitate at the interface, while the PHP remains in the aqueous phase. In some cases, it may be necessary to employ the Sevag reagent multiple times to ensure complete removal of proteins until they are entirely eliminated from the solution (Zhang et al., 2014).

The trichloroacetic acid (TCA) method offers another effective approach for eliminating proteins from polysaccharides. In this method, a TCA solution is introduced to the mixture of polysaccharides and proteins, causing the proteins to denature and precipitate as a result of the acid's protein-denaturing properties (Georgiou et al., 2018). Following an incubation period, the mixture is typically subjected to centrifugation to separate the protein precipitate from the supernatant containing both polysaccharides and proteins. However, it is important to be aware that the acidic environment generated by TCA may potentially impact the stability of sulfated PHP and could lead to the degradation of polysaccharides through the hydrolysis of glycosidic bonds (Wang et al., 2021). Another frequently employed approach for deproteinization involves the use of enzymatic methods, which offer a precise and selective means of removing proteins while safeguarding the integrity of PHP. In this technique, specific proteolytic enzymes like proteases, pepsin, and papain are employed to target and break down proteins while preserving PHP's structural integrity (Hong, Fan, Chalamaiah, & Wu, 2019). For instance, a solution containing 600 U/mL of alkaline protease was introduced to the mixture and incubated at 50 °C for 3 h to facilitate deproteinization (Ji et al., 2022). Alternatively, papain (at a concentration of 0.1%, *w/v*) was applied to cleave the protein components within the polysaccharide-protein mixture, resulting in protein-free PHP (Wu et al., 2020). This enzymatic process allows for precise control, permitting adjustments to digestion time and enzyme concentration as needed.

3.3. Chromatographic purification

Chromatographic purification techniques are indispensable for achieving high-purity fractions due to their exceptional ability to eliminate impurities while safeguarding the structural integrity of polysaccharides. These techniques play a critical role in various applications, including biological assays of PHP and their incorporation into

pharmaceuticals, biotechnology products, and functional foods, where the purity and specificity of PHP are of paramount importance (Xu, Huang, & Cheong, 2017). The realm of chromatographic purification techniques offers a variety of methods, each with distinct advantages in terms of selectivity and purity. Notably, gel filtration chromatography and ion-exchange chromatography methods are frequently employed in the purification process of PHP (Cheong, Qiu, Du, Liu, & Khan, 2018; Wang et al., 2023).

Gel filtration chromatography proves to be a suitable method for fractionating PHP based on their diverse molecular weights. This technique relies on the utilization of porous gel beads packed within a column, with various commercially available gel bead options (Gaborieau & Castignolles, 2011). The process entails introducing the PHP mixture into the column, where, as it traverses the gel matrix, molecules undergo separation based on their respective sizes. Larger polysaccharides are the first to elute, as they cannot penetrate the small pores within the gel beads, while smaller molecules move more slowly through the porous network and elute later (Ji et al., 2023). For instance, the commercial available Sephadex G-100 gel filtration was employed to purify a PHP fraction, resulting in a molecular weight of 523 kDa (Wang, Lin, et al., 2023). In another investigation conducted by Chen et al., Sephacryl S-400 HR gel filtration was utilized to isolate the PHP2 fraction, characterized by a backbone structure of →4)Gα(1 → 6)G4Sβ(1 → 4)Glc(1 → (Chen et al., 2023). Gel filtration chromatography offers numerous advantages, including its simplicity, the preservation of polysaccharide integrity through gentle purification conditions, and the capability to separate polysaccharides from other macromolecules such as proteins, nucleic acids, and low molecular weight compounds.

Ion exchange chromatography represents a valuable method for purifying PHP based on their ionic properties. This technique leverages the charged characteristics of PHP and their impurities (Lizeng Cheng, Wang, He, & Wei, 2018). Within a column filled with an ion exchange resin, PHP selectively bind and separate according to their ionic properties. Negatively charged polysaccharides adhere to positively charged ion exchange resins, while neutral polysaccharides pass through the column more rapidly (Zhang et al., 2004). Precise adjustments to the pH, ionic strength, and buffer conditions fine-tune the separation process. For instance, DEAE-cellulose 52 was employed to purify the PHP fraction. Initial elution with distilled water effectively removed neutral polysaccharides and proteins that did not bind to the gels. Subsequently, sulfated PHP was eluted using a 2.0 mol/L NaCl solution, resulting in purified PHP with sulfated and uronic acid contents of 14.26% and 14.02%, respectively (Shi et al., 2015). In another study by Chen et al., an ion exchange chromatograph DEAE-Sepharose FF column was employed to fractionate PHP. Elution was performed stepwise with 0.5 mol/L and 1.0 mol/L NaCl solutions, yielding PHP0.5 and PHP1.0 fractions with contents of 69.3 μg/mg and 50.7 μg/mg, respectively (Chen et al., 2021).

Chromatographic purification techniques assume a pivotal role in achieving elevated levels of purity for PHP, rendering them suitable for applications in industries like pharmaceuticals, biotechnology, and functional foods, where purity and specificity hold utmost importance.

3.4. Membrane filtration

Membrane filtration plays a pivotal role in the purification of polysaccharides, relying on semipermeable membranes with specific pore sizes to segregate molecules based on their molecular weight and size. This technique effectively rids the solution of impurities, especially those of smaller molecular size, by allowing them to permeate through the membrane. Researchers have the flexibility to tailor the filtration process by selecting membranes with the appropriate molecular weight cut-off. For instance, the polysaccharide extraction solution underwent 72 h of dialysis through a membrane with a 3500 Da molecular weight cut-off. This process effectively eliminated low molecular weight impurities, resulting in the isolation of PHP (Yun-Tao Wu et al., 2020). In

another study conducted by Chen et al., two distinct membranes were employed, one with a 3500 Da molecular weight cut-off and another with a 10,000 Da molecular weight cut-off. Dialysis for a day with these membranes removed low molecular weight impurities, leading to the isolation of PHP1 and PHP2 fractions, respectively (Chen, Tong, et al., 2021). Membrane filtration is highly regarded for its scalability, cost-effectiveness, and its ability to maintain the structural integrity of polysaccharides. As a result, it proves to be an invaluable tool for research at both laboratory-scale and industrial applications across various sectors such as food, pharmaceuticals, and biotechnology.

4. Characterization of PHP

The process of structural characterization plays a crucial role in acquiring a deeper understanding of the composition and properties of PHP. It provides valuable insights that expand our comprehension of their potential applications and bioactivity. This method encompasses the use of diverse analytical techniques to investigate elements such as monosaccharide composition, chemical structure, functional groups, molecular weight, and chain conformation within these complex biomolecules.

The utilization of a combination of analytical techniques, including High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), Fourier Transform Infrared spectroscopy (FT-IR), nuclear magnetic resonance (NMR) spectroscopy, and mass spectrometry (MS), has led to the elucidation of the distinctive chemical structure of PHP. Analysis of the monosaccharide composition, typically conducted through HPLC or GC, has revealed that PHP is primarily composed of higher levels of galactose and anhydro-galactose, while containing lower concentrations of glucose, fucose, and xylose. Dong et al. utilized a method involving the conversion of monosaccharides into their corresponding 1-phenyl-3-methyl-5-pyrazolone derivatives, followed by detection using HPLC. Their findings revealed molar ratios for PHP, galactose, glucose, and fucose, with a ratio of 76.2:2.1:1 (Dong et al., 2020). In another study, the molar ratio of galactose to fucose was reported to be approximately 92:8 (Quanbin Zhang et al., 2004). PHP derived from marine environments exhibits differences in monosaccharide composition compared to PHP originating from intestinal medicinal plants. The latter may include monosaccharides like glucose, fructose, mannose, rhamnose, and xylose. For example, garlic and onion polysaccharides predominantly consist of fructans, primarily composed of fructose (Wang & Cheong, 2023). *Aloe vera* polysaccharides, on the other hand, are primarily composed of mannose and glucose (Liu et al., 2019). Furthermore, red seaweed differs from other seaweed types due to the presence of anhydrogalactose, a key bioactive monosaccharide. Anhydrogalactose has gained recognition for its remarkable biological activities, particularly its antioxidant properties and potential for skin-whitening applications in the cosmetic industry (Cheong et al., 2018; Yun et al., 2013). The anhydro-galactose to galactose molar ratios within different species of red seaweed polysaccharides vary. For instance, *P. haitanensis*, *Gracilaria chouae*, and *Gracilaria blodgettii* polysaccharides exhibit molar ratios of approximately 1.0:1.4–1.6, while *Gelidium amansii* polysaccharides have a molar ratio of 1.0:1.0. Some red seaweeds contain relatively low amounts of anhydro-galactose, such as *Bangia fusco-purpurea* polysaccharide (Jiang et al., 2019), and *Eucheuma galetinae* has a molar ratio of 1.0:3.0 (Xie, Zhang, Liu, Chen, & Cheong, 2020).

The functional groups found in PHP, particularly the sulfate groups, play a pivotal role in mediating the interactions of these polysaccharides with biological systems, contributing to their pharmaceutical activity. FT-IR analysis is instrumental in identifying these functional groups within PHP by measuring the absorption and interaction of infrared radiation with the sample. For PHP, FT-IR analysis has successfully revealed critical chemical groups, including hydroxyl, carbonyl, and sulfate groups. Notably, distinct infrared signals in PHP were observed at 3422, 1639, 1419, 1225, 1155, 1073, 930, and 817 cm^{-1}. Specifically,

the signals at 1225 cm^{-1} and 819 cm^{-1} were attributed to the asymmetric stretching vibration of the sulfate group (Malairaj et al., 2023; Zhang et al., 2009), while the signal at 1637 cm^{-1} corresponded to the carboxyl group in uronic acid (Chen & Xue, 2019). Furthermore, the content of these functional groups can be quantified using a colorimetric method. The sulfate content of PHP can be determined through the barium sulfate turbidimetric approach, using potassium sulfate as a standard, typically ranging from 6.48% to 11.96% (Wu et al., 2020). The content of uronic acid can be ascertained using the sulfuric acid-carbazole method with glucuronic acid as the standard, and it typically falls within the range of 9.67% to 12.33% (Zheng et al., 2023).

The determination of the molecular weight of PHP using high-performance size exclusion chromatography is a fundamental aspect of polysaccharide characterization. High-performance size exclusion chromatography segregates molecules based on their hydrodynamic volume and size, enabling the determination of PHP's molecular weight (Xu et al., 2017). S Multiple studies have reported PHP molecular weights within the approximate range of 10–100 kDa. For example, Wang et al. employed an Ultrahyrogel™100 high-performance size exclusion column to determine three polysaccharide fractions, PHP-1, PHP-2, and PHP-3, using a series of standard calibration curve methods. Their findings showed molecular weights of 567.05 kDa, 414.09 kDa, and 323.80 kDa, respectively (Ji et al., 2022). Additionally, Gong et al. utilized a TSK-GEL G3000PWXL column to determine various PHP products (PHPD-I–IV), resulting in molecular weights of 329 kDa, 203 kDa, 128 kDa, and 10 kDa, respectively. (Gong et al., 2020). High molecular weights have also been reported, such as PHP0.5–1-UF and PHP1.0–1-UF, with values of 2.06 × 10^6 Da and 6.68 × 10^6 Da, respectively, determined and separated using the SB-806 M HQ column (Chen, Xu, et al., 2021). The precise determination of PHP's molecular weight assumes a crucial role in upholding quality control and consistency, ensuring that PHP-based products maintain their designated properties and efficacy.

Nuclear magnetic resonance (NMR) spectroscopy is a potent tool for unraveling the glycosidic linkages and backbone structure of intricate polysaccharides, including PHP. This methodology offers insights into the anomeric configurations, ring conformations, and branching points within the polymer. Moreover, 2D NMR techniques, such as correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond correlation spectroscopy (HMBC), are particularly valuable in discerning atomic connectivity and inter-nuclear correlations within PHP, thereby providing an intricate overview of its chemical structure (Cheng & Neiss, 2012). The chemical structure of PHP, which was previously reported, has been summarized in Fig. 2. For example, PHP1's primary linkage types were identified as →3)-β-D-galactose-4-sulfate-(1 → 3)-β-D-galactose-(1 → 6)-β-D-galactose-4-sulfate-(1 → 4)-L-Arabinose-(1 → 6)-β-D-galactose-4-sulfate-(1→, with elucidation achieved through a combination of ^{1}H NMR, ^{13}C NMR, TOCSY, COSY, and HSQC techniques (Chen, Tong, et al., 2021). Similarly, PHPD-IV-4 displayed a similar backbone, comprising repeating units of →3)-β-D-galactose-(1 → 4)-3,6-anhydro-α-L-galactose-(1→, and →3)-β-D-galactose-(1 → 4)-α-L-galactose-6-sulfate-(1→, confirmed through ^{1}H NMR, ^{13}C NMR, HSQC, and TOCSY methods (Gong et al., 2020). Reports have described the chemical backbone of PHP normally as featuring alternating (1 → 4)-linked 3,6-anhydro-α-L-galactopyranose units or (1 → 4)-linked α-L-galactose 6-sulfate units (Qiu et al., 2020; Qiu, Jiang, Fu, Ci, & Mao, 2021). However, Chen et al. introduced a notably distinct PHP type characterized as a sulfate glucogalactan, with a backbone structure of →4)-β-D-galactose-(1 → 6)-β-D-galactose-4-sulfate-(1 → 4)-β-D-glucose-(1 → and a mannose-(1 → 6)-glucose side chain, elucidated through the application of ^{1}H NMR, ^{13}C NMR, HSQC, COSY, and TOCSY techniques (Chen et al., 2023).

