Master's Thesis Report

"Optimization of Solid-State Fermentation process for brown seaweed using LAB and characterization of the products."

January 2023 – June 2023

SWETA SINGH

LUNDS
UNIVERSITET

Examiner: Prof. Eva Nordberg Karlsson
Supervisor: Dr. Supansa Westman

Table of content

I. ACKNOWLEDGEMENT 4

II. ABBREVIATIONS 5

III. SUMMARY 6

IV. AIM 7

1 INTRODUCTION 8

1.1 Fermentation (history) 8
1.2 Benefits of Fermentation 8
1.3 Importance of fermentation 8
1.4 Products of fermentation 9
1.4.1 Yogurt 9
1.4.2 Indian - Idli and Dosa 9
1.4.3 Kimchi 9
1.4.4 Kombucha 9
1.4.5 Sauerkraut 10
1.4.6 Sourdough 10
1.4.7 Kefir 10
1.4.8 Cheese 10
1.5 Process explanation 11
1.5.1 Aerobic fermentation 11
1.5.2 Anaerobic fermentation 11
1.5.3 Lactic acid fermentation 11
1.6 Solid state fermentation 13
1.7 Lactic Acid Bacteria 13
1.8 *Lactobacillus plantarum* 13
1.9 Seaweeds 14
1.9.1 Types of seaweed 14
1.10 Importance of seaweed fermentation for human Consumption 16

2 MATERIALS & METHODOLOGIES 17

2.1 Materials 17
2.1.1 Substrate (Seaweed biomass) preparation 17
2.1.2 Lactic acid bacteria 18
2.1.3 Chemicals 18
2.2 Methodologies 18
2.2.1 Media Preparation 18
2.2.2 Inoculum preparation 18
2.2.3 Fermenter preparation 19
2.2.4 Solid-State Fermentation of seaweed 19
2.2.5 pH measurement 20
2.2.6 Total colony plate count 21
2.2.7 Short chain fatty acid analysis 22
2.2.8 Sample preparation for proximate analysis (Total solid, Moisture, Oven Dry Weight and Total ash content). 23

3 RESULTS 25

3.1 Microbiological Profiling via CFU Determination 25
3.2 Seaweed Fermentation 25
3.2.1 pH Trends and Variability during Fermentation 25
3.2.2 Determination of presence of SCFA by HPLC 27
3.2.3 Proximate Analysis of seaweed after fermentation 29

4 DISCUSSION 30

4.1 ROLE OF IN *COMMERCIAL LACTOBACILLUS* SEAWEED FERMENTATION 30
4.1.1 CFU count of inoculum Commercial lactobacillus *30*
4.2 SOLID-STATE FERMENTATION OF *ALARIA ESCULENTA* AND *SACCHARINA LATISSIMA* 30
4.3 PH TRENDS DURING SEAWEED FERMENTATION 32
4.3.1 Experiment 2 ..... *32*
4.3.2 Experiment 3 ..... *32*
4.3.3 Experiment 4 ..... *33*
4.4 SHORT CHAIN FATTY ACID QUANTIFICATION 33
4.5 PROXIMATE ANALYSIS – TOTAL SOLID, MOISTURE, ODW, TOTAL ASH CONTENT 34
5 CONCLUSION 36
6 FUTURE WORK 37
7 REFERENCES 38
8 APPENDICES 46

I. Acknowledgement

This master's thesis was performed in collaboration with Division of biotechnology at Lund University and Aventure AB in Sweden. The project was conducted for 22 weeks from January 2023 to June 2023.

I would like to acknowledge and express my sincere gratitude to my supervisor, Dr. Supansa Westman, who made this work possible and gave me advice and encouraging input throughout my research. I also like to thank my examiner, Prof. Eva Nordberg Karlsson, for giving me the chance to work at Lund University's Division of Biotechnology.

Additionally, I would like to express my sincere gratitude to Olof Böök of Aventure AB for giving me the wonderful opportunity to join the company as a master's thesis student. For me, it was a wonderful opportunity to learn.

Finally, I would want to thank everyone at the department who assisted me with my thesis and my friends who encouraged me to finish this project.

II. Abbreviations

1. Spp - species
2. SCOBY - symbiotic culture of bacteria and yeast
3. NAS - National Ocean Service
4. SSF – solid-state fermentation
5. LAB – lactic acid bacteria
6. pH – potential of hydrogen
7. UB pH meter – ultrabasic pH meter
8. Hrs – hours
9. % - percentage
10. ℃ - degree Celsius
11. Gm - grams
12. OD – optical density
13. m – Meter
14. µm – Micrometre
15. mL – Milli Litre
16. mg – milli gram
17. µL – Micro Litre
18. mL/min – milli litre per minute
19. RPM – rotation per minute
20. Min - minutes
21. ODW – Oven Dry Weight
22. MRS – De Man, Rogosa and Sharpe
23. DW – Dry weight
24. w/w – weight by weight
25. CFU – colony forming unit
26. Nm – Nanometre
27. M – molar
28. HPLC – High performance liquid chromatography
29. HPAEC-PAD - high-performance anion-exchange chromatography with pulsed amperometric detection
30. SCFA – short chain fatty acids
31. NG – Non – grounded sample
32. G – Grounded sample
33. C – control
34. V3 – strain *Commercial lactobacillus*
35. St. dev – standard deviation

III. Summary

Fermentation has played a vital role in human history, offering solutions for food preservation, culinary diversity, and the creation of diverse and flavorful foods and beverages. Its importance continues today, both in traditional practices and in modern industrial applications.

This report outlines the outcomes of an investigation into and the optimization of the solid-state fermentation process for Alaria esculenta and Saccharina latissima seaweeds using lactic acid bacteria (LAB) as an inoculum. Considering brown seaweeds have unique biochemical characteristics and are regarded as a valuable source of sustainable food in European culture, brown seaweeds are specifically researched in this investigation. Cultures of *Commercial lactobacillus* strain, a commercially acquired freeze-dried lactic acid bacteria was used for the process of fermentation. Analysis was also done on 2 types of particulate size state namely grounded and non-grounded to study the effect of surface area for fermentation.

In the process of fermentation, lactic acid bacteria (LAB) engage with seaweed specimens, instigating diverse biochemical reactions yielding valuable compounds, augment nutritional profiles, or modify texture and flavour of seaweed. The examination of the fermentation process across various seaweed species, enables comparison of their fermentation characteristics, assessment of microbial activities, and potential identification of disparities in both process and final product. exemplified by Alaria esculenta and Saccharina latissima, notable differences for fermentation process to reach desirable pH was not same and significant colour change was observed on prolonged fermentation.

The process helps in breakdown of carbohydrate substrate, mannitol, inherent in seaweeds undergo enzymatic breakdown by the applied strain, yielding a spectrum of short-chain fatty acids (SCFAs) namely lactic acid, propionic acid, formic acid, butyric acid, to name a few. High-Performance Liquid Chromatography (HPLC) analysis revealed lactic acid and formic acid as the predominant SCFAs. Notably, lactic acid exhibited a substantial quantitative predominance over formic acid.

Within the investigations, comprehensive analyses were conducted for all samples , encompassing assessments of total solids, moisture content, organic dry weight (ODW), and total ash.
These examinations hold significance in food product characterization, serving as critical metrics for industrial setting to evaluation of product quality, surveillance of fermentation efficacy, quantification of moisture diminution, and the determination of nutrient concentration and uniformity in the ultimate product.

IV. Aim

The aim of my master thesis is to optimize solid-state fermentation process using two seaweed species (*Alaria esculenta* and *Saccharina latissima)* and characterizing produced Products."

During my analysis, It is to be examined if seaweed state – grounded and non-grounded and the environment temperature affects the process of solid-state fermentation.

In the first stage *Commercial lactobacillus* strain (lactic acid bacteria-LAB) will be grown in De Man, Rogosa and Sharpe (MRS) media in anaerobic condition using freeze-dried strain. After successfully growing the LAB strain, the next step involves using the strain to ferment both the seaweed samples in their different forms – grounded and non-grounded and at different temperature.

The second stage of the experiment will entail the analysis of the fermented sample using HPLC, which implies the detection of various short chain fatty acids created during the fermentation. The investigation of SCFA manufacturing was the goal.

Later, the total solid content, ODW, total moisture content and ash content of the seaweed samples was analyzed.

1 Introduction

1.1 Fermentation (history)

Fermented foods and beverages are staples in human diet and have been produced and consumed since emergence of human civilizations [1]. Fermentation techniques has been used for centuries to extend the shelf life of food during harsh seasons, for ritual feasts and to enhance the sensory properties [2]. Beverage like fermentation of milk, cereals and other substances are indigenous to different regions of Asia, Africa, Europe, the Middle East and South America. These fermented foods and beverages are often well-preserved and are known for their health promoting qualities as they contain stable sources of proteins, vitamins, and other nutrients [3]. Despite these similar benefits, there are a number of variations in substrates, end products, and species of microbes used in the production of fermented products as per regions and availability of resources. These variations are result of geographical distribution and industrialization, both of which have an impact on fermented food production [4] [5].

1.2 Benefits of Fermentation

Fermented foods are of great significance in civilizations throughout history. Fermented foods are defined as consumable products produced through controlled microbial growth, and conversion of food components through enzymatic action [6]. The below listed are few benefits in human nutrition: -

1. Fermentation enriches human dietary in terms of flavors, aromas, and textures in food namely fermented milk; cheese; wines, meat-based sauces and pastes; and fish-based sauces and pastes [7].
2. Preservation of food through lactic acid, alcoholic, acetic acid, and alkaline fermentations for survival in harsh conditions like winters in arctic regions (Vikings).
3. Biological enrichment of food substrates with protein, essential amino acids, essential fatty acids, and vitamins like in Indonesian tape Ketan fermentation [8].
4. Detoxification during fermentation processing.
5. Reduction in cooking time and fuel needs [9].

1.3 Importance of fermentation

From earliest available data and evidences from surveys in the past, it is clear that nutritional diseases are one of the major health problems around the world especially in developing and under developed countries. Nutritional diseases refer to abnormalities caused by absence, insufficient or excess of one or more nutrients[10]. The continuous growth in the global population, coupled with climate change and unequal access to adequate nutrition are few factors that further add impact to different kinds of nutritional problems [11]. Fermented foods can have direct or indirect impact on nutrition-related disorders since it increases protein content; vitamins content such as thiamine, riboflavin, folic acid. Nevertheless, as microorganisms employed in fermentations consume carbohydrates, by which availability of calories is reduced [12]. Fermented dairy products with high nutritional value are considered “The pearls in crown of the dairy industry” [13]. According to a report from ReportLiner, a market research company, it is expected that the global commercial seaweed market to grow at a compound annual growth rate of 9.7% between 2020 and 2025 [14].

1.4 Products of fermentation

1.4.1 Yogurt

It has a smooth, semi fluid with a subtle walnut taste and is perhaps most common type of fermented food. It is mild acidic fermented milk, where sub species namely *Streptococcus thermophilus* and *Lactobacillus delbrueckii* are employed*. Bulgaricus* work together in symbiotic cultures to ferment the milk to form yogurt[15]. Both species helps each other grow until stable equilibrium is reached, together they work to transform lactose naturally present in milk into lactic acid, creating yogurt as end product. The amount of lactose that is converted to lactic acid determines how acidic yogurt will be [16]. When milk is sufficiently acidified, caseins (milk proteins), start to clump together, changing the milk's consistency to become yogurt. The bacterial strain in yogurt inhibits the growth of other germs that would typically contaminate milk and cause it to deteriorate. Hence, fermentation has been proven to be a way of conservation [17]. It preserves nutrients like protein, minerals, and wide range of vitamins present in milk. It is prepared from cow, goat, or buffalo milk [18]. It has been produced and enjoyed throughout middle east, Europe and Asia and has since become an important part of global diet for people [19].

1.4.2 Indian - Idli and Dosa

Both are made by lactic fermentation of a thick batter made from grounded polished rice and dehulled black gram dhal, a pulse (*Phaseolus mungo*). This is example of natural fermentation and does not require yeast as a starter culture. *L. mesenteroides* and *S. faecalis* develop during soaking, then continue to multiply following grinding. Idli are cupcakes which are soft, moist, and spongy and have a pleasant sour flavour. Dosa, is a closely related product, which is made from the same ingredients, and both are finely grounded. The batter is spread thin onto a heated pan, it resembles pancake but sour in flavour [20].

1.4.3 Kimchi

It is a traditional and well-known ethnic fermented food in Korea. It is a fermented vegetable-based food containing probiotic lactic acid bacteria that is made at low temperature to promote proper ripening and preservation [21]. Kimchi has a characteristic taste, which imparts sour, sweet, and carbonated flavors. It is of different varieties depending upon the types of raw material which can be cabbage, radish, cucumber and garlic [22]. *Leuconostoc, Weissella,* and *Lactobacillus* genera of *lactic acid bacteria* are major contributors to fermentation of kimchi [23]. An essential element used is fermented fish sauce that supplies enzymes during the process. Types of microflorae developed in kimchi is influenced by addition of salt, garlic, and red pepper. Preservation of vegetables is maintained during storage period by production of organic acids at expense of carbohydrates and resulting pH drop [24].

1.4.4 Kombucha

Kombucha is fermented tea beverage reported to have originated in Northeast China around 220 BC. Upon gaining popularity, similar fermented tea beverages have subsequently become popular in Russia and eastern Europe. It is associated with different health benefits and can protect against a number of metabolic and infectious diseases [25]. Kombucha is produced

by fermentation of Black tea / green tea (belongs to *Theaceae family)* with white sugar [26]. Traditional kombucha are produced aerobically using symbiotic culture of bacteria and yeast (SCOBY). The bacteria and yeast work together to produce this beverage [27]. In this process of fermentation, yeast converts sucrose to ethanol and byproducts namely organic acids and carbon dioxide. Acetic acid bacteria then use these organic acids to produce acetaldehyde and acetic acid [28]. Acetic acid bacteria *(Acetobacter, Gluconobacter),* yeasts *(Saccharomyces, Zygosaccharomyces),* and lactic acid bacteria *(Lactobacillus, Lactococcus)* are the most common bacterial and fungal species that make up the SCOBY [29] [30].

