THE POTENTIAL OF SELECTED MARINE SEAWEEDS AS PREBIOTIC

YVONNE CHWEE LI YAN

THESIS SUBMITTED IN PARTIAL FULFILLMENT FOR THE BACHELOR DEGREE OF FOOD SCIENCE WITH HONOURS (FOOD SCIENCE AND NUTRITION)

SCHOOL OF FOOD SCIENCE AND NUTRITION
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2012
BORANG PENGENESAHAN STATUS TESIS

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30 May 2012

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Yvonne Chwee Li Yan
30 May 2012
The study was aimed to evaluate the prebiotic potential of acid-hydrolyzed water-soluble polysaccharides derived from brown, red and green seaweeds (Sargassum polycystum, Kappaphycus alverazii and Caulerpa lentillifera). The polysaccharides were extracted and hydrolyzed using acetic acid and sulphuric acid with three different hydrolysis times. Acid hydrolyzed substrates were evaluated for prebiotic activity based on the growth of Lactobacillus acidophilus within 24h. Growth pattern of L. acidophilus was determined from the acid hydrolyzed substrates every 4h interval. Prolonged fermentation at 37°C and cold storage (4°C) was performed to evaluate the effect of seaweeds substrates on the survivability of L. acidophilus. K. alverazii has the highest extraction yield (59.4% dry weight), followed by C. lentillifera (3.8%) and S. polycystum (2.55%). Most of the seaweed substrates were found to be able to positively stimulate the growth of L. acidophilus. The results indicated that all three species of seaweed polysaccharides hydrolyzed by using 1.38M sulphuric acid could give pronounced effect on the growth of L. acidophilus, both in the growth rate and the total count, even higher than the commercial prebiotic inulin. Prolonged fermentation showed that acetic acid hydrolyzed polysaccharides would give more protective effect on probiotic compared to sulphuric acid hydrolyzed polysaccharides while all seaweeds samples managed to enhance survivability of probiotic during refrigerated storage. Overall, the current results show that acid-hydrolyzed polysaccharides from seaweeds have prebiotic potential and different degree of hydrolysis for different polysaccharides gave different effect on prebiotic activity. Further studies should be done to evaluate the prebiotic potential by inoculating with faecal/intestinal material, which could assess the seaweed polysaccharides effect in modulation of gut microbiota.
ABSTRAK

POTENSI RUMPAI LAUT TERTENTU SEBAGAI PREBIOTIK

Penyelidikan ini bertujuan untuk menilai potensi prebiotik polisakarida larut air daripada rumpai laut perang, merah dan hijau (Sargassum polycystum, Kappaphycus alverazii dan Caulerpa lentillifera) yang telah dihidrolisiskan dengan asid. Ekstrak polisakaride dihidrolisis dengan menggunakan asid asetik dan asid sulfurik dengan tiga masa hidrolisis yang berbeza. Substrak yang telah dihidrolisiskan dengan asid difermentasikan dengan menggunakan Lactobacillus acidophilus. Substrak yang menunjukkan potensi fermentasi yang tinggi dipilih untuk mengkaji lengkungan penumbuhan L. acidophilus. Fermentasi pemanjangan pada 37°C and penyimpanan sejuk (4°C) dijalankan untuk menilai efek ekstrak rumpai laut pada kebolehhidupan L. acidophilus. K.alverazii mempunyai hasil pengekstrakan yang paling tinggi (59.4% berat kering), seterusnya C.lentillifera (3.8%) dan S.polycystum (2.55%). Kebanyakan substrak rumpai laut dapat merangsang penumbuhan L. acidophilus. Polisakarida daripada ketiga-tiga jenis species rumpai laut yang dihidrolisis dengan 1.38M asid sulfurik dapat memberi penumbuhan yang baik kepada L.acidophilus dari segi kadar penumbuhan dan jumlah penumbuhan, lebih baik daripada komersial prebiotik, inulin. Fermentasi pemanjangan menunjukkan bahawa polisakarida yang dihidrolisiskan oleh asid acetik dapat memberi efek perlindungan kepada probiotik, lebih baik daripada polisakarida yang telah dihidrolisiskan oleh asid sulfuric sementara semua sample rumpai laut dapat meningkatkan kebolehhidupan semasa penyimpanan sejuk (4°C). Keputusan menunjukkan bahawa polisakarida yang dihidrolisiskan oleh asid mempunyai potensi prebiotik dan darjah hidrolisis yang berlainan memberi kesan aktiviti prebiotik yang berlainan. Kajian pada masa depan harus dijalankan dengan menilai efek prebiotik dengan najis/bahan intestin. Ini dapat menunjukkan keberkesanan polisakarida rumpai laut dalam modulasi mikrobiota saluran gastrousus.
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LIST OF SYMBOLS