This in-depth structural characterization lays the foundation for understanding the PHP's properties, bioactivity, and their potential applications in diverse industries, ultimately driving further research and biotechnological advancements in this field.
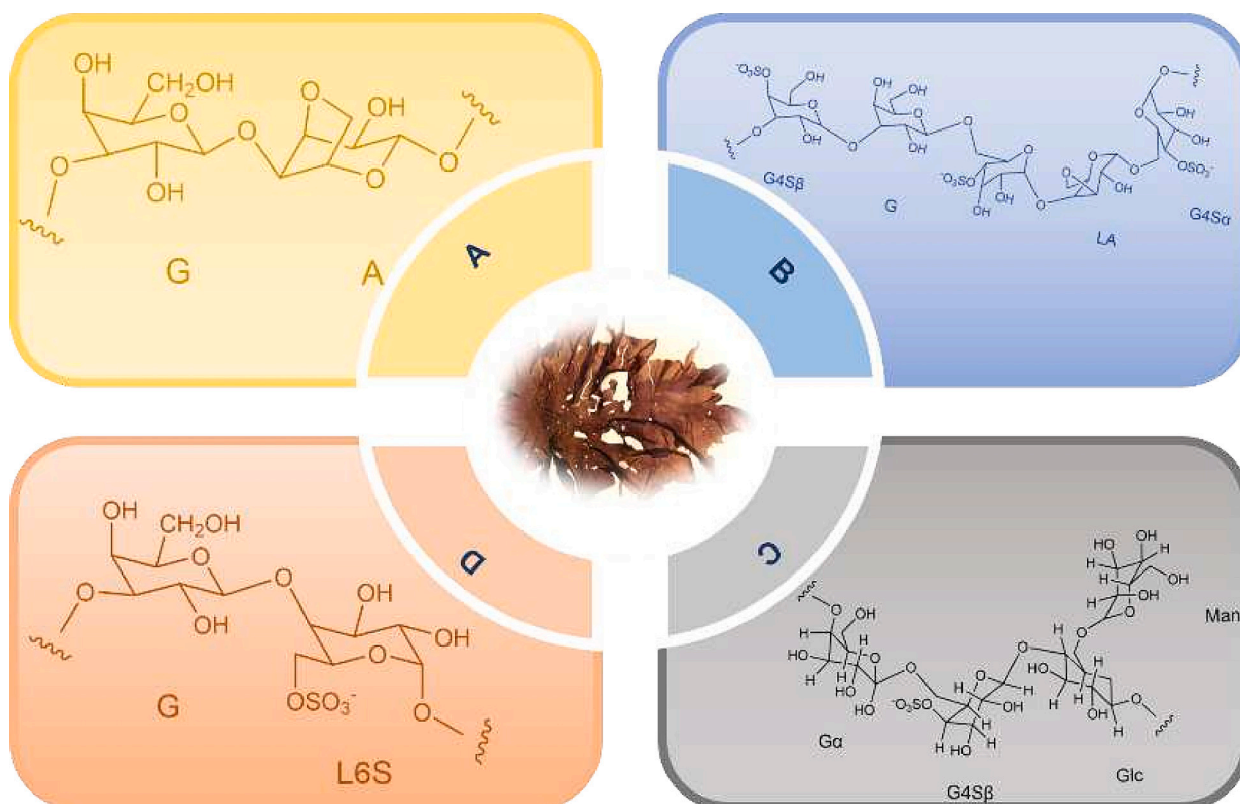

Fig. 2. Representative chemical structure unit of PHP as reported by (A) Chen et al. (B) Chen et al. (C) Gong et al. (D) Chen et al.

5. Beneficial effect of PHP on the gastrointestinal tract

5.1. PHP remains indigestible and maintains its structural integrity during the upper gastrointestinal tract's digestion

The human gastrointestinal system is a complex and indispensable part of the body, consisting of a series of organs and structures responsible for the digestion, absorption, and transport of food and essential nutrients. Its primary role is to break down ingested food into vital components like carbohydrates, proteins, and fats, which are then absorbed into the bloodstream to provide energy and necessary nutrients for bodily functions. Besides its primary nutrient absorption
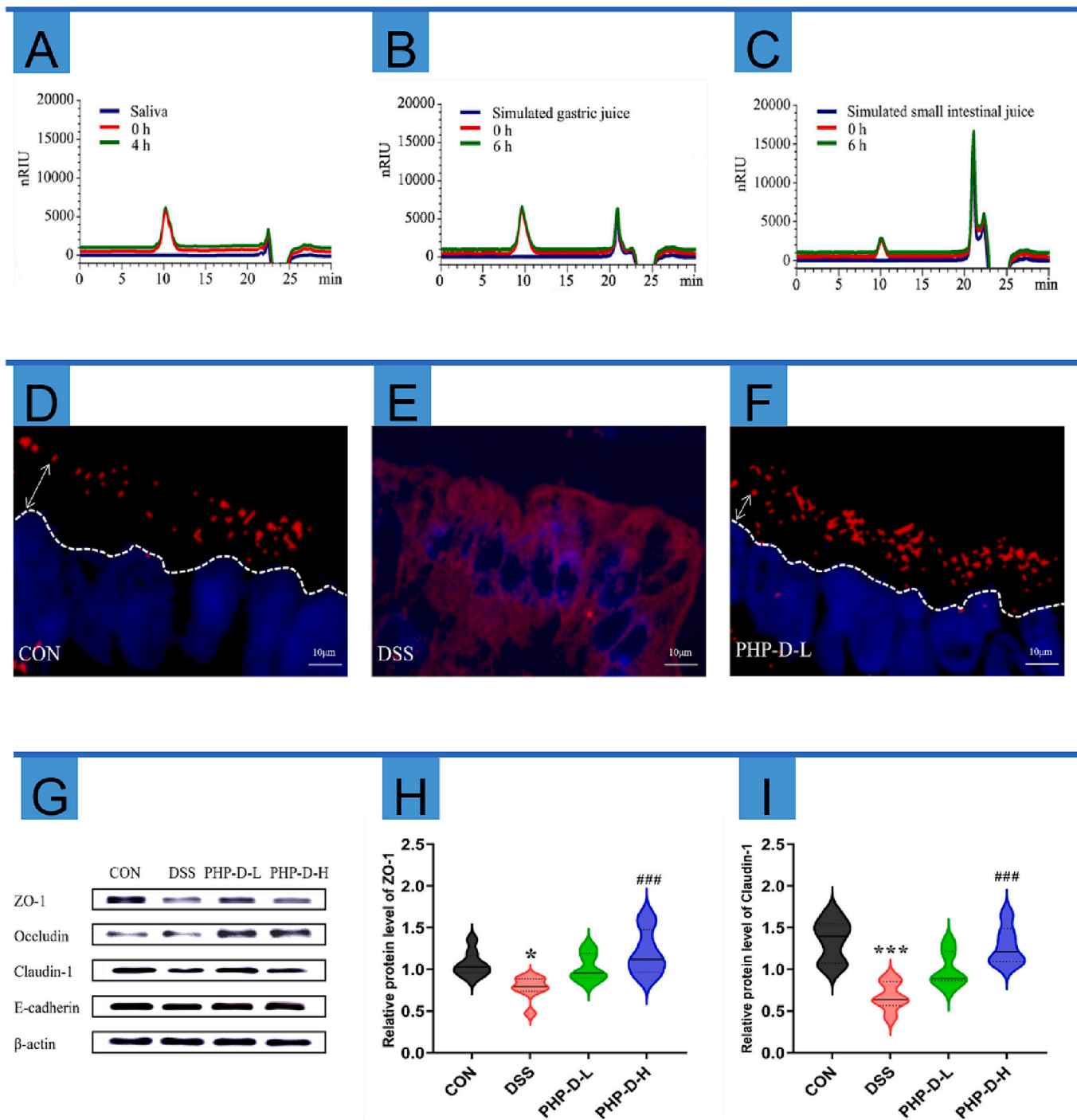
Fig. 3. PHP improves the colonic mucus barrier function. (A) PHP after digestion in saliva; (B) Simulated gastric fluid; (C) Simulated small intestinal fluid (Xu et al., 2020). "Reprinted from International Journal of Biological Macromolecules, 152, Shu-Ying Xu, Xian-Qiang Chen, Yang Liu, Kit-Leong Cheong, Ultrasonic/microwave-assisted extraction, simulated digestion, and fermentation in vitro by human intestinal flora of polysaccharides from *Porphyra haitanensis*, 748-756. Copyright 2023, with permission from Elsevier. " (D—F) Representative images of fluorescent in situ hybridization. Host nuclei are colored in blue (4′,6-diamidino-2-phenylindole, DAPI) and FISH positive bacteria are colored in red (EUB338-Cy3 probe). Scale bars are 10 μm; (G-I) Protein levels of ZO-1, occludin, claudin-1, and E-cadherin in colonic tissue are determined by Western blotting. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$ compared with the CON group. #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$, and ####$p < 0.0001$ compared with the DSS group. "Adapted with permission from (Yu et al., 2023). Copyright 2023 American Chemical Society." (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

function, the gastrointestinal system is intricately interconnected with the immune system and hosts a diverse community of gut microbiota (Obata & Pachnis, 2016; Patra, Amasheh, & Aschenbach, 2019). This complex interplay between the digestive system and overall health emphasizes the critical significance of maintaining the optimal functioning of the gastrointestinal tract (Monteiro & Batterham, 2017). Consequently, comprehending how dietary components, including PHP, can positively influence this essential system has profound implications for our general well-being.

PHP possesses a distinctive macromolecular characteristic in that it withstands digestion in the upper gastrointestinal tract (Xu et al., 2020). While simpler carbohydrates like monosaccharides and disaccharides are broken down and absorbed in the stomach and small intestine, PHP remains structurally intact. It exhibits remarkable resistance to digestion by key enzymes such as amylase and trypsin, and remains unaffected by the acidic environment of the stomach (Lovegrove et al., 2017) (Fig. 3A-C). Amylase, produced in the salivary glands and pancreas, primarily focuses on breaking down starches into glucose and maltose (Brownlee, Gill, Wilcox, Pearson, & Chater, 2018). However, PHP, with its complex monosaccharide composition mainly consisting of galactose and anhydrogalactose, evades the action of amylase (Zhang et al., 2022). Furthermore, in the stomach, the acidic environment is primarily dedicated to protein digestion rather than carbohydrate breakdown. The in vitro simulated digestion experiments involving human saliva, gastric, and small intestinal juices confirm that PHP remains structurally unaltered (Xu et al., 2020). This suggests that PHP largely preserves its integrity as it travels through the upper gastrointestinal tract, eventually reaching the large intestine. Here, it can undergo enzymatic degradation by specialized enzymes and fermentation by gut bacteria, further highlighting its unique role in gastrointestinal health and nutrition.

5.2. Effect of PHP on the gut microbiota

The gut microbiota consists of a diverse array of microorganisms inhabiting the gastrointestinal tract, making it an integral facet of human biology. This vibrant and multifaceted community includes bacteria, viruses, archaea, fungi, and a plethora of microorganisms, collectively playing a pivotal role in the maintenance of overall health (Sartor & Wu, 2017). Its constitution and variety are susceptible to influence by numerous factors, encompassing genetics, dietary habits, age, and environmental exposures (Gao et al., 2022). Unhealthy dietary patterns prevalent in contemporary lifestyles wield a profound influence on the gut microbiota, often yielding detrimental repercussions for our well-being (Kendig, Leigh, & Morris, 2021). The contemporary diet, characterized by the excessive consumption of processed foods teeming with sugars, unhealthy fats, and deficient in dietary fiber, has been correlated with a substantial transformation in the composition and diversity of the gut microbiota (Ye, Xu, & Liu, 2021). This transformation, commonly denoted as dysbiosis, involves a decline in beneficial microorganisms and an upsurge in potentially deleterious species (Petersen & Round, 2014).