1.4.5 Sauerkraut

Sauerkraut means sour cabbage. It is a common and oldest forms of preserving cabbage and can be traced back as food to 4th century BC. It contains large quantity of lactic acid, tyramines, vitamins, and has low on calories [31]. Shredded cabbage along with 2.3%−3.0% salt is left to undergo spontaneous fermentation which results in sauerkraut. Species namely *Leuconostoc* spp*., Lactobacillus* spp*., and Pediococcus* spp. takes part in the fermentation [32].

1.4.6 Sourdough

It is a mixture of wheat flour and water fermented with yeasts & lactic acid bacteria (LAB). The process is characterized by acid production, aroma, and leavening [33]. Sourdough bread has traditionally been used as leavening agent in bread making. Sourdough is used in industry to improve bread quality, structure and stability of baked goods and eliminate additives [34]. Fermentation during making of sourdough influences nutritional quality by decreasing or increasing levels of compounds, enhancing or retarding bioavailability of nutrients. [35]. Through enzymatic breakdown of proteins, sourdough also increases the organoleptic and nutritional quality of baked goods, facilitates better digestion, and reduces the immunogenicity of dough [35]. Additionally, it has positive effects on gut health through a variety of ways, including dietary fiber complex modulation and fermentation pattern that results, production of exopolysaccharides with prebiotic characteristics, and others [36].

1.4.7 Kefir

It is fermented milk drink with creamy texture, sour taste and subtle effervescence which originated in Caucasus Mountains. A starter culture known as "kefir grains" is added to milk to start the process of fermentation. Kefir grains are made up of lactic and acetic acid producing bacteria, symbiotic lactose-fermenting yeasts (such as *Kluyveromyces marxianus*), non-lactose fermenting yeasts (such as *Saccharomyces cerevisiae*, and *Saccharomyces unisporus*), housed within polysaccharide and protein matrix called kefiran [37]. The byproducts of fermentation contribute to the organoleptic qualities of kefir include lactic acids, flavor-producing substances (such as acetaldehyde), ethanol, and carbon dioxide [38].

1.4.8 Cheese

It is ancient traditional fresh or fermented dairy product with long history of production [39]. Each cheese has its unique flavoured compound composition [40].Feta cheese, is one of the significant and popular dairy products in Greece with worldwide acceptance. It is a soft, white cheese that is typically brine-ripened [13]. In order to make cheese, raw materials namely milk, cream, or partially skimmed buttermilk is first curdled before whey separation. In

general, it is made by fermenting milk, which converts proteins (mostly casein), carbohydrates, and lipids, and right amount of lactic acid bacteria (LAB) starter and rennet. The curdled solids are then left to set for long durarions to mature after separated from whey.

1.5 Process explanation

Fermentation is metabolic process in which carbohydrates are consumed in absence of oxygen, and transformation of these chemical components by microbes (bacteria or yeast) creates energy, acid, or alcohol [41]. The kind of fermentation is determined by the microorganisms used for process and respective byproducts produced. There are three main types of fermentation, namely Lactic acid fermentation, Acetic acid fermentation and Alcoholic fermentation. All types of fermentation have their significant impact on humans [42].

1.5.1 Aerobic fermentation

It is a common type of fermentation that normally occurs in beginning of all fermentations, in the presence of oxygen [43]. Oxygen is an essential element for proliferation and development of microorganisms, such as yeast, bacteria etc. [44]. It is commonly used in production of products like beer wine, bread, and yogurt[45]. Aerobic fermentation is shorter and more intense process in compared to anaerobic fermentation [46].

1.5.2 Anaerobic fermentation

Anaerobic fermentation occurs in absence of oxygen environment [45]. Microbes are employed to produce energy from carbohydrates or other organic compounds, through anaerobic fermentation [47]. Lactic acid & ethanol fermentation are two types of anaerobic fermentation [47]. The oxygen is replaced with N_2, CO_2 or any other fermentation byproduct during the process. [48]. It is a slower process compared to aerobic fermentation.[48].

1.5.3 Lactic acid fermentation

Lactic acid fermentation is a metabolic process takes place in some microorganisms, such as bacteria and yeast, where there is no presence of oxygen [49]. Lactic acid is main byproduct of this process, which is produced by breakdown of glucose or other carbohydrates [49]. It is also referred to as lacto-fermentation. It is one of common and easily followed practice in home preservation [50]. Before refrigeration and contemporary canning techniques were developed, lactic acid fermentation was one of the techniques used to preserve dairy goods, vegetables, and meat for lengthy periods of time [50] It is still employed in wide variety of products in food and beverage industrial setups today [50]. The lactic acid fermentations are typically inexpensive and frequently require little to no heat during preparation, making them very energy-efficient [20]. Every continent in world relies heavily on lactic acid-fermented foods to feed its population demands [20]. It is used in production of wide range of fermented food products with cabbage (sauerkraut, Korean kimchi), cucumbers, cereal yogurt (Nigerian ogi, Kenyan uji); sourdough bread and bread-like products made without wheat or rye flours (Indian idli, Philippine puto); fermented milks (yogurts and cheeses); fermented milk-wheat mixtures (Egyptian kishk, Greek trahanas); and many more [20].

During lactic acid fermentation, simple carbohydrates are broken down to create lactic acid, along with carbon dioxide, ethanol, and occasionally acetic acid by the bacteria through a series of reactions [51].The lactic acid fermentation begins with the glycolysis, which starts with glucose being phosphorylated, which produces glucose-6-phosphate [52]. This molecule is further transformed into fructose-6-phosphate, which is then converted to fructose-1,6-bisphosphate [52]. From this point, phosphoglyceraldehyde (PGAL) and dihydroxyacetone phosphate (DHAP), which can be changed into one another through a reversible reaction, are produced [52]. The next stages of anaerobic glycolysis require PGAL, it is engaged in oxidation of inorganic phosphate and NAD, which produces 1,3-bisphosphoglycerate and NADH as a byproduct [52]. When 1,3-bisphosphoglycerate is transformed into 3-phosphoglycerate and then 2-phosphoglycerate, mechanism continues, and one ATP molecule is simultaneously produced [52]. Following dehydrogenation of one water molecule, the 2-phosphoglycerate produces phosphoenolpyruvate (PEP) [52]. Anaerobic fermentation of glucose results in conversion of PEP to pyruvate along with creation of another ATP molecule, which produces energy [52], [53]. In above glycolysis process two molecules of energy currency adenosine triphosphate (ATP), and two molecules NADH is produced in the process [53]. The conversion of pyruvate, an intermediate product, into lactic acid is last stage in this, this step is catalyzed by enzyme lactate dehydrogenase [54] [49]. Pyruvate is converted to lactate by receiving electrons from NADH (nicotinamide adenine dinucleotide), which is produced during glycolysis [53]. This reaction regenerates NAD+ (nicotinamide adenine dinucleotide), allowing glycolysis to continue in the absence of oxygen [53], [49].

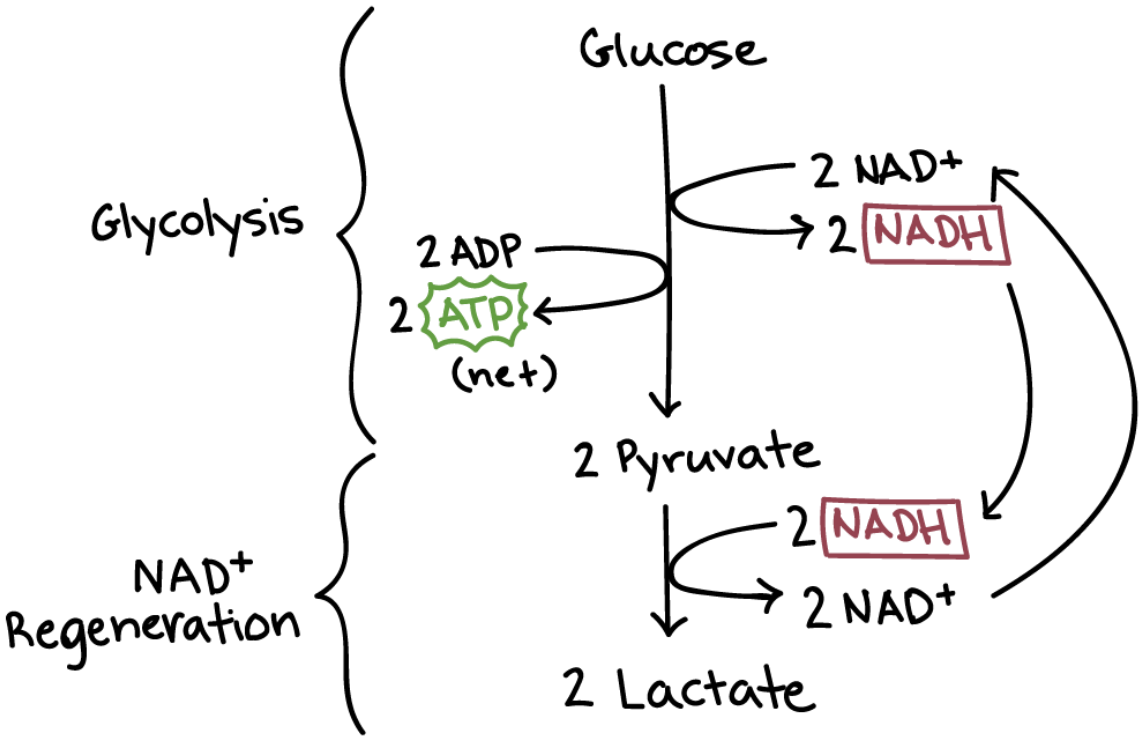

Figure 1 metabolism of lactic acid fermentation

1.6 Solid state fermentation

The broad definition of solid-state fermentation (SSF) is development of microbes on moist solid materials in lack or close to absence of free water [55] . However, substrate needs to have enough moisture for microbial growth and metabolism [55]. SSF has been used ambiguously as solid-state fermentation or solid-substrate fermentation [55]. In recent years, it has shown great promise in development of numerous bioprocesses and products [56]. Solid-state fermentation (SSF) can improve functional characteristics of waste or byproducts [57]. Compared to submerged fermentation (SmF), solid-state fermentation (SSF) offers numerous advantages, encompassing enhanced yields and productivities, extended product stability, reduced production costs, mitigated protein breakdown, minimized contamination risks, decreased energy requirements, lower sterilization energy costs, reduced fermenter volume, and diminished or absent catabolite repression [58]. Moreover, SSF enables a broader range of opportunities for utilizing unprocessed agro-industrial wastes and by-products as raw materials, which holds significant importance for ensuring economic viability, considering that raw materials often represent a substantial portion of operational expenses in enzyme production processes [58]. Despite of all the advantages, there is one major challenge related to characteristics of heterogeneous systems, where it is practically impossible to take equal samples in different places during fermentation to determine concentrations of biomass, substrate, and products [59].

1.7 Lactic Acid Bacteria

Lactic acid bacteria (LAB) are gram-positive, non-spore forming fermentative bacteria which grows anaerobically [60]. *LAB* is one of the most significant probiotic microorganisms which is commonly found in the human GI tract [60]. They are generally fastidious on artificial media, but they grow easily in most food substrates and quickly lower pH to a point where competing organisms cannot grow [20]. They excel at breaking down lactose found in milk it to lactic acid and byproducts. Lactic acid bacteria transform milk into completely different product by altering its flavor and texture [61].

1.8 *Lactobacillus plantarum*

Lactobacillus plantarum is one of probiotic bacteria that is present in oral cavity and digestive trachea in human digestive system [62]. It is common and easily adaptable type of lactic acid bacterium [63]. *Lactobacillus plantarum* are gram-positive, non-motile, and non-spore-forming bacteria [64]. The *Lactobacillus. plantarum* cell are straight rods with rounded ends, that measures 3.0-8.0 μm and found alone, in pairs, or in short chains [64]. *Lactobacillus. plantarum* is employed in fermentations, such as yogurt, kimchi, sauerkraut, and kefir. This bacteria helps to improve the sensory quality and shelf life of the fermented products [65]. A few strains are also employed as probiotics in animals and human applications [65]. It aids in digestion, nutrient absorption, and management of "bad" gastrointestinal microbes that could otherwise lead to disease [66].

1.9 Seaweeds

seaweeds are multicellular, macroscopic, eukaryotic, and autotrophic organisms [67]. According to National Ocean Service (NAS), numerous species of marine plants and algae, which thrive in ocean, river, lake as well as other water bodies are collectively referred as "seaweed". Seaweeds as a whole comprise of different sizes; microscopic like phytoplankton, medium size like red, green, brown or black algae or enormous like giant kelp [68]. Seaweeds have many benefits like sustainability and cost-effectiveness such as, high biomass production, quick growth rate, positive carbon balance, and absence of rivalry with ecologic flora and fauna. In terms of dry weight (DW), seaweeds are 80–90% water, of which 50% (carbohydrates), 1% (lipids), and 7–38% (minerals). In cases where 10 to 47% DW, protein concentration varies substantially amongst groups and is primarily made up of important amino acids [55].

1.9.1 Types of seaweed

Based on color of thallus, seaweeds are taxonomically divided into 3 types namely Chlorophyta (green algae), Rhodophyta (red algae), and Phaeophyta (brown algae). In process to store energy, they build up starch and polysaccharides inside cells [67]. Due to their size and ease of collection, brown seaweed has drawn more study attention than other types of algae and are used more commonly for animal feeding. Due to their high mineral content and low protein content (up to about 14%), brown algae have a lower nutritional value than other species [69]. Despite this, brown algae do contain many bioactive chemicals [69]. The crude protein content of red seaweeds can reach up to 50%, and protein content of green seaweeds can reach up to 30% [69]. Brown seaweed has a higher iodine content than red and green seaweed, and kelps can have extremely high levels [70].