%  Percent
€  Euro
µg  Microgram
Ca  Calcium
CFU  Colony Forming Unit
cm  Centimeter
d  day
g  Gram
h  Hour
HCl  Hydrochloride acid
H₂SO₄  Sulphuric acid
m  Meter
M  Mol
mg  Milligram
mins  Minutes
ml  Milliliter
N  Normality
n.d  Not detected
NaOH  Sodium hydroxide
nm  Nanometer
°C  Degree Celsius
p  Significant level
RM  Ringgit Malaysia
rpm  Rotary per minute
t  Time
v  Volume
wt  Weight
x  Unknown
X  times
y  Unknown
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CHAPTER 1

INTRODUCTION

Marine macroalgae, commonly known as seaweeds, are increasingly gaining interest as potential sources of bioactive compounds with immense importance in pharmaceutical, biomedical and nutraceutical (Veena et al., 2006). Seaweeds are a good source of healthy food due to their low content in lipids, high concentration in polysaccharides, natural richness in minerals, polyunsaturated fatty acids, vitamins, and bioactive molecules (Gupta and Abu-Ghannam, 2011). Since seaweed species are rich in beneficial nutrients, they have been commonly utilized in human alimentation (since ancient times) in countries such as China, Japan, and Korea (Lahaye, 1991). The major usage of seaweeds in Western countries is on the extraction of compounds to be used by pharmaceutical, cosmetics, and food industries as a source of phycocolloids, thickening and gelling agent (Mabeau and Fleurence, 1993; Jiménez-Escrig and Sánchez-Muniz, 2000). However, in recent decades, there has been an increase in the direct consumption of seaweed as food in Western countries and most recently as components of functional foods (Shahidi, 2009).

Seaweeds possess a wealth of bioactive compounds that could be potentially exploited as functional ingredients for both human and animal health application (Gupta and Abu-Ghannam, 2011). In recent years, food and nutritional science has progressed from identifying and improving nutritional deficiency to designing foods that are beneficial to promote optimal health and preventing the risk of diseases (Holdt and Kraan, 2011). Studies focus on bioactive compounds of seaweeds include evaluation of antioxidant activities (Zhang et al., 2010; Kim et al., 2007; Wu et al., 2005), antimicrobial activity (Damonte et al., 2004; Harden et al., 2009; Ohta et al., 2009), anticoagulant activity (Chandia and Matsuhiro, 2008; Hayakawa et al., 2000; Mao et al., 2009), and antiproliferation activities (Yuan and Walsh, 2006; Yang et al., 2008). Recently, it has been suggested that marine
seaweeds could have had prebiotic potential (Sullivan et al., 2010; Gupta and Abu-Ghannam, 2011; Mussatto and Mancilha, 2007).

Seaweeds contain large amounts of polysaccharides and are particularly rich in the soluble fraction, which mostly are not digestible by human because the gastrointestinal tract does not produce the required degradation enzymes (Gómez-Ordóñez et al., 2010). Since the polysaccharides are non-digestible, they can be regarded as dietary fiber. The seaweed polysaccharides are mainly used by the food industry as texture modifiers due to their high viscosity and gelling properties (Jiménez-Escrig and Sánchez-Muniz, 2000). Dietary fibres are classified as either soluble or insoluble. Soluble fibre can be characterized by its ability to form viscous gels in the intestinal tract while insoluble fibre is characterized by its faecal-bulking capacity. Both fibres have the ability to bind water or mineral cations and may be used by colonic microflora as fermentable substrate (Davidson and McDonald, 1998). Hence, it would be of great interest to determine whether seaweed polysaccharides have prebiotic potential.