A well-balanced gut microbiota has been associated with a reduced susceptibility to chronic inflammatory conditions, such as inflammatory bowel disease and autoimmune disorders (Tlaskalová-Hogenová et al., 2011). PHP has exhibited its capability to foster this equilibrium and significantly elevate the diversity of the gut microbial community, making it a pivotal factor in nurturing a more robust and resilient gut microbiota. In an in vitro fermentation analysis of PHP using the rat intestinal microbiome, the evaluation of microbial diversity through 16S rRNA gene sequencing unveiled notable increases in both the Shannon index and Simpson index (Chen, Tong, et al., 2021). These indices are instrumental in assessing microbial diversity by taking into account not only species richness but also the uniformity of their distribution (Nielsen et al., 2020), and they were significantly higher in the PHP group compared to the control group. Moreover, when examining the abundance-based coverage estimator (ACE) and Chao indexes of the two

distinct dosage groups of PHP, they outperformed not only the high-fat-diet-induced mice group but also the control group (Gong et al., 2020). Diminished microbial diversity and richness, as typically observed in individuals following high-fat diets, have been closely associated with an elevated risk of metabolic disorders, inflammation, and obesity (Araújo, Tomas, Brenner, & Sansonetti, 2017). These outcomes affirm that PHP can amplify microbial diversity and richness, characteristics often correlated with enhanced metabolic well-being, resilient immune system functionality, and a lowered risk of microbial imbalances or dysbiosis.

The dominant phyla in the gut microbiota encompass Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, and Verrucomicrobia, with Firmicutes and Bacteroidetes collectively constituting >90% of the gut microbial population (Soma Ghosh & Pramanik, 2021; Senghor, Sokhna, Ruimy, & Lagier, 2018). Bacteroidetes are particularly renowned for their specialized capability to enzymatically break down and ferment complex polysaccharides. Recent research has shown that both in vitro PHP fermentation and in vivo PHP administration lead to an increased relative abundance of Bacteroidetes within the gut microbiota (Xu et al., 2019; Zeng et al., 2023). This phenomenon can be attributed to Bacteroidetes' proficiency in metabolizing PHP. Firmicutes are also actively involved in the process of breaking down polysaccharides. However, they have developed distinct strategies to thrive in the dynamic gut environment, often competing with Bacteroidetes. Wang et al. investigated the impact of PHP on the modulation of colonic microbiota in obese mice, uncovering an increase in the relative abundance of Bacteroidetes and a decrease in Firmicutes (Wang et al., 2022). This shift in microbial composition, characterized by elevated Firmicutes and reduced Bacteroidetes, has been observed in both obese mice and humans and is reversed following weight loss induced by dietary changes (Magne et al., 2020). This observation has led to the hypothesis that an altered microbial composition, specifically a decreased presence of Bacteroidetes, may be associated with increased energy extraction from the diet and a heightened susceptibility to weight gain (Basak, Banerjee, Pathak, & Duttaroy, 2022).

PHP has been shown to enhance the population of probiotics, including *Bifidobacteria*, *Lactobacillus*, and *Lactobacilli* (Seong et al., 2019). Often referred to as beneficial bacteria, these probiotics are known for their ability to positively influence the presence of advantageous microorganisms in the gut, which, in turn, can yield a multitude of health benefits. While these beneficial bacteria may not be the primary degraders of complex polysaccharides like *Bacteroidetes*, they can still derive advantages from the presence of *Bacteroidetes*, responsible for the breakdown of these complex carbohydrates. As Bacteroidetes degrade polysaccharides, they release simpler sugar molecules and produce SCFAs as byproducts. These SCFAs not only function as an energy source for the gut microbiota but also create an environment conducive to the proliferation and activity of *Bifidobacteria* and *Lactobacillus*. These advantageous genera thrive in the presence of SCFAs and can utilize these compounds, in addition to other substrates, to support their own growth and metabolic functions. Moreover, the presence of a flourishing and diverse consortium of beneficial bacteria can effectively outcompete and supplant deleterious microorganisms. In this regard, PHP has been documented to reduce the relative prevalence of *Helicobacter* (Wang, Dong, et al., 2022) and *Escherichia-Shigella* (Chen, Tong, et al., 2021). While *Escherichia coli* is a common and generally harmless resident of the human gut, certain pathogenic strains can cause food poisoning and other gastrointestinal infections (Kaper, Nataro, & Mobley, 2004). *Shigella* species are accountable for the transmission of shigellosis, an exceptionally communicable diarrheal ailment (Bengtsson et al., 2022).

5.3. Enhancing the production of short-chain fatty acids: Functional metabolites

Intact PHP reaching the large intestine becomes a valuable substrate for various gut bacteria, many of which possess an extensive array of

carbohydrate-active enzymes (CAZymes) within their genetic makeup (Kaoutari, Armougom, Gordon, Raoult, & Henrissat, 2013). This feature is especially prominent among specific bacterial groups, such as those belonging to the phyla Bacteroidetes, Firmicutes, Actinobacteria, and Clostridia, renowned for their robust repertoire of CAZymes (Wardman, Bains, Rahfeld, & Withers, 2022). These enzymes play a crucial role in breaking down dietary fibers and complex carbohydrates, contributing not only to the host's energy metabolism but also fostering the production of SCFAs, including acetate, propionate, and butyrate (Zmora, Suez, & Elinav, 2019). The results from in vitro fermentation assays provide compelling evidence that PHP, when subjected to fermentation by human gut microbes, leads to the generation of elevated concentrations of SCFAs. The total SCFA concentration increased from 19.37 mmol/L to 32.32 mmol/L, notably featuring heightened levels of acetic, propionic, and butyric acids (Xu et al., 2019). Furthermore, in an in vitro fermentation test employing rat fecal microbiota, PHP was observed to yield substantial amounts of major SCFAs. After 24 h of fermentation, the concentrations of these SCFAs in the PHP2 fermentation group exhibited a remarkable increase ($p < 0.05$), approximately 10–15 times higher compared to the control bacterial mother liquor group (Chen et al., 2023). This favorable impact of PHP on SCFA production extends beyond in vitro experiments, as in vivo studies provide additional support for this effect. Mice administered with PHP demonstrated increased SCFA concentrations, leading to a lower pH environment. Notably, the PHP (300 mg/kg) group exhibited significantly higher concentrations of acetic acid and butyric acid compared to the control group ($p < 0.05$) (Malairaj et al., 2023).

SCFAs assume a pivotal role in diverse aspects of gastrointestinal health and overall well-being. They serve as essential energy sources for the cells lining the colon. Of these, butyrate is particularly favored as an energy substrate for colonic epithelial cells (Salvi & Cowles, 2021). Its availability is essential for maintaining the integrity of the gut barrier, which is critical in preventing the permeability of the gut lining and, subsequently, reducing the risk of toxins and harmful substances entering the bloodstream, a situation that could lead to inflammation and various gastrointestinal disorders. Furthermore, SCFAs make a significant contribution to the stability and diversity of the gut microbiome. Produced through the fermentation of polysaccharides, they establish a favorable environment characterized by a low pH, creating conditions conducive to the flourishing of beneficial gut bacteria (Deleu, Machiels, Raes, Verbeke, & Vermeire, 2021). This, in turn, aids in outcompeting potentially harmful microorganisms and helps maintain a balanced ecosystem within the gut.

Additionally, emerging research indicates that short-chain fatty acids (SCFAs) exert influence over lipid metabolism and energy utilization by operating through the gut-liver axis (Pabst et al., 2023). In this context, PHP demonstrates its potential by mitigating the effects of a high-fat diet, primarily by boosting the production of butyric acid within both the colon and liver. This augmentation has been associated with the upregulation of genes linked to phosphatidylcholine metabolites and fatty acid transport, including those involved in fatty acid transport (CD36), fatty acid oxidation (Acacb), and peroxisome proliferator-activated receptor (PPAR) gamma within the liver (Zeng et al., 2023). PPAR represents a pivotal target gene associated with the advantageous outcomes of SCFAs in addressing liver metabolic syndrome, underscoring the potential regulatory role of SCFAs (Oh, Visvalingam, & Wahli, 2019). In practical terms, PHP has demonstrated the capacity to alleviate the effects of a high-fat diet in mice, leading to reduced fat accumulation in serum, liver, and adipose tissues. This effect is attributed to the activation of PGC-1α expression (Wang, Dong, et al., 2022). It is noteworthy that SCFAs have also been reported to activate PGC-1α expression, thereby promoting improved mitochondrial function and increased energy expenditure (Zhang et al., 2023). Collectively, these mechanisms contribute to an enhanced lipid metabolism.

5.4. PHP improves the colonic mucus barrier function

The intestinal barrier encompasses several essential components, including the chemical barrier provided by the mucus layer, the physical barrier maintained by the epithelial cell layer, and the immune barrier found within the lamina propria (Dahlgren & Lennernäs, 2023). Among these constituents, mucus takes on the role of the foremost line of defense, serving as a vital guardian that restricts exposure to a multitude of threats targeting the epithelium. Originating as a gel-like secretion from specialized cells in the gastrointestinal tract, mucus serves as a versatile and protective shield. A fundamental component of this mucus layer is mucin 2 (MUC2), primarily synthesized by intestinal goblet cells situated within the epithelial cell layer (Yao, Dai, Dong, Dai, & Wu, 2021).

During colitis, inflammation often results in damage to goblet cells responsible for producing mucus. This damage can trigger changes in the composition and properties of the mucus layer, reducing its ability to maintain the intestinal barrier's integrity. Particularly in a dextran sulfate sodium (DSS)-induced colitis mouse model, mucosal tissue, crypt structures, and goblet cells are prone to damage. The reduction in mucus layer thickness in DSS-induced mice heightens the exposure of gut microbes to the immune system (Ahl et al., 2016). Interestingly, PHP has demonstrated its potential to alleviate mucosal damage, especially in the DSS model. PHP facilitates the restoration of the mucosal layer and promotes the regeneration of goblet cells (Yu et al., 2023) (Fig. 3D-F). This, in turn, leads to an increased goblet cell count, facilitating mucin replenishment and subsequent thickening of the mucus layer (Knoop & Newberry, 2018). This fortified mucus layer acts as a robust protective barrier against harmful bacteria, offering significant benefits in reducing inflammation, maintaining gut health, and preventing the recurrence of gastrointestinal disorders.

5.5. PHP enhances tight junction and adherent junction to seal the intestinal barrier

Tight junctions are critical for maintaining the integrity of the intestinal barrier, serving as barriers between adjacent epithelial cells that line the gastrointestinal tract. They act as gatekeepers, carefully regulating the passage of molecules and substances through the intercellular spaces (Balkovetz, 2006; Zihni, Mills, Matter, & Balda, 2016). In inflammatory bowel disease, any compromise to these tight junctions worsens the disruption of these structures, resulting in increased intestinal permeability of the gut lining (Schulzke et al., 2009). This heightened permeability can allow for the uncontrolled passage of bacteria, antigens, and toxins from the gut into the bloodstream, subsequently triggering an abnormal immune response and leading to chronic inflammation (Suzuki, 2013). Notably, PHP has demonstrated a positive impact on the expression and assembly of tight junction proteins. Immunochemistry investigations have revealed that mice fed PHP exhibited a significant increase in the expression of ZO-1 and occludin compared to control mice (Malairaj et al., 2023) (Fig. 3G-I). ZO-1, occludin, and claudin are pivotal proteins involved in the establishment and preservation of tight junctions within epithelial cells. ZO-1 is an essential cytoplasmic protein, which acts as a bridge connecting transmembrane proteins like occludin and claudin to the actin cytoskeleton, thereby forming a robust linkage between neighboring cells (Kuo, Odenwald, Turner, & Zuo, 2022). Elevating the levels of ZO-1 and occludin assumes considerable significance in upholding the integrity of tight junctions, thereby contributing to the overall well-being of the intestinal barrier.

Additionally, scratch wound-healing assays performed using intestinal epithelial cells-6 have shown that PHP can enhance cell migration and proliferation while also promoting the suppression of E-cadherin. These effects have been attributed to the activation of PKCβII by PHP, ultimately leading to enhanced wound healing (Hua-Mai Qiu et al., 2020). E-cadherin is a fundamental transmembrane protein crucial for fostering cell adhesion and preserving the structural integrity of tissues

(Lommel et al., 2013). It is predominantly concentrated within epithelial tissues, where it establishes adherens junctions connecting adjacent cells. E-cadherin molecules extend from one cell's surface to establish robust adhesive connections with E-cadherin molecules on neighboring cells, significantly contributing to the structural integrity of the digestive tract's lining (Liu et al., 2023).