1.9.1.1 Alaria esculenta

Alaria esculenta, a type of brown sea kelp, is common and grows well as winter crop in chilly climates with strong water exchange [71]. Due to its structure of mid rib and wavy membrane it is named winged kelp. Figure 2 [71]. Typically, winged kelp can reach lengths of 2-3 meters. On either side of midrib, it branches into wavy leaves that can reach width of 7 cm. It grows at pace of 5.5% each day, which is higher compared to other similar species. These can hold about 2 kg of wet weight per square meter [71]. Winged kelp is abundant in calcium and includes a wide range of vitamins, namely vitamins A, K, C, and numerous B vitamins. It has healthy minerals including nitrogen, boron, radium, rubidium, cadmium, cobalt, magnesium, iron, potassium, and iodine and nickel as well as high levels of dietary and soluble fiber [71]. *Alaria esculenta* also contains enzymes, chlorophyll, beta carotene, carbohydrates, and protein [71]. It is notably high in fucoxanthin, a compound being researched with benefits like regulating blood sugar levels, encourage weight loss, and cancer fighting enzymes. Alginic acid makes up to 42 % of the *Alaria esculenta* [71].

Figure 2 Alaria esculenta

1.9.1.2 Saccharina latissima

It is brown algae of *Laminariaceae* family, also known as sugar kelp, sea belt, and Devil's apron [72]. Kelp has a single, 40 cm-wide frond that can reach a maximum length of 7 meters Figure 3 [73]. It is situated in safe areas with little wave activity. It has colors like rich golden brown in December to light brown in February to green in March and April. Beta-carotene, iron, potassium, calcium, sodium, and magnesium are all abundant in kelp. Mannitol, a sugar alcohol, can be found in up to 14% of kelp [73]. Alginates, which can be utilized as thickening agent, are abundant in kelp [73]. Kelp is simple to use in cooking and has sweet flavor with hint of liquorice flavor [73]. The presence of rich umami flavor of dry kelp makes it fantastic flavor enhancer [73]. These kelps are young in early January or end of December and harvested when they are golden brown in appearance. During the season, these leave's colors shift from brown to green. Prior to integrity of its blades being compromised by other organisms' epiphytic growth, fresh sugar kelp is recommended to be harvested as early as possible in April. When kept in a dry environment, it has a long shelf life [73].

Figure 3 Seaweed, Saccharina latissima

1.10 Importance of seaweed fermentation for human Consumption

Seaweed is known as marine macroalgae low in calories and high in potential health-promoting micro &macronutrients, bioactive components namely antioxidants, flavonoids, and phenolic compounds [74]. Seaweeds have gained considerable attention in recent years due to their potential as a sustainable source of functional ingredients for food [75]. It presents itself in a compelling avenue for development of innovative food and nutraceutical products, boasting high bioactivity and sensory quality [75][76]. Moreover, sensory enhancements achieved through fermentation facilitate integration of seaweed-derived ingredients into various food matrices [75]. Continued research and development in this field hold significant potential for expansion of functional food industry and promotion of sustainable dietary choices [75]. Some studies also indicates that fermented seaweed extracts possess a potent hypolipidemic action, experimental findings reveal that fermented seaweed extracts exhibited a noteworthy ability to bind bile acids [76]. Moreover, a significant inhibitory effect against pancreatic lipase was observed [76]. These above findings suggest seaweed being potential utility in development of functional foods aimed at combating cardiovascular diseases [76].

2 Materials & Methodologies

2.1 Materials

2.1.1 Substrate (Seaweed biomass) preparation

Brown seaweed, winged kelp, *Alaria esculenta* harvested on18/05/2021 and sugar kelp, *Saccharina latissima* harvested on12/05/2021 which was used as substrate for this experiment, was procured from "Seaweed solutions" which was harvested from a spring in Norway. The seaweed batches were stored in freezer at -80°C during whole tenure. The seaweed was samples in two forms, one as non-grounded (NG) while other was grounded (G) using menuett meat mincer (köttkvarn) shown in Figure 4 and Figure 5. To prevent loss of targeted monosaccharide during grinding, frozen seaweed was grounded and then weighed, for the fermenters.

Figure 4: Non-ground Saccharina latissima (before fermentation) and Menuett meat mincer (köttkvarn)

Figure 5: Ground Alaria esculenta (before fermentation)

2.1.2 Lactic acid bacteria

The Freeze-dried commercial *lactobacilli* culture, which was obtained from Aventure AB was used as a pre-culture . Freeze dried culture was stored at -80°C till being used for the experiment.

2.1.3 Chemicals

MRS medium and MRS agar were used for inoculum preparation. Both were obtained from Sigma life sciences and Sigma Aldrich. Milli-Q water used in procedures was passed through 0.2µm filter for refinement. All chemical solutions used during media and agar preparation were autoclaved at 121℃. Short chain fatty acids were determined with a solution prepared from 98% sulfuric acid. Lactic acid, butyric acid, acetic acid, propionic acid and formic acid were used as standards for HPLC analysis.

2.2 Methodologies

2.2.1 Media Preparation

MRS medium was prepared by dissolving 52.2 gm of MRS broth in 1 L of distilled water. Then, 30 mL of MRS broth was transferred to serum glass bottle followed by sealing with rubber stopper and aluminum cap. Anaerobic condition was achieved in serum glass bottles by flushing with pure nitrogen gas. Later, all flushed glass bottles with MRS medium were autoclaved at 121℃ for 15 minutes and were stored in freezer 4℃ for further use.

2.2.2 Inoculum preparation

Stock culture, *(shown in* Figure 6*)* was thawed at room temperature, and later 1 mL of stock culture was used to inoculate in MRS medium (30 mL) (Figure 6) at room temperature (25℃). The culture was incubated at 37℃ for 16 hours. After 16 hours (Figure 7), 2% of the previously inoculated medium is transferred to 30 mL of new MRS medium. The new medium was incubated for 5 – 6 hours until optical density (OD) of pre-culture reached to 0.2 – 0.3 at 620 nm. The OD of sample was measured using WPA Biowave II spectrophotometer. Once desired OD (0.2 – 0.3) was reached, 2% (0.6 mL) of the inoculum was transferred to fermenters containing 100 gm of each seaweed species.

Figure 6 30 mL MRS medium (on the left side of the photo) with the freeze dried lactic acid bacteria (on the right side of the photo)

Figure 7 MRS medium after 16 hrs of inoculation with (present on the right side of the photo); freshly inoculated MRS medium with (present on the central of the photo); not inoculated MRS media (present on the left side of the photo)

2.2.3 Fermenter preparation

The procedure for fermentation begins with preparation of fermenters. This required the use of 100 mL clear-top screw glass bottles obtained from Simax Czech Republic, as well as green-colored screw caps with an aperture in the cap. A grey-colored body cap, equipped with dual openings was interposed between fermenter bottle and green-colored screw cap. The opening of body cap was closed with a single transparent tube, which were opened during time of oxygen flushing (Fig. 6).

2.2.4 Solid-State Fermentation of seaweed

For all experiments, 100 gm of ground and non-grounded seaweed was measured from 6kg (*Alaria esculenta)* and 10kg (*Saccharina latissima)* received from Seaweed solutions and transferred into 12 different fermenters of 6 each fermenter. In total, 6 bottles consist of grounded seaweed while the rest of 6 fermenters consists of non-grounded seaweed. Out of 6 fermenters, 3 bottles were inoculated with, while 3 fermenters were control samples. No

water was added to bottles since the process was designed as solid-state fermentation. Fermenters were added with 2% (0.6 mL – approximately $3.02x10^8$ colony forming unit) of inoculum and mixed thoroughly with help of L shaped rod and then closed tightly with screw caps. The CFU was calculated using CFU measurement, described later in the report. In order to ensure anaerobic condition inside fermenters, glass vials were flushed with nitrogen gas passed through VWR branded filter of size 0.45µm (shown in Figure 8) for…min. Finally, fermenters contained seaweed samples under anaerobic condition were placed in incubator at 37 ℃ and room temperature, as experiment plan. Samples were retrieved for pH measurement and returned once the measurement was recorded and flushed every time after it was opened. This procedure was carried out till pH reached 4.5.

Figure 8 Fermenter and anaerobic condition set up .

2.2.5 pH measurement

pH assessments were conducted at 3-day intervals post-inoculation until pH of seaweed reached below 4.5. This pH attainment took 6 days for *Alaria esculenta* and 14 days for *Saccharina latissima*. The pH measurement was done by using UB-5 pH meter from Denver instruments. The sample preparation for pH measurement was employed based on procedure from research paper ([77],[78]). About 3 gm of seaweed sample was mixed with 30mL of milli Q water in 50 mL falcon tubes. Then, it was mixed thoroughly, and then pH measurement was recorded after 30 min (shown in Figure 9).

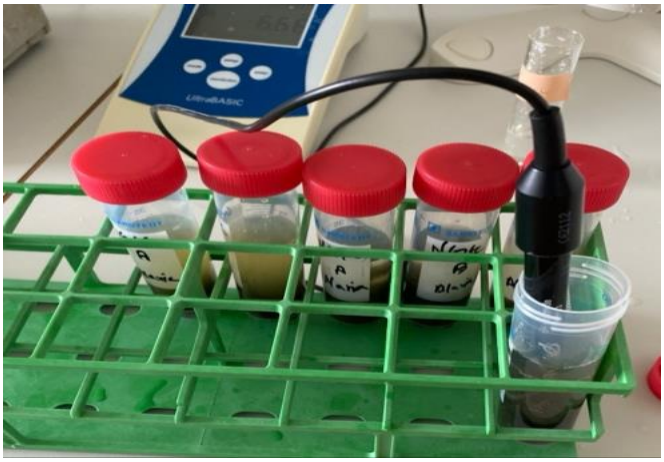

Figure 9 pH measurement of the sample

2.2.6 Total colony plate count

2.2.6.1 Media preparation

The MRS agar plates was prepared by mixing 68.2 gm of commercial MRS agar powder into 1 L of distilled water. The mixture was further mixed properly using magnetic stirrer and was autoclaved at 121℃ for 10 min. Agar plates were prepared by transferring 10- 20 mL of sterile MRS media into the petri plate. The agar was kept for settling before inoculated with the LAB.

2.2.6.2 CFU measurement

Serial dilution was used to dilute initial solution to prepare the 10^6 and 10^7 dilutions. Once agar was completely settled in the petri plates, aliquots of with dilution of 10^6 and 10^7 were spread on agar plate using L shaped glass rod. The agar plates were kept for incubation at 37℃ for 3 days (Figure 10). After 3rd day, number of colonies were counted (Figure 11).

Figure 10) agar plates are placed in air tight container for incubation

Figure 11 colony formation of lactic acid bacteria used for experiment after 3 days of incubation in the air tight container

2.2.7 Short chain fatty acid analysis

The short chain fatty acids (SCFAs) produced during solid-state fermentation process of seaweed was analyzed using high- performance liquid chromatography (HPLC) (Thermo Fisher Scientific) (Figure 12). Bio-Rad Aminex 87-HPX column along with 0.5mm as a mobile phase was used for fatty acid analysis. The flow rate through column was set to 0.5 mL/min and column compartment temperature was maintained at 50℃.

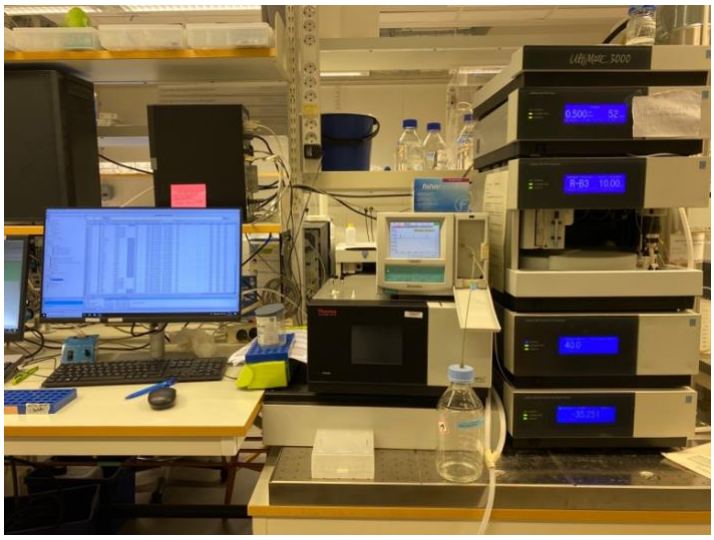

Figure 12 HPLC set up

Sample preparation

The sample for HPLC was prepared by taking 2 mL of liquid samples from fermented seaweed in Eppendorf tube Figure 13. These samples were then centrifuged at 13000 rpm for 5 min using legend micro 17 centrifuge from Thermo scientific. 1 mL of supernatant was transferred to new Eppendorf without disturbing the pellet. The supernatant was diluted with milli Q water to reach 10% dilution . Then 20 µL of 20 % (v/v) sulfuric acid was added to diluted sample. The samples were kept at +4℃ in fridge for 30 min and then transferred to 1.5 mL

HPLC glass vials after filtering through 0.45µm filter (VWR, Avantor) (Figure 13). Finally, the samples were stored in fridge until further used for analysis.

Figure 13 Liquid samples for further analysis

2.2.8 Sample preparation for proximate analysis (Total solid, Moisture, Oven Dry Weight and Total ash content).

The samples were prepared by following protocols from Technical Report NREL/TP-5100-60957 [79]. Sample preparation for all above analyses (total, solid, moisture, oven dry weight and total ash content) began with freeze drying seaweed sample using Labconco Freeze Dry System Lyph-lock 12 and vacuum pump (Figure 14). Seaweed of 20 gram portions from all the samples; grounded, non-grounded, control and inoculated; was weighed and placed in transparent zip-lock plastic bags. Then, bags were placed in the freeze drier after being punctured with tiny needle holes. It should be noted that samples were kept frozen throughout the procedure. The samples were lyophilized for a total of three days.

Figure 14 freeze-drying seaweed sample using Labconco Freeze Dry System Lyph-lock 12.