The gastrointestinal microbiota is made up of diverse and complex microbial communities including bacteria, fungi and protozoa which play a key role in host’s overall health (Possemiers et al., 2009). The gut microbiota involved in metabolic activities and physiological regulation such as promotion of nutrient absorption, synthesis of bioactive compounds, improvement of intestinal barrier function, motilility, resistance to pathogens or modulation of the immune system (Grimoud et al., 2010). Gut bacteria can be categorized as either beneficial or potentially pathogenic. Undesirable changes on the composition of microbiota may leads to some direct or indirect digestive pathologies such as infectious diseases and chronic inflammation (Packey and Sartor, 2009), metabolic disorders (Cani and Delzenne, 2009) or atopic diseases (Penders et al., 2007). Thus, recognition of health-promoting properties of certain gut microorganisms has advocate dietary-based modulation of the human intestinal microbiota towards a more beneficial composition and metabolism (Gibson and McCartney, 1998). This has led to the development of probiotics and prebiotics which aim to restore or to maintain the intestinal ecosystem. The probiotic approach advocates the use of living organisms.
while prebiotic aim at increasing the amount of health-promoting bacteria in the gut by the intake of certain non-digestible carbohydrate (Wallace et al., 2011).

The market for prebiotics in food is growing rapidly with oligofructose dominating the prebiotics market in terms of volume sales with 871,000 tonnes globally in 2009 (Euromonitor, 2010). A prebiotic is "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host wellbeing and health" (Gibson et al., 2004). This definition considers the microflora changes in the whole gastrointestinal tract and thus, extrapolates the definition into other areas that may benefit from a selective targeting of particular microorganisms. The target genera are lactobacilli and bifidobacteria but prebiotic success has predominantly been with bifidobacteria. This is probably because there are usually more bifidobacteria in the human colon than lactobacilli and they exhibit a preference for oligosaccharides (Gibson et al., 2010). For a food component to be considered as prebiotic, it must fulfill the criteria for prebiotic classification. The criteria are resistance to digestive processes in the upper part of the gastrointestinal tract, fermentation by intestinal microflora, and selective stimulation of the growth and/or activity of a limited number of the health-promoting bacteria in the microbiota (Gibson et al., 2004).

Any food ingredient that is non-digestible and enters the large intestine is a candidate prebiotic. Non-digestible food ingredients include polysaccharide-type carbohydrates such as resistant starch and dietary fibre, and also proteins and lipids. However, current prebiotics are limited to non-digestible oligosaccharides, many of which seem to confer the degree of fermentation selectivity that is required (Gibson et al., 2010). Oligosaccharides are defined as molecules with a degree of polymerization (DP) between 2 and 10 residues, but DP up to 20-25 are often considered as oligosaccharides (Courtois, 2009). Not all dietary nondigestible carbohydrates and dietary fibre are prebiotic. Candidate prebiotic should be tested using standardized methodologies to obtain reliable and biologically meaningful data to demonstrate fulfillment of the criteria for prebiotic classification.
(Roberfroid, 2007) which include extensive in vitro and in vivo testing across a number of subject species, including human beings (Douglas and Sanders, 2008).

Commercially available prebiotic fibres include inulin, fructooligosaccharides, polydextrose, some granular resistant starches, xanthan gum and pectin (Euromonitor, 2010). There are some oligosaccharides that fulfill the prebiotic criteria and are confirmed prebiotics, namely fructans such as inulin-type fructans, galactans such as trans-galactooligosaccharides and lactulose. For most confirmed prebiotic, both in vitro and animal data are demonstrated. There are also some dietary ingredients that are candidate prebiotics but evidence in fulfilling the prebiotic criteria is not as convincing as fructans or galactans. Several independent human trials from different researchers may be needed to form definitive assessment of prebiotic effect of the candidate prebiotics. Examples of candidate prebiotics are polydextrose, soybean oligosaccharides, lactosucrose, isomaltooligosaccharides, glucans, and xylooligosaccharides (Gibson et al., 2010).

Some prebiotics occur naturally in several foods such as leeks, asparagus, chicory, Jerusalem artichokes, garlic, onions, wheat, oats, and soybeans (Manning and Gibson, 2004). Numerous studies have reported prebiotic activity from plant-derived oligosaccharides such as pectic oligosaccharides extracted from bergamot peel (Mandalari et al., 2007), oligosaccharides from durum wheat (Napolitano, 2009), almond seeds (Mandalari et al., 2008), blueberry extracts (Molan et al., 2009), honey oligosaccharides (Sanz et al., 2005), and oligosaccharides from pitaya flesh (Wichienchot et al., 2010). However, few studies have specifically examined the prebiotic potential of seaweed-derived oligosaccharides (Ramnani et al., 2011; Wu et al., 2007; Deville et al., 2007; Michel et al., 1996). Relatively little is known on the fermentation characteristics and potential beneficial effects of seaweeds oligosaccharides and whether they exhibit similar properties to the current confirmed prebiotic such as inulin and galacto-oligosaccharides. While prebiotic activity must be ultimately determined in vivo, in vitro studies are useful for preliminary screening of candidate compounds (Sullivan et al., 2010). The aim of this study is to select the variety of seaweeds with highest prebiotic potential after performing in vitro test. The success of the investigation could recognize the
candidate compounds to be used for further study of prebiotic activity such as in animal study and human trial. If prebiotic activity is found, it would add to the value of seaweeds as a functional food. Besides, seaweeds’ oligosaccharides may be a new ingredient contributing to the growing prebiotic industry. The specific objectives are:

