CLASSIFICATION AND BIOCHEMICAL CHARACTERIZATION OF SPIRULINA (*Arthrospira* sp.) STRAINS USING FOCAL PLANE ARRAY FT-IR IMAGING

SINAPHON CHAIRAT

A Thesis Submitted in Partial Fulfillment of the Requirements for the
Degree of Master of Science in Biotechnology
Suranaree University of Technology
Academic Year 2024

การจัดจำแนกสายพันธุ์และการศึกษาคุณลักษณะทางชีวเคมีของสไปรูลินา (*Arthrospira* sp.) ด้วย Focal Plane Array FT-IR Imaging

นายศินพล ไชยรัตน์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเทคโนโลยีชีวภาพ
มหาวิทยาลัยเทคโนโลยีสุรนารี
ปีการศึกษา 2567

CLASSIFICATION AND BIOCHEMICAL CHARACTERIZATION OF SPIRULINA (*Arthrospira* sp.) STRAINS USING FOCAL PLANE ARRAY FT-IR IMAGING

Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for a Master's Degree.

Thesis Examining Committee

Kanjana Thumanu
.....
(Dr. Kanjana Thumanu)
Chairperson

.....
(Asst. Prof. Dr. Panwong Kuntanawat)
Member (Thesis Advisor)

.....
(Assoc. Prof. Dr. Mariena Ketudat-Cairns)
Member

.....
(Assoc. Prof. Dr. Panlada Tittabutr)
Member

.....
(Assoc. Prof. Dr. Pakpoom Boonchuen)
Member

Yupaporn Ruksakulpiwat
.....
(Assoc. Prof. Dr. Yupaporn Ruksakulpiwat)
Vice Rector for Academic Affairs
and Quality Assurance

N. Teaumroong
.....
(Prof. Dr. Neung Teaumroong)
Dean of Institute of Agricultural
Technology

ศินพล ไชยรัตน์: การจัดจำแนกสายพันธุ์และการศึกษาคุณลักษณะทางชีวเคมีของสไปรูลินา (*Arthrospira* sp.) ด้วย Focal Plane Array FT-IR Imaging (CLASSIFICATION AND BIOCHEMICAL CHARACTERIZATION OF SPIRULINA (*Arthrospira* sp.) STRAINS USING FOCAL PLANE ARRAY FT-IR IMAGING) อาจารย์ที่ปรึกษา: ผศ.ดร.พันธุ์วงค์ คุณธนะวัฒน์, 76 หน้า

คำสำคัญ: สไปรูลิน่า/เอฟทีไออาร์ สเปกโทรสโกปี/ไบโอเคมิคัล โพรไฟลิง/พีแอลเอส–ดีเอ/เควชาร์

สไปรูลิน่า (*Arthrospira platensis*) เป็นไซยาโนแบคทีเรียที่มีความสำคัญทางเศรษฐกิจและถูกใช้ในอุตสาหกรรมอาหารและเภสัชกรรมโดยทั่วไปผู้บริโภคอาจรับรู้ว่าสไปรูลินาที่มีขายในท้องตลาดมีลักษณะเป็นผลิตภัณฑ์ชนิดเดียวกันที่ทดแทนกันได้อย่างไรก็ตามการศึกษาทางวิทยาศาสตร์พบว่าสไปรูลินามีความหลากหลายทางสายพันธุ์สูงและมีองค์ประกอบทางชีวเคมีแตกต่างกันทั้งชนิดและปริมาณซึ่งยังสามารถแปรผันได้ตามเงื่อนไขการเพาะเลี้ยงคุณลักษณะนี้ทำให้สไปรูลินามีศักยภาพในการพัฒนาเป็นผลิตภัณฑ์เฉพาะทางที่มีมูลค่าสูงการระบุอัตลักษณ์ของสไปรูลินาตามสายพันธุ์และโปรไฟล์ทางชีวเคมีจึงเป็นกุญแจสำคัญต่อการสร้างความแตกต่างของผลิตภัณฑ์งานวิจัยนี้มีวัตถุประสงค์เพื่อพิสูจน์แนวคิดในการนำ FTIR imaging มาใช้ในการจำแนกสายพันธุ์สไปรูลินาจากองค์ประกอบทางชีวเคมีและศึกษาผลกระทบของปัจจัยการเพาะเลี้ยงต่อโปรไฟล์องค์ประกอบทางชีวเคมีดังกล่าว

การวิเคราะห์ด้วย Partial Least Squares Discriminant Analysis (PLS-DA) จากสัญญาณ FTIR ของสายพันธุ์ SB, C005H และ C005L พบค่าสัมประสิทธิ์ความสัมพันธ์ (R^2) เท่ากับ 0.914, 0.945 และ 0.914 ตามลำดับโดยมีค่า RMSECV เท่ากับ0.112, 0.140 และ 0.140 แบบจำลอง 3 แบบจำลอง ที่สร้างขึ้นจากสัญญาณเพื่อจัดจำแนกสไปรูลินา 3 สายพันธุ์ พบว่ามีความแม่นยำ (accuracy) ในการจำแนกสายพันธุ์เท่ากับ 97%, 93% และ 81% ตามลำดับขณะที่ค่าความไว (sensitivity) อยู่ที่ 100% ทั้งสามแบบจำลอง

การเปรียบเทียบปัจจัยการเพาะเลี้ยงพบว่าระยะเวลารับแสงที่นานขึ้น (8, 12 และ 24 ชั่วโมง) ส่งผลให้สัญญาณโปรตีนสูงขึ้นการใช้น้ำบาดาลเพิ่มสัญญาณโพลีแซคคาไรด์มากกว่าน้ำประปาและการเพาะเลี้ยงในห้องปฏิบัติการด้วยแสงสังเคราะห์ให้สัญญาณโปรตีนที่สูงกว่าในขณะที่การเพาะเลี้ยงกลางแจ้งให้สัญญาณโพลีแซคคาไรด์สูงกว่ากว่าผลลัพธ์นี้แสดงให้เห็นว่าโปรไฟล์ทางชีวเคมีของสไปรูลินาถูกกำหนดโดยปัจจัยสิ่งแวดล้อมและมีความเป็นไปได้ในเชิงแนวคิดว่า

FTIR imaging เป็นเครื่องมือที่มีศักยภาพในการจำแนกสายพันธุ์และติดตามสภาวะการเพาะเลี้ยง ซึ่งสามารถนำไปสู่การสร้างอัตลักษณ์และมูลค่าเพิ่มของผลิตภัณฑ์สไปรูลินาในอนาคต

สาขาวิชาเทคโนโลยีชีวภาพ
ปีการศึกษา 2567

ลายมือชื่อนักศึกษา.....
ลายมือชื่ออาจารย์ที่ปรึกษา.....

SINAPHON CHAIRAT: CLASSIFICATION AND BIOCHEMICAL CHARACTERIZATION SPIRULINA (*Arthrospira* sp.) STRAINS USING FOCAL PLANE ARRAY FT-IR IMAGING. THESIS ADVISOR: Asst. Prof. Panwong Kuntanawat, Ph.D., 76 PP.

Keyword: Spirulina/FTIR spectroscopy/Biochemical profiling/PLS-DA/Quasar

Spirulina (*Arthrospira platensis*) is a cyanobacterium of significant economic importance widely used in the food and pharmaceutical industries. Consumers generally perceive commercially available Spirulina products as undifferentiated and interchangeable. However, scientific studies have revealed that spirulina exhibits high strain diversity, with varying biochemical compositions in both type and quantity. Furthermore, these compositions can further fluctuate under different cultivation conditions. This characteristic provides spirulina with strong potential for development into high-value, specialized products. Therefore, identifying spirulina based on its strain and biochemical profile is a key factor in creating product differentiation. This study aimed to conceptually demonstrate the feasibility of using FTIR imaging to classify *spirulina* strains based on their biochemical compositions and to examine the effects of cultivation parameters on these biochemical profiles.

Partial Least Squares Discriminant Analysis (PLS-DA) of FTIR spectra from strains SB, C005H, and C005L yielded coefficients of determination (R^2) of 0.914, 0.945, and 0.914, respectively, with RMSECV values of 0.112, 0.140, and 0.140. The three models developed for strain classification achieved accuracies of 97%, 93%, and 81%, respectively, with all three models showing sensitivity of 100%.

Cultivation parameter comparisons revealed that longer light exposure (8, 12, and 24 hours) increased protein signal intensity. Groundwater cultivation enhanced polysaccharide signals compared to tap water. While indoor laboratory cultivation under artificial light produced stronger protein signals, outdoor cultivation resulted in higher polysaccharide signals. These findings indicate that the biochemical profile of spirulina is strongly influenced by environmental factors and conceptually highlight the potential of FTIR imaging as a tool for strain discrimination and indirect

monitoring of cultivation conditions. This approach could pave the way for establishing strain-specific identities and enhancing the market value of spirulina products.

School of Biotechnology
Academic Year 2024

Student's Signature.....
Advisor's Signature.....

ACKNOWLEDGEMENTS

The thesis project has been possible through the kind support and guidance of Asst. Prof. Dr. Panwong Kuntanawat provided valuable advice and knowledge on biotechnology, which facilitated the successful completion of the research and experiments during the study and review of this project.

I would like to thank Dr. Kanjana Thumanu for her advice on the use of the equipment and programs used in the research as well as suggestions on how to solve various problems during the research.

I would like to thank Assoc. Prof. Mariena Ketudat-Cairns for kind guidance, helpful suggestions, and support in developing my academic skills.

I would like to thank Assoc. Prof. Dr. Panlada Tittabutr, for kindly providing the most thorough and transparent perspective on analyzing special problems as well as various knowledge skills. Microbiology that is used in research.

I would like to thank Assoc. Prof. Dr. Pakpoom Boonchuen for kindly accepting as a committee and providing knowledge, and teaching the knowledge that is very necessary for conducting research.

Thank you for the OROG Scholarship for providing the opportunity for me to develop and increase my skills and knowledge in biotechnology

Thank you to Phoochit W. for stand by me. And Synchrotron Light Research Institute (Public Organization) for tools for research as well as training on data analysis used in research.

Sinaphon Chairat

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LITS OF TABLE

LITS OF FIGURES

LITS OF FIGURES (Continued)

Page

LITS OF FIGURES (Continued)

Page

LIST OF ABBREVIATIONS

HPLC	=	High Performance Liquid Chromatography
®	=	Registered Trademark
-	=	minus
™	=	trademark
%	=	percent
°C	=	Degree Celsius
µmol	=	Micromole
µL	=	Microliter
ANSI	=	American National Standards Institute
BMAA	=	beta-Methylamino-L-alanine
C	=	Carbon
CAPP	=	Cold Atmospheric Pressure Plasma
CO_2	=	Carbon Dioxide
Cu^{2+}	=	Copper (II) ion
ESA	=	European Space Agency
EU	=	European Union
FDA	=	Food and Drug Administration
GC-MS	=	Gas Chromatography–Mass Spectrometry
HACCP	=	Hazard Analysis and Critical Control Point
HPLC	=	High Performance Liquid Chromatography
ICP-AES	=	Inductively Coupled Plasma Atomic Emission Spectrometry
ICP-MS	=	Inductively Coupled Plasma Mass Spectrometry
ISO	=	International Organization for Standardization
IR	=	Infrared light
K	=	Potassium
LED	=	Light Emitting Diode
OD	=	Optical Density

LIST OF ABBREVIATIONS (Continued)

TCA Cycle	=	Tricarboxylic Acid Cycle
ToF-SIMS	=	Time-of-Flight Secondary Ion Mass Spectrometry
FAO	=	Food and Agriculture Organization of the United Nations
FTIR	=	Fourier Transform Infrared Spectroscopy
GMO	=	Genetically Modified Organism
GMP	=	Good Manufacturing Practice
P	=	Phosphorus
PBRs	=	Photobioreactors
PCA	=	Principal Component Analysis
PCs	=	Principal Components
pH	=	Positive Potential of the Hydrogen ions
PLS-DA	=	Partial Least Squares Discriminant Analysis
PS I	=	Photosystem I
PS II	=	Photosystem II
m^2	=	Square Meter
Mg^{2+}	=	Magnesium (II) ion
N	=	Nitrogen
NASA	=	National Aeronautics and Space Administration
Ni^{2+}	=	Nickel (II) ion
R^2	=	Coefficient of Determination
ROS	=	Reactive Oxygen Species
RMSEC	=	Root Mean Square of Calibration
RMSECV	=	Root Mean Square Error of Cross Validation
s	=	second
SDS-PAGE	=	Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis
sp.	=	species
SB	=	Super Blue
SBL	=	Super Blue Long
SL	=	Super Long

LIST OF ABBREVIATIONS (Continued)

SUT	=	Suranaree University of Technology
USDA	=	United States Department of Agriculture
XAFS	=	X-ray Absorption Fine Structure
XPS	=	X-ray photoelectron spectroscopy
XRD	=	X-ray Diffraction
ZM	=	Zarrouk's medium
Zn^{2+}	=	Zinc (II) ion

CHAPTER I
INTRODUCTION

1.1 Introduction

Spirulina (*Arthrospira* spp.) is a photosynthetic, multicellular, filamentous cyanobacteria that has a long history of utilization. It has been traditionally consumed for centuries as human food by various cultures which call by different names, such as dihé in Africa and tecuitlatl in Mexico (Henrikson 1994; Abdulqader et al., 2000). Countless scientific studies highlight the fact that Spirulina is a rich source of nutrients and of high-value compounds, as it has a very high contents of macro- and micronutrients, essential amino acids, up to 62.7% protein content, 15.6% carbohydrates, 8.1% fat, 3.1% fiber, vitamins, minerals, and anti-oxidants (Aouir et al., 2017).

Nowadays, Spirulina is commercially produced worldwide and is mainly cultivated for nutritional biomass as human food and supplement, as well as for animal feed (Priyadarshani et al., 2012). High-value natural pigments such as phycocyanin, which is exclusively found in cyanobacterial cells, can be obtained from Spirulina. In the past few decades, Spirulina made its name in the food supplement market as one of the gold standards. Unfortunately, regardless of its manifold nutritional and health benefits and despite its great business potential, Spirulina has gradually lost its shine and is not in the global nutritional spotlight anymore. Based on the market research conducted by Siambiota Co., Ltd (unpublished study), the main reason for this loss of interest towards Spirulina over the past decades resides in an avoidable marketing mistake: manufacturers constantly advertise Spirulina as a single universal product. Spirulina can indeed be purchased under a variety of forms (i.e. tablets, powder, and capsules) but variations of the product itself (i.e. strains, cultivars, cultivation methods) have not been mention. In addition, the health benefits of Spirulina have been marketed with unclear directions and overclaimed. This gradually generalized Spirulina

products, creating a biased customer perception that Spirulina is one single agricultural product with indifferent yet arguable properties. This perception has been scientifically proven to be false, as the health benefits of Spirulina are indeed significant in different model organisms and between clinical trials (Marles et al., 2011), but they also vary greatly depending on Spirulina species, strains, and the way they have been cultured (Van Eykelenburg and Fuchs, 1980). As the trends of plant-based protein food and holistic nutritional supplements have recently come into fashion, it is believed that Spirulina may regain its position in the market again. However, in order to achieve and make a Spirulina-based sustainable business, its marketing has to be strategic, notably by showcasing the varieties of the Spirulina produced (i.e. species, strains, and the ways they are cultivated) with claimable benefits that are specific to a given variety. The validation of the Spirulina varieties is however a challenging task for manufacturers, but it is also a crucial step for the determination of varieties, customer recognition, and therefore for the successful strategic marketing of Spirulina. Unlike other agricultural products such as durians, mangoes, or rice of certain cultivars, ways of farming, and regions whose characteristics may be observed through ordinary senses such as visible appearance, scent, and taste, differences in the characteristics of Spirulina varieties can only be confirmed at a biomolecular/biofunctional level. Biochemical analysis equipment such as HPLC (High Performance Liquid Chromatography), Raman spectroscopy and gas analyzers may be used to characterize such biochemical differences. Although these methodologies can deliver accurate results, they are less practical for repeated use on an industrial scale due to their requirements for complicated sample preparation, the time spent to proceed with the analysis, demand for technical skills to operate and operational costs. Alternative methodologies, such as UV-Vis spectrophotometry and FTIR/UV-Vis spectroscopy (Fourier Transform Infrared Spectroscopy) are less time-consuming, less complicated, and much more cost-effective. These techniques allow for the optical responses of certain constitutional biomolecules, which are specific to broad scanned wavelengths, to be semi-quantitatively measured. The profile of the optical response over the range

of scanned wavelengths is therefore specific to a sample of a unique biochemical constitution. In other words, this profile can be used as a "fingerprint" of a biochemically distinct sample. A similar technique has been previously implemented in characterizing and identifying agricultural products such as yeast, grassland plant species, and also chicken (Rana et al., 2018; Shapaval et al., 2019; Katemala, S., 2022). In this particular study, our aim is to investigate the relationship between the biochemical profiles obtained from UV-Vis spectrophotometry and FTIR spectroscopy and link it to a single variety of Spirulina (i.e. strains and the ways they are cultivated), which will ultimately allow for the construction of the standard biochemical fingerprints of specific Spirulina products. Finally, we aim to test these fingerprints for their ability to determine strains and ways of cultivation of unknown Spirulina samples.

1.2 Research Objective

1. To create and validate standard biochemical profiles base on FTIR analysis for the classification of Spirulina strains

2. To characterize biochemical profile with FTIR specific to the species or standard culture.

1.3 Research hypothesis

The biochemical profile of Spirulina is generated from FTIR that can determine the difference of species and culture in terms of different condition factors. These biochemical profiles can be used to determine the species and culture, water quality, origin of species, and type of media used to grow Spirulina.

1.4 Scope of research

This research focuses on studying and determining the SB, C005H and C005L species (previously developed at SUT) and standard culture in terms of quality of water, duration of light, indoor and outdoor culture with the aim of creating biochemical profiles based FTIR analysis results. This method aims to be effective both for known and unknown samples.

CHAPTER II

LITERATURE REVIEWS

2.1 Spirulina Cultivation and Environmental Influences

Spirulina is a multicellular and filamentous cyanobacterium whose open helix cylindrical shape undergoes binary fission. It is classified within the phylum known as blue-green algae. It has been reported to exist in lakes, ponds, and lagoons in Kenya, Egypt, India, Thailand, France, etc. (Rich 1931; Vareschi 1982; El-Bestawy et al., 1996).