I. Determination of total solids in Algal biomass

Total solid in biomass was determined using method from Technical Report NREL/TP-5100-60956 [80]. The freeze-dried samples are used for determination of total solids. crucibles are pre-treated overnight in muffle furnace at temperature of 575°C, to ensure removal of any combustible contaminants. Following conditioning, crucibles were kept in desiccator under vacuum, to bring it to room temperature after which their weight was recorded. Then 100 ± 5 mg of prepared freeze-dried seaweed samples are weighed in pre-weighed crucibles. These

crucibles along with biomass were kept in oven for drying at 105℃ for 24 hrs. The samples were transferred to desiccator to cool down to room temperature, and later weights were recorded. The total solid content of biomass was calculated using the formula below.

$$\%\ Total\ Solids = \frac{(weight_{crucibles+dry\ sample} - weight_{crucibles})}{weight_{sample\ received}} \times 100 \quad \textit{(Equation i)}$$

$$\%\ Moisture = 100 - \%\ Total\ solids \quad \textit{(Equation ii)}$$

$$ODW_{sample} = \frac{Weight_{air\ dried\ sample} - \%\ Total\ solids}{100} \quad \textit{(Equation iii)}$$

2.2.8.1 Determination of ash content in algal biomass

To determine ash content of algal biomass, method mentioned in Technical Report NREL/TP-5100-60956 was employed [80]. The pre oven dried samples were incinerated in muffle furnace equipped with ramping program. The starting temperature was 105℃ and ending temperature was 575℃ for 180 min. The incinerated samples were transferred to desiccator from muffle furnace and was let to cool down to room temperature. Upon cooling to room temperature, weights were recorded. The ash content of algal biomass was calculated using formula as below.

$$\%\ Ash\ content = \frac{Weight_{crucible+ash} - Weight_{crucible}}{ODW_{sample}} \quad \textit{(Equation iv)}$$

3 Results

Table 1 List of Experiments performed during this research:

S.No.	Experiment	Seaweed species	Temperature
1	Exp 1	*Alaria esculenta*	37°C
2	Exp 2	*Saccharina latissima*	37°C
3	Exp 3	*Alaria esculenta*	37°C
4	Exp 4	*Alaria esculenta*	25°C (Room Temperature)

3.1 Microbiological Profiling via CFU Determination

The CFU count analysis was conducted to determine population of *commercial lactobacillus strain* in the inoculum, and results are summarized in the Table 2.

Table 2 Total CFU count of Commercial lactobacillus strain

Dilution (Inoculum)	Volume of culture plated (mL)	Dilution factor	Average number of colonies	CFU/mL	Bacterial concentration (per mL)
10^{-6}	0.2	10^{6}	92	$4.6x10^{8}$	$2.3\ x10^{15}$
10^{-6}	0.1	10^{6}	50.33	$5.03\ x10^{8}$	$5.03\ x10^{14}$

3.2 Seaweed Fermentation

3.2.1 pH Trends and Variability during Fermentation

The pH measurement of fermented seaweed was conducted throughout experiment and results are presented in Figure 15, Figure 16, and Figure 17**Error! Reference source not found.**. All experiments (1, 2, 3, 4) were conducted till fermented seaweed reached desired pH of 4.5, while ensuring absence of mold growth by checking through the fermenter bottles. The graph plotted illustrates results of experiment 2, 3 and 4 as the contamination was observed during the first experiment (experiment – 1). are comparisons of pH levels between control samples and inoculated samples containing *Commercial lactobacillus strain*. Furthermore, impact of grounded (G) and non-grounded (NG) states of samples on fermentation process were also analyzed. In graph depicted below shown in**Error! Reference source not found.Error! Reference source not found.** Figure 15, Figure 16, and Figure 17 blue and grey bars represent control samples of *Saccharina latissima* and *Alaria esculenta* seaweed, respectively in grounded and NG states. On other hand, orange and yellow bars represent samples inoculated with *Commercial lactobacillus* strain, in their G and NG states, respectively.

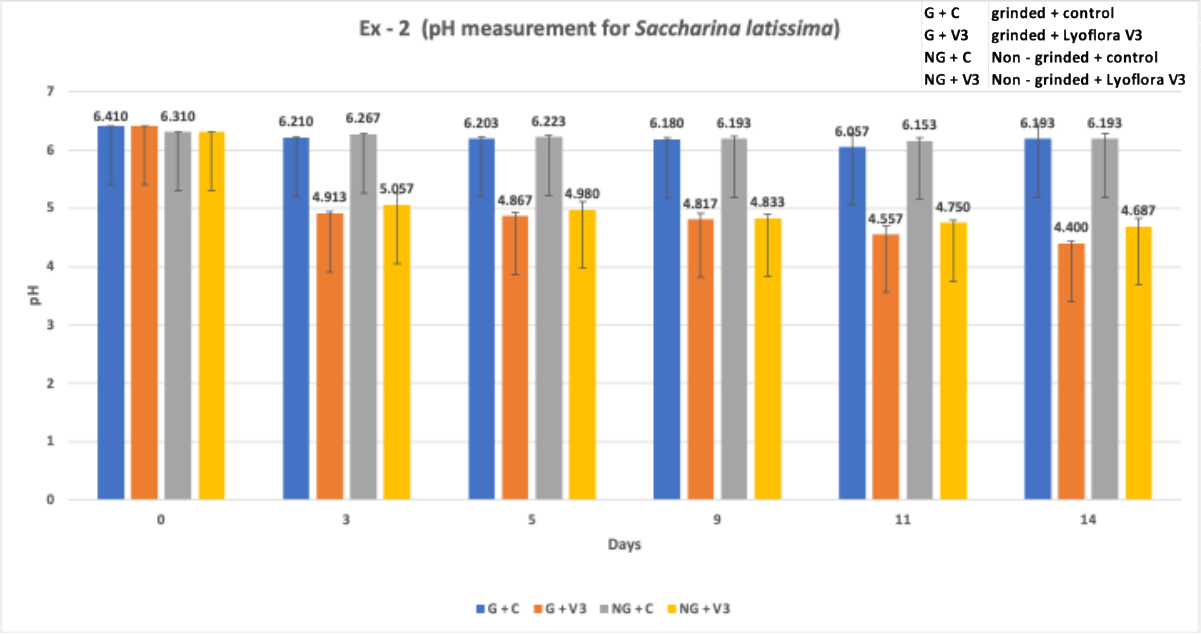

Figure 15 Recorded pH for Saccharina latissima throughout the fermentation for 14 days at 37°C.

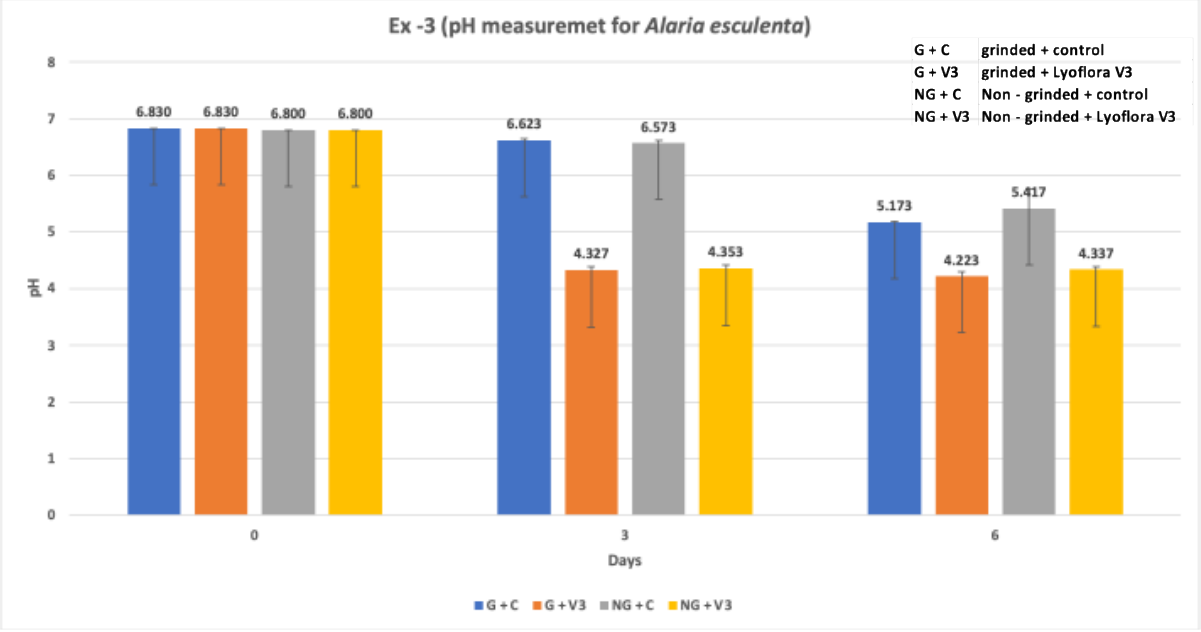

Figure 16 Recorded pH for Alaria esculenta throughout the fermentation for 6 days at 37°C.

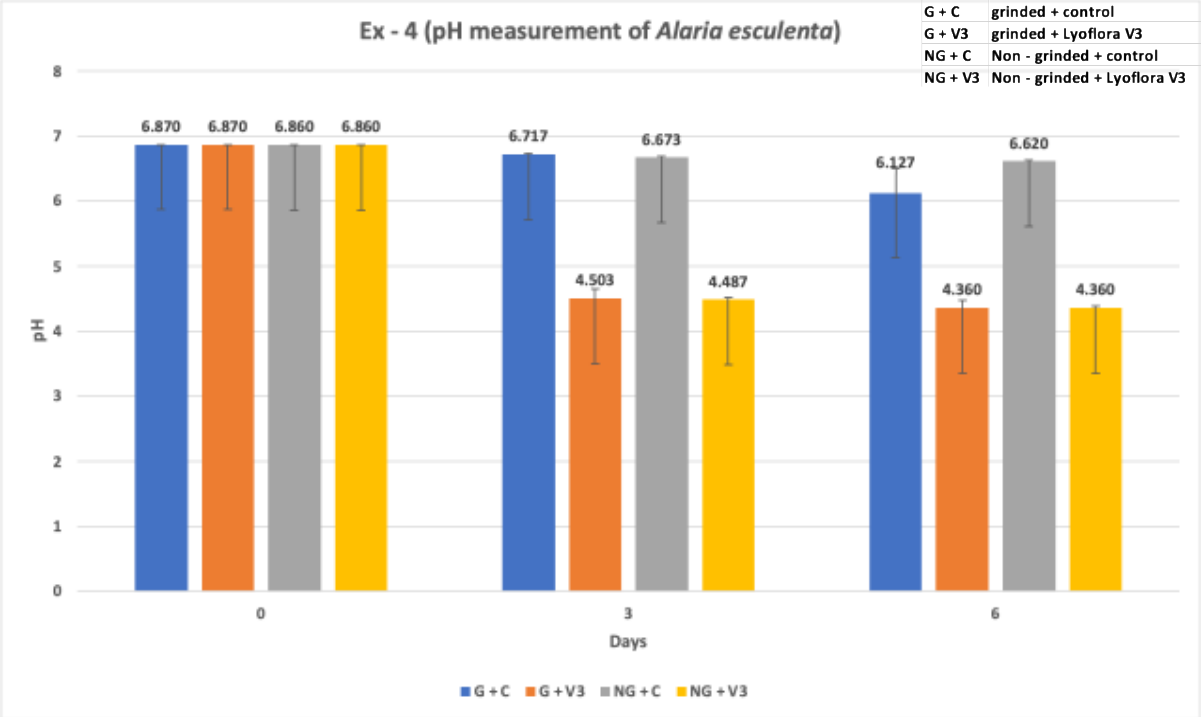

Figure 17 Recorded pH for Alaria esculenta throughout the fermentation for 6 days at 22℃ - room temperature.

3.2.2 Determination of presence of SCFA by HPLC

HPLC was employed to analyze short chain fatty acids and investigate variation in composition of acids before and after fermentation of seaweed. The results of analysis are depicted in Figure 18, Figure 19, Figure 20 along with corresponding**Error! Reference source not found.** Table 7, Table 8, and Table 9 elucidate differences in acid content. The results from HPLC analysis depicted in graphs illustrate concentration of lactic and formic acids in *Saccharina latissima* and *Alaria esculenta* seaweed, both before and after fermentation.

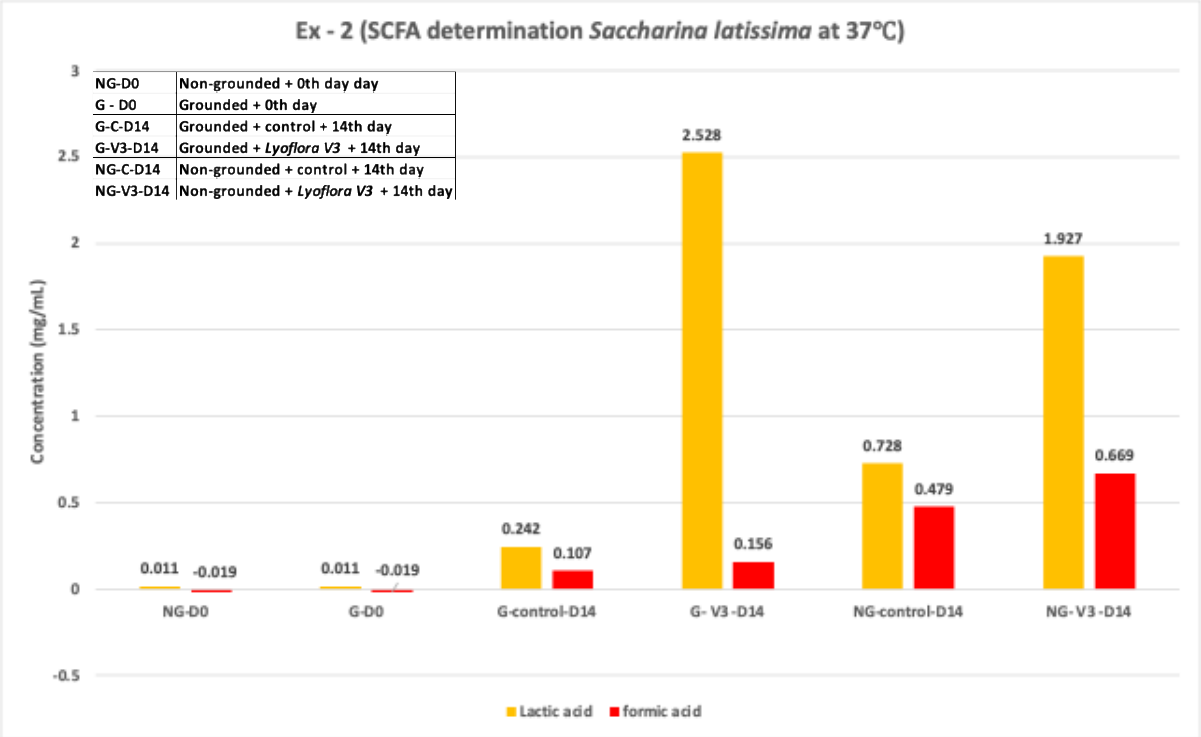

Figure 18 Measured concentration of short chain fatty acids – lactic acid and formic acid, before (day – 0) and after (day - 14) fermentation of Saccharina latissima at 37°C, detected by HPLC)