5.6. Anti-inflammation effect of PHP within the gastrointestinal tract

The gastrointestinal immune barrier is notable for its dual role in both pathogen defense and immune tolerance maintenance. Polysaccharides have been the subject of extensive research due to their capacity to directly modulate the immune system, influencing the release of pro-inflammatory cytokines and enhancing the activity of anti-inflammatory molecules (Hou, Chen, Yang, & Ji, 2020; Yuan, Li, Huang, Fu, & Dong, 2023). Likewise, PHP has exhibited immunomodulatory properties. Notably, PHP enhances the phagocytic activity of RAW264.7 macrophages and increases the secretion of interleukin (IL)-6, IL-10, and tumor necrosis factor-alpha (TNF-α) (Liu et al., 2017). The mechanism by which PHP induces the production of nitric oxide in RAW264.7 macrophages involves the activation of the Jun N-terminal kinase (JNK) and Janus kinase (JAK2) signaling pathways (Liu et al., 2017).

Anti-inflammatory molecules such as interleukin-10 (IL-10), transforming growth factor-beta (TGF-β), and various regulatory T cells (Tregs) play a crucial role in curbing excessive immune responses and preventing autoimmunity. A prior investigation revealed that PHP not only significantly elevates the levels of cytokines IL-2, TNF-α, and IFN-γ but also modulates Th1 and Th2 responses while promoting the proliferation of the $CD4^+CD25^+$ Treg subpopulation in the spleen (Fu et al., 2019). PHP demonstrates robust immunomodulatory activity by influencing immunocyte maturation and differentiation through the NF-κB-dependent pathway (Fu et al., 2019). The pivotal role of $CD4^+$ T cells lies in their interactions with antigen-presenting cells, notably dendritic cells, for the recognition of foreign antigens or pathogens. This recognition initiates a sequence of immune responses, culminating in the activation of other immune cell types, including B cells and $CD8^+$ cytotoxic T cells. Growing evidence suggests that $CD4^+$ T lymphocytes are often hyperactive in the context of colitis, thereby contributing to intestinal inflammation (Shale, Schiering, & Powrie, 2013). Studies have revealed an increased presence of activated $CD4^+$ cells in the colons of individuals with colitis (Leung et al., 2014). In a study conducted by Yu et al., the administration of PHP-D to mice with DSS-induced colitis significantly reduced the levels of activation when compared to those in the DSS group (Yu et al., 2023).

PHP significantly contributes to the enhancement of the gastrointestinal immune barrier through indirect yet influential interactions with the gut microbiota, profoundly impacting immune function. PHP has the potential to stimulate the growth of beneficial prebiotic strains, including *Lactobacillus*, *Lactobacilli*, and *Bifidobacterium* (Seong et al., 2019; Wei et al., 2023). These specific prebiotic strains have been shown to produce antimicrobial peptides and anti-inflammatory factors, such as interleukin-10 (IL-10), within the gastrointestinal tract (Hrdý et al., 2020). These antimicrobial peptides not only target pathogenic invaders but also exert notable anti-inflammatory effects (Underwood et al., 2012). The production of IL-10 by *Bifidobacterium*, which aids in mitigating inflammation, acts as a counterbalance to pro-inflammatory signals, ultimately contributing to reduced inflammation (Yan et al., 2020). Additionally, the functional metabolite known as SCFAs functions as potent immune modulators, playing a pivotal role in regulating immune cell activity and cytokine production (Corrêa-Oliveira, Fachi, Vieira, Sato, & Vinolo, 2016). SCFAs possess the capacity to dampen excessive immune responses and decrease inflammation within the gastrointestinal tract (Li et al., 2018). This immune system modulation is instrumental in maintaining a balanced and responsive immune function, preventing unnecessary inflammation, and potentially alleviating

autoimmune conditions and allergies.

6. Preventive effect of oxidative stress on organ damage

Oxidative stress refers to a physiological condition referred by an imbalance in the generation of reactive oxygen species (ROS), including superoxide anions and hydrogen peroxide (Checa & Aran, 2020). ROS, highly reactive oxygen-containing molecules, naturally arise as byproducts of various metabolic processes in the human body. While they serve essential functions in cell signaling and immune defense, excessive ROS production, surpassing the body's antioxidant capacity, can lead to damage at the cellular and molecular levels (Apak, Özyürek, Güçlü, & Çapanoğlu, 2016). This damage disrupts normal cell functioning and can contribute to a wide array of health problems. The development of long-lasting medical conditions like cardiovascular diseases, neurodegenerative disorders, cancer, diabetes, and inflammatory conditions is intricately linked to the gradual accumulation of oxidative damage (Shatadal Ghosh, Banerjee, & Sil, 2015; Sivandzade, Prasad, Bhalerao, & Cucullo, 2019). Polysaccharides play a pivotal role in counteracting the adverse effects of ROS and in promoting overall health as part of the battle against oxidative stress (Lu, Tan, Zhong, & Cheong, 2023; Wang, Xue, & Mao, 2020).

ROS comprise a spectrum of both free radical and non-free radical oxygenated molecules, including hydrogen peroxide (H_2O_2), superoxide ($O_2\bullet^-$), singlet oxygen ($^1O_2$), and the hydroxyl radical ($\bullet OH$) (Shu Liu, Oshita, Kawabata, Makino, & Yoshimoto, 2016). Moreover, there are reactive species involving nitrogen, iron, copper, and sulfur (Mishanina, Libiad, & Banerjee, 2015). Within the realm of antioxidant research, assays that assess radical scavenging activities are fundamental. These assays often employ stable free radicals such as DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) as chemical markers for evaluating a substance's antioxidant potential (Arranz, Cert, Pérez-Jiménez, Cert, & Saura-Calixto, 2008). PHP exhibited notable antioxidant activity, with a ABTS acid radical scavenging activity of approximately 53.16% at 2 mg/mL, DPPH radical scavenging efficacy of around 34.63% at 2 mg/mL, and a hydroxyl radical scavenging potential of roughly 23.80% at 2 mg/mL (Khan et al., 2020). Polysaccharides extracted from various origins and harvest periods of *P. haitanensis* demonstrated DPPH radical scavenging activity, hydroxyl radical scavenging, and remarkable scavenging capabilities (Fig. 4A-C). Notably, at a concentration of 6 mg/mL, both PHPR and PHPX displayed scavenging rates of 83.62% and 82.86%, respectively, underscoring their exceptional scavenging abilities against reactive radicals (Ji et al., 2022).

Polysaccharides offer a notable mechanism for mitigating oxidative stress through the upregulation of endogenous antioxidants. The human body boasts a sophisticated network of these internal antioxidants, which encompass enzymes like superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), as well as non-enzymatic antioxidants like glutathione (Raish et al., 2018). These internal protectors act as the body's initial line of defense against the detrimental effects of ROS. PHP has the ability to bolster the body's resistance to oxidative stress by enhancing the production and activity of these internal defenders. To illustrate, when RAW264.7 cells were exposed to H_2O_2, there was a significant reduction in the enzyme activity of GSH-Px, SOD, and CAT ($p < 0.05$). Intriguingly, PHP with molecular weights of 524 kDa and 217 kDa were found to enhance the activities of intracellular SOD, GSH-Px, and CAT (Yin-Ting Li et al., 2020) (Fig. 4D-F). The aging process is often marked by a gradual reduction in the production of antioxidant enzymes. This decline is closely linked to the aging of cellular components, including the mitochondria, which become progressively less efficient and can lead to increased production of ROS (Trifunovic & Larsson, 2008). Significantly, PHP has demonstrated the ability to enhance the overall antioxidant capacity and the activities of key enzymes such as SOD and GSH-Px in aging mice (Zhang et al., 2004). Specifically, the administration of PHP at a dosage of 50 mg/kg increased the maximal
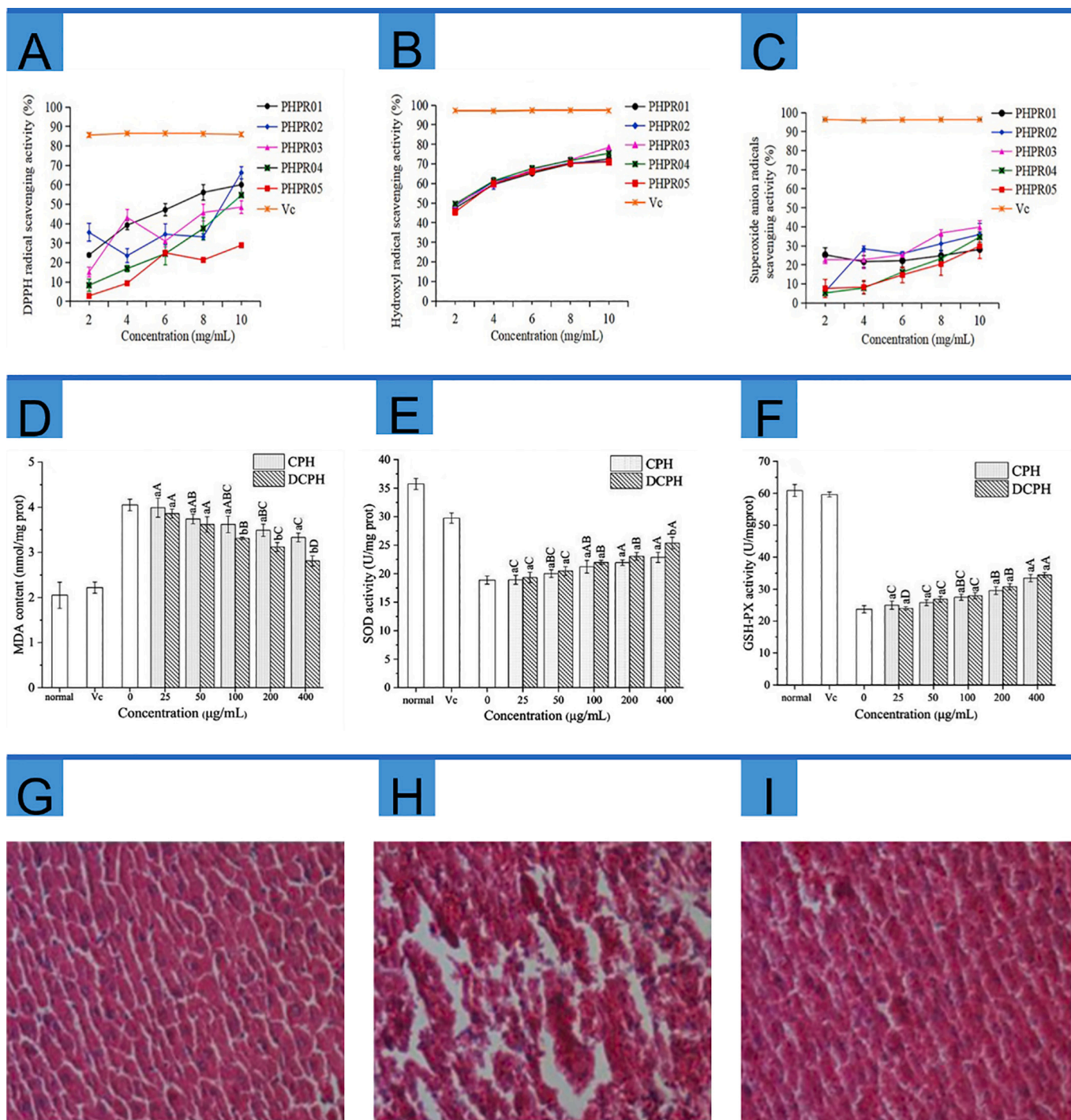
Fig. 4. Antioxidant activity of PHP. (A-C) were DPPH, hydroxyl, and superoxide of PHP in different harvest periods. "Reprinted with permission from (Ji et al., 2022). Copyright 2023 Wiley." (D) Effect of CPH and DCPH on MDA level; (E) SOD activity; (F) GSH-Px activity. "Reprinted with permission from (Li et al., 2020). Copyright 2023 Wiley." (G-H) Histopathological features of liver in the experiments. Liver tissues were stained with H&E (400 ×). (G) Normal group; (H) Model group; (F) High-dose group (Cao et al., 2016). "Reprinted from Bioactive Carbohydrates and Dietary Fibre, 8, Chunjie Cao, Meizhen Chen, Bin Liang, Jingyan Xu, Tianwen Ye & Zufeng Xia, Hypoglycemic effect of abandoned *Porphyra haitanensis* polysaccharides in alloxan-induced diabetic mice, 1-6. Copyright 2023, with permission from Elsevier. ".

activity of GSH-Px in the livers and brains of aging mice, while a dosage of 100 mg/kg maximally increased GSH-Px activity in the hearts of aging mice. Furthermore, the administration of PHP within a dose range of 100–200 mg/kg successfully inhibited lipid peroxidation, evident in the reduced production of malondialdehyde (MDA) in the livers, hearts, and brains of aging mice (Zhang et al., 2004). In diabetes, increased ROS production within liver cells can lead to oxidative stress and subsequent liver damage. In a previous study demonstrated that the administration of PHP led to an enhancement in the activities of antioxidant enzymes (SOD, GSH-Px, and GSH) in the livers of alloxan-induced diabetic mice, signifying an amelioration (Cao et al., 2016). Moreover, the histopathological examination of the liver using H&E staining in diabetic mice revealed indications of cellular damage, heightened inflammation, and potential lipid accumulation, all suggestive of oxidative stress effects.