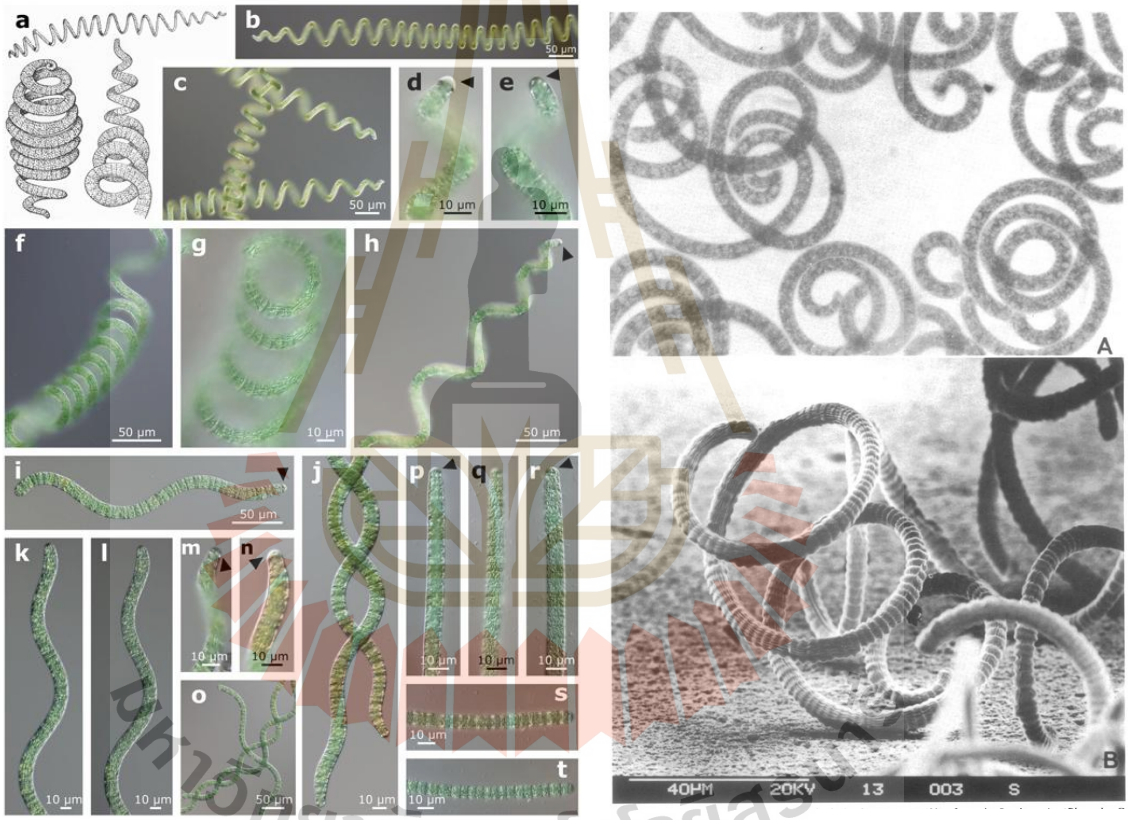

Figure 2.1 Microscopic examination of Spirulina under light microscope (Nowicka-Krawczyk, et al 2019) and scanning electron microscope (Aissaoui, O., et al 2017)

The growth conditions for Spirulina depend on nitrogen, potassium and bicarbonate concentrations, an optimal pH and culture salinity. Furthermore, since Spirulina is autotrophic, it has a very high content of macro and micronutrients, essential amino acids, proteins, carbohydrates, fats, fiber, vitamins, minerals, and anti-

oxidants (Aouir, A. et al., 2017), and produces various pigments such as phycocyanin, lutein, and beta-carotene (Leema et al., 2010).

Table 2.1 Typical analysis of the composition of Spirulina (per 3 grams of dry biomass) (Shao, W., et al 2019)

Items	Amount	Items	Amount
General Composition		**Phytonutrients**	
Carbohydrates	17-25%	Chlorophyll	30 mg
Protein	53-62%	Total carotenoids	15 mg
Lipids	4-6%	β-carotene	6.8 mg
Minerals	8-13%	Total phycocyanins	519 mg
Vitamins		C-phycocyanin	240 mg
Vitamin A	11,250 IU	Zeaxanthin	9 mg
Vitamin B1	3.5 µg	Superoxide dismutase	1080 units
Vitamin B2	140 µg	**Minerals**	
Vitamin B3	400 µg	Calcium	10 mg
Vitamin B6	30 µg	Magnesium	15 mg
Vitamin B12	9.0 µg	Iron	6.5 mg
Vitamin E	285 µg	Phosphorus	33 mg
Inositol	1.7 µg	Potassium	60 mg
Biotin	0.5 µg	Sodium	30 mg
Folic acid	6.2 µg	Manganese	400 µg
Pantothenic acid	4.5 µg	Zinc	90 µg
Vitamin K1	60 µg	Boron	22 µg
Vitamin K2	15 µg	Copper	20 µg
		Selenium	0.9 µg
		Iodine	15 µg

Therefore, Spirulina has been used in food for a long time and is thought to be safe for human consumption based on the latest scientific findings. Additionally, to meet their protein needs, African tribes incorporate Spirulina biomass into their local cuisine (Beshi, M. 2021). It has been selected as one of the main meals for extended space travel by the National Aeronautics and Space Administration (NASA) and the European Space Agency (ESA). Spirulina is exported as a nutrition product in Bangladesh, Spain, France and Ireland just to name a few (Ihsanullah et al., 2017). Spirulina has gained popularity in the aquaculture, food, and health sectors due to its ease of harvesting, processing, and water-based growth. It has a high market value for use in the creation of value-added products and supplemental foods because of its

high protein content and nutrient bioavailability. The growth of Spirulina is affected by various environmental factors. Physical factors are necessary for the industrial growth and production of Spirulina. Such as pH, temperature, light, and nutrients, which directly affect the photosynthesis process. (Abu et al., 2007; Colla et al., 2007; Madkour et al., 2012; Costa et al., 2003). Another significant factor influencing cell size, growth rate, and biochemical makeup is temperature. Low temperatures, however, have an impact on CO_2 synthesis, leading to photoinhibition, which lowers PSII efficiency and protein D1 repair (Vonshak, A., et al 2014). It also influences the production of carotenoid pigments. This is in charge of absorbing light energy and preventing damage to chlorophyll. When it comes to light quality and intensity, red light has the greatest effects on growth and protein accumulation because it directly absorbs chlorophyll a, phycocyanin, and allophycocyanin (Wang et al., 2007). Compared to other light conditions, blue light results in lower growth rates but encourages high protein production. Furthermore, the dry weight production efficiency is in the following order: Green > Yellow > Blue > White > Red. Excessive light levels (greater than 2500 μmol/(m^2·s)) can cause ROS production and harm the PSII system. (Y. Zhang et al., 2024). Chlorophyll a and protein accumulation are increased by prolonged exposure to light (Posten, 2009). In addition to temperature and light levels, the makeup of nutrients and exposure to heavy metals have a variety of effects on cells. According to Muwafq and Bernd (2006), copper (Cu^{2+}) is extremely toxic, destroys chlorophyll, and promotes the production of ROS. Although it has less of an impact than copper, zinc (Zn^{2+}) promotes the production of plant paints (El-Maghrabi, 2002). Although it has less of an impact than copper and zinc, nickel (Ni^{2+}) stimulates the formation of partial pigmentation. Magnesium (Mg^{2+}): Enhances pH and encourages the synthesis of proteins and chlorophyll (Nyabuto et al., 2015). Macronutrients (N, P, C): At 2.5% salinity, formulas with $NaNO_3$, K_2HPO_4, and $NaHCO_3$ produce the highest yield (Nyabuto et al., 2015). Salinity influences these differences, affecting the growth, productivity, and size structure of Spirulina strains (Lao & Edullantes, 2025).

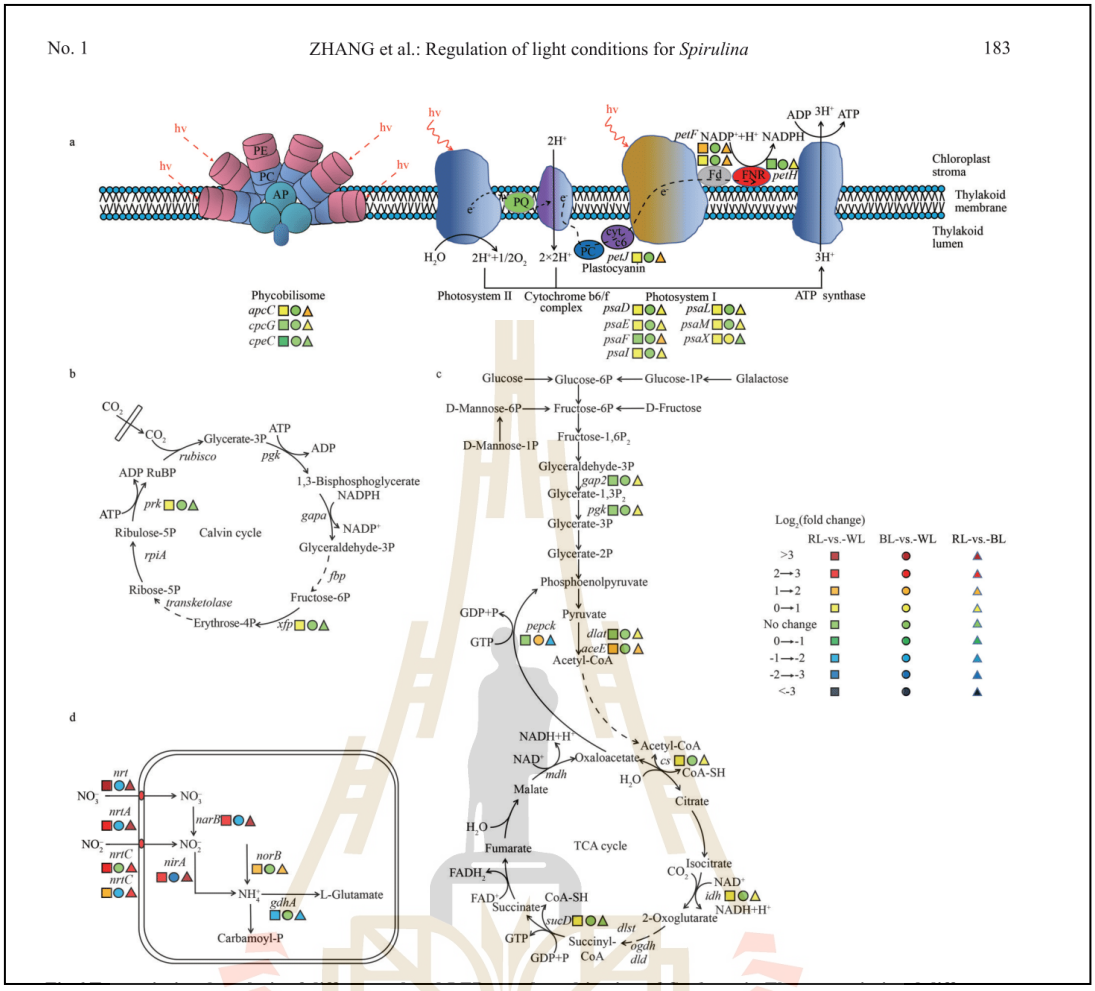

Figure 2.2 Regulation of light conditions for Spirulina **Source:** Zhang, Y., et al. (2024)

Spirulina can be grown in open systems, such as lakes, lagoons, and ponds, or in closed systems, like vertical column photobioreactors (PBRs) and polybags (Singh and Sharma, 2012). Although minor, space requirement has measurable effects on growth efficiency. Limited space can lead to uneven light distribution and reduced growth efficiency in dense cultures. And overcrowding limits light penetration and nutrient availability. The evaporation in open systems or high-temperature environments, water loss through evaporation can alter medium concentration. This may lead to increased salinity, affecting Spirulina's metabolic activity and osmotic balance. Carbon dioxide (CO_2) is essential for photosynthesis. But it decreases via surface exchange or degassing reduces carbon availability. In case of Temperature (Weather Dependence). Outdoor systems are highly sensitive to daily and seasonal temperature fluctuations. Hydrodynamic Stress may be caused by mixing, aeration, or turbulence in reactors. Moderate agitation promotes gas exchange and prevents settling, but excessive stress can damage filaments and reduce productivity (Sili, C., et al 2012). A closed system (e.g., vertical column PBR, poly bags, etc.) or an open system (e.g., raceway pound, attached culture, etc.) can be used to cultivate industrial Spirulina. An open system has the advantages of being ideal for mass algae cultivation, reasonably priced, and simple to clean up after cultivation. Open culture systems have several drawbacks, including less control over culture conditions, trouble maintaining algal cultures for extended periods of time, low productivity, taking up a lot of land, being limited to a small number of strains of algae, and being easily contaminated. High mass transfer, good mixing with low shear stress, a high potential for scalability, ease of sterilization, good immobilization of algae, decreased photoinhibition and photo-oxidation, and the convenience of gas supply are the benefits of a closed system (Soni et al., 2021).

However, even in most controlled culture systems, differences between and within Spirulina strains continue to occur. The most recent research on Spirulina found both intra- and interspecific differences in chemical composition between *Arthospira*

platensis and *Arthospira maxim*a, when cultivated under different light conditions (Milia et al., 2022)

However, even in the most controlled culture systems, differences between and within Spirulina strains continue to occur. Recent research has confirmed both intraspecific and interspecific variations in chemical composition and morphology, for example between *Arthrospira platensis* and *Arthrospira maxima* under different light conditions (Milia et al., 2022; Chaiyasitdhi, 2018). More recent publications also report increasing evidence of strain-specific differences, including taxonomic reclassification of commercially grown taxa into the new genus *Limnospira* (Nowicka-Krawczyk et al., 2019; Sinetova et al., 2024). These findings emphasize that both genetic background and cultivation conditions contribute to variability among Spirulina strains.

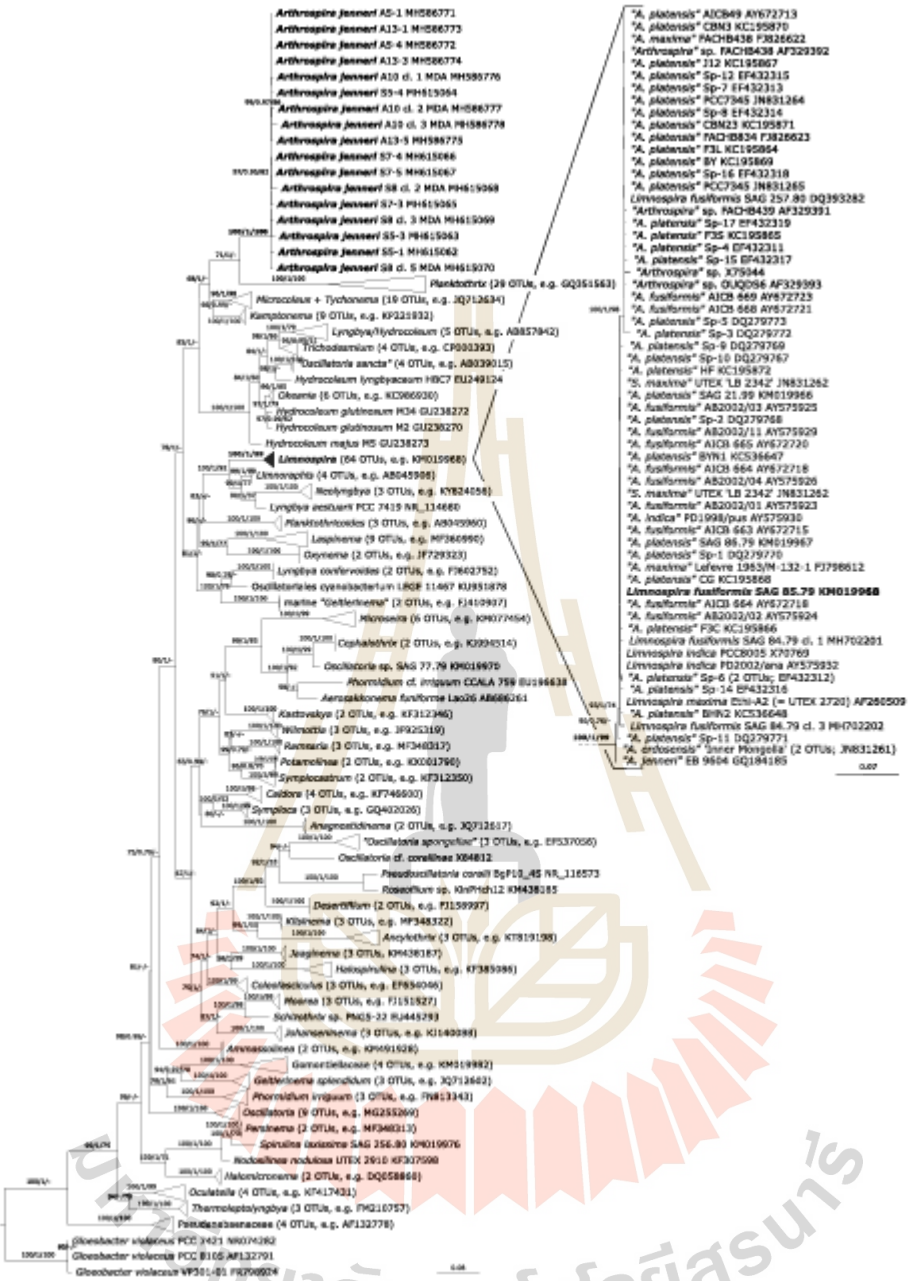

Figure 2.3 Phylogenetic tree of *Arthrospira* and *Limnospira*. Source: Nowicka-Krawczyk, P., et al 2019

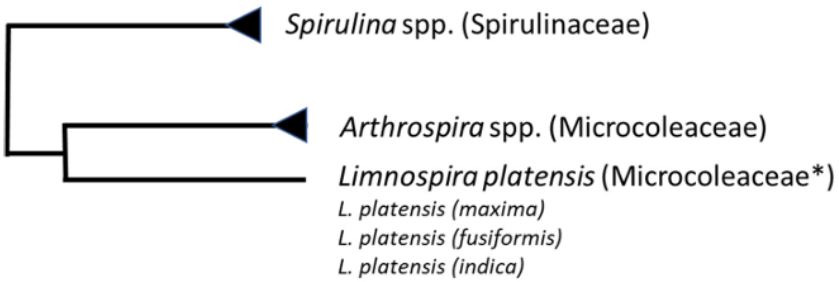

Figure 2.4 A simplified scheme of taxonomic positioning of Spirulina. Source: Sinetova, M. A., et al 2024

Beyond its biological structure, the increasing demand for Spirulina has led to widespread commercial production and innovation across the global topics that are explored in the following section.

2.1.1 Trends in the uses of Spirulina microalgae

Commercialized Spirulina is increasingly being grown and used as food and feed globally, including in Europe, due to its high nutritional value and health benefits as previous information. Regional Production and Consumption Patterns: Commercialized Spirulina is increasingly being grown and used as food and feed globally, including in Europe, due to its high nutritional value and health benefits. The FAO estimates that annual biomass production levels have reached roughly 30,000 tons, and its use has nearly doubled globally over the last 20 years. Spirulina is one well-known, secure, and nutritious food item. It has been officially classified as "Marine Algae" since 2017 and is included in the EU Food Catalog. As a result, it is now governed by EU Organic Products Regulation RCE 889/2008.