1. To determine the effect of acid hydrolysis on polysaccharides extracted from Sargassum polycystum, Kappaphycus alverazii and Caulerpa lentillifera

2. To evaluate the prebiotic activity of acid-hydrolyzed seaweed polysaccharides using pure culture growth experiments

3. To evaluate the effects of acid-hydrolyzed seaweed polysaccharides on the survivability of Lactobacillus acidophilus after prolonged fermentation and cold storage
2.1 Seaweed Industry

Around 500 species of marine algae with mostly seaweeds have been used for centuries for human food, medicinal purpose and indirectly by the phycocolloid industry (agars, carrageenans, and alginates). Seaweeds are involved in multibillion-dollar enterprise that is very diversified, including food, brewing, textile, pharmaceutical, animal feed, bioactive and antiviral compounds, and biotechnological sectors (Chopin and Sawhney, 2009). In 2008, the highest production of cultured seaweed was of Japanese kelp (*Laminaria japonica*, 4.8 million tonnes) which are mostly used as food, second highest production is *Eucheuma* seaweeds (*Kappaphycus alverazii* and *Eucheuma* spp., 3.8 million tonnes) which are mostly used for phycocolloids extraction, followed by Wakame (*Undaria pinnatifida*, 1.8 million tonnes), *Gracilaria* spp. (1.4 million tonnes) and *Nori* (*Porphyra* spp., 1.4 million tonnes) (FAO, 2010). Table 2.1 shows the biomass, products and value of the main components of world’s seaweed-derived industry in 2006. Vast majority of seaweed species has yet to be screened for various applications, and their extensive diversity ensures that many new algal products and processes will be discovered (Chopin and Sawhney, 2009).

World total production of aquatic plants in 2008 is 15.8 million tonnes (live weight equivalent), with a total estimated value of US$ 7.4 billion. Of the world total production of aquatic plant, 93.8% came from aquaculture where the production is mostly dominated by seaweeds (99.6% by quantity and 99.3% by value in 2008). Seaweed culture production is dominated by countries in East and Southeast Asia with 99.8% by quantity and 99.5% by value in 2008. The major seaweed producer is China which accounted for 62.9% of the world's aquaculture production of seaweeds by quantity. Other major seaweed producers included Indonesia (13.7%), Philippines (10.6%), North Korea (5.9%), Japan (2.9%) and South Korea (2.8%). Indonesia replaced Philippines as the world’s second-largest
seaweed producer in 2007. Almost all cultured seaweed species in East Asia are for human consumption while seaweed farming in Southeast Asia is mainly to produce raw material for carrageenan extraction. Outside Asia, Chile is the most important seaweed culturing country by producing 21,700 tonnes in 2008. Africa produced 14,700 tonnes of farmed seaweeds in 2008 (FAO, 2010).

Table 2.1: Biomass, products and value of the main components of world’s seaweed-derived industry in 2006