However, following the administration of PHP to diabetic mice, these symptoms were alleviated. (Cao et al., 2016) (Fig. 4G-I).

7. Future perspective

The future outlook in the field of PHP is exceptionally promising, poised to drive scientific innovation and practical utilization. Sustainable methods for extracting and preparing PHP compounds are gaining significant prominence. With the increasing demand for these valuable substances, the development of extraction techniques that blend high efficiency with environmental responsibility is of paramount importance. A particularly encouraging strategy involves the adoption of green methods, characterized by their non-toxic and energy-efficient processes, which effectively mitigate the environmental impact associated with traditional extraction techniques. Moreover, the incorporation of high-efficiency extraction methods, such as ultrasonic or microwave-assisted extraction, can notably enhance the sustainability of PHP production. Concurrently, researchers are delving into closed-loop extraction systems to curtail waste and energy consumption, thus contributing to a more ecologically conscious and economically viable industry. Additionally, there is a growing emphasis on exploring the recycling and repurposing of extraction byproducts for diverse applications, such as biofuel production or agriculture, aimed at optimizing resource efficiency. Persistent research into the structural diversity of PHP and its connection to biological activities will remain a central focus, enabling structural modifications that enhance bioactivity or tailor these compounds for specific applications.

Moreover, the food industry stands at the threshold of incorporating PHP as natural nutraceutical and fiber enhancements, a highly promising development. The integration of PHP into the food and nutraceutical sectors holds vast potential, with these compounds serving as natural additives and ingredients, elevating the nutritional profiles and functional attributes of an extensive array of food products. As PHP is sourced from natural resources, it offers an avenue to replace synthetic additives in processed foods. This transition not only results in cleaner ingredient lists but also aligns with the surging consumer demand for natural products. In the nutraceutical domain, PHP is currently being investigated for their diverse health benefits, encompassing antioxidant, immunomodulatory, and prebiotic properties. Significantly, PHP plays a pivotal role in endorsing gut health and sustaining gut microbiota, making them invaluable inclusions in the realm of functional foods. Future research endeavors may uncover more specific health applications, further reinforcing their position in the development of functional foods and dietary supplements. As public awareness of the health benefits linked to seaweed-derived compounds continues to expand, the food and nutraceutical industries are strategically positioned to unlock the potential of marine seaweed polysaccharides in the formulation of innovative, health-enhancing products. This fosters the ongoing advancement of healthier and more functionally rich dietary options.

8. Conclusion

In conclusion, this review has shed light on the extraction and characterization of PHP, highlighting their impressive biological activities, particularly in the realms of promoting intestinal well-being and providing protection against oxidative stress. PHP have unequivocally showcased their ability to positively influence gut health by fostering a harmonious microbiome and mitigating inflammation. Furthermore, their robust antioxidant properties make them indispensable in counteracting oxidative stress, thereby reducing the susceptibility to various chronic diseases. As our understanding of the intricate interplay between gut health and oxidative stress continues to progress, the potential for leveraging polysaccharides in preventive and therapeutic approaches takes on ever greater significance. The horizon for PHP appears promising, with ongoing research poised to unravel the intricate structures and relationships that underpin their functionality.

CRediT authorship contribution statement

Kit-Leong Cheong: Writing – original draft, Investigation, Conceptualization. **Keying Liu:** Writing – review & editing, Investigation. **Wenting Chen:** Writing – review & editing, Investigation. **Saiyi Zhong:** Supervision, Project administration, Funding acquisition. **Karsoon Tan:** Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

References

Ahl, D., Liu, H., Schreiber, O., Roos, S., Phillipson, M., & Holm, L. (2016). *Lactobacillus reuteri* increases mucus thickness and ameliorates dextran sulphate sodium-induced colitis in mice. *Acta Physiologica, 217*(4), 300–310. https://doi.org/10.1111/apha.12695

Ameer, K., Shahbaz, H. M., & Kwon, J.-H. (2017). Green extraction methods for polyphenols from plant matrices and their byproducts: A review. *Comprehensive Reviews in Food Science and Food Safety, 16*(2), 295–315. https://doi.org/10.1111/1541-4337.12253

Apak, R., Özyürek, M., Güçlü, K., & Çapanoğlu, E. (2016). Antioxidant activity/capacity measurement. 3. Reactive oxygen and nitrogen species (ROS/RNS) scavenging assays, oxidative stress biomarkers, and chromatographic/Chemometric assays. *Journal of Agricultural and Food Chemistry, 64*(5), 1046–1070. https://doi.org/10.1021/acs.jafc.5b04744

Araújo, J. R., Tomas, J., Brenner, C., & Sansonetti, P. J. (2017). Impact of high-fat diet on the intestinal microbiota and small intestinal physiology before and after the onset of obesity. *Biochimie, 141*, 97–106. https://doi.org/10.1016/j.biochi.2017.05.019

Arranz, S., Cert, R., Pérez-Jiménez, J., Cert, A., & Saura-Calixto, F. (2008). Comparison between free radical scavenging capacity and oxidative stability of nut oils. *Food Chemistry, 110*(4), 985–990. https://doi.org/10.1016/j.foodchem.2008.03.021

Balkovetz, D. F. (2006). Claudins at the gate: Determinants of renal epithelial tight junction paracellular permeability. *American Journal of Physiology. Renal Physiology, 290*(3), F572–F579. https://doi.org/10.1152/ajprenal.00135.2005

Basak, S., Banerjee, A., Pathak, S., & Duttaroy, A. K. (2022). Dietary fats and the gut microbiota: Their impacts on lipid-induced metabolic syndrome. *Journal of Functional Foods, 91*, Article 105026. https://doi.org/10.1016/j.jff.2022.105026

Bengtsson, R. J., Simpkin, A. J., Pulford, C. V., Low, R., Rasko, D. A., Rigden, D. J., … Baker, K. S. (2022). Pathogenomic analyses of *Shigella* isolates inform factors limiting shigellosis prevention and control across LMICs. *Nature Microbiology, 7*(2), 251–261. https://doi.org/10.1038/s41564-021-01054-z

Blouin, N. A., Brodie, J. A., Grossman, A. C., Xu, P., & Brawley, S. H. (2011). *Porphyra*: A marine crop shaped by stress. *Trends in Plant Science, 16*(1), 29–37. https://doi.org/10.1016/j.tplants.2010.10.004

Brownlee, I. A., Gill, S., Wilcox, M. D., Pearson, J. P., & Chater, P. I. (2018). Starch digestion in the upper gastrointestinal tract of humans. *Starch - Stärke, 70*(9–10), Article 1700111. https://doi.org/10.1002/star.201700111

Cao, C., Chen, M., Liang, B., Xu, J., Ye, T., & Xia, Z. (2016). Hypoglycemic effect of abandoned *Porphyra haitanensis* polysaccharides in alloxan-induced diabetic mice. *Bioactive Carbohydrates and Dietary Fibre, 8*(1), 1–6. https://doi.org/10.1016/j.bcdf.2016.05.001

Charoensiddhi, S., Lorbeer, A. J., Lahnstein, J., Bulone, V., Franco, C. M. M., & Zhang, W. (2016). Enzyme-assisted extraction of carbohydrates from the brown alga *Ecklonia radiata*: Effect of enzyme type, pH and buffer on sugar yield and molecular weight profiles. *Process Biochemistry, 51*(10), 1503–1510. https://doi.org/10.1016/j.procbio.2016.07.014

Checa, J., & Aran, J. M. (2020). Reactive oxygen species: Drivers of physiological and pathological processes. *Journal of Inflammation Research, 13*(null), 1057–1073. https://doi.org/10.2147/JIR.S275595

Chemat, F., Abert Vian, M., Fabiano-Tixier, A.-S., Nutrizio, M., Režek Jambrak, A., Munekata, P. E. S., … Cravotto, G. (2020). A review of sustainable and intensified

techniques for extraction of food and natural products. *Green Chemistry, 22*(8), 2325–2353. https://doi.org/10.1039/C9GC03878G

Chen, P., Liu, L., Cheng, Z., Zhang, Y., Zheng, B., Hu, X., & Zeng, H. (2023). Structure elucidation and *in vitro* rat intestinal fermentation properties of a novel sulfated glucogalactan from *Porphyra haitanensis*. *Food Science and Human Wellness, 12*(2), 596–606. https://doi.org/10.1016/j.fshw.2022.07.062

Chen, P., Tong, M., Zeng, H., Zheng, B., & Hu, X. (2021). Structural characterization and *in vitro* fermentation by rat intestinal microbiota of a polysaccharide from *Porphyra haitanensis*. *Food Research International, 147*, Article 110546. https://doi.org/10.1016/j.foodres.2021.110546

Chen, P., Xu, Y., Yang, S., Chang, Q., Zheng, B., Zhang, Y., Hu, X., & Zeng, H. (2021). Application of X-ray diffraction and energy dispersive spectroscopy in the isolation of sulfated polysaccharide from Porphyra haitanensis and its antioxidant capacity under in vitro digestion. *Journal of the Science of Food and Agriculture, 101*(15), 6452–6462. https://doi.org/10.1002/jsfa.11316

Chen, Y.-Y., & Xue, Y.-T. (2019). Optimization of microwave assisted extraction, chemical characterization and antitumor activities of polysaccharides from *porphyra haitanensis*. *Carbohydrate Polymers, 206*, 179–186. https://doi.org/10.1016/j.carbpol.2018.10.093

Cheng, H. N., & Neiss, T. G. (2012). Solution NMR spectroscopy of food polysaccharides. *Polymer Reviews (Philadelphia, PA, United States), 52*(2), 81–114. https://doi.org/10.1080/15583724.2012.668154

Cheng, L., Wang, Y., He, X., & Wei, X. (2018). Preparation, structural characterization and bioactivities of se-containing polysaccharide: A review. *International Journal of Biological Macromolecules, 120*, 82–92. https://doi.org/10.1016/j.ijbiomac.2018.07.106

Cheong, K.-L., Qiu, H.-M., Du, H., Liu, Y., & Khan, B. M. (2018). Oligosaccharides derived from red seaweed: Production, properties, and potential health and cosmetic applications. *Molecules, 23*(10), 2451. https://doi.org/10.3390/molecules23102451

Cheong, K.-L., Yu, B., Chen, J., & Zhong, S. (2022). A comprehensive review of the Cardioprotective effect of marine algae polysaccharide on the gut microbiota. *Foods, 11*(22), 3550. https://doi.org/10.3390/foods11223550

Corrêa-Oliveira, R., Fachi, J. L., Vieira, A., Sato, F. T., & Vinolo, M. A. R. (2016). Regulation of immune cell function by short-chain fatty acids. *Clinical & Translational Immunology, 5*(4), Article e73. https://doi.org/10.1038/cti.2016.17

Dahlgren, D., & Lennernäs, H. (2023). Review on the effect of chemotherapy on the intestinal barrier: Epithelial permeability, mucus and bacterial translocation. *Biomedicine & Pharmacotherapy, 162*, Article 114644. https://doi.org/10.1016/j.biopha.2023.114644

Deleu, S., Machiels, K., Raes, J., Verbeke, K., & Vermeire, S. (2021). Short chain fatty acids and its producing organisms: An overlooked therapy for IBD? *eBioMedicine*, 66. https://doi.org/10.1016/j.ebiom.2021.103293

Dong, M., Jiang, Y., Wang, C., Yang, Q., Jiang, X., & Zhu, C. (2020). Determination of the extraction, physicochemical characterization, and digestibility of sulfated polysaccharides in seaweed—*Porphyra haitanensis*. *Marine Drugs, 18*(11), 539. https://doi.org/10.3390/md18110539

Flórez, N., Conde, E., & Domínguez, H. (2015). Microwave assisted water extraction of plant compounds. *Journal of Chemical Technology and Biotechnology, 90*(4), 590–607. https://doi.org/10.1002/jctb.4519