France's Paris. In 1974, Mr. Durand Chastel, the French manager of Sosa Texcoco, established the first large-scale commercial Spirulina biomass production plant after determining that Spirulina was a resource that was hindering the carbonate evaporation process at the carbonate production site in Mexico owned by the Sosa Texcoco Company. Microalgae cultivation has a long history, dating back to early international research conducted in the US, Japan, Germany, and other countries. Bur

Lew's 1953 book "Algal culture, from laboratory to pilot plant" went into great detail about this. The first pilot plant was established in Japan as a result of this research, and in 1960 a commercial *Chlorella* production facility was constructed there. In order to develop microalgae cultivation for protein products in Germany, a global effort was gathered at that time (Soeder and Binsack 1978).

Large-scale Spirulina production has also expanded to several nations, most notably the US and China, which currently account for more than half of global production. A global average of 20,000 tons annually is frequently reported, but Asian production volumes, which can range greatly from 10,000 tons annually to more, are frequently overlooked. (Wurmann et al., 2016; Virginin et al., 2022; Vieira et al., 2025).

Table 2.2 Trends in the uses of Spirulina microalgae (Soeder and Binsack 1978:Wurmann, C., et al 2016:Virgin, I., et al 2022.:Vieira, V. V., et al 2025)

Region	Major product	Consumption Trend	Market Characteristics
North America	USA, Mexico	High supplement consumption, growing natural food coloring market	Premium product positioning, well-developed distribution
Europe	France, Spain, Germany	Strong demand for natural ingredients, strict regulations on synthetic additives	Focus on sustainability, organic certification
Asia	China, India, Thailand, Japan	Rapidly growing production, increasing domestic consumption	Cost-effective production, traditional usage history
Latin America	Brazil, Chile	Growing production capacity, export-oriented	Favorable cultivation conditions
Africa	Kenya, Ethiopia, South Africa	Emerging production, focus on nutritional applications	Addressing malnutrition, development initiatives

2.1.2 Commercial Applications

Pigments in Spirulina gained attention in the field of food and human nutrition due to their health benefits and fluorescence under infrared light. As a result, they have been increasingly incorporated into various food and beverage products, and the market for this segment is anticipated to grow significantly by 2030, although the nutraceutical segment held the largest market share in 2021 (Desai, S. S., & Mane, V. K. 2024). Among other things, they have been added to ice creams, snacks, muffins, crackers, bars, cookies, bread, pasta and noodles, yoghurts, jelly gums, and smoothies and other drinks (Alfadhly, N.K.Z. et al 2022). Spirulysat® and Spirugrass® have been successfully registered by Algo Source. The first is a well-known extract with a high phycocyanin content that is enhanced with amino acids, polysaccharides, and other substances. However, Spirugrass®, a biorefining byproduct of Spirulina, is distinguished by its high content of beta-carotenes, iron, vitamin K, and amino acids. (T. Dalmonte, 2024)

Aquarists and Aquaculture The total production of aquaculture animals was 87.5 million tons in 2020, valued at USD 264.8 billion, and is projected to grow to 106 million tons in 2030. To meet the rising demand for aquatic animal foods, aquaculture development must continue to be innovative and sustainable. Microalgae can be added to aquaculture in two different ways: first, as food for zooplankton, which in turn provides food for fish and their larvae; second, as a component of feed for adult fish, replacing fish meal or fish oil. Aquaculture companies usually have their own microalgae production systems for feeding zooplankton.

Additionally, fish fed copepod fortified with *Chlorella* sp. and Spirulina (*Arthrospira*) sp. showed the highest growth and survival rates of *Betta splendens*. These copepods have the potential to replace fish oil and fish meals in diets, improving meat quality and growth, boosting immunity, and improving pigmentation in a variety of fish species. (Ahmad, M.T. et al 2020)

Bioactive compounds and lipids obtained from microalgae are becoming more widely acknowledged as viable substitutes for traditional synthetic

ingredients in the cosmetic and skin care industries. A variety of cosmetic products, such as eyeliners, lipsticks, eye shadows, moisturizers, face cleansers, shampoos, sunscreens, and beauty masks, can be made using microalgae or particular bioactive compounds. The anti-aging and wrinkle-reducing properties of bioactive compounds from *Chlorella* sp. and Spirulina (*Arthrospira*) sp., which have antioxidant and free radical scavenging properties, make them useful in skincare and cosmetic products. In creams and sunscreens, carotenoids and peptides provide superior UV protection, while polysaccharides are best suited for moisturizing and supporting the reservation of the skin's oil balance and water barrier. Triacylglycerides, waxes, ceramides, phospholipids, sterols, as well as hydrogenated, esterified, and oxidized lipids, are varieties of microalgal lipids that are frequently utilized in cosmetics. With the growing demand for safe and environmentally friendly cosmetics and skin care products, ingredients derived from *Chlorella* sp. and Spirulina (*Arthrospira*) sp. are expected to play a significant role in the industry. Each of these lipids contributes distinct properties to cosmetic formulations, making them valuable and versatile ingredients. their capacity to offer sustainable and effective substitutes. Algenist used Spirulina to create the Blue Algae Vitamin C™, an active blue form of L-ascorbic acid. Spiruderm®, a liquid Spirulina extract with a high phycocyanin concentration, was created by AlgoSource and is used as an active ingredient to moisturize, re-densify, and smooth out fine lines on the skin. (Zhuang, D. et al. 2022: Ragusa, I. et al. 2021).

Feed for Animals Following extensive web research, it was discovered that there are very few animal feed products. Adding Spirulina to the diets of pigs and poultry has the potential to replace less sustainable protein sources like fishmeal while having no discernible effect on animal productivity or product quality. Spirulina has also been shown to have a number of positive effects on poultry, such as lowered cholesterol, better growth, and strengthened immunity. Additionally, it raises glutathione peroxidase, oxidant capacity, oxidative stability, and high-density lipoproteins in chickens. (B. A. Altmann & S. Rosenau, 2022)

Farming Global agricultural practices today heavily rely on synthetic

pesticides and fertilizers, which has negative effects on the environment and human health. The growth of green gram (*Vigna radiata*), including the length of its shoots and roots as well as its weight at the flowering stage, was enhanced by biofertilizer extracts of *C. vulgaris* and Spirulina. Along with improving the plant's physical traits like its ability to absorb water and oil, these treatments also had a positive impact on the pH, EC, and mineral content of the soil. *Arthrospira* sp., or Spirulina, has gained interest recently as a sustainable source of agricultural biostimulants. This microalgae is probably the result of a combination of bioactive substances, including vitamins, amino acids, polysaccharides, and phytohormones. Thus, biostimulants derived from Spirulina may offer a sustainable substitute for chemical pesticides, fertilizers, and growth regulators. Furthermore, six of the nine fungal pathogen strains tested were successfully inhibited by the Spirulina-based biostimulants, which also decreased the growth of the pathogens. When the effects of two biostimulants Spirulina and Egyptian clover (*Trifolium alexandrinum*) on the soil characteristics, growth, and yield of a pea (*Pisum sativum* L.) plant were assessed, it was discovered that applying these biofertilizers separately or in combination greatly enhanced plant growth and yield. (A. L. Gonçalves, 2021).

Pharmaceutical Substances Numerous bioactive substances with a variety of biological characteristics, such as anti-inflammatory, anticoagulant, antioxidant, antimicrobial, anticancer, and neuroprotective effects, are produced by microalgae. The bioactive substances found in Spirulina (*Arthrospira*) sp. and *Chlorella* sp. may aid in the creation of novel medications for both humans and animals. (A. P. Abreu et al., 2023)

Biopolymers The rapid rate of global industrialization, which is currently endangering global stability, necessitates urgent attention to the replacement of petroleum-based products and polymers. The ability of Spirulina to produce PHA through induced nitrogen deficiency has been investigated. Coelho et al. and Costa et al., for example, demonstrated yields of 30.7% PHA (w/w dry biomass) and 12.0% PHA (w/w dry biomass), respectively. A study by Corrêa et al. from 2021 also used Spirulina

to produce PHA. (S. G. Mastropetros et al., 2022).

Since *Chlorella* sp. and Spirulina (*Arthrospira*) sp. can treat a wide range of wastewater types, including municipal, aquaculture, swine, olive oil milling, distillery, confectionary, brine tapioca, tofu, rubber, paper mill, dye wastewaters, agricultural runoffs, and groundwaters, wastewater treatment by these two species has been documented. *Chlorella* sp. and Spirulina (*Arthrospira*) sp. biomass can be generated and then valorized during wastewater treatment. It should be mentioned that microalgae-based wastewater systems are different from traditional wastewater treatment methods because they are inexpensive and offer two main advantages: (1) wastewater purification and (2) simultaneous biomass harvesting. Furthermore, a recent study demonstrated that the wastewater treated by microalgae could be used again for irrigation, which is thought to be the primary use of freshwater and is highly advantageous for the environment and economy. (A. P. Abreu et al., 2023)

Table 2.3 Industrial Applications and Cultivation (Ahmad,M.T. et al 2020: Gonçalves, A. L. 2021: Ragusa, I. et al 2021: Altmann, B. A., & Rosenau, S. 2022: Mastropetros, S. G., et al 2022:Zhuang, D. et al 2022:Alfadhly, N.K.Z. et al 2022: Abreu, A. P., et al 2023: Desai, S. S., & Mane, V. K. 2024: Dalmonte, T. 2024)

Industry Sector	Applications	Growth Driver
Food & Beverages	Natural food colorant (blue), nutritional supplements, protein-enriched foods, bakery products, beverages, confectionery, dairy	Clean-label trend, plant-based diet growth, natural colorant demand
Nutraceuticals	Dietary supplements (tablets, capsules, powders), functional ingredients, protein supplements	Health consciousness, preventive healthcare, aging population

Personal Care & Cosmetics	Natural pigments, antioxidant formulations, anti-aging products, skincare, hair care	Clean beauty trend, demand for natural ingredients
Feed for Animals	Aquaculture feed, poultry feed, pet food supplements	Sustainable farming practices, demand for natural feed additives
Agriculture	Biofertilizers, biostimulants, plant growth enhancers	Organic farming growth, sustainable agriculture
Pharmaceuticals & Biotechnology	Therapeutic proteins, bioactive peptides, genetic engineering platforms	Research advancements, biomedical applications
Environmental Applications	Wastewater treatment, carbon capture, bioremediation	Climate change mitigation, environmental regulations
Cultivation	Open raceway ponds, Photobioreactors, Thin-layer cascade systems	Commercial biomass production
Harvesting	Filtration, Centrifugation, Flocculation	Biomass recovery
Drying	Spray drying, Sun drying, Freeze drying	Powder production
Extraction	Ultrasound-assisted, Enzymatic, Supercritical CO_2	High-value compound isolation
Formulation	Encapsulation, Tablet compression, Blending	Product development
Biorefinery	Fractionation, Sequential extraction	Multiple product streams

Table 2.4 Product Forms and Their Industrial Utilization (Payne, E. 2023: Sabat, S., et al 2025)

Product Form	Primary Industries
Powder	Food, Nutraceuticals, Cosmetics
Tablets & Capsules	Nutraceuticals, Pharmaceuticals
Phycocyanin	Food coloring, Cosmetics, Biotechnology
Liquid Extract	Beverages, Skincare
Flakes & Granules	Food, Animal Feed

The integration of exogenous genes into the Spirulina chromosome through markerless homologous recombination and the stable, high-level expression of therapeutic proteins, such as bioactive peptides, single-chain antibodies, enzymes, signaling proteins, and vaccine antigens, are examples of genetic engineering techniques for Spirulina. (B. W. Jester et al., 2022).

According to recent research, algae hold promise as a biomass source for the production of bioplastics because they provide a number of benefits, including lower carbon dioxide emissions, less food waste, and lower energy consumption. The high protein content of microalgae is a key component of the polymer's advantageous characteristics. Numerous studies on microalgae have taken advantage of their biomass potential for the production of biofuel, biochemicals, and substitute foods. (W. Y. Cheah et al., 2023).

Biorefineries based on Spirulina are a good way to improve both environmental sustainability and economic viability. The current review examines the difficulties and potential future developments related to the biorefining of Spirulina to produce protein and c-phycocyanin, with the goal of advancing a circular bioeconomy. (B. Thevarajah et al., 2022).

The potential health benefits of certain bioactive peptides derived from Spirulina, including their antimicrobial, antiallergic, antihypertensive, antitumor, and immunomodulatory qualities, are being investigated. (C. A. Ovando et al. 2018).

The most prevalent organic carbon sources that increase the biomass yield in Spirulina are glucose and acetate, as has been extensively documented. Therefore, it is possible that the organic materials (such as lactose, fats, proteins, and perhaps other additives) commonly found in wastewater from dairy processing could promote Spirulina cell growth. Furthermore, research has shown that the organic carbon content may help cells grow during the later stages of a growth cycle when the light-restricted cultures' photosynthetic activity is at its lowest. (W. T. Chang and others, 2013)

Table 2.5 Recent Industrial Innovations and Emerging Applications (Chang, W. T., et al 2013: Ovando, C. A., et al 2018: Thevarajah, B., et al 2022: Jester, B. W., et al 2022: Cheah, W. Y., et al 2023)

Innovation	Industrial Sector
Genetic engineering of Spirulina for therapeutic proteins	Biotechnology, Pharmaceuticals
Spirulina-based bioplastics	Packaging, Materials
Spirulina biorefinery systems	Multiple industries
Spirulina as therapeutic protein production platform	Pharmaceuticals
Spirulina-derived peptides as functional ingredients	Food, Cosmetics
Carbon-capture utilization with Spirulina	Environmental, Energy

2.1.3 Assessment of Spirulina Quality

Microbiological testing is an essential procedure for preventing and identifying health, safety, and well-being crises. It can identify contaminants like pesticides and heavy metals (cadmium, lead, arsenic, and mercury) as well as harmful bacteria like *Salmonella*, mold, yeast, and *E. coli*. (M. A. Pfaller et al., 2004).

In contrast, physical quality testing is a more accessible method, as it involves evaluating external characteristics such as the color, odor, and texture of Spirulina. In recent years, several physical quality testing methods have been further developed, particularly through the application of CAPP (Cold Atmospheric Pressure Plasma): Boosts lipid production in *Chlorella vulgaris* and lowers pH from 7.8 to 2.4 in 6 minutes (J.Q.M. Almarashi et al., 2020). Multi-needle gas-liquid hybrid discharge reactor that uses a gas stream combined with oxygen and air to inhibit algae (N.N. Aye et al., 2012). And non-thermal plasma: Disrupting the cell wall of Nannochloropsis gaditana for better lipid extraction (A.P. Matos et al., 2019) Cold plasma oxidation: Removal of harmful algae and BMAA toxins in water bodies while reducing pH to 2.5–3.5 (B. Nisol et al., 2019)

In commercial production, production standards is symbols displayed on the product to ensure the quality and standards that the product has passed the inspection in accordance with the requirements of the responsible authority such as, Certified by USDA Organic, Non-GMO Project, Naturland, FDA, ANSI, and more. GMP standard and HACCP, ISO system Environmental Monitoring Water quality, pH, temperature, light, and nutrient levels. It is found that product quality standards often focus mainly on preventing potential harm to consumers, such as the HACCP system that emphasizes risk control from the production process to consumption. However, if the product does not meet the claim, such as components or properties that do not match the stated product, the product will not be used as a product for the first time. FDA standards will play a role in consumer protection. Especially in the field of supervision, advertising and health references of new dietary supplements and food products (novel foods). While the validation of species used in the food industry can

be done with biomolecular techniques such as SDS-PAGE, which is used to specifically identify green seaweed species Ulva and Enteromorpha that are allowed to be used in the food industry (Grobbelaar, J. U. 2003). Nutritional Analysis, there are a variety of analysis methods by technique, with destructive methods and experimental examples including HPLC, Macro-Bradford Assay, Phenol sulfuric acid method, and Bigogno method. Non-destructive is a technique that performs the analysis without much deterioration of the sample. Chemical analysis will be ICP-AES, ICP-MS techniques in the case of mineral samples, XRD techniques, surface analysis will be XPS, ToF-SIMS techniques, and molecular units, valences, and coordination environments will be Raman, FTIR, Mossbauer, XAFS techniques.

FTIR and Raman will be popular. Through samples at lower concentrations or weaker signals, such as the band at 2713 cm^{-1} attributed to the aldehyde (O=) C-H stretch and the band at 1702 cm^{-1} attributed to the carbonyl group, FTIR will yield more sensitive data than Raman in terms of quick analysis and less preparation (Yan, B., Gremlich, H. U., et al 1999). However, FTIR has been shown in numerous studies to be a rapid indicator of the content of biomolecules (Sharma, V. et al. 2020: AlShikaili, T. Y., et al. 2022). No difficult sample preparation Consequently, the expense of analysis is reduced. The sample won't deteriorate if it is analyzed again during the process. It is therefore the best option for production or industries that need constant quality control, like rapidly expanding microorganism production lines. However, the technique and the specifics of fat cannot be analyzed by FTIR. destructive techniques like GC-MS or HPLC, but they can also be used as an effective biochemical trend indicator, particularly when tracking changes in the composition of biomolecules.

2.2 FTIR (Fourier Transform Infrared Spectroscopy)

In FTIR, it shoots IR radiation from a Glowbar source through the sample material and then to a beam splitter, which splits half of the beam of radiation that has passed through the sample material to a rotating glass. Both the reflected beam and

the transmitted beam are reflected back to the beam splitter, which stores all the wavenumber data at once, and because there are so many data points, a computer must be used as an important component in the FTIR spectrometer to analyze the data

The FTIR has several advantages: first, it has a high signal-to-noise value, which helps to provide a clear signal. Therefore, multiple scans can be done quickly and the data is combined. The signal from the true peak is positive, and it usually occurs at the same frequency when it shines. The peak of the noise is both positive and negative and occurs at a random number of waves, so the signal of the true peak is repeated many times, but the signal of the interference wave is canceled, so we can obtain a clear IR spectrum and use a small sample (1 milligram or less) and obtain a spectrum of good quality as used. Because the frequency is compared to the He-Ne laser's peak, it also has the benefit of being accurate.

Absorption band (cm^{-1})	Assignment
~3,010	trans-CH=CH-
~2,955	υ_{asym} CH_3 in lipids
~2,920	υ_{asym} CH_2 in lipids
~2,875	υ_{sym} CH_3 in lipids
~2,850	υ_{sym} CH_2 in lipids
~1,740	C=O stretching in lipids and fatty acids
~1,650	Amide I: C=O vibration
~1,546	Amide II: N–H and C–N vibration
~1,455	CH_2/CH_3 in lipids and proteins
~1,240	υ_{asym} PO_2^- in nucleic acids or phospholipids
~1,200–900	υ(C–O–C) of polysaccharides

υ_{asym}, asymmetric stretch; υ_{sym}, symmetric stretch.