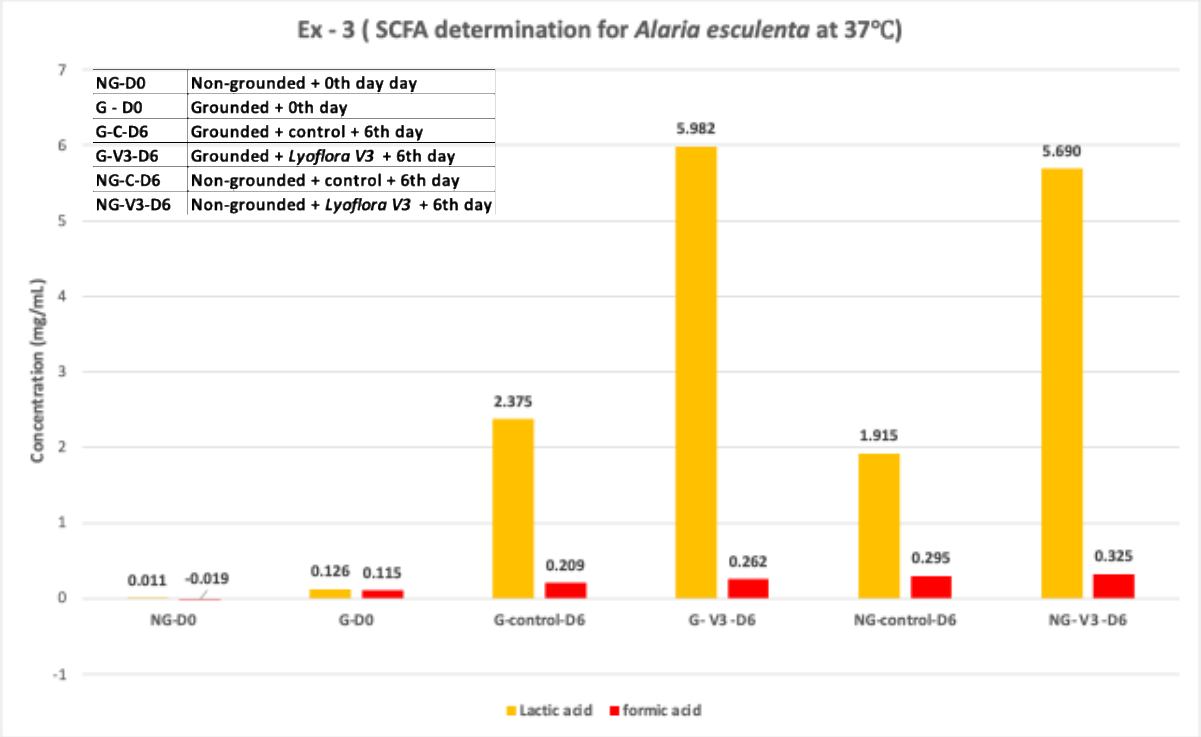

Figure 19 Measured concentration of short chain fatty acids – lactic acid and formic acid, before (day – 0) and after (day - 6) fermentation of Alaria esculenta at 37°C, detected by HPLC

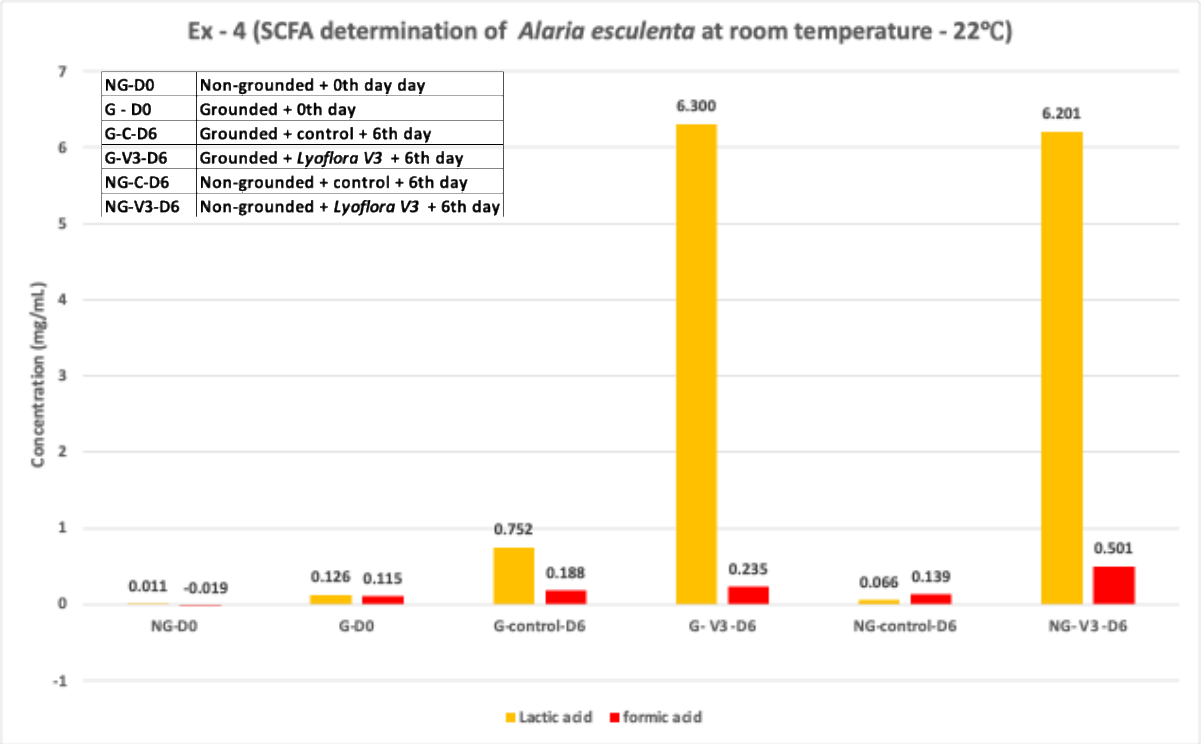

Figure 20 Measured concentration of short chain fatty acids – lactic acid and formic acid, before (day – 0) and after (day - 6) fermentation of Alaria esculenta at 22℃ - room temperature, detected by HPLC

3.2.3 Proximate Analysis of seaweed after fermentation

The proximate analysis results for *Alaria esculenta* and *Saccharina latissima* seaweed species are presented in Table 3.

Table 3 Results of total solid, moisture, ODW & total ash

	Alaria esculenta	*Saccharina latissima*
% Total Solid	93.92 ± 0.9	94.11 ± 0.42
% moisture	5.88± 1.2	5.59 ± 1.1
% ODW	5.99 ± 0.42	5.46 ± 0.35
% Total Ash	5.24 ± 1.16	7.61 ± 1.5

4 Discussion

4.1 Role of commercial lactobacillus strain Seaweed fermentation

For inoculum freeze dried sample of commercial lactobacillus strain *was used* [81]. Freeze-drying bacterial strain is a useful preservation technique. This involves suspending the bacteria culture in a lyophilization media or buffer, putting them through the freeze-drying process, and then appropriately storing them after that [81]. A properly freeze-dried bacterial strains can be stored for extended periods of 30 years or beyond and reactivated by rehydrating them when needed for experimental analysis [82]. By using freeze-dried strains of LAB, ensure consistent and viable bacterial populations for fermentation [66]. commercial lactobacillus strain was selected as an inoculum was based on previous studies, it had shown multiple advantages on seaweed fermentation, one of which was reduced fermentation time [83].

4.1.1 CFU count of inoculum commercial lactobacillus strain

The main objective of this analysis was to determine the initial concentration of a viable bacterial colony that was present in the inoculum [84]. For the success of solid-state fermentation, concentration of microorganisms that will be used as an inoculum. CFU count is a test to measure it is one of the key elements [85] This test is based on fact that all colonies are separated and are started by viable microbial cell [86]. CFU/mL is standard unit used for measurement of CFU. The dilution plates with colony count of 25 – 250 are used for counting and plates with more than 250 are considered Too Numerous To Count (TNTC) [84]. The dilution of 10^{-6} was selected since number of colonies was 92 (in 0.2 mL volume of sample transferred)) and 50.33 (in 0.1 mL volume of sample transferred) which lies within range of 25 – 250. Total CFU/mL was calculated to be 4.6×10^{8} and concentration of bacteria in original same was found to be 4.60×10^{14}. So, it can be concluded that 4.60×10^{14} is the number of bacteria present in per mL of prepared inoculum.

4.2 Solid-state fermentation of *Alaria esculenta* and *Saccharina latissima*

Saccharina latissima and *Alaria esculenta* possess immense potential as food source in Europe [87]. Their rich nutritional composition, including iodine, proteins, polyphenols, carbohydrates, and micronutrients, along with their favorable sensory attributes, make them promising ingredients for food industry. The utilization of *Saccharina latissima* and *Alaria esculenta* in various food applications can contribute to improved nutrition, culinary innovation, and sustainability, thereby opening up new avenues for integrating seaweeds into European diet. These seaweeds are abundant in cold waters of North Atlantic, particularly Norway. These are the reasons for selection of *Saccharina latissima* and *Alaria esculenta* for this study [87].

The fermentation of seaweed, *Alaria esculenta* and *Saccharina latissima* with resulted in noticeable difference in color compared to control group as shown in Figure 21. Although any measurement was not done to prove this analysis, but it was visible on the last day of fermentation process. The lighter color observed in experimental group suggests a influence of commercial lactobacillus strains on color development during fermentation. However,

reason behind this is inconclusive and this finding highlight need for further research to elucidate underlying mechanisms and explore potential applications of commercial lactobacillus strains in seaweed fermentation processes. It needs to be further investigated, if strain is the only responsible factor for this color change or is there any other factors that is influencing it like pH, temperature or others.

Figure 21 colour difference between the fermented and control samples was observed. left - inoculated with the commercial lactobacillus *strain, right – control*

Contamination presents one of significant challenges encountered during fermentation process, as it can adversely affect end product. Contamination refers to introduction of undesirable microorganisms, which can compete with or inhibit growth of desired microbial cultures, ultimately leading to altered fermentation outcomes. During initial batch (ex – 1) of *Alaria esculenta* fermentation, contamination was observed (**Error! Reference source not found.**). Potential factors contributing to this contamination could be inadequate adherence to aseptic procedures and insufficient oxygen removal through nitrogen flushing, which may have facilitated proliferation of unwanted microorganisms like, moulds contamination. Consequently, moulds contamination in solid-state fermentation (SSF) can affect the growth of LAB, a crucial component in *Alaria esculenta* fermentation as it can have various detrimental effects, including competition for nutrients, pH alteration as well as end product quality and safety. [88].

Figure 22 Contamination observed during first batch of fermentation (whitish in colour)

4.3 pH trends during seaweed fermentation

The main goal of these set of experiments was to examine solid-state fermentation process of two types of seaweed, *Alaria esculenta* and *Saccharina latissima*, using commercial lactobacillus strain, to reach a desired pH of 4.5. The pH level drop to 4.5 is critical as it signifies that, growth of most of endogenous spoiling bacteria and fungi are prevented, as they grow in environment where the pH is above 4.6 [89] [90]. The results obtained from all conducted experiments (experiment 1, 2, 3 and 4) indicate that freeze-dried commercial strain of lactic acid bacteria (LAB) effectively fermented both types of seaweed, causing a reduction in pH below 4.5. The experiments were performed to check if

4.3.1 Experiment 2

In experiment 2**Error! Reference source not found.**, fig Figure 15 of bar graphs is used to compare pH of *Saccharina latissima* seaweed during incubation at temperature of 37℃. When examining *Saccharina latissima*, it was observed that pH levels for control group in both non-grounded (NG) and grounded (G) forms decreases a very little throughout 14-day fermentation period. The pH dropped to 6.15 and 6.06 on 11th day and later increases to 6.19 in both NG and G on 14th day. Although a good pH drop was observed in seaweed inoculated with Commercial lactobacillus strain. Interestingly, there was no substantial difference in final pH between G and NG forms of algal biomass, despite their different states. However, a notable difference of drop in pH was observed during the fermentation period for both G and NG states. In G state, pH dropped to 4.91 by 3rd day of fermentation, whereas in NG state, pH reached 4.98 on 5th day. Furthermore, the pH drop occurred slightly faster in G state compared to NG state. By final day of fermentation, pH in G state was considerably lower than that in NG state.

4.3.2 Experiment 3

Figure 16 presents a bar graph depicting pH drop in *Alaria esculenta* biomass during incubation at temperature of 37℃. The experiment conducted with *Alaria esculenta* revealed that on 3rd day, pH of 4.33 was achieved in grounded (G) state of seaweed, while non-

grounded (NG) state reached pH of 4.35 respectively at the same time. Subsequently, there was further decrease in pH in grounded state, reaching a final pH to 4.22. However, NG state showed a minimal pH drop, with final pH attained was 4.34. In comparison with control, it was observed to be higher in control samples, both in the NG and G states, reaching 5.42 and 5.17, respectively. It is important to note that these pH levels were still significantly higher than the desired target pH.

4.3.3 Experiment 4

In bar graph Figure 17, **Error! Reference source not found.** illustrates pH drop in *Alaria esculenta* at room temperature (22℃). In this particular experiment, it was observed that there was minimal or negligible pH drop in control samples, in both G and NG states. The final pH attained for control samples were found to be 6.13 in G state and 6.62 in NG state. Conversely, samples inoculated with commercial lactobacillus strain exhibited significant pH drop, reaching pH of 4.5 as early as 3rd day in both G and NG states. Subsequently, pH continued to decrease in all samples, reaching final pH of 4.36 in both G and NG states.