<table>
<thead>
<tr>
<th>Industry component</th>
<th>Raw material (wet tonnes)</th>
<th>Product (tonnes)</th>
<th>Value (US $)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sea vegetables</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kombu (Laminaria)</td>
<td>8.59 million</td>
<td>1.42 million</td>
<td>5.29 billion</td>
</tr>
<tr>
<td>Nori (Porphyra)</td>
<td>4.52 million</td>
<td>1.08 million</td>
<td>2.75 billion</td>
</tr>
<tr>
<td>Wakame (Undaria)</td>
<td>1.40 million</td>
<td>141,556</td>
<td>1.34 billion</td>
</tr>
<tr>
<td></td>
<td>2.52 million</td>
<td>166,320</td>
<td>1.02 billion</td>
</tr>
<tr>
<td>Phycocolloids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carrageenans</td>
<td>1.26 million</td>
<td>70,630</td>
<td>650 million</td>
</tr>
<tr>
<td>Alginites</td>
<td>528,000</td>
<td>33,000</td>
<td>300 million</td>
</tr>
<tr>
<td>Agars</td>
<td>600,000</td>
<td>30,000</td>
<td>213 million</td>
</tr>
<tr>
<td></td>
<td>127,167</td>
<td>7,630</td>
<td>137 million</td>
</tr>
<tr>
<td>Phycosupplements</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil additives</td>
<td>1.22 million</td>
<td>242,600</td>
<td>53 million</td>
</tr>
<tr>
<td>Agrichemicals (Fertilizers, biostimulants)</td>
<td>1.10 million</td>
<td>220,000</td>
<td>30 million</td>
</tr>
<tr>
<td>Animal feeds (supplements, ingredients)</td>
<td>20,000</td>
<td>2,000</td>
<td>10 million</td>
</tr>
<tr>
<td>Pharmaceuticals nutraceuticals, botanicals, cosmeceuticals, pigments, bioactive compounds, antiviral agents, brewing, etc.</td>
<td>100,000</td>
<td>20,000</td>
<td>10 million</td>
</tr>
<tr>
<td></td>
<td>3,000</td>
<td>600</td>
<td>3 million</td>
</tr>
</tbody>
</table>

Source: Chopin and Sawhney (2009)

The farming of seaweed has expanded rapidly as demand has exceeded the supply available from natural resources (McHugh, 2003). As demand increases, natural populations often become overexploited and the need for cultivation of appropriate species emerges. Cultivation technology can be low-tech, but still extremely successful with highly efficient and simple culture techniques, coupled with intensive labour at low cost or can become highly advanced and mechanized, requiring on-land cultivation system for seeding some phases of the life history
before growth-out at open-sea aquaculture sites. Cultivation technology would also depend on the selected species, their biology, life history, level of tissue specialization and the socioeconomic situation of the region where it is developed (Chopin and Sawhney, 2009).

**2.1.1 Potential Growth of Seaweed Industry in Malaysia**

Seaweed cultivation has increasingly become an economically important natural resource for Malaysia, particularly for Sabah since Sabah is only state that produces seaweeds in Malaysia. Seaweed-farming activities in Sabah are essential not only to improve the income of fishermen or an effective tool for poverty eradication, but also for the conservation of coral reefs (Sade *et al.*, 2006). Table 2.2 shows the cultivation area, estimated aquaculture production and estimated wholesale value of seaweeds in Malaysia from year 2004 to year 2009. From the table, it shows that the cultivation area, production and value of seaweeds are steadily increased almost every year.

**Table 2.2: Cultivation area, estimated aquaculture production and estimated wholesale value of seaweeds in Malaysia**

<table>
<thead>
<tr>
<th>Year</th>
<th>Culture area (hectares)</th>
<th>Production, wet weight (tonnes)</th>
<th>Value (RM, million)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>986.02</td>
<td>30,956.90</td>
<td>15.48</td>
</tr>
<tr>
<td>2005</td>
<td>2,368.89</td>
<td>31,426.20</td>
<td>15.71</td>
</tr>
<tr>
<td>2006</td>
<td>5,949.37</td>
<td>43,200.00</td>
<td>6.91</td>
</tr>
<tr>
<td>2007</td>
<td>6,684.19</td>
<td>90,268.50</td>
<td>18.05</td>
</tr>
<tr>
<td>2008</td>
<td>7,730.57</td>
<td>111,298.20</td>
<td>22.26</td>
</tr>
<tr>
<td>2009</td>
<td>7,538.46</td>
<td>138,855.90</td>
<td>27.77</td>
</tr>
</tbody>
</table>


Sabah is well-placed for seaweed farming due to its strategic location in the Coral Triangle together with Indonesia and Philippines. This triangle supplies nearly 80% of Kappaphycus seaweed (PEMANDU, 2010). In 2010, around 20,000 metric tonne of dried seaweed value at RM 60 million was produced and are the source of income for 1,200 families at the coastal area of Sabah (Nor *et al.*, n.d). Under the 10th Malaysia Plan, seaweed farming has been identified as one of the Entry Point Project (EPP) to be given focus under the National Key Economic Area (NKEA). The aim is to transform the seaweed industry to a high-yielding and commercial scale
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