Figure 2.5 Assignments of the main absorption bands in the FTIR spectrum of Spirulina. Source: Liu, J., et al 2022

Biochemical analyses, however, can identify species- or even strain-specific composition differences. Raman spectroscopy for instance is an inelastic light scattering technique that is workable with liquid samples. In contrast, this technique is expensive and the information collected is often biased by machine noise. The HPLC technique is commonly used in the separation of substances and can clearly identify the type and quantity of given organic compounds. but this technique requires to prepare chemicals and solvents and is even more expensive.

An alternative, far less expensive method for determining a sample's infrared spectrum of absorption, emission, and photoconductivity is FTIR/UV-Vis. It can also be used to identify various molecular functional groups. The biochemical profiles of

chicken, grassland plant species, yeast, and *Cannabis sativa* L. have all been examined using this technique in the past (Shapaval et al., 2019; Rana et al., 2018; Katemala, S., et al 2022; Siano et al., 2018).

Moreover, FTIR/UV-Vis is inexpensive, quick, and requires little sample preparation. It is therefore an appropriate technique in terms of determining species-specific or standard culture compounds, both in the industry and in start-ups that require quality control measurements without the need of elaborate and expensive equipment.

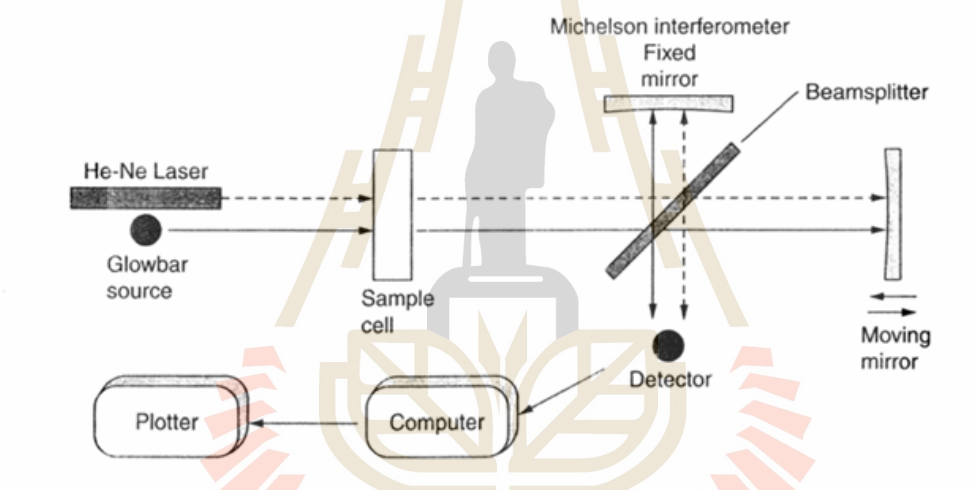

Figure 2.6 Infrared absorption frequencies of various functional groups

Source: http://old-book.ru.ac.th/e-book/c/CM328/CM328-10.pdf

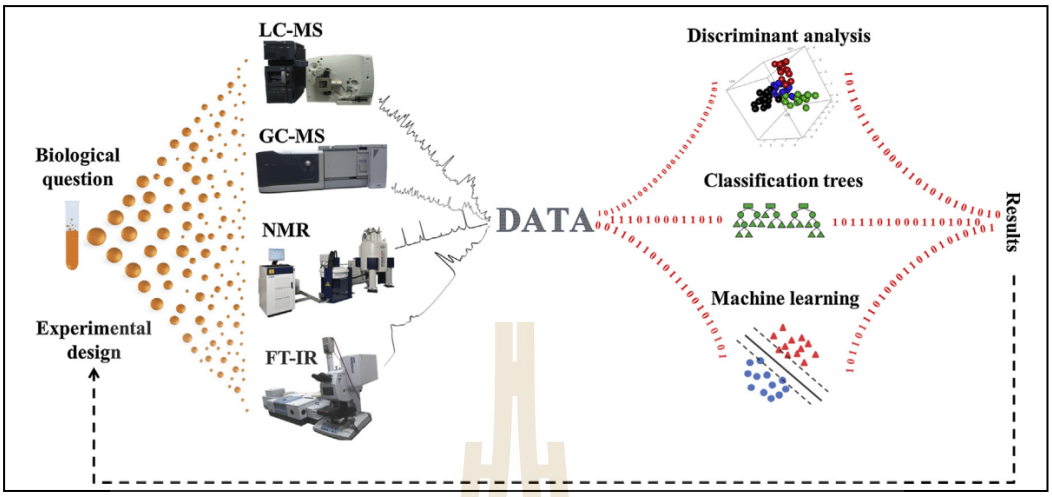

Figure 2.7 A graphical representation of the different analytical approaches and informatics techniques employed in metabolomics studies. Source: Gromski, P. S., et al 2015

2.3 Partial Least Squares (PLS-DA)

One of the most widely used classification methods in chemometrics, and particularly in the metabolomics category, is PLS-DA. However, PLS-DA is still frequently a subject of debate and interpretation in spite of the Metabolomics Standards Initiative's (MSI) guidelines for reporting research findings. The need for effective and trustworthy data analytics tools is growing as the bioinformatics (such as genomics, molecular phylogenetics, metabolomics, proteomics, chemoinformatics, and drug design) market is predicted to reach US$12.48 billion in 2020. The field of metabolomics has seen a sharp rise in research publications over the last ten years due to its recognition as a valuable tool for choosing attributes and classifications, particularly in data sets with high and complex data dimensions, like data from chemometrics and omics. Discriminant analysis using partial least squares (PLS-DA) A chemometrics method called PLS-DA is used to identify differences between sample

groups, which are the relationship between two matrix data, X (raw data) and Y (groups, class membership, etc.).

Through the identification of plot scores with few dimensions, this technique makes complex data sets visually interpretable and illustrates the division between the groups. as depicted in the figure. Validation data predictions should be near 1, and if they are near 0.5, the model is not accurate enough and should not be used for predictions (Gromski, P. S., et al 2015).

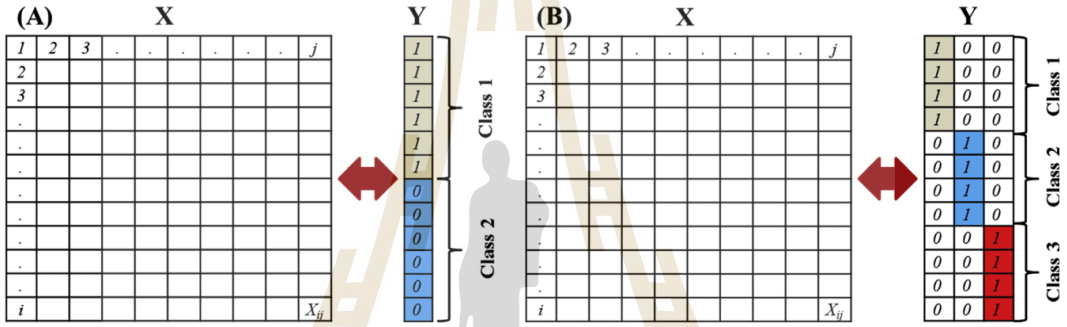

Figure 2.8 An illustration of partial least squares-discriminant analysis (PLS-DA)

Source: Gromski, P. S., et al 2015

2.4 Quasar

Quasar is an open-source software tool designed to make machine learning accessible for analyzing biospectroscopy data, particularly infrared spectroscopy. In biomedical and biochemical spectroscopy research, data quantities are often too large to interpret effectively, particularly when multiple replicates and analytical techniques are required for reliable results. The commercial software users face the fact that currently available data analysis tools suffer from poor user-friendliness, limited capabilities, and difficult access, while problem-specific software or scripts are often highly specialized or simply too hard to use. As machine learning techniques increasingly involve data analysis in natural sciences, there is a growing demand for

user-friendly and flexible tools that can effectively combine machine learning with spectroscopy datasets. The open-source software with strong community engagement is the way forward to counter these problems. Quasar was developed as a user-friendly solution that promotes reproducibility and community contribution while remaining flexible enough to work with various machine learning techniques. Currently, 35 scientific publications have successfully used Quasar. (Toplak, M., et al 2021).

CHAPTER III
RESEARCH METHODOLOGY

3.1 Materials

In this study, Spirulina (*Arthrospira platensis*) strains (H53, Rev, SL, SBL, SB, C005H, and C005L) were obtained from the Suranaree University of Technology (SUT), which was isolated and thoroughly purified.

3.2 Growth medium and culture method

The Spirulina (A. *platensis*) was cultivated with 12 hours light and 12 hours dark photoperiod in Zarrouk's medium at 25 ℃, pH 10. They were kept in 500 mL working-volume transparent plastic bottles. The culture bottles were cultured in a white LED fluorescent light shelf with an intensity of 4,500-5,000 lux (Lamptan, Thailand), and an air pump system for the transparent plastic bottles with air tubes. The tube was all set vertically from the top to the to maintain the desired light intensity setting. The BioTek Epoch microplate reader (Winooski, VT, USA) was used to measure the optical density (OD) values at 560 nm in order to calculate the growth rate. The sample was harvested for the FT-IR experiment in the mid-log phase after it had been cultured.

3.3 Photoperiod lengths treatment

The Spirulina cultured in Zarrouk’s medium using distilled water, adjusted to pH 10 with NaOH, under cycles of 8, 12, and 24 hours photoperiod.

3.4 Water Source treatment

The Spirulina was cultured in Zarrouk's medium using groundwater and tap water, adjusted to pH 10 with NaOH, under a cycle of 12 hours light/12 hours dark photoperiod.

3.5 Indoor and Outdoor cultured conditions

The indoor Spirulina was cultured in Zarrouk's medium using distilled water, adjusted to pH 10 with NaOH, under a cycle of 12 hours light/12 hours dark photoperiod. The strain culture in plastic bottles with a working volume of 500 mL. The outdoor Spirulina was culture in in Zarrouk's medium using distilled water, adjusted to pH 10 with NaOH, in green house. Illumination is provided by natural sunlight. The strain culture in 100 L. photobioreactor and an air pump system

3.6 Focal Plane Array (FPA) FT-IR spectroscopy

The Synchrotron Light Research Institute's (SLRI, Nakhon Ratchasima, Thailand) infrared microspectroscopy beamline BL4.1 IR Spectroscopy and Imaging was used to record the samples' infrared spectra. A Vertex 70 FTIR/UV-Vis spectrometer (Bruker Optics, Ettlingen, Germany) connected to an IR microscope (Hypersion 2000, Bruker) with a liquid nitrogen-cooled MCT detector will be used to acquire the spectra.

3.7 FTIR spectroscopy measurements

Mid-log phase Spirulina samples were collected and transferred in 500 µL aliquots into 1.5 mL Eppendorf tubes. The samples were centrifuged at 1200 rpm for 10 minutes. Following centrifugation, the samples were washed three times with a 0.9% (w/v) saline and three times with deionized water (DI water), respectively. Subsequently, 5 µL of each sample was deposited onto a BaF_2 window measuring 13

x 1 mm. Before being analyzed, the windows were kept in a desiccator for a number of hours while being vacuumed. FT-IR microspectroscopy and an FPA detector were used to gather the spectra. The absorbance was measured by rationing the single beam spectrum against the window background. For twelve hours, the samples on the window were dried in a desiccator. 64 scans with a spectral resolution of 6 cm^{-1} were used to record infrared absorption spectra in the 4000-400 cm^{-1} range. Absorbance values were obtained from the measured spectral values.

3.8 Multivariate statistical analysis

The instrument system was controlled and FT-IR spectral data was obtained using OPUS 7.5 software (Bruker Optics Ltd., Ettlingen, Germany). Principal Component Analysis (PCA) was used to identify the samples' spectra using the Unscrambler X 10.5 software's variability (CAMO Software AS, Oslo, Norway) and Quasar. A spectrum is obtained by the FT-IR/UV-Vis Spectrometer for additional analysis.

CHAPTER IV
RESULTS AND DISCUSSION

4.1 Spirulina Strain Classification by FTIR and PLS-DA

Discovery of Spirulina morphology. In the study of strain classification, we observed the differentiate of helical and the length that similar like other Spirulina (Wang, Z. P., & Zhao, Y. 2005: Nowicka-Krawczyk, P., et al 2019: Sinetova, M. A., et al 2024). The Spirulina strains (H53, SB, SL, SBL, Rev, C005L, and C005H) were observed under light microscopy. Strain H53 presented an open helix cylindrical shape typical of Spirulina. Also, strain SB showed visibly thicker trichomes than strain H53, while strain SL had slightly longer filaments but a coil structure similar to that of H53 strain. The SBL strain consisted of SB traits with longer filaments. Similarly, strain Rev showed a reversed helical as opposed to the H53 stain. Distinctly, C005L consists of straight, non-helical filaments, unlike all other strains. Also, the C005H strain stood out with notably long trichomes.

The growth curves of all seven Spirulina strains (H53, R, SBL, SL, C005L, C005H, and SB) cultured in Zarrouk's medium at 25°C were observed with the identification of their stationary phases (Figure 4.1). The mid-log phase of each strain was reached at different time points. Strain H53 reached this phase earliest on day 7, followed by strains R, SB, SL, C005H, and C005L on day 8. Strain SBL exhibited the latest mid-log phase on day 9. These showed different growth patterns with optical density (OD_{560}). These results were aligned with previous studies on S. platensis cultivation (Shi et al., 2016; da Silva et al., 2016). These strains were appropriate representatives for FTIR analysis due to their biochemical composition during the stationary phase.

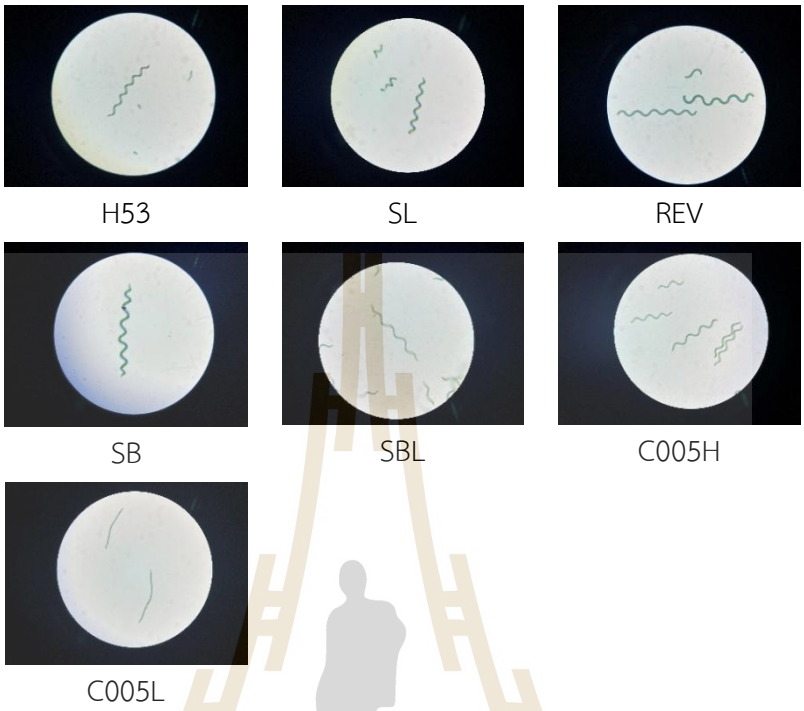

Figure 4.1 Morphological comparison of seven Spirulina strains (H53, SB, SL, SBL, Rev, C005L, C005H) under light microscopy

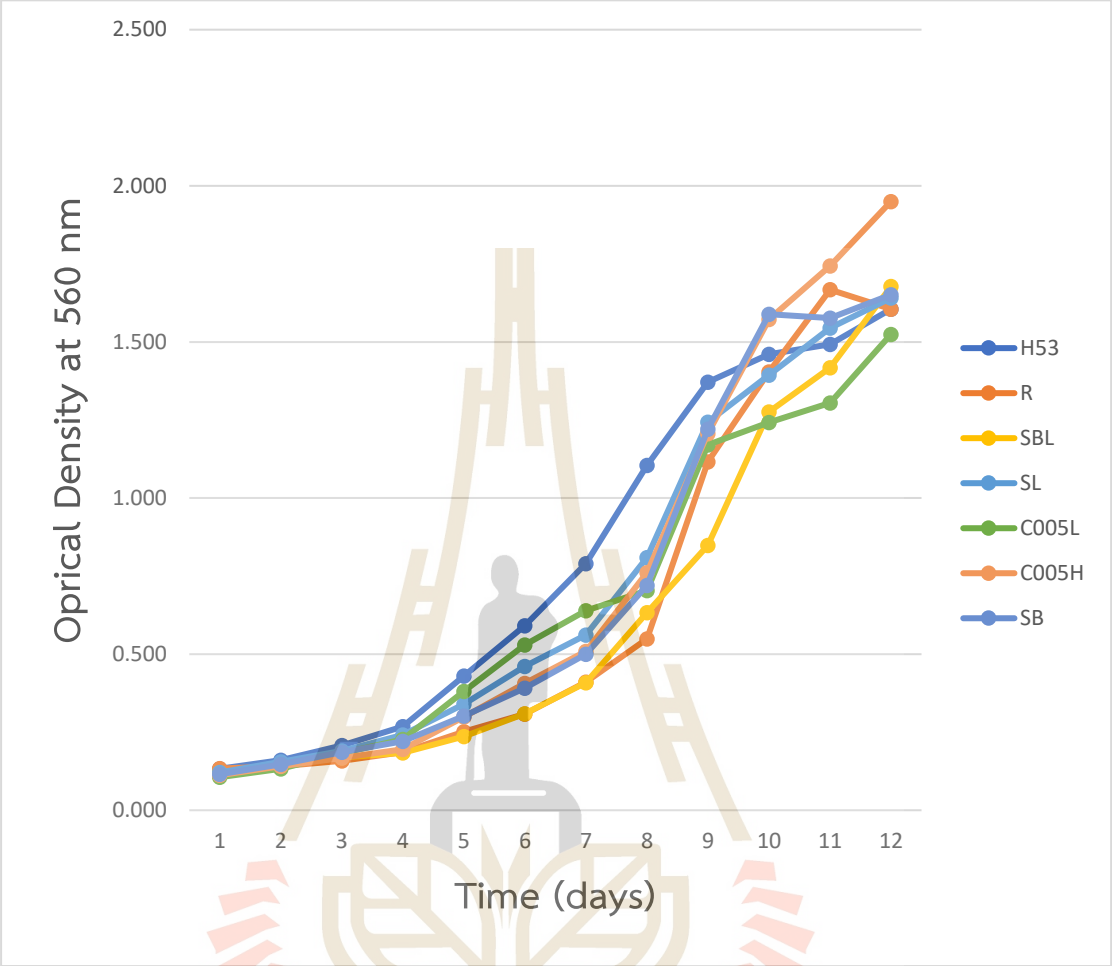

Figure 4.2 Growth curves of seven Spirulina strains measured by optical density at 560 nm (OD_{560}) over the cultivation period

Fourier transform infrared (FTIR) spectroscopy, it is a technique used to identify and classify microbes Since 1991 (Wenning et al., 2013). The mid-IR spectroscopy uses wave numbers between approximately 400 and 4000 cm^{-1}. The principle in the FTIR technique is a spectroscopy method used to identify variations in microorganisms total composition by detecting changes in functional groups in biomolecules including proteins, carbohydrates, lipids, and nucleic acids, absorb infrared light at particular wavelengths. Multivariate statistical analysis is used in the data evaluation process.

therefore, this method has rapid screening and potential approach for determine the correlation of biochemical profile in Spirulina. Because it is a technique that specific analyzed, time efficiency, minimal sample requirement, It is safe for humans and the environment and does not react with samples, making it an effective tool for rapid screening and precise high energy throughput characterization while safeguarding public health, the environment and product. (Wu, T. et al., 2018; Mazivila, et al., 2015) by this technique has been used in identification and characterization of some species of Cyanobacteria, Chlorophyta and Bacillariophyta (Özer, T., et al., 2016)

The study revealed that FTIR spectroscopy could determine biochemical profile of Spirulina among 7 strains such as H53, Reverse, SB (super blue), SBL (super blue long), SL (super long), C005H and C005L. The Strain was analyzed. Characteristic bands showed cellular components that were observed in all samples. Especially, the peak region at 3,100-2,800 cm-1 aligned with the vibrational of CH=CH in lipids region and carotenoids, the peak region at 1,700–1,500 cm^{-1} was characterized by amide bands in proteins and the peaks at the 1,200–900 cm^{-1} range were primarily linked to C–O–C stretching vibrations. That has been found in polysaccharides. The dendrogram depicts dissimilarity that exists among the 7 strains sample that difference between their functional group ratio. The results revealed that protein, lipid and carbohydrate are the characteristics peak that found differently ratio in each strain.