Alaria esculenta was fermented at room temperature (22℃) to assess if commercial lactobacillus strain can ferment subjected seaweeds even at lower temperature. Encouragingly, results were positive and inoculated samples exhibited a pH drop to 4.5 without any signs of mold growth. To facilitate a comparative analysis, control groups were included, consisting of uninoculated seaweed biomass subjected to same fermentation conditions. Control is seaweed biomass which was received and doesn't contain any inoculation from outside. The seaweed specie *Alaria esculenta* experiments lasted for 6 days at both 37℃ and room temperature of 22℃, while other seaweed specie *Saccharina latissima* experiment required 14 days to achieve desired pH level. Notably, in case of *Saccharina latissima*, pH of control group remained at an average of 6 even on the 14th day. However, for *Alaria esculenta*, a slight lower pH was observed for control group on 6th day when incubated at 37℃. Conversely, when incubated at room temperature, pH of *Alaria esculenta* control group remained steady at an average of 6. These results emphasize ability of LAB to effectively lower pH of seaweed samples in solid-state fermentation, thereby exhibiting potential for pathogen inhibition and preservation purposes. During our investigation Even with the addition of starting culture, *Saccharina latissima* showed a slow pH reduction than *Alaria esculenta*; this is probably because commercial lactobacillus strain growth was restricted in *Saccharina latissima,*[91].

It is worth noting that there is drop in pH in control samples for both *Alaria esculenta* and *Saccharina latissima*. And since samples were not autoclaved, there are chances that sample already contains some bacteria which are working in collaboration to breakdown sugars present in sample to produce acids Thus, this observation suggests and supports that fresh seaweeds inherently possess a low initial population of lactic acid bacteria (LAB) at approximately (102 CFU/g) [92] [93].

4.4 Short chain fatty acid quantification

The carbohydrate substrate, mannitol, present in, *Alaria esculenta* and *Saccharina latissima* is broken down by commercial lactobacillus strain into short chain fatty acids (SFCA) such as lactic acid, propionic acid, formic acid, butyric acid, among many others [91]. High-

performance liquid chromatography (HPLC) was employed for detection and quantification of SCFAs and values are show in the graph Figure 18, Figure 19, Figure 20.

As seen in graph Figure 18 short chain fatty acids are produced in fermented sample of *Saccharina latissima* when fermentation was carried at 37℃. The data reveals that lactic acid and formic acid were predominant short-chain fatty acids formed. Notably, lactic acid production was higher than formic acid production. A noteworthy observation was substantial increase in lactic acid concentration during fermentation process, compared to relatively low levels present in seaweed prior to fermentation. Furthermore, G state of *Saccharina latissima* exhibited a higher yield of lactic and formic acid compared to NG state.

It was also observed that lactic acid content was higher in control of NG compared to G state for *Saccharina latissima* specie. The reason behind this disparity remains inconclusive; however, it is plausible that presence of microbes in seaweed biomass may contribute to this variation.

Similarly,**Error! Reference source not found.** graphs Figure 19 depict production of lactic acid and formic acid during fermentation of *Alaria esculenta*. Consistent with observations similar to *Saccharina latissima*, lactic acid was predominant SCFA produced, exceeding quantity of formic acid even in *Alaria esculenta*. Although it was noticed that when fermenting *Alaria esculenta* at temperature of 37℃, control sample exhibited a slightly lower lactic acid production compared to sample fermented at room temperature, reason behind this is inconclusive. Additionally, as observed in *Saccharina latissima*, G state form of *Alaria esculenta* displayed higher SCFA production compared to NG state. This phenomenon can be attributed to influence of surface area on solid-state fermentation, where smaller substrate particles provide a larger surface area for microbial activity. However, larger particle sizes offer improved respiration and aeration efficiency due to increased interparticle space, but provide limited surface area for microbial colonization [94]. Additionally, HPLC analysis revealed an increase in production of SCFAs in control samples of both *Saccharina latissima* and *Alaria esculenta* seaweeds over fermentation period. This observation also suggests and supports that fresh seaweeds inherently possess a low initial population of lactic acid bacteria (LAB) at approximately (102 CFU/g) [92] [93].

Furthermore, the presence of an unidentified peak with a retention time of 12050 was observed in both fermented and non-fermented samples of *Alaria esculenta* and *Saccharina latissima*. These peak warrants further investigation in subsequent studies to determine the identity and properties of the unknown acid.

4.5 Proximate analysis – Total solid, moisture, ODW, Total ash content

The common term used to refer to combined analysis of total ash, moisture, organic dry weight (ODW), and ash content is proximate analysis [95]. Proximate analysis provides valuable information about major components of sample, including amount of moisture, ash, and organic matter present [95]. It is commonly used in various fields like food science, agriculture, and environmental studies to assess the quality and composition of different materials [95].

Fermentation is characterized by breakdown of carbohydrates and other soluble components, resulting in generation of various metabolic byproducts. These byproducts, such as organic acids and gases, contribute to changes in total solid content of fermented food. Total solids and moisture content analysis in fermented foods is essential for quality assessment, monitoring fermentation progress, evaluating shelf stability, determining nutritional composition and overall characteristics of fermented food products [96]. Total solids refer to sum of all solid components present in food, including both soluble and insoluble substances [96]. Secondly, measuring total solids allows for assessment of moisture loss during fermentation, providing insights into preservation and shelf stability of final fermented *Alaria esculenta* and *Saccharina latissima* products. Furthermore, reduction in moisture content plays a crucial role in inhibiting microbial growth and enzymatic activity, thus prolonging product's shelf life and ensuring its safety for human consumption [96].

The % totals solids, ODW, moisture content for *Saccharina latissima* and *Alaria esculenta* is discussed in Table 3. The results, reveal a higher percentage of total solids in commercial lactobacillus strain-inoculated sample compared to control sample in both *Saccharina latissima* and *Alaria esculenta*. It is plausible that variations in total solids could be arised from differences in freeze-drying process, specifically extent to which samples were dried [97]. Secondly, another possible explanation for disparity in total solids between fermentation samples lies in inclusion of bacterial biomass. It is conceivable that during centrifugation prior to freeze-drying, bacterial biomass may have precipitated alongside algal biomass. Consequently, total solids measurements could encompass both algal and bacterial biomass. Further analysis is needed to elucidate role of bacterial-algal interactions in substrate utilization.

It is to be noted that moisture content of fermented seaweed is subject to uncertainty as drying process may not exclusively just remove water. Extensive research has shown that seaweed contains volatile compounds that can also potentially vaporize during drying [98]. These volatile compounds play a crucial role in conferring aromatic properties to seaweed [98]. To conduct a more comprehensive analysis of volatile components, gas chromatography-mass spectrometry (GC-MS) can be employed. It is worth noting that green seaweed generally possesses a higher abundance of volatile compounds compared to brown and red seaweed. [98].

5 Conclusion

This report has provided significant insights into the potential applications of seaweed, specifically *Alaria esculenta* and *Saccharina latissima*, through a comprehensive literature survey. Furthermore, the report has highlighted the significance of fermentation, particularly solid-state fermentation, as a viable process for the utilization of these seaweed species. The successful application of the commercial lactobacillus strain in the fermentation of both *Alaria esculenta* and *Saccharina latissima* has been demonstrated, resulting in a reduction of pH to 4.5 for both species, although variations in fermentation time was observed between the two Additionally, it was observed that there are slight changes in the fermentation period between the grounded and non-ground states of the seaweed. On the 11th day of fermentation, the pH of the grounded *Saccharina latissima* reached 4.56, whereas the non-grounded sample reached a pH of 4.69 even on the 14^{th} day. On the third day of fermentation, the pH of the *Alaria esculenta* sample, which was in the grounded state, reached 4.33 while the pH of the non-grounded sample reached 4.35, did not exhibit as much variance.
Furthermore, it was discovered that temperature differences had a significant impact on the Alaria esculenta control sample. At 37℃, the control sample's pH value dropped to 5.17 (grounded) and 5.42 (non-grounded), but at 22℃, the pH drop was not as great, reaching 6.13 (grounded) and 6.62 (non-grounded).

During the literature review it was found that commercial lactobacillus strain holds promise for the fermenting *Alaria esculenta* and *Saccharina* latissima & and the same was found during the experiment. This strain exhibits the capability to efficiently ferment the seaweed samples and effectively lower the pH to the desired level. The successful application of this fermentation process signifies its potential for the production of value-added products derived from seaweed.

6 Future work

Further research is recommended to optimize the fermentation parameters, including fermentation time, temperature, and nutrient composition, to enhance the efficiency and consistency of the fermentation process. Additionally, evaluating the biochemical composition and functional properties of the fermented products would provide valuable insights into their potential applications in various industries, such as food, pharmaceuticals, and biofuels. Since currently the concentration of the cell is high, we can try in future to decrease that. Also, we can try with the new batch for the fermentation. only very limited evidence on the effectiveness of most fermented foods in gastrointestinal health, so that can be also done.

7 References

[1] "Microbiology and Technology of Fermented Foods - Robert W. Hutkins - Google Books." Accessed: Apr. 14, 2023. [Online]. Available: https://books.google.se/books?hl=en&lr=&id=CvIl2k8jGgMC&oi=fnd&pg=PR7&ots=S2ood3Roa-&sig=WoLSTGb8OvbWOUOL43fJZKd2NmE&redir_esc=y#v=onepage&q&f=false

[2] M. Mannaa, G. Han, Y. S. Seo, and I. Park, "Evolution of Food Fermentation Processes and the Use of Multi-Omics in Deciphering the Roles of the Microbiota," *Foods*, vol. 10, no. 11, Nov. 2021, doi: 10.3390/FOODS10112861.

[3] A. J. Marsh, C. Hill, R. P. Ross, and P. D. Cotter, "Fermented beverages with health-promoting potential: Past and future perspectives," *Trends Food Sci Technol*, vol. 38, no. 2, pp. 113–124, Aug. 2014, doi: 10.1016/J.TIFS.2014.05.002.

[4] J. P. Tamang *et al.*, "Fermented foods in a global age: East meets West," *Compr Rev Food Sci Food Saf*, vol. 19, no. 1, pp. 184–217, Jan. 2020, doi: 10.1111/1541-4337.12520.

[5] F. A. Oguntoyinbo, "Safety Challenges Associated with Traditional Foods of West Africa," *Food Reviews International*, vol. 30, no. 4, pp. 338–358, 2014, doi: 10.1080/87559129.2014.940086.

[6] E. Dimidi, S. R. Cox, M. Rossi, and K. Whelan, "Fermented Foods: Definitions and Characteristics, Impact on the Gut Microbiota and Effects on Gastrointestinal Health and Disease," *Nutrients*, vol. 11, no. 8, Aug. 2019, doi: 10.3390/NU11081806.

[7] Frank. Kosikowski, "Cheese and fermented milk foods," p. 711, 1982, Accessed: Apr. 25, 2023. [Online]. Available: https://books.google.com/books/about/Cheese_and_Fermented_Milk_Foods.html?id=iB9tAAAAMAAJ

[8] K. Steinkraus, *Handbook of Indigenous Fermented Foods Food Science and Technology*. 1995. Accessed: Apr. 25, 2023. [Online]. Available: https://www.routledge.com/Handbook-of-Indigenous-Fermented-Foods-Revised-and-Expanded/Steinkraus/p/book/9780824793524

[9] K. H. Steinkraus, "Nutritional significance of fermented foods," *Food Research International*, vol. 27, no. 3, pp. 259–267, Jan. 1994, doi: 10.1016/0963-9969(94)90094-9.

[10] "Insect Pathology V1: An Advanced Treatise - Google Books." Accessed: Apr. 25, 2023. [Online]. Available: https://books.google.se/books?hl=en&lr=&id=LmYQx-XORW4C&oi=fnd&pg=PA133&dq=nutritional+diseases+&ots=NpAfpFewGi&sig=iOHfmpnmy9vlKeQBug05pZbpFd4&redir_esc=y#v=onepage&q=nutritional%20diseases&f=false

[11] "MRC review of nutrition and human health research – UKRI." Accessed: Apr. 25, 2023. [Online]. Available: https://www.ukri.org/publications/mrc-review-of-nutrition-and-human-health-research/

[12] K. H. Steinkraus, "Nutritional significance of fermented foods," *Food Research International*, vol. 27, no. 3, pp. 259–267, Jan. 1994, doi: 10.1016/0963-9969(94)90094-9.