Fu, L., Qian, Y., Wang, C., Xie, M., Huang, J., & Wang, Y. (2019). Two polysaccharides from Porphyra modulate immune homeostasis by NF-κB-dependent immunocyte differentiation. *Food & Function, 10*(4), 2083–2093. https://doi.org/10.1039/C9FO00023B

Gaborieau, M., & Castignolles, P. (2011). Size-exclusion chromatography (SEC) of branched polymers and polysaccharides. *Analytical and Bioanalytical Chemistry, 399*(4), 1413–1423. https://doi.org/10.1007/s00216-010-4221-7

Gao, S., Khan, M. I., Kalsoom, F., Liu, Z., Chen, Y., & Chen, Z. (2022). Role of gene regulation and inter species interaction as a key factor in gut microbiota adaptation. *Archives of Microbiology, 204*(6), 342. https://doi.org/10.1007/s00203-022-02935-5

Generalić Mekinić, I., Šimat, V., Rathod, N. B., Hamed, I., & Čagalj, M. (2023). Algal carotenoids: Chemistry, sources, and application. *Foods, 12*(14), 2768. https://doi.org/10.3390/foods12142768

Georgiou, C. D., Zisimopoulos, D., Argyropoulou, V., Kalaitzopoulou, E., Salachas, G., & Grune, T. (2018). Protein and cell wall polysaccharide carbonyl determination by a neutral pH 2,4-dinitrophenylhydrazine-based photometric assay. *Redox Biology, 17*, 128–142. https://doi.org/10.1016/j.redox.2018.04.010

Gharibzahedi, S. M. T., Marti-Quijal, F. J., Barba, F. J., & Altintas, Z. (2022). Current emerging trends in antitumor activities of polysaccharides extracted by microwave- and ultrasound-assisted methods. *International Journal of Biological Macromolecules, 202*, 494–507. https://doi.org/10.1016/j.ijbiomac.2022.01.088

Ghosh, S., Banerjee, S., & Sil, P. C. (2015). The beneficial role of curcumin on inflammation, diabetes and neurodegenerative disease: A recent update. *Food and Chemical Toxicology, 83*, 111–124. https://doi.org/10.1016/j.fct.2015.05.022

Ghosh, S., & Pramanik, S. (2021). Structural diversity, functional aspects and future therapeutic applications of human gut microbiome. *Archives of Microbiology, 203*(9), 5281–5308. https://doi.org/10.1007/s00203-021-02516-y

Gong, G., Dang, T., Fang, J., Deng, Y., Liu, Q., Dai, W., Sun, J., Wang, L., Liu, Y., Sun, T., Song, S., Fan, L., Huang, L., & Wang, Z. (2020). Preparation, structural characterization, and bioactivity of PHPD-IV-4 derived from *Porphyra haitanensis*. *Food Chemistry, 329*, Article 127042. https://doi.org/10.1016/j.foodchem.2020.127042

Gong, G., Zhao, J., Wang, C., Wei, M., Dang, T., Deng, Y., Sun, J., Song, S., Huang, L., & Wang, Z. (2018). Structural characterization and antioxidant activities of the degradation products from *Porphyra haitanensis* polysaccharides. *Food Science and Human Wellness, 74*, 185–193. https://doi.org/10.1016/j.procbio.2018.05.022

Holkar, C. R., Jadhav, A. J., Pinjari, D. V., & Pandit, A. B. (2019). Cavitationally driven transformations: A technique of process intensification. *Industrial & Engineering Chemistry Research, 58*(15), 5797–5819. https://doi.org/10.1021/acs.iecr.8b04524

Hong, H., Fan, H., Chalamaiah, M., & Wu, J. (2019). Preparation of low-molecular-weight, collagen hydrolysates (peptides): Current progress, challenges, and future perspectives. *Food Chemistry, 301*, Article 125222. https://doi.org/10.1016/j.foodchem.2019.125222

Hou, C., Chen, L., Yang, L., & Ji, X. (2020). An insight into anti-inflammatory effects of natural polysaccharides. *International Journal of Biological Macromolecules, 153*, 248–255. https://doi.org/10.1016/j.ijbiomac.2020.02.315

Hrdý, J., Alard, J., Couturier-Maillard, A., Boulard, O., Boutillier, D., Delacre, M., Lapadatescu, C., Cesaro, A., Blanc, P., Pot, B., Ryffel, B., Chamaillard, M., & Grangette, C. (2020). *Lactobacillus reuteri* 5454 and *Bifidobacterium animalis* ssp. *lactis* 5764 improve colitis while differentially impacting dendritic cells maturation and antimicrobial responses. *Scientific Reports, 10*(1), 5345. https://doi.org/10.1038/s41598-020-62161-1

Huang, Y., Chen, H., Zhang, K., Lu, Y., Wu, Q., Chen, J., Li, Y., Wu, Q., & Chen, Y. (2022). Extraction, purification, structural characterization, and gut microbiota relationship of polysaccharides: A review. *International Journal of Biological Macromolecules, 213*, 967–986. https://doi.org/10.1016/j.ijbiomac.2022.06.049

Ji, C., Pan, C., Huang, H., Tao, F., Lin, S., Chen, S., Qi, B., Hu, X., & Yang, X. (2022). Effects of origin and harvest period on characterisation, structure and antioxidant activity of polysaccharides derived from *Porphyra haitanensis*. *International Journal of Food Science & Technology, 57*(1), 123–136. https://doi.org/10.1111/ijfs.15349

Ji, X., Peng, B., Ding, H., Cui, B., Nie, H., & Yan, Y. (2023). Purification, structure and biological activity of pumpkin polysaccharides: A review. *Food Reviews International, 39*(1), 307–319. https://doi.org/10.1080/87559129.2021.1904973

Jiang, Z., Yu, G., Liang, Y., Song, T., Zhu, Y., Ni, H., Yamaguchi, K., & Oda, T. (2019). Inhibitory effects of a sulfated polysaccharide isolated from edible red alga *Bangia fusco-purpurea* on α-amylase and α-glucosidase. *Bioscience, Biotechnology, and Biochemistry, 83*(11), 2065–2074. https://doi.org/10.1080/09168451.2019.1634515

Kaoutari, A. E., Armougom, F., Gordon, J. I., Raoult, D., & Henrissat, B. (2013). The abundance and variety of carbohydrate-active enzymes in the human gut microbiota. *Nature Reviews Microbiology, 11*(7), 497–504. https://doi.org/10.1038/nrmicro3050

Kaper, J. B., Nataro, J. P., & Mobley, H. L. T. (2004). Pathogenic *Escherichia coli*. *Nature Reviews Microbiology, 2*(2), 123–140. https://doi.org/10.1038/nrmicro818

Kendig, M. D., Leigh, S.-J., & Morris, M. J. (2021). Unravelling the impacts of western-style diets on brain, gut microbiota and cognition. *Neuroscience & Biobehavioral Reviews, 128*, 233–243. https://doi.org/10.1016/j.neubiorev.2021.05.031

Khan, B. M., Qiu, H.-M., Xu, S.-Y., Liu, Y., & Cheong, K.-L. (2020). Physicochemical characterization and antioxidant activity of sulphated polysaccharides derived from *Porphyra haitanensis*. *International Journal of Biological Macromolecules, 145*, 1155–1161. https://doi.org/10.1016/j.ijbiomac.2019.10.040

Knoop, K. A., & Newberry, R. D. (2018). Goblet cells: Multifaceted players in immunity at mucosal surfaces. *Mucosal Immunology, 11*(6), 1551–1557. https://doi.org/10.1038/s41385-018-0039-y

Kuo, W.-T., Odenwald, M. A., Turner, J. R., & Zuo, L. (2022). Tight junction proteins occludin and ZO-1 as regulators of epithelial proliferation and survival. *Annals of the New York Academy of Sciences, 1514*(1), 21–33. https://doi.org/10.1111/nyas.14798

Leung, J. M., Davenport, M., Wolff, M. J., Wiens, K. E., Abidi, W. M., Poles, M. A., … Loke, P. (2014). IL-22-producing CD4+ cells are depleted in actively inflamed colitis tissue. *Mucosal Immunology, 7*(1), 124–133. https://doi.org/10.1038/mi.2013.31

Li, M., van Esch, B. C. A. M., Wagenaar, G. T. M., Garssen, J., Folkerts, G., & Henricks, P. A. J. (2018). Pro- and anti-inflammatory effects of short chain fatty acids on immune and endothelial cells. *European Journal of Pharmacology, 831*, 52–59. https://doi.org/10.1016/j.ejphar.2018.05.003

Li, Y.-T., Huo, Y.-F., Wang, F., Wang, C., Zhu, Q., Wang, Y.-B., Fu, L.-L., & Zhou, T. (2020). Improved antioxidant and immunomodulatory activities of enzymatically degraded *Porphyra haitanensis* polysaccharides. *Journal of Food Biochemistry, 44*(5), Article e13189. https://doi.org/10.1111/jfbc.13189

Liu, B., Liu, Q.-M., Li, G.-L., Sun, L.-C., Gao, Y.-Y., Zhang, Y.-F., Liu, H., Cao, M.-J., & Liu, G.-M. (2019). The anti-diarrhea activity of red algae-originated sulphated polysaccharides on ETEC-K88 infected mice. *RSC Advances, 9*(5), 2360–2370. https://doi.org/10.1039/c8ra09247h

Liu, C., Cui, Y., Pi, F., Cheng, Y., Guo, Y., & Qian, H. (2019). Extraction, purification, structural characteristics, biological activities and pharmacological applications of Acemannan, a polysaccharide from *Aloe vera*: A review. *Molecules, 24*(8), 1554. https://doi.org/10.3390/molecules24081554

Liu, Q.-M., Xu, S.-S., Li, L., Pan, T.-M., Shi, C.-L., Liu, H., Cao, M.-J., Su, W.-J., & Liu, G.-M. (2017). *In vitro* and *in vivo* immunomodulatory activity of sulfated polysaccharide from *Porphyra haitanensis*. *Carbohydrate Polymers, 165*, 189–196. https://doi.org/10.1016/j.carbpol.2017.02.032

Liu, R., Armstrong, E., Constable, S., Buchanan, L. B., Mohammadi, A., Galiwango, R. M., … Kaul, R. (2023). Soluble E-cadherin: A marker of genital epithelial disruption. *American Journal of Reproductive Immunology, 89*(3), Article e13674. https://doi.org/10.1111/aji.13674

Liu, S., Oshita, S., Kawabata, S., Makino, Y., & Yoshimoto, T. (2016). Identification of ROS produced by Nanobubbles and their positive and negative effects on vegetable seed germination. *Langmuir, 32*(43), 11295–11302. https://doi.org/10.1021/acs.langmuir.6b01621

Lommel, M., Winterhalter, P. R., Willer, T., Dahlhoff, M., Schneider, M. R., Bartels, M. F., … Strahl, S. (2013). Protein O-mannosylation is crucial for E-cadherin–mediated cell adhesion. *Proceedings of the National Academy of Sciences, 110*(52), 21024–21029. https://doi.org/10.1073/pnas.1316753110

Lovegrove, A., Edwards, C. H., De Noni, I., Patel, H., El, S. N., Grassby, T., … Shewry, P. R. (2017). Role of polysaccharides in food, digestion, and health. *Critical Reviews in Food Science and Nutrition, 57*(2), 237–253. https://doi.org/10.1080/10408398.2014.939263

Lu, S.-Y., Tan, K., Zhong, S., & Cheong, K.-L. (2023). Marine algal polysaccharides as future potential constituents against non-alcoholic steatohepatitis. *International Journal of Biological Macromolecules, 250*, Article 126247. https://doi.org/10.1016/j.ijbiomac.2023.126247

Magne, F., Gotteland, M., Gauthier, L., Zazueta, A., Pesoa, S., Navarrete, P., & Balamurugan, R. (2020). The Firmicutes/Bacteroidetes ratio: A relevant marker of gut Dysbiosis in obese patients? *Nutrients, 12*(5), 1474. https://doi.org/10.3390/nu12051474

Malairaj, S., Veeraperumal, S., Yao, W., Subramanian, M., Tan, K., Zhong, S., & Cheong, K.-L. (2023). Porphyran from *Porphyra haitanensis* enhances intestinal barrier function and regulates gut microbiota composition. *Marine Drugs, 21*(5), 265. https://doi.org/10.3390/md21050265

Mirzadeh, M., Arianejad, M. R., & Khedmat, L. (2020). Antioxidant, antiradical, and antimicrobial activities of polysaccharides obtained by microwave-assisted extraction method: A review. *Carbohydrate Polymers, 229*, Article 115421. https://doi.org/10.1016/j.carbpol.2019.115421

Mishanina, T. V., Libiad, M., & Banerjee, R. (2015). Biogenesis of reactive sulfur species for signaling by hydrogen sulfide oxidation pathways. *Nature Chemical Biology, 11* (7), 457–464. https://doi.org/10.1038/nchembio.1834

Monteiro, M. P., & Batterham, R. L. (2017). The importance of the gastrointestinal tract in controlling food intake and regulating energy balance. *Gastroenterology, 152*(7). https://doi.org/10.1053/j.gastro.2017.01.053, 1707–1717.e1702.