Recently, consumers' perspective is lost faith in food product safety and quality (Lobstein, T. 2019). Thus, this study holds considerable potential for Spirulina quality control methodologies through FTIR technique, offering an alternative approach to assess biochemical composition and distinguish among cultivation conditions. FTIR technique can support the improvement of analytical quality to provide products and consumer confidence in the future. Additionally, the FTIR technique has been used to classify species and examine biochemical profile. (Özer, TF., et al., 2016; Nalimova, et al., 2005).

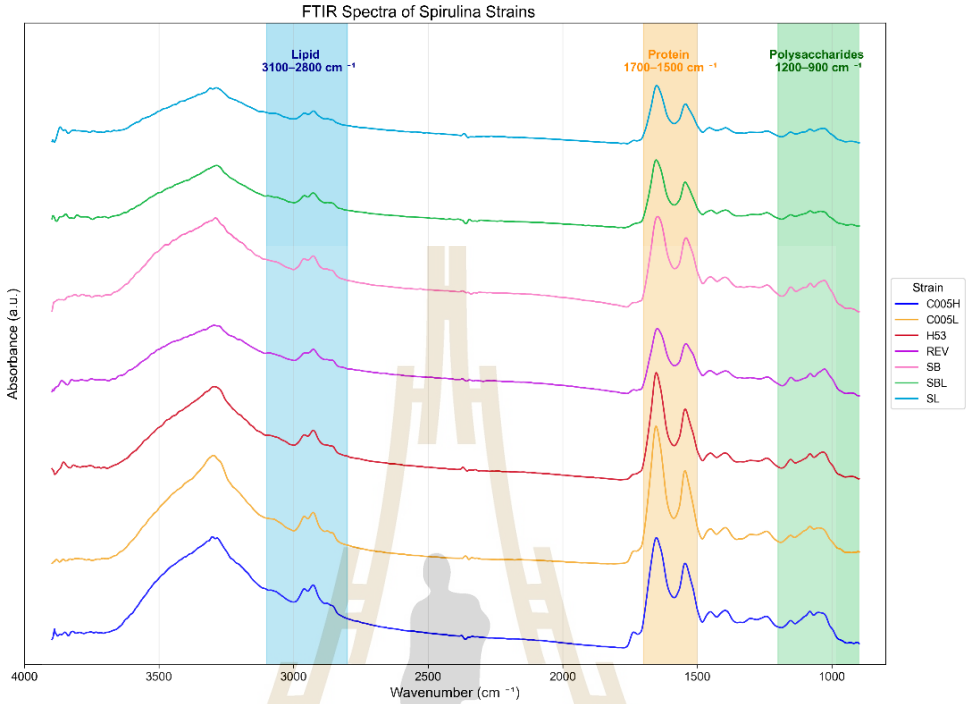

Figure 4.3 Average spectrum obtained from FTIR spectroscopy of 7 strains (H53, SB, SL, SBL, Rev, C005L, C005H)

In this study, the biochemical profile of Spirulina with independent variants, namely H53, Reverse, SB (Super Blue), SBL (Super Blue Long), SL (Super Long), C005H and C005L in controlled environment factors were studied. The biochemical profiles of the 7 species are shown in the figure (Figure 4.3). In the biomolecular level, it was found that the optimal three strains of Spirulina SB, C005L and C005H, which are align with previous research (Mróz et al 2024) that have successfully classified Spirulina. The peaks showing distinct differences among the three Spirulina strains appeared in the loading plot in the region of 1662–1513 cm^{-1} (proteins) and around 1020 cm^{-1} (polysaccharides), as presented in Figure 4.5.

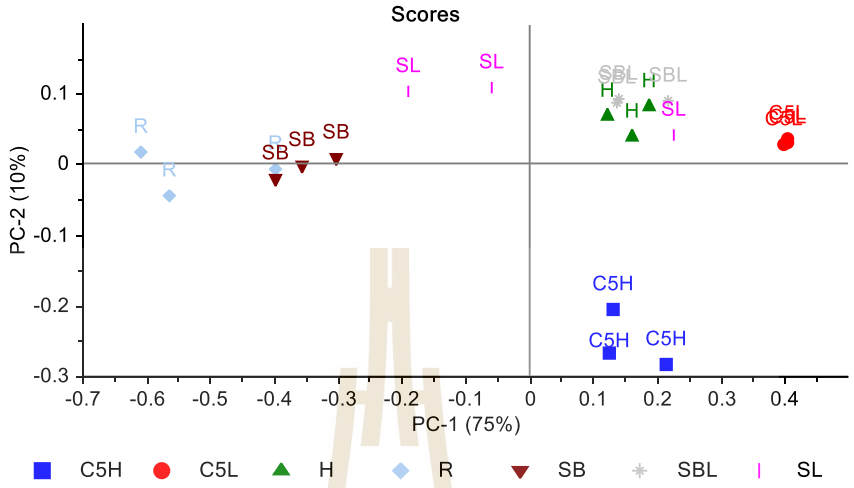

Figure 4.4 PCA score plot based on average FTIR spectra of 7 strains (H53, SB, SL, SBL, Rev, C005L, C005H)

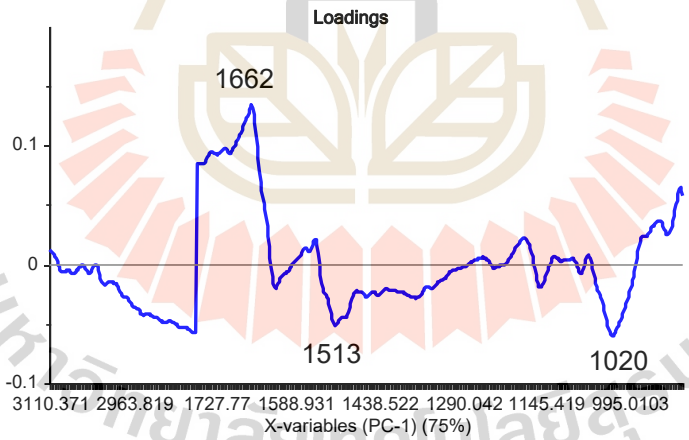

Figure 4.5 PCA loading plot (PC1) of FTIR spectra from C005H, C005L and SB strains

The model was created from the data of this experiment and consist of the coefficients of determination (R^2) were 0.945, 0.914, and 0.914 for SB, C005L, and

C005H-specific models, and the root mean squares error for the cross-validation (RMSECV) was 0.112, 0.140, and 0.140 for SB, C005L, and C005H models, respectively. This value is close to the value reported in the published research. (Wu, T., et al., 2018; Tarighat, M. A., et al 2022) The accuracy of models such as C005H achieved 81%, C005L achieved 93%, and SB achieved 97%. And these models had sensitivity value of 100%. Spectral data analysis from the FTIR technique was combined with PLS-DA data analysis in culture in the same control variable in this experiment (light, temperature, pH, it was suggested that FTIR in combination with PLS-DA was effective in classifying Spirulina strains.

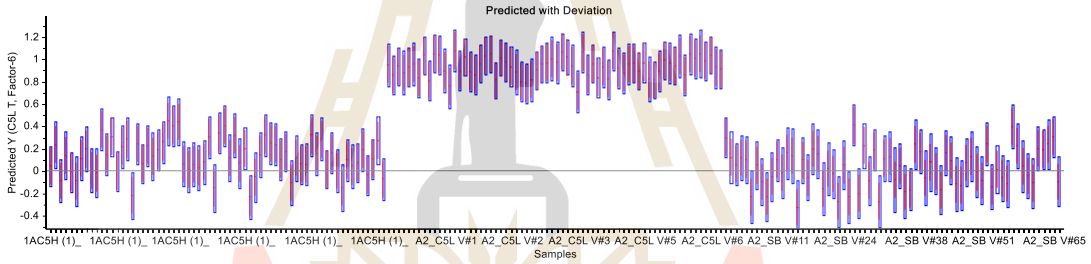

Figure 4.6 Distributions of reference and predicted values for the calibration sample sets from the C005H strains, C005L strains, and SB strains. Distributions of the reference and predicted values determined by the PLS-DA model

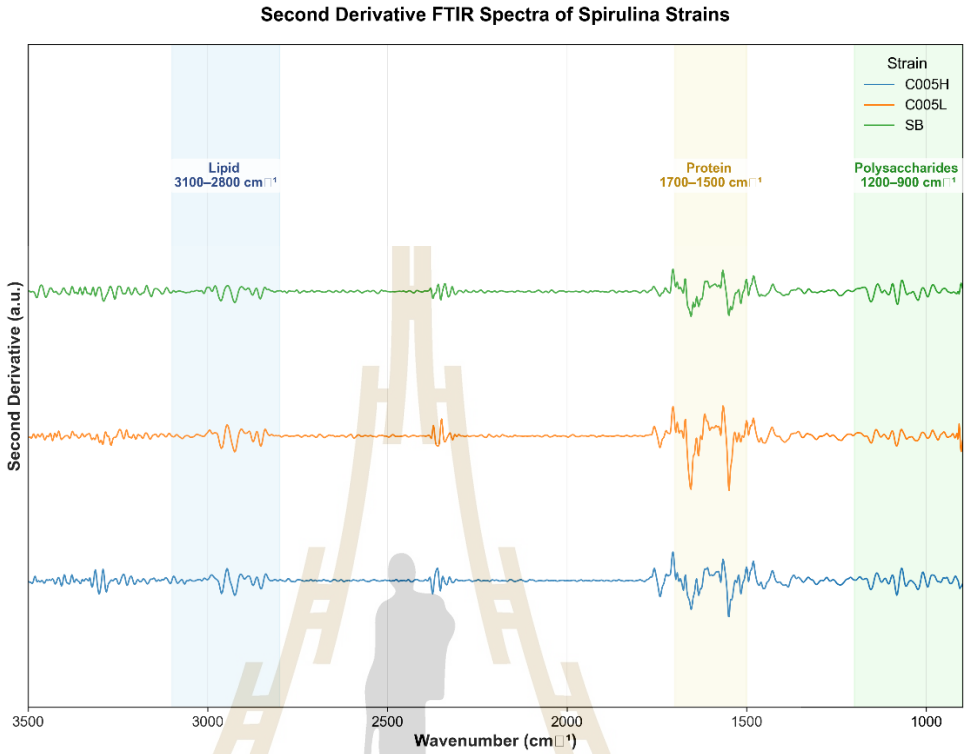

Figure 4.7 Second derivative of C005H, C005L and SB strains

The spectra in the lipid region showed similarity among the strains (Figure 4.8), which corresponds to the loading plot results.

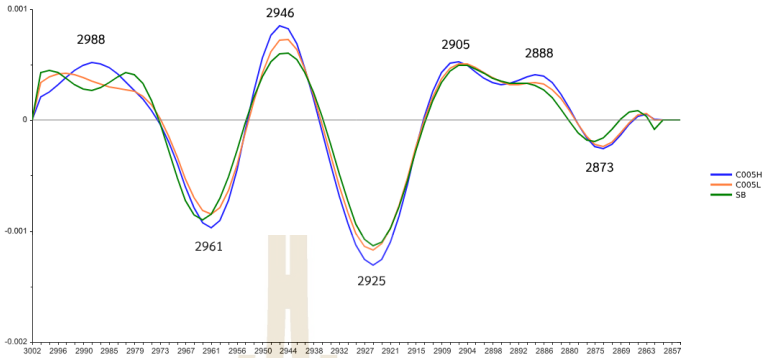

Figure 4.8 Second derivative FTIR spectra of C005H, C005L, and SB strains in the lipid region

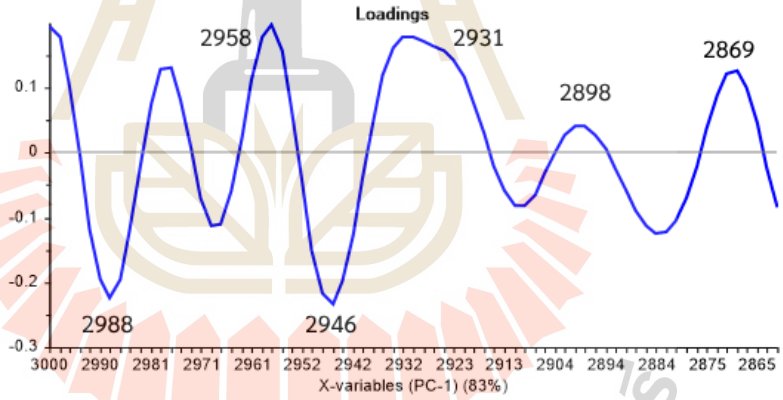

Figure 4.9 PCA loading plot (PC1) of FTIR spectra from of C005H, C005L, and SB strains in the lipid region

Based on the spectral data, the protein region showed the characteristic of peaks that among the strains. The main spectral difference was observed around 1656 cm^{-1}.

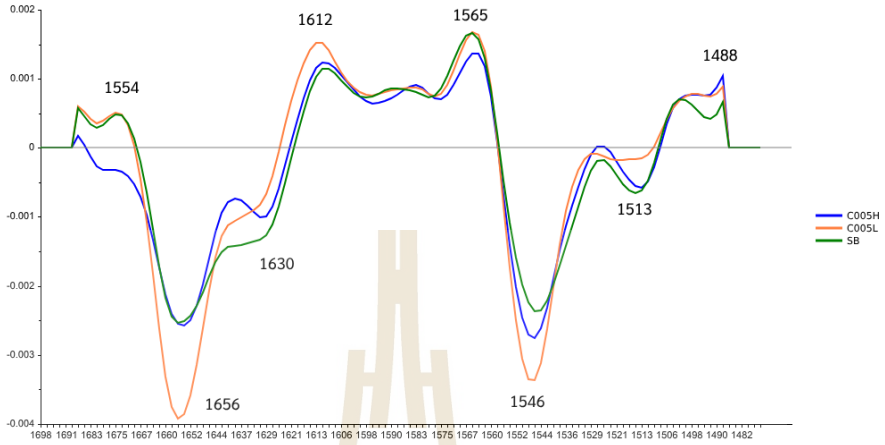

Figure 4.10 Second derivative FTIR spectra of C005H, C005L, and SB strains in the protein region

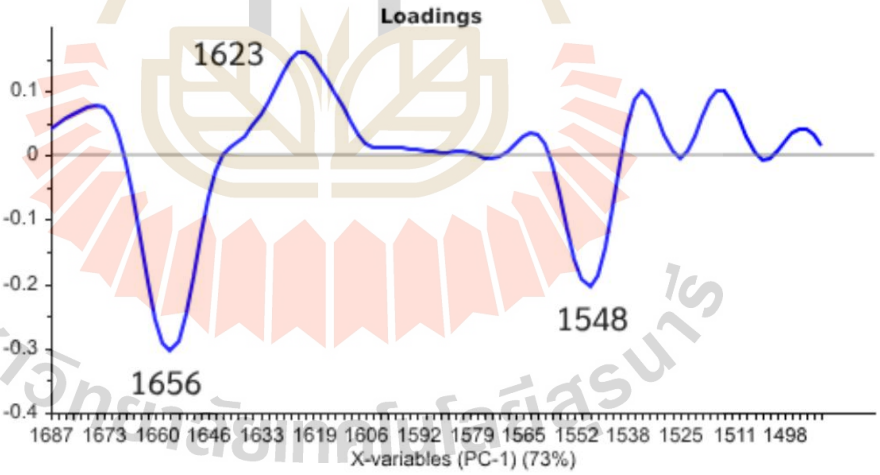

Figure 4.11 PCA loading plot (PC1) of FTIR spectra from of C005H, C005L, and SB strains in the protein region

In the polysaccharide region, several peaks showed noticeable differences among the strains, with the most distinct variation appearing around 1023 cm^{-1}.