[13] Y. Kourkoutas, L. Bosnea, S. Taboukos, C. Baras, D. Lambrou, and M. Kanellaki, "Probiotic Cheese Production Using Lactobacillus casei Cells Immobilized on Fruit

Pieces," *J Dairy Sci*, vol. 89, no. 5, pp. 1439–1451, May 2006, doi: 10.3168/JDS.S0022-0302(06)72212-3.
[14] "Commercial Seaweed Market - Growth, Trends, COVID-19 Impact, and Forecasts (2021 - 2026)." Accessed: Apr. 25, 2023. [Online]. Available: https://www.reportlinker.com/p06195680/Commercial-Seaweed-Market-Growth-Trends-COVID-19-Impact-and-Forecasts.html
[15] "Encyclopedia of Dairy Sciences | ScienceDirect." Accessed: Apr. 26, 2023. [Online]. Available: https://www.sciencedirect.com/referencework/9780123744074/encyclopedia-of-dairy-sciences
[16] "Fermentation of Yogurt and the Chemistry Behind It." Accessed: Apr. 27, 2023. [Online]. Available: https://www.foodunfolded.com/article/the-chemistry-behind-the-fermentation-of-yogurt
[17] S. Sieuwerts, "Microbial Interactions in the Yoghurt Consortium: Current Status and Product Implications Open Access Mini Review," 2016, Accessed: Apr. 27, 2023. [Online]. Available: www.symbiosisonline.orgwww.symbiosisonlinepublishing.com
[18] "Encyclopedia of Food Sciences and Nutrition | ScienceDirect." Accessed: Apr. 26, 2023. [Online]. Available: https://www.sciencedirect.com/referencework/9780122270550/encyclopedia-of-food-sciences-and-nutrition
[19] J. P. Tamang *et al.*, "Fermented foods in a global age: East meets West," *Compr Rev Food Sci Food Saf*, vol. 19, no. 1, pp. 184–217, Jan. 2020, doi: 10.1111/1541-4337.12520.
[20] N. R. C. (US) P. on the A. of B. to T. F. Foods, "Lactic Acid Fermentations," 1992, Accessed: Apr. 29, 2023. [Online]. Available: https://www.ncbi.nlm.nih.gov/books/NBK234703/
[21] K. Y. Park, H. Y. Kim, and J. K. Jeong, "Kimchi and Its Health Benefits," *Fermented Foods in Health and Disease Prevention*, pp. 477–502, Jan. 2017, doi: 10.1016/B978-0-12-802309-9.00020-0.
[22] D. Y. Kwon, E. Nyakudya, and Y. S. Jeong, "Fermentation: Food Products," *Encyclopedia of Agriculture and Food Systems*, pp. 113–123, Jan. 2014, doi: 10.1016/B978-0-444-52512-3.00155-8.
[23] B. S. Noh, H. Y. Seo, W. S. Park, and S. Oh, "Safety of Kimchi," *Regulating Safety of Traditional and Ethnic Foods*, pp. 369–380, Jan. 2016, doi: 10.1016/B978-0-12-800605-4.00019-0.
[24] C. H. Lee and G. I. Lee, "Safety of Food and Beverages: Safety of Regional Specialities – Korean Fermented Foods," *Encyclopedia of Food Safety*, vol. 3, pp. 462–469, Jan. 2014, doi: 10.1016/B978-0-12-378612-8.00307-3.
[25] I. Vina, P. Semjonovs, R. Linde, and I. Deniņa, "Current Evidence on Physiological Activity and Expected Health Effects of Kombucha Fermented Beverage," *https://home.liebertpub.com/jmf*, vol. 17, no. 2, pp. 179–188, Feb. 2014, doi: 10.1089/JMF.2013.0031.
[26] "Introduction: botany and importance. | The mango: botany, production and uses." Accessed: Apr. 28, 2023. [Online]. Available: https://www.cabidigitallibrary.org/doi/abs/10.1079/9781845934897.0001
[27] C. H. Liu, W. H. Hsu, F. L. Lee, and C. C. Liao, "The isolation and identification of microbes from a fermented tea beverage, Haipao, and their interactions during

Haipao fermentation," *Food Microbiol*, vol. 13, no. 6, pp. 407–415, Dec. 1996, doi: 10.1006/FMIC.1996.0047.
[28] J. Reiss, "Influence of different sugars on the metabolism of the tea fungus," *Z Lebensm Unters Forsch*, vol. 198, no. 3, pp. 258–261, Mar. 1994, doi: 10.1007/BF01192606.
[29] M. Coton *et al.*, "Unraveling microbial ecology of industrial-scale Kombucha fermentations by metabarcoding and culture-based methods," *FEMS Microbiol Ecol*, vol. 93, no. 5, May 2017, doi: 10.1093/FEMSEC/FIX048.
[30] A. J. Marsh, O. O'Sullivan, C. Hill, R. P. Ross, and P. D. Cotter, "Sequence-based analysis of the bacterial and fungal compositions of multiple kombucha (tea fungus) samples," *Food Microbiol*, vol. 38, pp. 171–178, Apr. 2014, doi: 10.1016/J.FM.2013.09.003.
[31] C. Raak, T. Ostermann, K. Boehm, and F. Molsberger, "Original Research Regular Consumption of Sauerkraut and Its Effect on Human Health: A Bibliometric Analysis 酸菜的定期消耗量及其对人类健康的影响：一项文献计量分析 Consumo regular de chucrut y su efecto sobre la salud humana: un análisis bibliométrico," *Global Adv Health Med*, vol. 3, no. 6, pp. 12–18, 2014, doi: 10.7453/gahmj.2014.038.
[32] M. Ranjan Swain, M. Anandharaj, R. Chandra Ray, and R. Parveen Rani, "Fermented Fruits and Vegetables of Asia: A Potential Source of Probiotics," 2014, doi: 10.1155/2014/250424.
[33] F. R. Dinardo, F. Minervini, M. De Angelis, M. Gobbetti, and M. G. Gänzle, "Dynamics of Enterobacteriaceae and lactobacilli in model sourdoughs are driven by pH and concentrations of sucrose and ferulic acid," *LWT*, vol. 114, p. 108394, Nov. 2019, doi: 10.1016/J.LWT.2019.108394.
[34] M. G. Gänzle, "Enzymatic and bacterial conversions during sourdough fermentation," *Food Microbiol*, vol. 37, pp. 2–10, Feb. 2014, doi: 10.1016/J.FM.2013.04.007.
[35] W. Fu *et al.*, "Sourdough yeast-bacteria interactions results in reduced immunogenicity by increasing depolymerization and hydrolysis of gluten," *Innovative Food Science & Emerging Technologies*, vol. 84, p. 103281, Mar. 2023, doi: 10.1016/J.IFSET.2023.103281.
[36] K. Poutanen, L. Flander, and K. Katina, "Sourdough and cereal fermentation in a nutritional perspective," *Food Microbiol*, vol. 26, no. 7, pp. 693–699, Oct. 2009, doi: 10.1016/J.FM.2009.07.011.
[37] F. Lopitz-Otsoa, A. Rementeria, N. Elguezabal, and J. Garaizar, "Kefir: A symbiotic yeasts-bacteria community with alleged healthy capabilities," *Rev Iberoam Micol*, vol. 23, no. 2, pp. 67–74, 2006, doi: 10.1016/S1130-1406(06)70016-X.
[38] D. D. Rosa, M. M. S. Dias, Ł. M. Grześkowiak, S. A. Reis, L. L. Conceição, and M. D. C. G. Peluzio, "Milk kefir: nutritional, microbiological and health benefits," *Nutr Res Rev*, vol. 30, no. 1, pp. 82–96, Jun. 2017, doi: 10.1017/S0954422416000275.
[39] "SYMPOSIUM ON MICROBIALLY INDUCED FLAVORS AND FERMENTED FOODS Introduction", Accessed: Apr. 28, 2023. [Online]. Available: https://pubs.acs.org/sharingguidelines
[40] X. Zheng, X. Shi, and B. Wang, "A Review on the General Cheese Processing Technology, Flavor Biochemical Pathways and the Influence of Yeasts in Cheese," *Front Microbiol*, vol. 12, p. 2186, Jul. 2021, doi: 10.3389/FMICB.2021.703284/BIBTEX.
[41] "The Fermentation Process - Emmi USA." Accessed: Apr. 28, 2023. [Online]. Available: https://www.emmiusa.com/the-fermentation-process/

[42] I. C. Taveira, K. M. V. Nogueira, D. L. G. de Oliveira, and R. do N. Silva, “Fermentation: Humanity’s Oldest Biotechnological Tool,” *Front Young Minds*, vol. 9, Oct. 2021, doi: 10.3389/FRYM.2021.568656.
[43] S. T. Yang, H. Huang, A. Tay, W. Qin, L. De Guzman, and E. C. S. Nicolas, “Extractive Fermentation for the Production of Carboxylic Acids,” *Bioprocessing for Value-Added Products from Renewable Resources: New Technologies and Applications*, pp. 421–446, Dec. 2006, doi: 10.1016/B978-044452114-9/50017-7.
[44] “Aerobic and Anaerobic Fermentation.” Accessed: Jun. 13, 2023. [Online]. Available: https://www.slideshare.net/HazemHussein8/aerobic-and-anaerobic-fermentation
[45] “Aerobic vs. Anaerobic & Kombucha Fermentation - Kombucha.com.” Accessed: Jun. 10, 2023. [Online]. Available: https://www.kombucha.com/aerobic-vs-anaerobic-fermentation/
[46] “Aerobic Fermentation Processing - Lee Enterprises Consulting.” Accessed: Jun. 13, 2023. [Online]. Available: https://lee-enterprises.com/aerobic-fermentation-processing/
[47] G. Sourvinos, “Methods Involved in Aerobic and Anaerobic Fermentation,” *J Probiotics Health*, vol. 10, no. 6, pp. 1–1, Jul. 2022, doi: 10.35248/2329-8901.22.10.277.
[48] W. C. Huang and I. C. Tang, “Bacterial and Yeast Cultures – Process Characteristics, Products, and Applications,” *Bioprocessing for Value-Added Products from Renewable Resources: New Technologies and Applications*, pp. 185–223, Jan. 2007, doi: 10.1016/B978-044452114-9/50009-8.
[49] “15.3: Lactic Acid Fermentation - Chemistry LibreTexts.” Accessed: Jun. 13, 2023. [Online]. Available: https://chem.libretexts.org/Courses/University_of_Kentucky/UK%3A_CHE_103_-_Chemistry_for_Allied_Health_(Soult)/Chapters/Chapter_15%3A_Metabolic_Cycles/15.3%3A_Lactic_Acid_Fermentation
[50] P. M. Malo and E. A. Urquhart, “Fermented Foods: Use of Starter Cultures,” *Encyclopedia of Food and Health*, pp. 681–685, Jan. 2016, doi: 10.1016/B978-0-12-384947-2.00282-8.
[51] “The Science of Lactic Acid Fermentation.” Accessed: Jun. 13, 2023. [Online]. Available: https://www.seriouseats.com/science-of-lactic-acid-fermentation-preservation
[52] “Anaerobic Fermentation - Biology Wise.” Accessed: Jun. 14, 2023. [Online]. Available: https://biologywise.com/anaerobic-fermentation
[53] “Lactic acid and alcohol fermentation (article) | Khan Academy.” Accessed: Jun. 13, 2023. [Online]. Available: https://www.khanacademy.org/science/in-in-class-12-biology-india/xc09ed98f7a9e671b:microbes-in-human-welfare/xc09ed98f7a9e671b:microbes-in-industrial-products/a/lactic-acid-and-alcohol-fermentation
[54] Y. Wang *et al.*, “Metabolism Characteristics of Lactic Acid Bacteria and the Expanding Applications in Food Industry,” *Front Bioeng Biotechnol*, vol. 9, p. 612285, May 2021, doi: 10.3389/FBIOE.2021.612285/BIBTEX.
[55] C. Vidal-Carou, M. Ferreira, J. M. Salgado, H. Fernandes, H. Peres, and I. Belo, “Potential of Red, Green and Brown Seaweeds as Substrates for Solid State Fermentation to Increase Their Nutritional Value and to Produce Enzymes,” *Foods*

2022, Vol. 11, Page 3864, vol. 11, no. 23, p. 3864, Nov. 2022, doi: 10.3390/FOODS11233864.

[56] "Fermentation Types: 8 Types of Fermentations| Industrial Biotechnology." Accessed: Apr. 29, 2023. [Online]. Available: https://www.biotechnologynotes.com/industrial-biotechnology/fermentation-process/fermentation-types-8-types-of-fermentations-industrial-biotechnology/13695

[57] A. Septembre-Malaterre, F. Remize, and P. Poucheret, "Fruits and vegetables, as a source of nutritional compounds and phytochemicals: Changes in bioactive compounds during lactic fermentation," *Food Research International*, vol. 104, pp. 86–99, Feb. 2018, doi: 10.1016/J.FOODRES.2017.09.031.

[58] A. Machado de Castro, A. Fragoso dos Santos, V. Kachrimanidou, A. A. Koutinas, and D. M. G. Freire, "Solid-State Fermentation for the Production of Proteases and Amylases and Their Application in Nutrient Medium Production," *Current Developments in Biotechnology and Bioengineering*, pp. 185–210, Jan. 2018, doi: 10.1016/B978-0-444-63990-5.00010-4.

[59] J. A. Rodríguez-León, J. C. de Carvalho, A. Pandey, C. R. Soccol, and D. E. Rodríguez-Fernández, "Kinetics of the Solid-State Fermentation Process," *Current Developments in Biotechnology and Bioengineering*, pp. 57–82, Jan. 2018, doi: 10.1016/B978-0-444-63990-5.00004-9.

[60] W. H. Holzapfel, P. Haberer, R. Geisen, J. Björkroth, and U. Schillinger, "Taxonomy and important features of probiotic microorganisms in food and nutrition," *Am J Clin Nutr*, vol. 73, no. 2, pp. 365s–373s, Feb. 2001, doi: 10.1093/AJCN/73.2.365S.

[61] "What is lactic acid fermentation and how does it work? | Arla." Accessed: Apr. 29, 2023. [Online]. Available: https://www.arla.com/articles/what-is-lactic-acid-fermentation-and-how-does-it-work/

[62] J. Zheng *et al.*, "A taxonomic note on the genus Lactobacillus: Description of 23 novel genera, emended description of the genus Lactobacillus beijerinck 1901, and union of Lactobacillaceae and Leuconostocaceae," *Int J Syst Evol Microbiol*, vol. 70, no. 4, pp. 2782–2858, 2020, doi: 10.1099/IJSEM.0.004107.

[63] A. Corsetti and S. Valmorri, "Lactic Acid Bacteria | Lactobacillus spp.: Lactobacillus plantarum," *Encyclopedia of Dairy Sciences: Second Edition*, pp. 111–118, Jan. 2011, doi: 10.1016/B978-0-12-374407-4.00263-6.

[64] A. Corsetti and M. Gobbetti, "LACTOBACILLUS spp. | Lactobacillus plantarum," *Encyclopedia of Dairy Sciences*, pp. 1501–1507, Jan. 2002, doi: 10.1016/B0-12-227235-8/00242-X.

[65] B. Mayo and A. B. Fiórez, "Lactic Acid Bacteria: Lactobacillus plantarum," *Encyclopedia of Dairy Sciences: Third edition*, vol. 4, pp. 206–217, Jan. 2022, doi: 10.1016/B978-0-08-100596-5.00856-8.

[66] J. Peiren *et al.*, "Improving survival and storage stability of bacteria recalcitrant to freeze-drying: a coordinated study by European culture collections," *Appl Microbiol Biotechnol*, vol. 99, no. 8, pp. 3559–3571, Apr. 2015, doi: 10.1007/S00253-015-6476-6.