More, P. R., Jambrak, A. R., & Arya, S. S. (2022). Green, environment-friendly and sustainable techniques for extraction of food bioactive compounds and waste valorization. *Trends in Food Science & Technology, 128*, 296–315. https://doi.org/10.1016/j.tifs.2022.08.016

Nadar, S. S., Rao, P., & Rathod, V. K. (2018). Enzyme assisted extraction of biomolecules as an approach to novel extraction technology: A review. *Food Research International, 108*, 309–330. https://doi.org/10.1016/j.foodres.2018.03.006

Nielsen, C. C., Gascon, M., Osornio-Vargas, A. R., Shier, C., Guttman, D. S., Becker, A. B., … Kozyrskyj, A. L. (2020). Natural environments in the urban context and gut microbiota in infants. *Environment International, 142*, Article 105881. https://doi.org/10.1016/j.envint.2020.105881

Obata, Y., & Pachnis, V. (2016). The effect of microbiota and the immune system on the development and Organization of the Enteric Nervous System. *Gastroenterology, 151* (5), 836–844. https://doi.org/10.1053/j.gastro.2016.07.044

Oh, H. Y. P., Visvalingam, V., & Wahli, W. (2019). The PPAR–microbiota–metabolic organ trilogy to fine-tune physiology. *The FASEB Journal, 33*(9), 9706–9730. https://doi.org/10.1096/fj.201802681RR

Ou, Y., Guo, Y., Chen, M., Lu, X., Guo, Z., & Zheng, B. (2023). Gut microbiome–serum metabolic profiles: Insight into the hypoglycemic effect of *Porphyra haitanensis* glycoprotein on hyperglycemic mice. *Food & Function, 14*(17), 7977–7991. https://doi.org/10.1039/d3fo02040a

Pabst, O., Hornef, M. W., Schaap, F. G., Cerovic, V., Clavel, T., & Bruns, T. (2023). Gut–liver axis: Barriers and functional circuits. *Nature Reviews Gastroenterology & Hepatology, 20*(7), 447–461. https://doi.org/10.1038/s41575-023-00771-6

Padayachee, A., Day, L., Howell, K., & Gidley, M. J. (2017). Complexity and health functionality of plant cell wall fibers from fruits and vegetables. *Critical Reviews in Food Science and Nutrition, 57*(1), 59–81. https://doi.org/10.1080/10408398.2013.850652

Pan, C., Ma, J., Tao, F., Ji, C., Zhao, Y., Chen, S., & Yang, X. (2021). Novel insight into the antioxidant proteins derived from laver (*Porphyra haitanensis*) by proteomics analysis and protein based bioinformatics. *Food Bioscience, 42*, Article 101134. https://doi.org/10.1016/j.fbio.2021.101134

Patra, A. K., Amasheh, S., & Aschenbach, J. R. (2019). Modulation of gastrointestinal barrier and nutrient transport function in farm animals by natural plant bioactive compounds – A comprehensive review. *Critical Reviews in Food Science and Nutrition, 59*(20), 3237–3266. https://doi.org/10.1080/10408398.2018.1486284

Petersen, C., & Round, J. L. (2014). Defining dysbiosis and its influence on host immunity and disease. *Cellular Microbiology, 16*(7), 1024–1033. https://doi.org/10.1111/cmi.12308

Picó, Y. (2013). Ultrasound-assisted extraction for food and environmental samples. *TrAC Trends in Analytical Chemistry, 43*, 84–99. https://doi.org/10.1016/j.trac.2012.12.005

Puri, M., Sharma, D., & Barrow, C. J. (2012). Enzyme-assisted extraction of bioactives from plants. *Trends in Biotechnology, 30*(1), 37–44. https://doi.org/10.1016/j.tibtech.2011.06.014

Qiu, H.-M., Veeraperumal, S., Lv, J.-H., Wu, T.-C., Zhang, Z.-P., Zeng, Q.-K., … Cheong, K.-L. (2020). Physicochemical properties and potential beneficial effects of porphyran from *Porphyra haitanensis* on intestinal epithelial cells. *Carbohydrate Polymers, 246*, Article 116626. https://doi.org/10.1016/j.carbpol.2020.116626

Qiu, S.-M., Aweya, J. J., Liu, X., Liu, Y., Tang, S., Zhang, W., & Cheong, K.-L. (2022). Bioactive polysaccharides from red seaweed as potent food supplements: A systematic review of their extraction, purification, and biological activities. *Carbohydrate Polymers, 275*, Article 118696. https://doi.org/10.1016/j.carbpol.2021.118696

Qiu, Y., Jiang, H., Fu, L., Ci, F., & Mao, X. (2021). Porphyran and oligo-porphyran originating from red algae Porphyra: Preparation, biological activities, and potential applications. *Food Chemistry, 349*, Article 129209. https://doi.org/10.1016/j.foodchem.2021.129209

Raish, M., Ahmad, A., Ansari, M. A., Alkharfy, K. M., Aljenoobi, F. I., Jan, B. L., … Ali, N. (2018). *Momordica charantia* polysaccharides ameliorate oxidative stress,

inflammation, and apoptosis in ethanol-induced gastritis in mucosa through NF-kB signaling pathway inhibition. *International Journal of Biological Macromolecules, 111*, 193–199. https://doi.org/10.1016/j.ijbiomac.2018.01.008

Rathore, A. S., Nikita, S., Thakur, G., & Mishra, S. (2023). Artificial intelligence and machine learning applications in biopharmaceutical manufacturing. *Trends in Biotechnology, 41*(4), 497–510. https://doi.org/10.1016/j.tibtech.2022.08.007

Rhein-Knudsen, N., Ale, M. T., & Meyer, A. S. (2015). Seaweed hydrocolloid production: An update on enzyme assisted extraction and modification technologies. *Marine Drugs, 13*(6), 3340–3359. https://doi.org/10.3390/md13063340

Salvi, P. S., & Cowles, R. A. (2021). Butyrate and the intestinal epithelium: Modulation of proliferation and inflammation in homeostasis and disease. *Cells, 10*(7), 1775. https://doi.org/10.3390/cells10071775

Sartor, R. B., & Wu, G. D. (2017). Roles for intestinal Bacteria, viruses, and Fungi in pathogenesis of inflammatory bowel diseases and therapeutic approaches. *Gastroenterology, 152*(2). https://doi.org/10.1053/j.gastro.2016.10.012, 327–339. e324.

Schulzke, J. D., Ploeger, S., Amasheh, M., Fromm, A., Zeissig, S., Troeger, H., … Fromm, M. (2009). Epithelial tight junctions in intestinal inflammation. *Annals of the New York Academy of Sciences, 1165*(1), 294–300. https://doi.org/10.1111/j.1749-6632.2009.04062.x

Senghor, B., Sokhna, C., Ruimy, R., & Lagier, J.-C. (2018). Gut microbiota diversity according to dietary habits and geographical provenance. *Human Microbiome Journal, 7-8*, 1–9. https://doi.org/10.1016/j.humic.2018.01.001

Seong, H., Bae, J.-H., Seo, J. S., Kim, S.-A., Kim, T.-J., & Han, N. S. (2019). Comparative analysis of prebiotic effects of seaweed polysaccharides laminaran, porphyran, and ulvan using *in vitro* human fecal fermentation. *Journal of Functional Foods, 57*, 408–416. https://doi.org/10.1016/j.jff.2019.04.014

Shakoor, H., Hussain, N., Younas, S., & Bilal, M. (2023). Novel strategies for extraction, purification, processing, and stability improvement of bioactive molecules. *Journal of Basic Microbiology, 63*(3–4), 276–291. https://doi.org/10.1002/jobm.202200401

Shale, M., Schiering, C., & Powrie, F. (2013). CD4+ T-cell subsets in intestinal inflammation. *Immunological Reviews, 252*(1), 164–182. https://doi.org/10.1111/imr.12039

Shi, C., Pan, T., Cao, M., Liu, Q., Zhang, L., & Liu, G. (2015). Suppression of Th2 immune responses by the sulfated polysaccharide from *Porphyra haitanensis* in tropomyosin-sensitized mice. *International Immunopharmacology, 24*(2), 211–218. https://doi.org/10.1016/j.intimp.2014.11.019

Sivandzade, F., Prasad, S., Bhalerao, A., & Cucullo, L. (2019). NRF2 and NF-κB interplay in cerebrovascular and neurodegenerative disorders: Molecular mechanisms and possible therapeutic approaches. *Redox Biology, 21*, Article 101059. https://doi.org/10.1016/j.redox.2018.11.017

Soni, A., Smith, J., Thompson, A., & Brightwell, G. (2020). Microwave-induced thermal sterilization- a review on history, technical progress, advantages and challenges as compared to the conventional methods. *Trends in Food Science & Technology, 97*, 433–442. https://doi.org/10.1016/j.tifs.2020.01.030

Sun, H., Li, C., Ni, Y., Yao, L., Jiang, H., Ren, X., Fu, Y., & Zhao, C. (2019). Ultrasonic/microwave-assisted extraction of polysaccharides from *Camptotheca acuminata* fruits and its antitumor activity. *Carbohydrate Polymers, 206*, 557–564. https://doi.org/10.1016/j.carbpol.2018.11.010

Suzuki, T. (2013). Regulation of intestinal epithelial permeability by tight junctions. *Cellular and Molecular Life Sciences, 70*(4), 631–659. https://doi.org/10.1007/s00018-012-1070-x

Tlaskalová-Hogenová, H., Štěpánková, R., Kozáková, H., Hudcovic, T., Vannucci, L., Tučková, L., … Funda, D. P. (2011). The role of gut microbiota (commensal bacteria) and the mucosal barrier in the pathogenesis of inflammatory and autoimmune diseases and cancer: Contribution of germ-free and gnotobiotic animal models of human diseases. *Cellular and molecular immunology, 8*(2), 110–120. https://doi.org/10.1038/cmi.2010.67

Trifunovic, A., & Larsson, N.-G. (2008). Mitochondrial dysfunction as a cause of ageing. *Journal of Internal Medicine, 263*(2), 167–178. https://doi.org/10.1111/j.1365-2796.2007.01905.x

Udepurkar, A. P., Clasen, C., & Kuhn, S. (2023). Emulsification mechanism in an ultrasonic microreactor: Influence of surface roughness and ultrasound frequency. *Ultrasonics Sonochemistry, 94*, Article 106323. https://doi.org/10.1016/j.ultsonch.2023.106323

Underwood, M. A., Kananurak, A., Coursodon, C. F., Adkins-Reick, C. K., Chu, H., Bennett, S. H., … Bevins, C. L. (2012). *Bifidobacterium bifidum* in a rat model of necrotizing enterocolitis: Antimicrobial peptide and protein responses. *Pediatric Research, 71*(5), 546–551. https://doi.org/10.1038/pr.2012.11

Vaes, N., Idris, M., Boesmans, W., Alves, M. M., & Melotte, V. (2022). Nerves in gastrointestinal cancer: From mechanism to modulations. *Nature Reviews Gastroenterology & Hepatology, 19*(12), 768–784. https://doi.org/10.1038/s41575-022-00669-9

Venkatraman, K. L., & Mehta, A. (2019). Health benefits and pharmacological effects of *Porphyra* species. *Plant Foods for Human Nutrition, 74*(1), 10–17. https://doi.org/10.1007/s11130-018-0707-9

Wang, C., Lin, W., Sun, Z., Sun, Y., Wang, Y., & Fu, L. (2023). *Porphyra haitanensis* polysaccharide (PH) attenuates cell hyperplasia via remodeling the cross-talk between hippo/YAP and mTOR pathways. *Food Science and Human Wellness, 12*(2), 424–430. https://doi.org/10.1016/j.fshw.2022.07.044

Wang, C., Ye, Z., Wang, Y., & Fu, L. (2022). Effect of the harvest period on the structure and anti-allergic activity of *Porphyra haitanensis* polysaccharides. *Food & Function, 13* (19), 10034–10045. https://doi.org/10.1039/d2fo01442d

Wang, M., & Cheong, K.-L. (2023). Preparation, structural characterisation, and bioactivities of Fructans: A review. *Molecules, 28*(4), 1613. https://doi.org/10.3390/molecules28041613