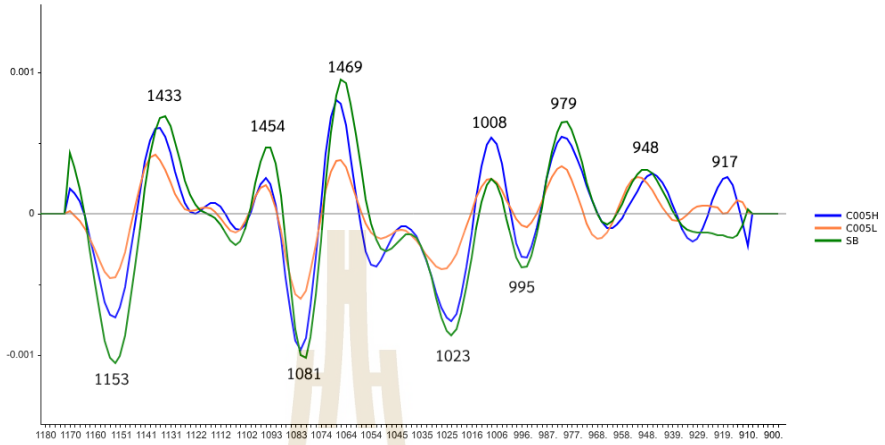

Figure 4.12 Second derivative FTIR spectra of C005H, C005L, and SB strains in the polysaccardes region

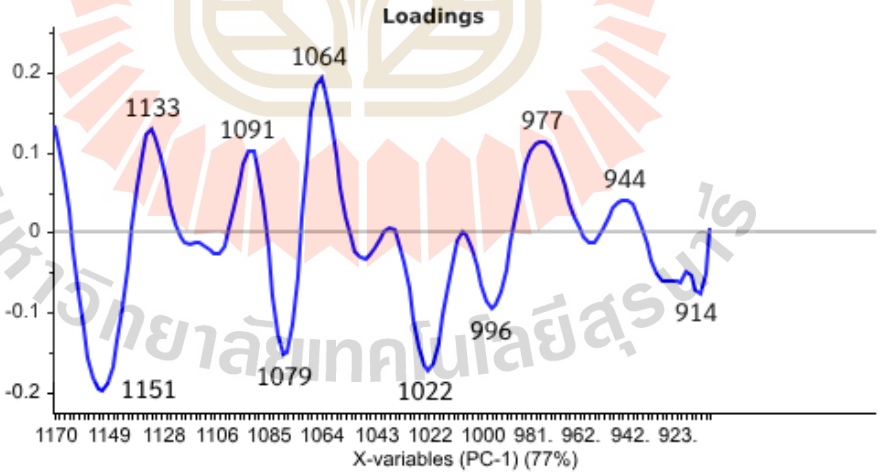

Figure 4.13 PCA loading plot (PC1) of FTIR spectra from of C005H, C005L, and SB strains in the polysaccardes region

The present tools for data analysis had lacked user-friendliness, Problem-specific software or scripts, or simply too hard to use. On the contrary, the Quasar evaluated supervised prediction methods, logistic regression and random experiment groups. The confusion matrix shows how well the SVM model can tell the difference between Spirulina strains. The numbers on the diagonal are the correctly identified samples, while the numbers off the diagonal show where the model got confused, meaning those strains have similar spectral patterns (Toplak, M., et al 2021).

The SVM focuses on the samples that are most similar to other strains, called support vectors. These points are used to create an optimal hyperplane or decision boundary, which helps the model decide the class of new observations in the future. This allows SVM to classify data efficiently and accurately.

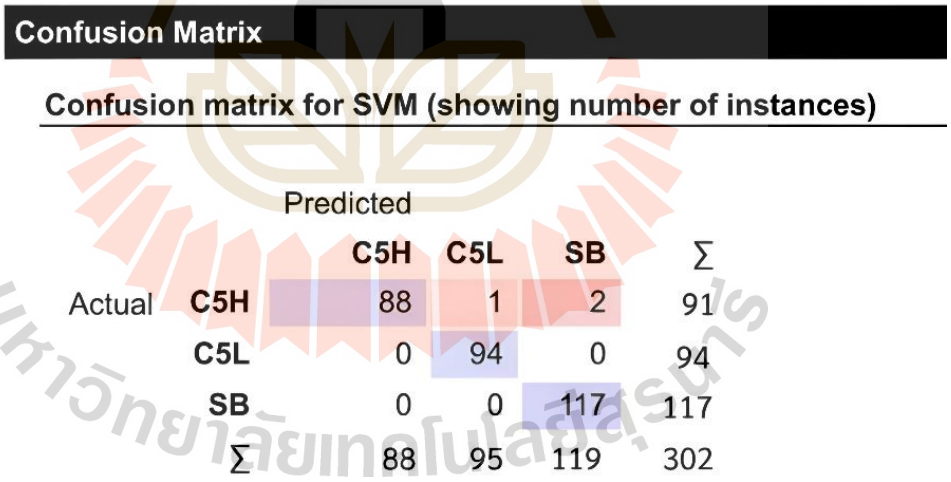

Figure 4.14 Confusion Matrix for Support Vector Machine (SVM) Classification of Astronomical Objects from Quasar Dataset

4.2 Effect of Light Conditions on Biochemical Profile of Spirulina H53

To investigate the biochemical profile effect of photoperiod length on the Spirulina H53, the strain was cultured under controlled indoor conditions using Zarrouk's medium, temperature (25°C) and pH 10, and three different light exposure durations (8, 12, and 24 hours). The FTIR spectra collected in the range of 4000–400 cm^{-1}. The treatment originally showed similar spectra signals among the treatments (Figure 4.15). The spectra determined peak regions corresponding to lipids, proteins, and polysaccharides. However, the spectra did not show visually distinguishable differences in the raw spectra.

There were noticeable differences after applying the second derivative transformation (Figure 4.16), especially in the protein region between 1700 and 1500 cm^{-1}. There was a noticeable difference at 1658 cm^{-1}, which corresponds to the amide I band of proteins but all three spectra showed consistent peak positions but differing intensities. This indicates that the length of the photoperiod influences the amount of protein compounds without changing their structure.

Further analysis showed loading line plots (Figure 4.22) revealed a considerable spectral region. These were found to be around 1685 to 1741 cm^{-1}. Protein signals are represented by this zone, which includes the amide I band, confirming that the main factor separating the light treatments was protein part.

The biochemical trends were visualized using cluster bar plots (Figure 4.18), which clearly show that protein signal quantity increased progressively with longer light exposure. This data corresponds with the numerical data in Table 4, where the protein content increased from 14.259 for 8 hours to 15.350 for 12 hours and 15.848 for 24 hours. This trend suggests a direct correlation between light exposure duration and protein synthesis.

Although lipid and polysaccharide signals showed some variation, they did not follow the same consistent trend as protein signal. While polysaccharide values rose at 12 hours (3.414) and slightly decreased at 24 hours (3.160), lipid values peaked at

12 hours (2.666) and then declined at 24 hours (2.184). Additionally, the 3D PCA model (Figure 4.19) supports the above findings by clearly separating the experimental groups based on their spectral profiles. The 8 hours data formed a distinct cluster, whereas the 12 and 24 hours groups were positioned closer to each other, indicating biochemical similarity in protein signal values. This result aligns with the protein data trends shown in Table 4 and the cluster bar plot, further confirming that protein part is the major factor influenced by variations in photoperiod.

The result can be explained biologically by increased activity in pathways linked to photosynthesis, including the Calvin cycle, nitrogen metabolism, and Photosystems I and II, when exposed to prolonged light. Zhang et al. (2024) claim that extended exposure to light activates pathways involved in protein biosynthesis, which is consistent with the spectral patterns found in this experiment.

Collectively, these results confirm that light exposure duration has an impact on the biochemical profile of Spirulina H53 strain, particularly in terms of protein content. This insight is crucial for optimizing cultivation conditions to enhance desired biochemical characteristics in Spirulina production, and may also be applied in the future to trace the origin and cultivation methods of Spirulina strains.

Figure 4.15 Average spectrum obtained from FTIR spectroscopy of Culture at 8, 12, and 24 hours under light conditions after smoothing, baseline correction, and extended multiplicative scatter correction (EMSC)

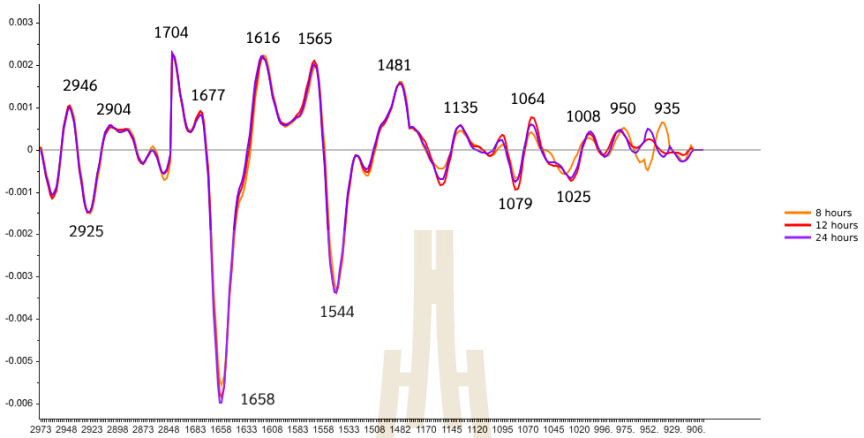

Figure 4.16 Second derivative of Photoperiod lengths treatment

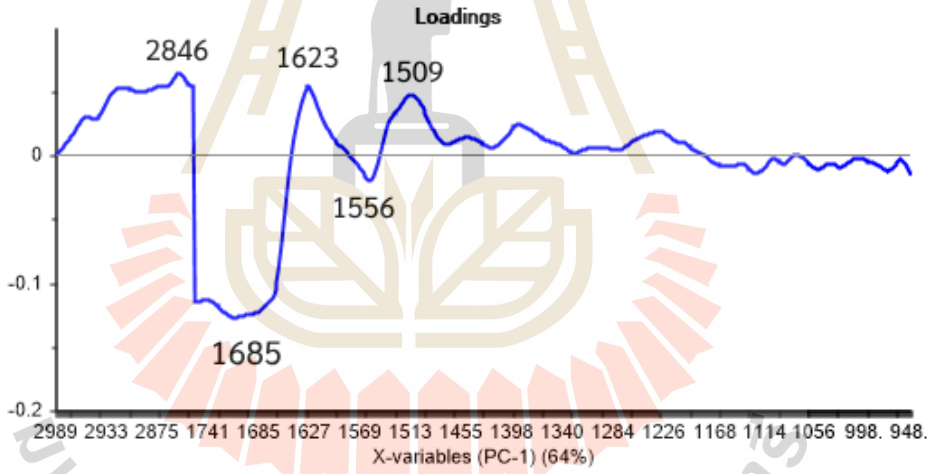

Figure 4.17 PCA loading plot (PC1) of FTIR spectra from photoperiod length treatments

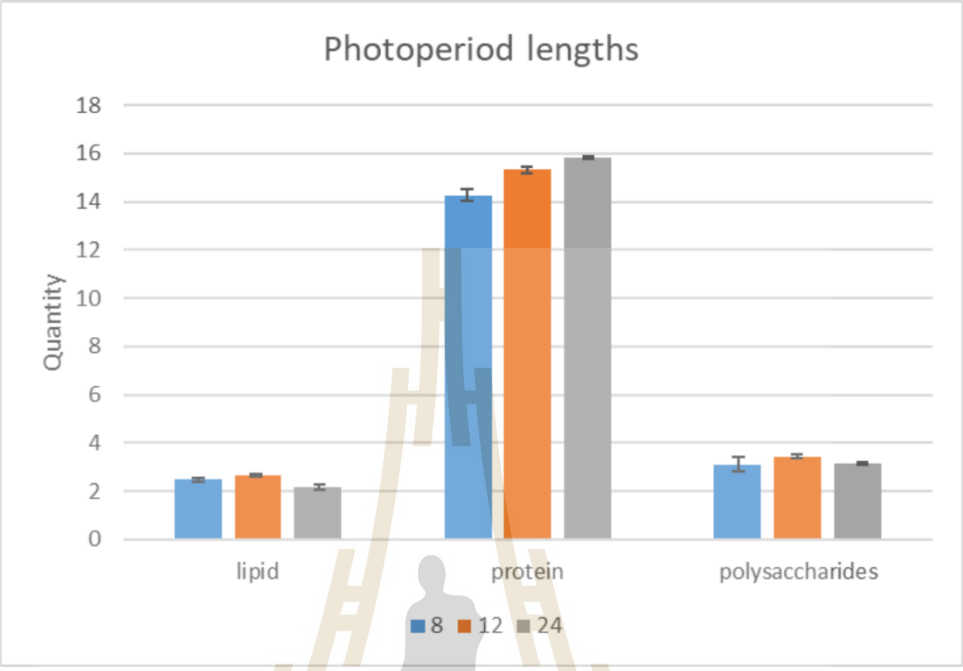

Figure 4.18 Biochemical composition under different photoperiod lengths

Table 4.1 The integral area of the average spectrum of Spirulina culture (H53 strain) from different photo periods

Biomolecule	photoperiod		
	8	12	24
C-H stretching of lipid	2.484 ± 0.08	2.666 ± 0.04	2.184 ± 0.10
C=O, N–H, C–N stretching of proteins	14.259 ± 0.26	15.350 ± 0.14	15.848 ± 0.04
C–O–C stretching of polysaccharides	3.099 ± 0.35	3.414 ± 0.08	3.16 ± 0.06

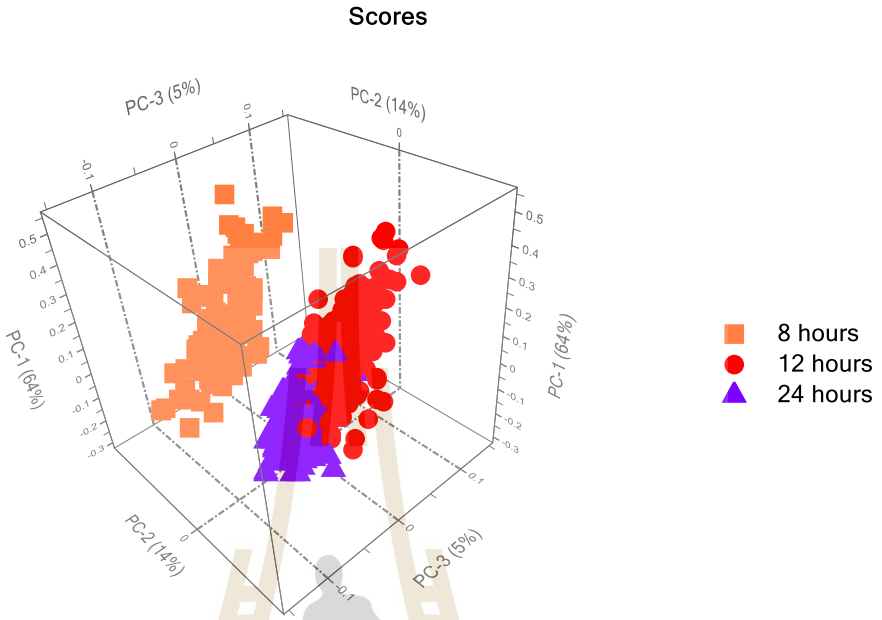

Figure 4.19 3D Model PCA of Spirulina Culture in Photoperiod lengths treatment

4.3 Effect of Water Types in Culture Medium on Biochemical Profile

Spirulina is well-known for the unique capacity to adapt to a wide range of conditions, including freshwater, brackish water, saltwater, and even soil (Ciferri, 1983). Spirulina H53 was cultivated indoors under controlled conditions using Zarrouk's medium with different water treatments, temperature (25°C), and pH 10 to investigate the influence of water source on biochemical composition. The variable in the culture medium consisted of two kinds of water: tap water and groundwater.

For both treatments, FTIR spectra in the 4000–400 cm^{-1} range were obtained (Figure 4.20). Visual inspection of the raw spectra showed an obvious difference in the carbohydrate region (1200–900 cm^{-1}), with higher intensity in the groundwater group. This implied a variation in the accumulation of polysaccharides. The protein region (1700–1500 cm^{-1}) and the carbohydrate region both showed stronger signal differences after applying second derivative transformation (Figure 4.21). These spectral regions

align with polysaccharide bands and amide bands, which are indicators of polysaccharides and proteins, respectively. The major peaks in various types of water were found in these two areas, as shown by the loading line plot (Figure 4.21). Polysaccharide-related signals near 1023 cm^{-1} were stronger in the groundwater group, whereas protein signals around 1648 cm^{-1} were greater in the tap water group.

These findings were supported by the cluster bar chart (Figure 4.23), which showed a noticeable increase in protein signal in the tap water condition, whereas the polysaccharide signal was markedly higher under the groundwater condition. Table 4 presents the quantitative absorbance values of major biomolecules: the protein region showed a higher average value in the tap water group (16.869 ± 0.06) compared to the groundwater group (13.885 ± 0.08), while the polysaccharide region showed the opposite trend, groundwater (10.272 ± 0.11) was significantly higher than tap water (4.805 ± 0.22). Lipid values were slightly higher in groundwater, but the variation was less pronounced (tap water: 2.349 ± 0.08; groundwater: 2.553 ± 0.04).

The cluster bar chart (Figure 4.23), which showed an obvious increase in the protein signal under tap water conditions while the polysaccharide signal was much greater under groundwater conditions, corroborated these findings. The quantitative absorbance values of the main biomolecules are shown in Table 4. The average value of the protein region was higher in the tap water group (16.869 ± 0.06) than in the groundwater group (13.885 ± 0.08), while the polysaccharide region displayed the opposite trend, with groundwater (10.272 ± 0.11) being significantly higher than tap water (4.805 ± 0.22). Although the difference was less noticeable, groundwater had slightly higher lipid values (tap water: 2.349 ± 0.08; groundwater: 2.553 ± 0.04).

In the cluster bar chart (Figure 4.23), the absorbance values of the major biomolecules are shown in Table 4. The value of the protein region of tap water (16.869 ± 0.06) was higher than the groundwater (13.885 ± 0.08), while the

polysaccharide region displayed the opposite trend, with groundwater (10.272 ± 0.11) being higher than tap water (4.805 ± 0.22). Groundwater had slightly higher lipid values. (groundwater: 2.553 ± 0.04; tap water: 2.349 ± 0.08)

The 3D PCA plot (Figure 4.24) shows a clear difference between the two water groups based on their spectral features, confirming that the type of water greatly influences the overall biochemical profile of Spirulina. The difference is mainly due to variations in protein and polysaccharide signals. The separation is mainly driven by differences in protein and polysaccharide signals.

The results imply that the different mineral compositions of tap and groundwater may be the cause of the biochemical variations. While tap water contains chlorine, fluoride, and leftovers from water treatment processes, groundwater typically has a wider variety of minerals, such as calcium, magnesium, bicarbonate, sulfate, chloride, and nitrate (Moreno-Merino et al., 2022). Groundwater's mineral content may cause osmotic or ionic stress, which would cause Spirulina to adapt its metabolism due to osmoregulation. Spirulina has been reported to accumulate carbohydrates, especially polysaccharides, under such stress (Kebede, 1997; Rosales et al., 2005).

As a result, the unique spectral patterns found in this study represent the biochemical profile of Spirulina H53 strain in response to variations in water quality. Such insights not only contribute to the improvement of cultivation strategies for targeted biochemical outcomes but also offer potential for future traceability of cultivation conditions based on strain signatures.