[67] A. Leandro, L. Pereira, and A. M. M. Gonçalves, "Diverse Applications of Marine Macroalgae," *Marine Drugs 2020, Vol. 18, Page 17*, vol. 18, no. 1, p. 17, Dec. 2019, doi: 10.3390/MD18010017.

[68] N. O. and A. A. US Department of Commerce, "What is seaweed?".

[69] H. P. S. Makkar *et al.*, “Seaweeds for livestock diets: A review,” *Anim Feed Sci Technol*, vol. 212, pp. 1–17, Feb. 2016, doi: 10.1016/J.ANIFEEDSCI.2015.09.018.
[70] “What’s The Difference Between Kelp & Seaweed? | Weed & Wonderful® Blog – Doctor Seaweed.” Accessed: Jun. 29, 2023. [Online]. Available: https://doctorseaweed.com/blogs/news/whats-the-difference-between-kelp-and-seaweed
[71] “Winged kelp | Wakame | Alaria Esculenta | Seaweed Solutions.” Accessed: Jun. 29, 2023. [Online]. Available: https://seaweedsolutions.com/winged-kelp-wakame-alaria-esculenta
[72] C. E. Lane, C. Mayes, L. D. Druehl, and G. W. Saunders, “A multi-gene molecular investigation of the kelp (Laminariales, Phaeophyceae) supports substantial taxonomic re-organization,” *J Phycol*, vol. 42, no. 2, pp. 493–512, Apr. 2006, doi: 10.1111/j.1529-8817.2006.00204.x.
[73] “Seaweed Species - Scandsea.” Accessed: Jun. 29, 2023. [Online]. Available: https://www.scandsea.se/seaweed-species/
[74] P. Cherry, C. O’hara, P. J. Magee, E. M. Mcsorley, and P. J. Allsopp, “Risks and benefits of consuming edible seaweeds,” *Nutr Rev*, vol. 77, no. 5, p. 307, May 2019, doi: 10.1093/NUTRIT/NUY066.
[75] J. Reboleira, S. Silva, A. Chatzifragkou, K. Niranjan, and M. F. L. Lemos, “Seaweed fermentation within the fields of food and natural products,” *Trends Food Sci Technol*, vol. 116, pp. 1056–1073, Oct. 2021, doi: 10.1016/J.TIFS.2021.08.018.
[76] Q. Yue *et al.*, “Hypolipidemic Effects of Fermented Seaweed Extracts by Saccharomyces cerevisiae and Lactiplantibacillus plantarum,” *Front Microbiol*, vol. 12, p. 772585, Nov. 2021, doi: 10.3389/FMICB.2021.772585/BIBTEX.
[77] R. Peng, Z. He, T. Gou, J. Du, and H. Li, “Detection of parameters in solid state fermentation of Monascus by near infrared spectroscopy,” *Infrared Phys Technol*, vol. 96, pp. 244–250, Jan. 2019, doi: 10.1016/J.INFRARED.2018.11.030.
[78] H. Jiang, G. Liu, C. Mei, S. Yu, X. Xiao, and Y. Ding, “Rapid determination of pH in solid-state fermentation of wheat straw by FT-NIR spectroscopy and efficient wavelengths selection,” *Anal Bioanal Chem*, vol. 404, no. 2, pp. 603–611, Aug. 2012, doi: 10.1007/S00216-012-6128-Y/FIGURES/6.
[79] S. Van Wychen and L. M. L. Laurens, “Determination of Total Carbohydrates in Algal Biomass: Laboratory Analytical Procedure (LAP),” 2013, Accessed: May 17, 2023. [Online]. Available: www.nrel.gov/publications.
[80] S. Van Wychen and L. M. L. Laurens, “Determination of Total Solids and Ash in Algal Biomass: Laboratory Analytical Procedure (LAP) (Revised),” 2013, Accessed: May 18, 2023. [Online]. Available: www.nrel.gov/publications.
[81] “Bacterial Freeze Drying Protocol.” Accessed: Jun. 14, 2023. [Online]. Available: https://opsdiagnostics.com/notes/ranpri/rpbacteriafdprotocol.htm
[82] “Freeze-drying service for long-term storage of bacterial cultures | BCCM Belgian Coordinated Collections of Microorganisms.” Accessed: Sep. 18, 2023. [Online]. Available: https://bccm.belspo.be/lmg/freeze-drying
[83] “https://lup.lub.lu.se/student-papers/record/9091598/file/9091606.pdf,” https://lup.lub.lu.se/student-papers/record/9091598/file/9091606.pdf. Accessed: Sep. 18, 2023. [Online]. Available: https://www.google.com/search?q=Fermentation+%2B+seaweed+%2B+Lyoflora+V3&rlz=1C5GCEM_enSE982SE983&ie=UTF-8#ip=1

[84] “Microbiology Writing Guide: Presenting Data | Writing Intensive Curriculum Program | Oregon State University.” Accessed: May 25, 2023. [Online]. Available: https://wic.oregonstate.edu/microbiology-writing-guide-presenting-data
[85] “Growth Curves, CFU and Optical Density Measurements | Microbiology | JoVE.” Accessed: May 25, 2023. [Online]. Available: https://www.jove.com/v/10511/growth-curves-generating-growth-curves-using-colony-forming-units
[86] “Colony Forming Unit (CFU) in Microbiology | Definition and Uses.” Accessed: May 27, 2023. [Online]. Available: https://www.toppr.com/guides/biology/microorganisms/colony-forming-unit-cfu-in-microbiology/
[87] “Effects of blanching, drying and fermentation on nutrient content and physio-chemical properties of Norwegian Saccharina latissima and Alaria esculenta for application in meat-based products | Lunds universitet.” Accessed: Jul. 01, 2023. [Online]. Available: https://www.lu.se/lup/publication/8993294
[88] B. K. Lonsane, N. P. Ghildyal, S. Budiatman, and S. V. Ramakrishna, “Engineering aspects of solid state fermentation,” *Enzyme Microb Technol*, vol. 7, no. 6, pp. 258–265, Jun. 1985, doi: 10.1016/0141-0229(85)90083-3.
[89] P. Dalgaard and O. Mejlholm, “Modeling Growth of Listeria and Lactic Acid Bacteria in Food Environments,” *Methods in Molecular Biology*, vol. 1918, pp. 247–264, 2019, doi: 10.1007/978-1-4939-9000-9_20/FIGURES/6.
[90] “Indigenous fermented foods,” 2007.
[91] J. S. Sørensen, S. K. Madsen, C. H. Bang-Berthelsen, and L. T. Hansen, “Quality and safety aspects in fermentation of winged kelp (Alaria esculenta) and sugar kelp (Saccharina latissima) by the natural microbiota with or without addition of a Lactiplantibacillus plantarum starter culture,” *Food Research International*, vol. 150, p. 110800, Dec. 2021, doi: 10.1016/J.FOODRES.2021.110800.
[92] A. Bruhn *et al.*, “Fermentation of sugar kelp (Saccharina latissima)—effects on sensory properties, and content of minerals and metals,” *J Appl Phycol*, vol. 31, no. 5, pp. 3175–3187, Oct. 2019, doi: 10.1007/S10811-019-01827-4/TABLES/4.
[93] C. Herrmann, J. FitzGerald, R. O’Shea, A. Xia, P. O’Kiely, and J. D. Murphy, “Ensiling of seaweed for a seaweed biofuel industry,” *Bioresour Technol*, vol. 196, pp. 301–313, Nov. 2015, doi: 10.1016/J.BIORTECH.2015.07.098.
[94] A. Pandey, C. Larroche, and C. R. Soccol, “General considerations about solid-state fermentation processes,” *Current Developments in Solid-state Fermentation*, pp. 13–25, 2008, doi: 10.1007/978-0-387-75213-6_2/COVER.
[95] K. Rohani-Ghadikolaei, E. Abdulalian, and W. K. Ng, “Evaluation of the proximate, fatty acid and mineral composition of representative green, brown and red seaweeds from the Persian Gulf of Iran as potential food and feed resources,” *J Food Sci Technol*, vol. 49, no. 6, p. 774, Dec. 2012, doi: 10.1007/S13197-010-0220-0.
[96] J. Yi, B. Dong, J. Jin, and X. Dai, “Effect of Increasing Total Solids Contents on Anaerobic Digestion of Food Waste under Mesophilic Conditions: Performance and Microbial Characteristics Analysis,” *PLoS One*, vol. 9, no. 7, p. e102548, Jul. 2014, doi: 10.1371/JOURNAL.PONE.0102548.
[97] “Evaluation of Novel Probiotics Acting on Seaweed Biomass | LUP Student Papers.” Accessed: Dec. 13, 2023. [Online]. Available: https://lup.lub.lu.se/student-papers/search/publication/9046733

[98] N. Narain, “Volatile Components in Seaweeds,” *Examines in Marine Biology & Oceanography*, vol. 2, no. 2, Aug. 2018, doi: 10.31031/EIMBO.2018.02.000535.

8 Appendices

Table 4 pH of the Saccharina latissima at 37 ℃ (Average + st. dev)

			Ex - 2 (*Saccharina latissimi)*					
	G+C		G + V3		NG + C		NG + V3	
Days	Average	St. dev	Average	St. dev	Average	St. dev	Average	St. dev
0	6.41	0.000	6.41	0.000	6.31	0.000	6.31	0.000
3	6.21	0.017	4.91	0.035	6.27	0.015	5.06	0.207
5	6.20	0.021	4.87	0.057	6.22	0.031	4.98	0.141
9	6.18	0.030	4.82	0.100	6.19	0.046	4.83	0.071
11	6.06	0.223	4.56	0.137	6.15	0.057	4.75	0.050
14	6.19	0.254	4.40	0.036	6.19	0.090	4.69	0.146

Table 5 pH of Alaria esculenta at 37℃ (Average + st. dev)

Ex - 3 *(Alaria esculenta)*								
	G + C		G + V3		NG + C		NG + V3	
Days	Average	St. dev	Average	St. dev	Average	St. dev	Average	St. dev
0	6.83	0.000	6.83	0.000	6.80	0.000	6.80	0.000
3	6.62	0.025	4.33	0.064	6.57	0.038	4.35	0.064
6	5.17	0.021	4.22	0.081	5.42	0.345	4.34	0.059

Table 6 pH of Alaria esculenta at room temperature (average + st. dev)

Ex - 4 *(Alaria esculenta)*								
	G + C		G + V3		NG + C		NG + V3	
Days	Average	St. dev	Average	St. dev	Average	St. dev	Average	St. dev
0	6.87	0.000	6.87	0.000	6.86	0.000	6.86	0.000
3	6.72	0.015	4.50	0.151	6.67	0.023	4.49	0.029
6	6.13	0.372	4.36	0.113	6.62	0.020	4.36	0.026

Table 7 Measured concentration of short chain fatty acids – lactic acid and formic acid, before (day – 0) and after (day - 14) fermentation of Saccharina latissima at 37℃, detected by HPLC

Ex - 2 (SCFA determination *Saccharina latissima at 37℃)*			
DAYS		LACTIC ACID	FORMIC ACID
0	NG-D0	0.011	-0.019
	G-D0	0.011	-0.019
6	G-control-D14	0.242	0.107
	G- V3 -D14	2.528	0.156
	NG-control-D14	0.728	0.479
	NG- V3 -D14	1.927	0.669

Table 8 Measured concentration of short chain fatty acids – lactic acid and formic acid, before (day – 0) and after (day - 6) fermentation of Alaria esculenta at 37℃, detected by HPLC

Ex - 3 (SCFA determination *Alaria esculenta 37℃*)			
DAYS		LACTIC ACID	FORMIC ACID
0	NG-D0	0.011	-0.019
	G-D0	0.126	0.115
6	G-control-D6	2.375	0.209
	G- V3 -D6	5.982	0.262
	NG-control-D6	1.915	0.295
	NG- V3 -D6	5.690	0.325

Table 9 Measured concentration of short chain fatty acids – lactic acid and formic acid, before (day – 0) and after (day - 6) fermentation of Alaria esculenta at 22℃ - room temperature, detected by HPLC

Ex - 4 (SCFA determination *Alaria esculenta at 22℃ room temperature*)			
DAYS		LACTIC ACID	FORMIC ACID
0	NG-D0	0.011	-0.019
	G-D0	0.126	0.115
6	G-control-D6	0.752	0.188
	G- V3 -D6	6.300	0.235
	NG-control-D6	0.066	0.139
	NG- V3 -D6	6.201	0.501

Table 10 Results of total solid, total moisture, ODW & total ash of fermented and unfermented Alaria esculenta sample

Ex - 3 & 4 (*Alaria*)						
	type	**Day**	**% Total Solids**	**% moisture**	**ODW**	**% Ash**
Ex - 3 & 4	**NG + C**	**0**	93.61	6.39	5.99	4.97
Ex - 3 & 4	**G + C**	**0**	93.01	6.99	6.42	4.81
Ex - 3	**G + C**	**6**	93.31	6.69	6.16	5.02
Ex - 3	**G + V3**	**6**	93.61	6.39	5.90	5.24
Ex - 3	**Ng + C**	**6**	93.92	6.08	5.73	4.90
Ex - 3	**Ng + V3**	**6**	94.01	5.99	5.45	5.32
Ex - 4	**Ng + V3**	**6**	94.51	5.49	6.14	4.83
Ex - 4	**Ng + C**	**6**	94.12	5.88	4.42	6.40
Ex - 4	**G + C**	**6**	93.31	6.69	6.16	5.02
Ex - 4	**G + V3**	**6**	93.51	6.49	5.98	5.16

Table 11 Results of total solid, total moisture, ODW & total ash of fermented and unfermented Saccharina latissima sample

Ex - 2 (*Saccharina*)						
	type	**Day**	**% Total Solids**	**% moisture**	**ODW**	**% Ash**
Ex - 2	**G + C**	**0**	93.31	6.69	5.13	7.05
Ex - 2	**NG + C**	**0**	93.80	6.20	5.25	7.61
Ex - 2	**NG + V3**	**14**	94.52	5.48	5.10	8.29
Ex - 2	**NG + C**	**14**	94.41	5.59	5.57	9.10
Ex - 2	**G + C**	**14**	94.11	5.89	5.46	6.63
Ex - 2	**G + V3**	**14**	94.31	5.69	5.28	6.85