Wang, S.-H., Huang, C.-Y., Chen, C.-Y., Chang, C.-C., Huang, C.-Y., Dong, C.-D., & Chang, J.-S. (2021). Isolation and purification of brown algae fucoidan from *Sargassum siliquosum* and the analysis of anti-lipogenesis activity. *Biochemical Engineering Journal, 165*, Article 107798. https://doi.org/10.1016/j.bej.2020.107798

Wang, W., Xue, C., & Mao, X. (2020). Radioprotective effects and mechanisms of animal, plant and microbial polysaccharides. *International Journal of Biological Macromolecules, 153*, 373–384. https://doi.org/10.1016/j.ijbiomac.2020.02.203

Wang, X., Dong, J., Liang, W., Fang, Y., Liang, M., Xu, L., … Li, X. (2022). Porphyran from *Porphyra haitanensis* alleviates obesity by reducing lipid accumulation and modulating gut microbiota homeostasis. *Frontiers in Pharmacology, 13*. https://doi.org/10.3389/fphar.2022.942143

Wang, X., He, L., Ma, Y., Huan, L., Wang, Y., Xia, B., & Wang, G. (2020). Economically important red algae resources along the Chinese coast: History, status, and prospects for their utilization. *Algal Research, 46*, Article 101817. https://doi.org/10.1016/j.algal.2020.101817

Wang, Z., Zhong, Z., Zheng, B., Zhang, Y., & Zeng, H. (2023). Effects of *Porphyra haitanensis* polysaccharides on gelatinization and gelatinization kinetics of starches with different crystal types. *International Journal of Biological Macromolecules, 242*, Article 125117. https://doi.org/10.1016/j.ijbiomac.2023.125117

Wardman, J. F., Bains, R. K., Rahfeld, P., & Withers, S. G. (2022). Carbohydrate-active enzymes (CAZymes) in the gut microbiome. *Nature Reviews Microbiology, 20*(9), 542–556. https://doi.org/10.1038/s41579-022-00712-1

Wei, Y.-J., Fang, R.-E., Liu, J.-S., Chen, Y.-C., Lin, H.-T. V., Pan, C.-L., & Huang, C.-H. (2023). Influence of *Porphyra*-derived polysaccharides and oligosaccharides on attenuating food allergy and modulating enteric microflora in mice. *Food and Agricultural Immunology, 34*(1). https://doi.org/10.1080/09540105.2023.2248419

Wells, M. L., Potin, P., Craigie, J. S., Raven, J. A., Merchant, S. S., Helliwell, K. E., … Brawley, S. H. (2017). Algae as nutritional and functional food sources: Revisiting our understanding. *Journal of Applied Phycology, 29*(2), 949–982. https://doi.org/10.1007/s10811-016-0974-5

Wu, G.-H., Hu, T., Huang, Z.-L., & Jiang, J.-G. (2013). Characterization of water and alkali-soluble polysaccharides from *Pleurotus tuber-regium* sclerotia. *Carbohydrate Polymers, 96*(1), 284–290. https://doi.org/10.1016/j.carbpol.2013.03.036

Wu, Y.-T., Huo, Y.-F., Xu, L., Xu, Y.-Y., Wang, X.-L., & Zhou, T. (2020). Purification, characterization and antioxidant activity of polysaccharides from *Porphyra haitanensis*. *International Journal of Biological Macromolecules, 165*, 2116–2125. https://doi.org/10.1016/j.ijbiomac.2020.10.053

Xie, X.-T., Zhang, X., Liu, Y., Chen, X.-Q., & Cheong, K.-L. (2020). Quantification of 3,6-anhydro-galactose in red seaweed polysaccharides and their potential skin-whitening activity. *3. Biotech, 10*(4), 189. https://doi.org/10.1007/s13205-020-02175-8

Xu, S.-Y., Aweya, J. J., Li, N., Deng, R.-Y., Chen, W.-Y., Tang, J., & Cheong, K.-L. (2019). Microbial catabolism of *Porphyra haitanensis* polysaccharides by human gut microbiota. *Food Chemistry, 289*, 177–186. https://doi.org/10.1016/j.foodchem.2019.03.050

Xu, S.-Y., Chen, X.-Q., Liu, Y., & Cheong, K.-L. (2020). Ultrasonic/microwave-assisted extraction, simulated digestion, and fermentation in vitro by human intestinal flora of polysaccharides from *Porphyra haitanensis*. *International Journal of Biological Macromolecules, 152*, 748–756. https://doi.org/10.1016/j.ijbiomac.2020.02.305

Xu, S.-Y., Huang, X., & Cheong, K.-L. (2017). Recent advances in marine algae polysaccharides: Isolation, structure, and activities. *Marine Drugs, 15*(12), 388. https://doi.org/10.3390/md15120388

Xu, S.-Y., Liu, J.-P., Huang, X., Du, L.-P., Shi, F.-L., Dong, R., Huang, X.-T., Zheng, K., Liu, Y., & Cheong, K.-L. (2018). Ultrasonic-microwave assisted extraction, characterization and biological activity of pectin from jackfruit peel. *LWT, 90*, 577–582. https://doi.org/10.1016/j.lwt.2018.01.007

Yan, Q., Mei, J., Li, D., & Xie, J. (2022). Application of sonodynamic technology and sonosensitizers in food sterilization: A review of developments, trends and challenges. *Critical Reviews in Food Science and Nutrition*, 1–20. https://doi.org/10.1080/10408398.2022.2108368

Yan, S., Yang, B., Ross, R. P., Stanton, C., Zhang, H., Zhao, J., & Chen, W. (2020). *Bifidobacterium longum* subsp. *longum* YS108R fermented milk alleviates DSS induced colitis via anti-inflammation, mucosal barrier maintenance and gut microbiota modulation. *Journal of Functional Foods, 73*, Article 104153. https://doi.org/10.1016/j.jff.2020.104153

Yao, D., Dai, W., Dong, M., Dai, C., & Wu, S. (2021). MUC2 and related bacterial factors: Therapeutic targets for ulcerative colitis. *eBioMedicine, 74*. https://doi.org/10.1016/j.ebiom.2021.103751

Yao, W.-Z., Veeraperumal, S., Qiu, H.-M., Chen, X.-Q., & Cheong, K.-L. (2020). Anti-cancer effects of *Porphyra haitanensis* polysaccharides on human colon cancer cells via cell cycle arrest and apoptosis without causing adverse effects in vitro. *3. Biotech, 10*(9), 386. https://doi.org/10.1007/s13205-020-02379-y

Ye, Z., Xu, Y.-J., & Liu, Y. (2021). Influences of dietary oils and fats, and the accompanied minor content of components on the gut microbiota and gut inflammation: A review. *Trends in Food Science & Technology, 113*, 255–276. https://doi.org/10.1016/j.tifs.2021.05.001

Yu, B., Wang, M., Teng, B., Veeraperumal, S., Cheung, P. C.-K., Zhong, S., & Cheong, K.-L. (2023). Partially acid-hydrolyzed Porphyran improved dextran sulfate sodium-induced acute colitis by modulation of gut microbiota and enhancing the mucosal barrier. *Journal of Agricultural and Food Chemistry, 71*(19), 7299–7311. https://doi.org/10.1021/acs.jafc.2c08564

Yu, P., & Zhang, Y. (2017). Separation and purification of *Porphyra haitanensis* polysaccharide and its preliminary structural characterization. *Separation Science and Technology, 52*(11), 1835–1842. https://doi.org/10.1080/01496395.2017.1296464

Yuan, D., Li, C., Huang, Q., Fu, X., & Dong, H. (2023). Current advances in the anti-inflammatory effects and mechanisms of natural polysaccharides. *Critical Reviews in Food Science and Nutrition, 63*(22), 5890–5910. https://doi.org/10.1080/10408398.2022.2025535

Yun, E. J., Lee, S., Kim, J. H., Kim, B. B., Kim, H. T., Lee, S. H., … Kim, K. H. (2013). Enzymatic production of 3,6-anhydro-L-galactose from agarose and its purification and in vitro skin whitening and anti-inflammatory activities. *Applied Microbiology and Biotechnology, 97*(7), 2961–2970. https://doi.org/10.1007/s00253-012-4184-z

Zeng, H., Chen, P., Wang, Z., Hu, X., Zhang, Y., & Zheng, B. (2023). *Porphyra haitanensis* polysaccharides attenuates blood lipid via gut-liver Axis in diet-induced high-fat Mesocricetus auratus through multiple integrated omics. *Molecular Nutrition & Food Research, 67*(5), Article 2200638. https://doi.org/10.1002/mnfr.202200638

Zhang, H., Jiang, F., Zhang, J., Wang, W., Li, L., & Yan, J. (2022). Modulatory effects of polysaccharides from plants, marine algae and edible mushrooms on gut microbiota and related health benefits: A review. *International Journal of Biological Macromolecules, 204*, 169–192. https://doi.org/10.1016/j.ijbiomac.2022.01.166

Zhang, H.-L., Cui, S.-H., Zha, X.-Q., Bansal, V., Xue, L., Li, X.-L., Hao, R., Pan, L.-H., & Luo, J.-P. (2014). Jellyfish skin polysaccharides: Extraction and inhibitory activity on macrophage-derived foam cell formation. *Carbohydrate Polymers, 106*, 393–402. https://doi.org/10.1016/j.carbpol.2014.01.041

Zhang, Q., Li, N., Liu, X., Zhao, Z., Li, Z., & Xu, Z. (2004). The structure of a sulfated galactan from *Porphyra haitanensis* and its in vivo antioxidant activity. *Carbohydrate Research, 339*(1), 105–111. https://doi.org/10.1016/j.carres.2003.09.015

Zhang, Q., Yu, P., Li, Z., Zhang, H., Xu, Z., & Li, P. (2003). Antioxidant activities of sulfated polysaccharide fractions from *Porphyra haitanensis*. *Journal of Applied Phycology, 15*(4), 305–310. https://doi.org/10.1023/A:1025137728525

Zhang, W., Kong, L., Zhong, Z., Lin, L., Li, J., & Zheng, G. (2023). Short chain fatty acids increase fat oxidation and promote browning through β3-adrenergic receptor/AMP-activated protein kinase α signaling pathway in 3T3-L1 adipocytes. *Journal of Functional Foods, 103*, Article 105488. https://doi.org/10.1016/j.jff.2023.105488

Zhang, Z., Wang, X., Han, S., Liu, C., & Liu, F. (2018). Effect of two seaweed polysaccharides on intestinal microbiota in mice evaluated by illumina PE250 sequencing. *International Journal of Biological Macromolecules, 112*, 796–802. https://doi.org/10.1016/j.ijbiomac.2018.01.192

Zhang, Z., Wang, X., Lv, F., Xie, X., Zhang, S., Cai, C., Jia, R., Pan, Y., & Liu, F. (2020). Anti-complementary activity of a degraded sulfated heterogalactan from red alga *Pyropia haitanensis*. *International Journal of Biological Macromolecules, 147*, 527–533. https://doi.org/10.1016/j.ijbiomac.2020.01.045

Zhang, Z., Zhang, Q., Wang, J., Shi, X., Song, H., & Zhang, J. (2009). In vitro antioxidant activities of acetylated, phosphorylated and benzoylated derivatives of porphyran extracted from *Porphyra haitanensis*. *Carbohydrate Polymers, 78*(3), 449–453. https://doi.org/10.1016/j.carbpol.2009.04.026

Zheng, M., Ma, M., Yang, Y., Liu, Z., Liu, S., Hong, T., Ni, H., & Jiang, Z. (2023). Structural characterization and antioxidant activity of polysaccharides extracted from *Porphyra haitanensis* by different methods. *International Journal of Biological Macromolecules, 242*, Article 125003. https://doi.org/10.1016/j.ijbiomac.2023.125003

Zia, S., Khan, M. R., Shabbir, M. A., Aslam Maan, A., Khan, M. K. I., Nadeem, M., … Aadil, R. M. (2022). An inclusive overview of advanced thermal and nonthermal extraction techniques for bioactive compounds in food and food-related matrices. *Food Reviews International, 38*(6), 1166–1196. https://doi.org/10.1080/87559129.2020.1772283

Zihni, C., Mills, C., Matter, K., & Balda, M. S. (2016). Tight junctions: From simple barriers to multifunctional molecular gates. *Nature Reviews Molecular Cell Biology, 17*(9), 564–580. https://doi.org/10.1038/nrm.2016.80

Zmora, N., Suez, J., & Elinav, E. (2019). You are what you eat: Diet, health and the gut microbiota. *Nature Reviews Gastroenterology & Hepatology, 16*(1), 35–56. https://doi.org/10.1038/s41575-018-0061-2