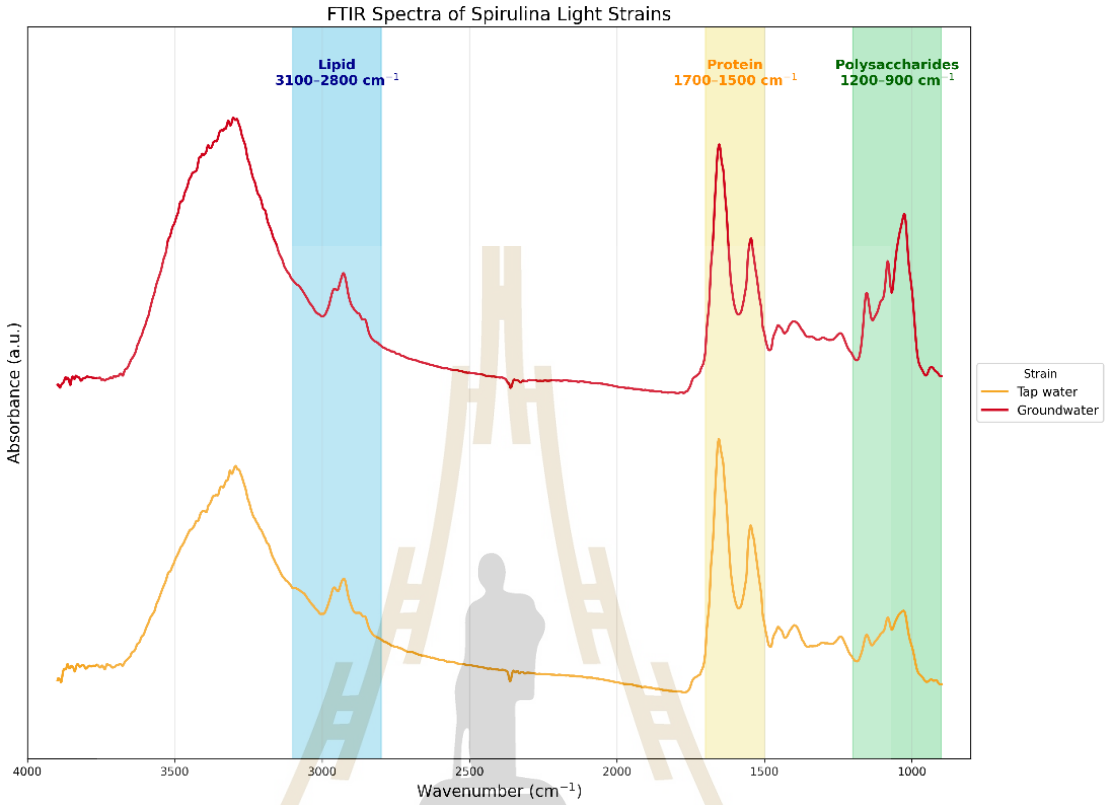

Figure 4.20 Average spectrum obtained from FTIR spectroscopy of Culture in Tap water and Groundwater after smoothing, baseline correction, and extended multiplicative scatter correction (EMSC)

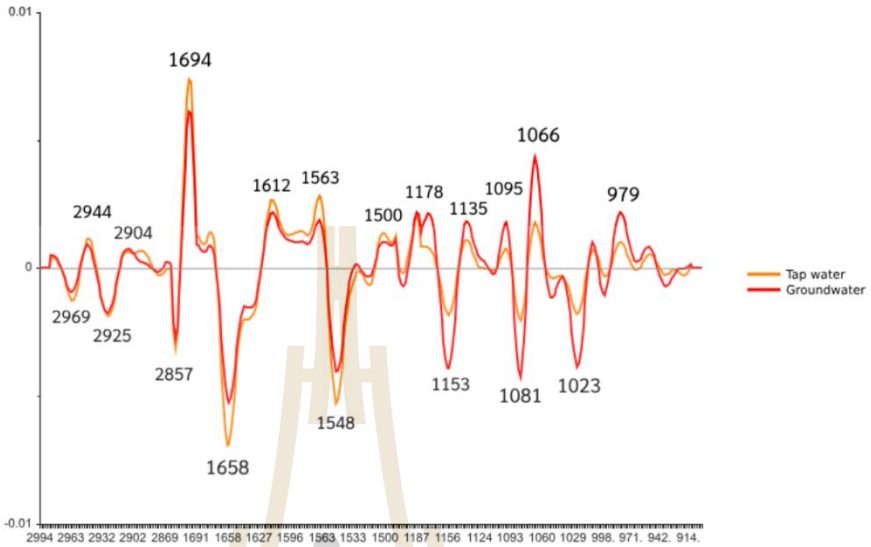

Figure 4.21 Second derivative of Photoperiod lengths treatment

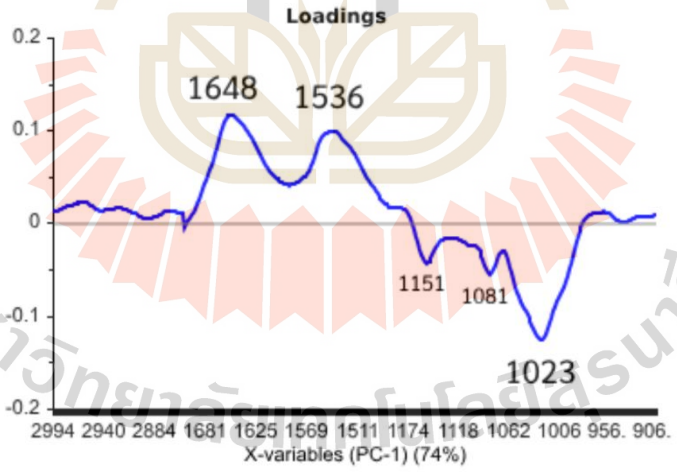

Figure 4.22 PCA loading plot (PC1) of FTIR spectra from Culture in Tap water and Ground water treatments

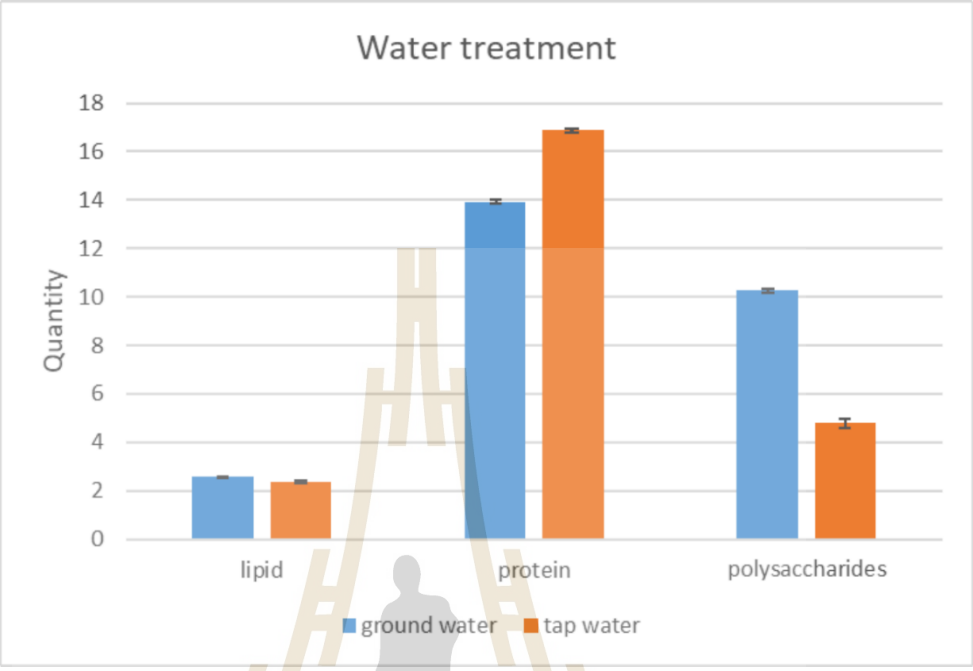

Figure 4.23 Biochemical composition under different water source treatment

Table 4.2 The integral area of average spectrum of Spirulina culture (H53 strain) from different water source treatment

Biomolecule	Water	
	Tap water	Ground water
C-H stretching of lipid	2.349 ± 0.08	2.553 ± 0.04
C=O, N–H, C–N stretching of proteins	16.869 ± 0.06	13.885 ± 0.08
C–O–C stretching of polysaccharides	4.805 ± 0.22	10.272 ± 0.11

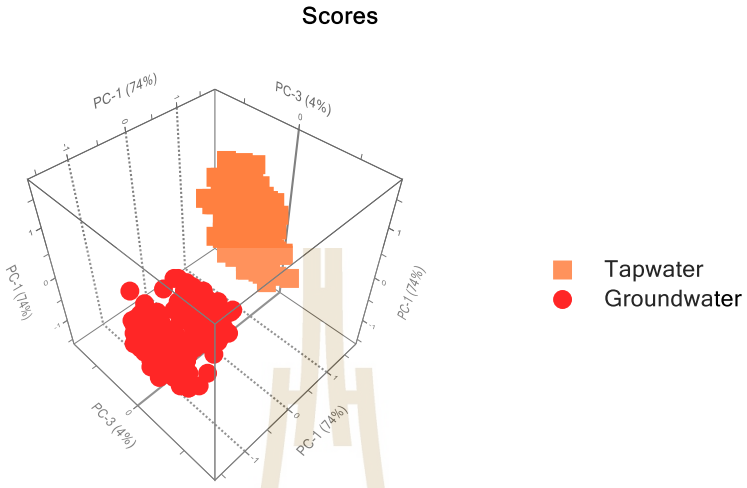

Figure 4.24 3D Model PCA of Spirulina Culture in water source treatment

4.4 Comparison of Indoor vs. Outdoor Cultivation Systems

The two main categories of Spirulina cultivation systems are indoor and outdoor, each with various environmental influences. In this study, 500 mL flasks were used to cultivate Spirulina H53 indoors under controlled conditions (Zarrouk's medium, temperature (25°C), and pH 10), while 100 L open systems were used in outdoor cultivation. The outdoor setting provided less control, like Zarrouk's medium, 100 L tank with air tube and Spirulina H53 strain. But the sunlight intensity, photoperiod, and temperatures are not controlled as in the indoor setting.

There were obvious differences between the two cultivation systems, especially in the polysaccharide region (1200–900 cm^{-1}), which appeared to be higher in the outdoor group, according to FTIR spectra obtained in the 4000–400 cm^{-1} range (Figure 4.25). According to previous results in water source comparisons, distinct differences were seen in the polysaccharide region (1200–900 cm^{-1}) and the protein region (1700–1500 cm^{-1}) following a process of second derivative transformation (Figure 4.26).

Two major peak zones for differentiating between indoor and outdoor cultivation were found by loading line analysis (Figure 4.27). These are the carbohydrate region, which is around 1022 cm^{-1} (polysaccharides), and the amide I region, which is around 1656 cm^{-1} (proteins). Cluster bar plots also showed that the polysaccharide signal was higher in outdoor cultures, and the protein signal was stronger in indoor cultures.

Table 4 provided quantitative data to support these observations. While the polysaccharide content showed the opposite trend. And outdoor cultures showed a higher value (5.517 ± 0.12) than indoor (3.781 ± 0.04). The protein absorbance was higher in indoor cultures (15.953 ± 0.11) than in outdoor cultures (15.21 ± 0.21). There were only minor variations in lipid levels between the two conditions.

It was found that environmental factors involving cultivation systems have an influence on the biochemical profiles by the 3D PCA model (Figure 4.29), which separated the indoor and outdoor groups. This supports previous results that stress situations, including photo-inhibition, can result from changes in the environment in outdoor systems, including intense light exposure, photoperiod variation, and temperature shifts (Juneja et al., 2013; Soni et al., 2017; Richmond et al., 1986).

It's interesting to realize that this trend aligns with previous results sections. In the photoperiod length treatment, the protein signals were the major variable. Also, the water source treatment, both protein and polysaccharide regions, was affected. And in this cultivation treatment comparison. The biochemical profile in protein and polysaccharides regions has variation. This pattern suggests that these two regions are major biochemical profiles that vary for environmental effects on Spirulina H53 strain.

These results support that Spirulina's biochemical profile is influenced by factors in cultivation systems, whether they are natural or controlled. More strategic cultivation planning is made possible by an understanding of how various systems affect biochemical profiles. These results may also help with traceability of cultivation conditions or uniquify Spirulina strains hereafter by using FTIR spectral fingerprints.

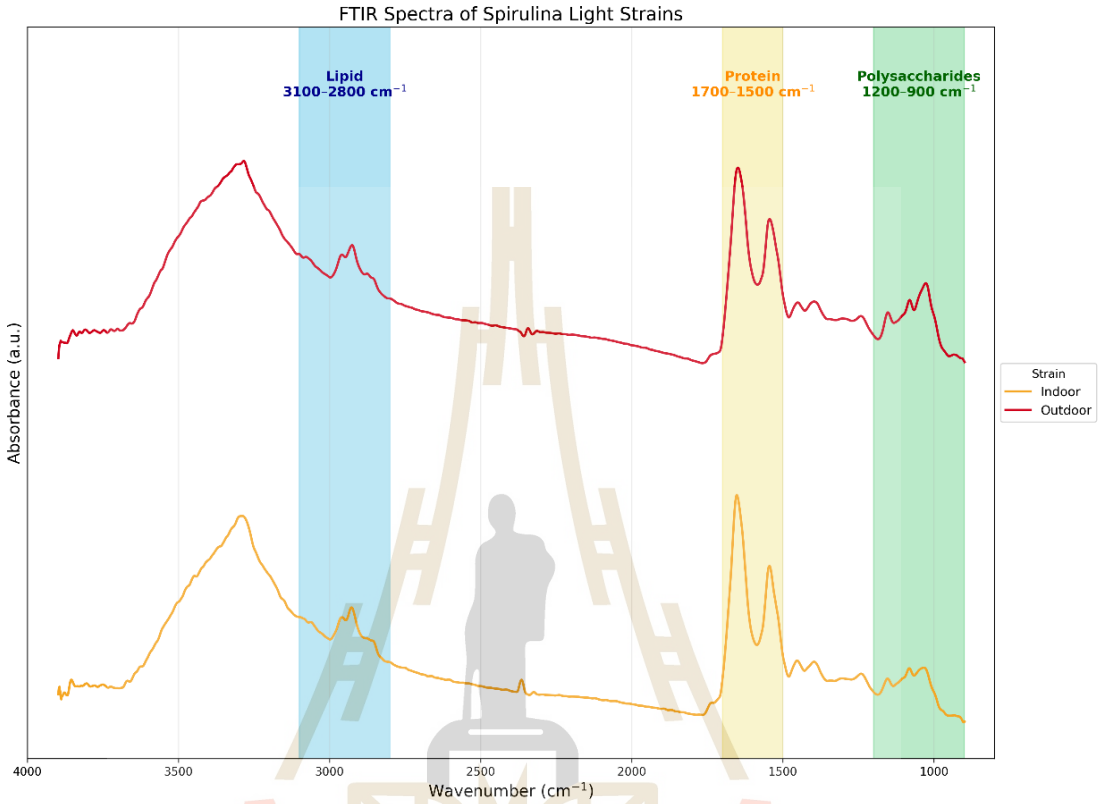

Figure 4.25 Average spectrum obtained from FTIR spectroscopy of Culture in indoor and outdoor conditions after smoothing, baseline correction, and extended multiplicative scatter correction (EMSC)

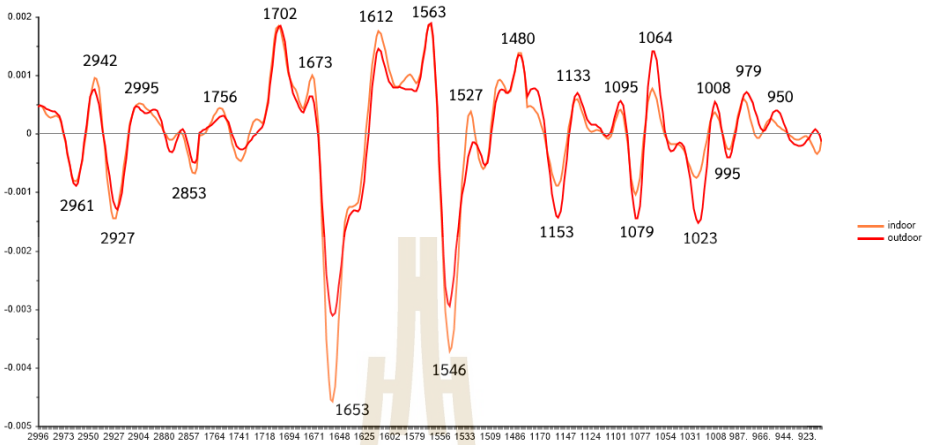

Figure 4.26 Second derivative of Culture in indoor and outdoor conditions

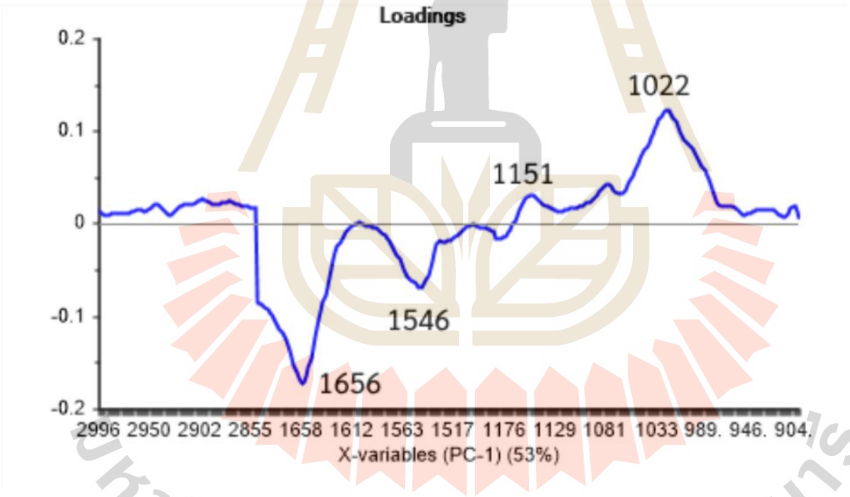

Figure 4.27 PCA loading plot (PC1) of FTIR spectra from Culture in indoor and outdoor conditions

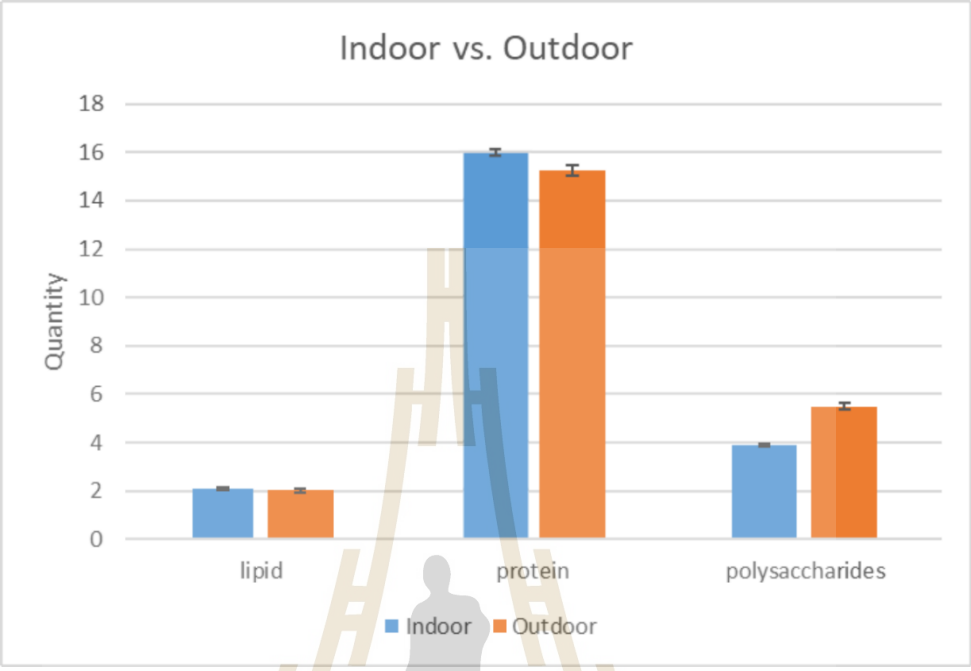

Figure 4.28 Biochemical composition under different Culture in indoor and outdoor conditions

Table 4.3 The integral area of average spectrum of Spirulina culture (H53 strain) from different indoor and outdoor conditions

Biomolecule	Culture	
	Indoor	Outdoor
C-H stretching of lipid	2.107 ± 0.05	2.039 ± 0.06
C=O, N–H, C–N stretching of proteins	15.953 ± 0.11	15.321 ± 0.21
C–O–C stretching of polysaccharides	3.781 ± 0.04	5.517 ± 0.12

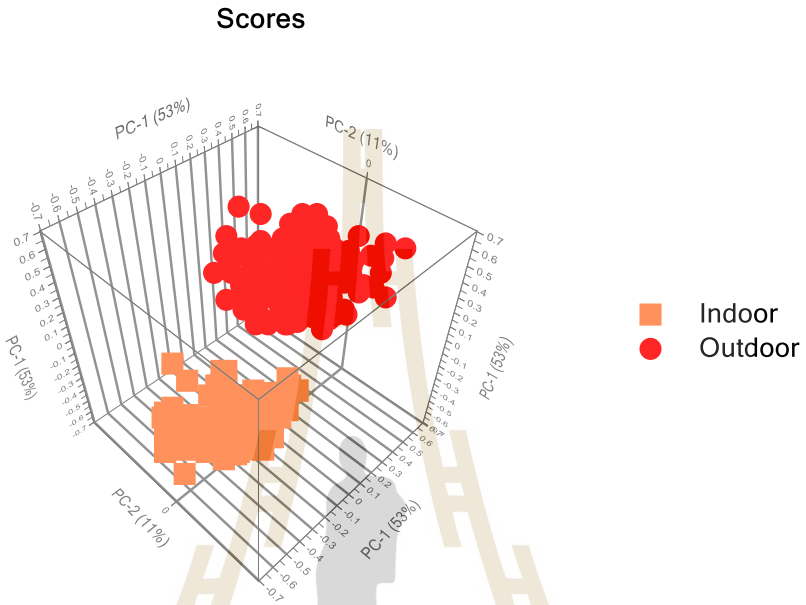

Figure 4.29 3D Model PCA of Spirulina Culture in Indoor and Outdoor cultured conditions

CHAPTER V
CONCLUSION

The combination of FTIR spectroscopy, Quasar and PLS-DA analytical model is effective in distinguishing Spirulina strains and can be developed as a proof of concept for strain classification with high reliability and satisfactory accuracy. This procedure is fast, non-destructive, and suitable for quality inspection of products in the market, protecting the rights of both producers and consumers. The study highlights the significant impact of environmental cultivation factors including photoperiod length, water source, and indoor versus outdoor cultivation systems on Spirulina biochemical profiles, particularly in protein and polysaccharide content. The research identified key spectral regions serving as biochemical fingerprints, with protein and polysaccharide regions emerging as primary markers responsive to environmental variations. In the future, this FTIR-based approach can be utilized to support identify the origin and cultivation methods of Spirulina products by analyzing their unique spectral signatures. This research paves the way for a more cost-effective and rapid method for distinguishing Spirulina samples and ensuring quality control in both laboratory and commercial algal production.

REFERENCES

Abdulqader, G., Barsanti, L., & Tredici, M. R. (2000). Harvest of *Arthrospira platensis* from Lake Kossorom (Chad) and its household usage among the Kanembu. *Journal of Applied Phycology*, *12*(3–5), 493–498. https://doi.org/10.1023/A:1008177925799

Abreu, A. P., Martins, R., & Nunes, J. (2023). Emerging applications of *Chlorella* sp. And Spirulina *(Arthrospira)* sp. *Bioengineering*, *10*(8), 955.

Abu, G. O., Ogbonda, K. H., & Aminigo, R. E. (2007). Optimization studies of biomass production and protein biosynthesis in a Spirulina sp. Isolated from an oilpolluted flame pit in the Niger Delta. *African Journal of Biotechnology*, *6*(22). https://www.ajol.info/index.php/ajb/article/view/58135

Ahmad, M. T., Shariff, M., Md. Yusoff, F., Goh, Y. M., & Banerjee, S. (2020). Applications of microalga *Chlorella vulgaris* in aquaculture. *Reviews in Aquaculture*, *12*(1), 328–346. https://doi.org/10.1111/raq.12320

AlFadhly, N. K., Alhelfi, N., Altemimi, A. B., Verma, D. K., Cacciola, F., & Narayanankutty, A. (2022). Trends and technological advancements in the possible food applications of Spirulina and their health benefits: A Review. *Molecules*, *27*(17), 5584.

Almarashi, J. Q., El-Zohary, S. E., Ellabban, M. A., & Abomohra, A. E.-F. (2020). Enhancement of lipid production and energy recovery from the green microalga *Chlorella vulgaris* by inoculum pretreatment with low-dose cold atmospheric pressure plasma (CAPP). *Energy Conversion and Management*, *204*, 112314.

AlShikaili, T. Y., Thomasson, J. A., Ge, Y., Brown, L., & Brown, J. (2022). FTIR transmission spectroscopy for measurement of algae neutral lipids.

Agricultural Engineering International: CIGR Journal, *24*(4). https://cigrjournal.org/index.php/Ejounral/article/view/5405

Altmann, B. A., & Rosenau, S. (2022). Spirulina as animal feed: Opportunities and challenges. *Foods*, *11*(7), 965.

Aouir, A., Amiali, M., Bitam, A., Benchabane, A., & Raghavan, V. G. (2017a). Comparison of the biochemical composition of different *Arthrospira platensis* strains from Algeria, Chad and the USA. *Journal of Food Measurement and Characterization*, *11*(2), 913–923. https://doi.org/10.1007/s11694-016-9463-4

Aouir, A., Amiali, M., Bitam, A., Benchabane, A., & Raghavan, V. G. (2017b). Comparison of the biochemical composition of different *Arthrospira platensis* strains from Algeria, Chad and the USA. *Journal of Food Measurement and Characterization*, *11*(2), 913–923. https://doi.org/10.1007/s11694-016-9463-4

Application of modified *Spirulina platensis* and *Chlorella vulgaris* powder on the adsorption of heavy metals from aqueous solutions | Request PDF. (n.d.). *ResearchGate*. https://doi.org/10.1016/j.jece.2019.103169

Araujo, G. S., Santiago, C. S., Moreira, R. T., Neto, M. P. D., & Fernandes, F. A. (2021). Nutrient removal by *Arthrospira platensis* cyanobacteria in cassava processing wastewater. *Journal of Water Process Engineering*, *40*, 101826.

Aye, N. N., Sun, B., Zhu, X. M., Gao, Z. Y., Song, Y. J., & Sato, M. (2012). Inactivation of algae with multi-needle gas-liquid hybrid discharge reactor by introducing oxygen and air. *Advanced Materials Research*, *365*, 389–395.

Beshi, M. (2021). OPTIMIZATION OF GROWTH PARAMETERS FOR THE PRODUCTION OF *ARTHROSPIRA* SPECIES IN INDOOR AND OUTDOOR CONDITIONS [PhD Thesis]. http://ir.bdu.edu.et/handle/123456789/12116

Bitam, A., & Aissaoui, O. (2020). Chapter 32—*Spirulina platensis*, oxidative stress, and diabetes. In V. R. Preedy (Ed.), *Diabetes (Second Edition)* (pp. 325–331). Academic Press. https://doi.org/10.1016/B978-0-12-815776-3.00033-4

Chaiyasitdhi, A., Miphonpanyatawichok, W., Riehle, M. O., Phatthanakun, R., Surareungchai, W., Kundhikanjana, W., & Kuntanawat, P. (2018). The biomechanical role of overall-shape transformation in a primitive multicellular organism: A case study of dimorphism in the filamentous cyanobacterium *Arthrospira platensis*. *PLOS ONE*, *13*(5), e0196383. https://doi.org/10.1371/journal.pone.0196383

Chang, W.-T., Lee, M., & Den, W. (2013). Simultaneous Carbon Capture, Biomass Production, and Diary Wastewater Purification by Spirulina *maxima* Photobioreaction. *Industrial & Engineering Chemistry Research*, *52*(5), 2046–2055. https://doi.org/10.1021/ie301932v

Cheah, W. Y., Er, A. C., Aiyub, K., Yasin, N. H. M., Ngan, S. L., Chew, K. W., Khoo, K. S., Ling, T. C., Juan, J. C., & Ma, Z. (2023). Current status and perspectives of algae-based bioplastics: A reviewed potential for sustainability. *Algal Research*, *71*, 103078.

Ciferri, O. (1983). Spirulina, the edible microorganism. *Microbiological Reviews*, *47*(4), 551–578. https://doi.org/10.1128/mr.47.4.551-578.1983

Colla, L. M., Reinehr, C. O., Reichert, C., & Costa, J. A. V. (2007). Production of biomass and nutraceutical compounds by *Spirulina platensis* under different temperature and nitrogen regimes. *Bioresource Technology*, *98*(7), 1489–1493.

Da Silva, M. F., Casazza, A. A., Ferrari, P. F., Perego, P., Bezerra, R. P., Converti, A., & Porto, A. L. F. (2016). A new bioenergetic and thermodynamic approach to batch photoautotrophic growth of *Arthrospira (*Spirulina*) platensis* in different photobioreactors and under different light conditions. *Bioresource Technology*, *207*, 220–228. https://doi.org/10.1016/j.biortech.2016.01.128

Dalmonte, T. (2024). Biochemical bases of the activity of phytoextracts and use of microalgae in veterinary medicine [Doctoral Thesis, Alma Mater Studiorum - Università di Bologna]. https://doi.org/10.48676/unibo/amsdottorato/11546

Desai, S. S., & Mane, V. K. (2024). Biotechnological Advances in Cosmetic Industry. In A. K. Patel & A. K. Sharma (Eds.), *Sustainable Production Innovations* (1st ed., pp. 103–135). Wiley. https://doi.org/10.1002/9781119792888.ch3

Detailed characterization of the *Arthrospira* type species separating commercially grown taxa into the new genus *Limnospira (Cyanobacteria) | Scientific Reports*. (n.d.). Retrieved July 5, 2025, from https://www.nature.com/articles/s41598-018-36831-0

El-Bestawy, E., Bellinger, E. G., & Sigee, D. C. (1996). Elemental composition of phytoplankton in a subtropical lake: X-ray microanalytical studies on the dominant algae *Spirulina platensis* (Cyanophyta) and *Cyclotella meneghiniana* (Bacillariophyceae). *European Journal of Phycology*, *31*(2), 157–166. https://doi.org/10.1080/09670269600651331

Gonçalves, A. L. (2021). The Use of Microalgae and Cyanobacteria in the Improvement of Agricultural Practices: A Review on Their Biofertilising, Biostimulating and Biopesticide Roles. *Applied Sciences*, *11*(2), 871. https://doi.org/10.3390/app11020871

Grobbelaar, J. U. (2003). Quality Control and Assurance: Crucial for the sustainability of the applied phycology industry. *Journal of Applied Phycology*, *15*(2–3), 209–215. https://doi.org/10.1023/A:1023820711706

Gromski, P. S., Muhamadali, H., Ellis, D. I., Xu, Y., Correa, E., Turner, M. L., & Goodacre, R. (2015). A tutorial review: Metabolomics and partial least squares-discriminant analysis–a marriage of convenience or a shotgun wedding. *Analytica Chimica Acta*, *879*, 10–23.

Growth, Productivity, and Size Structure of Spirulina Strain Under Different Salinity Levels: Implications for Cultivation Optimization. (n.d.). Retrieved October 29, 2025, from https://www.mdpi.com/2673-9410/5/3/31

Harvey, B. J., Soto, D., Carolsfeld, J., Beveridge, M. C. M., & Bartley, D. M. (Eds.). (2017). Planning for aquaculture diversification: The importance of climate change

and other drivers : FAO Technical Workshop, 23-25 June 2016, FAO Rome, Italy. Food and Agriculture Organization of the United Nations.

Henrikson, Microalgae *Spirulina* super alimento del futuro. Ediciones S. A. Urano. - *Google Scholar*. (n.d.). Retrieved June 12, 2025, from https://scholar.google.com/scholar_lookup?hl=en&publication_year=1994&pages=222&author=R.+Henrikson&title=Microalga+Spirulina%2C+superalimento+del+futuro.+Ronore+Enterprises

http://old-book.ru.ac.th/e-book/c/CM328/CM328-10.pdf - Google. (n.d.). Retrieved June 12, 2025, from https://www.google.com/search บทที่ 2 : สเปกโทรสโกปี

Ihsanullah, I., & Rashid, A. (2017). Current activities in food irradiation as a sanitary and phytosanitary treatment in the Asia and the Pacific Region and a comparison with advanced countries. *Food Control*, *72*, 345–359. https://doi.org/10.1016/j.foodcont.2016.03.011

Jester, B. W., Zhao, H., Gewe, M., Adame, T., Perruzza, L., Bolick, D. T., Agosti, J., Khuong, N., Kuestner, R., & Gamble, C. (2022). Development of Spirulina for the manufacture and oral delivery of protein therapeutics. *Nature Biotechnology*, *40*(6), 956–964.

Juneja, A., Ceballos, R. M., & Murthy, G. S. (2013). Effects of environmental factors and nutrient availability on the biochemical composition of algae for biofuels production: A review. *Energies*, *6*(9), 4607–4638.

Kaamoush, M., El-Agawany, N., Salhin, H. E., & El-Zeiny, A. (2022). Monitoring effect of nickel, copper, and zinc on growth and photosynthetic pigments of *Spirulina platensis* with suitability investigation in Idku Lake. *Environmental Science and Pollution Research*, *29*(52), 78942–78959. https://doi.org/10.1007/s11356-022-21328-1

Katemala, S., Molee, A., Thumanu, K., & Yongsawatdigul, J. (2022a). A comparative study of meat quality and vibrational spectroscopic properties of different chicken breeds. *Poultry Science*, *101*(6), 101829.

Katemala, S., Molee, A., Thumanu, K., & Yongsawatdigul, J. (2022b). A comparative study of meat quality and vibrational spectroscopic properties of different chicken breeds. *Poultry Science*, *101*(6), 101829.

Kebede, E. (1997). Response of *Spirulina platensis* (*Arthrospira fusiformis*) from Lake Chitu, Ethiopia, to salinity stress from sodium salts. *Journal of Applied Phycology*, *9*(6), 551–558. https://doi.org/10.1023/A:1007949021786

Lao, I. K. M., & Edullantes, B. (2025). Growth, Productivity, and Size Structure of *Spirulina* Strain Under Different Salinity Levels: Implications for Cultivation Optimization. *Phycology*, *5*(3), 31. https://doi.org/10.3390/phycology5030031

Leema, J. M., Kirubagaran, R., Vinithkumar, N. V., Dheenan, P. S., & Karthikayulu, S. (2010). High value pigment production from *Arthrospira (Spirulina) platensis* cultured in seawater. *Bioresource Technology*, *101*(23), 9221–9227.

Liu, J., Zhu, C., Li, Z., & Zhou, H. (2022). Screening of Spirulina strains for high copper adsorption capacity through Fourier transform infrared spectroscopy. *Frontiers in Microbiology*, *13*, 952597.

Lobstein, T. (2019). Why consumers have lost confidence in the food industry. *International Food Safety Handbook*, 673–699.

Madkour, F. F., & Nasr, H. S. (2012). Production and nutritive value of *Spirulina platensis* in reduced cost media. *Egyptian Journal of Aquatic Research*, *38*(1), 51–57.

Marles, R. J., Barrett, M. L., Barnes, J., Chavez, M. L., Gardiner, P., Ko, R., Mahady, G. B., Dog, T. L., Sarma, N. D., Giancaspro, G. I., Sharaf, M., & Griffiths, J. (2011). United States Pharmacopeia Safety Evaluation of Spirulina. *Critical Reviews in Food Science and Nutrition*, *51*(7), 593–604. https://doi.org/10.1080/10408391003721719

Mastropetros, S. G., Pispas, K., Zagklis, D., Ali, S. S., & Kornaros, M. (2022). Biopolymers production from microalgae and cyanobacteria cultivated in wastewater: Recent advances. *Biotechnology Advances*, *60*, 107999.

Matos, Â. P., Matos, A. C., & Moecke, E. H. S. (2019). Polyunsaturated fatty acids and nutritional quality of five freshwater fish species cultivated in the western region of Santa Catarina, Brazil. *Brazilian Journal of Food Technology*, *22*, e2018193.

Milia, M., Corrias, F., Addis, P., Chini Zitelli, G., Cicchi, B., Torzillo, G., Andreotti, V., & Angioni, A. (2022). Influence of different light sources on the biochemical composition of *Arthrospira* spp. Grown in model systems. *Foods*, *11*(3), 399.

Mohammed, M., & Markert, B. (2006). Toxicity of Heavy Metals on Scenedesmus quadricauda (Turp.) de Brébisson in Batch Cultures (7 pp). *Environmental Science and Pollution Research*, *13*(2), 98–104. https://doi.org/10.1065/espr2005.07.274

Moreno-Merino, L., Aguilera, H., & De la Losa Román, A. (2022). Are bottled mineral waters and groundwater for human supply different? *Science of The Total Environment*, *835*, 155554.

Mróz, M., Parchem, K., Jówik, J., Domingues, M. R., & Kusznierewicz, B. (2024). The impact of different drying methods on the metabolomic and lipidomic profiles of *Arthrospira platensis*. *Molecules*, *29*(8), 1747.

Nalimova, A. A., Popova, V. V., Tsoglin, L. N., & Pronina, N. A. (2005). The effects of copper and zinc on *Spirulina platensis* growth and heavy metal accumulation in its cells. *Russian Journal of Plant Physiology*, *52*(2), 229–234. https://doi.org/10.1007/s11183-005-0035-4

Nisol, B., Watson, S., Leblanc, Y., Moradinejad, S., Wertheimer, M. R., & Zamyadi, A. (2019). Cold plasma oxidation of harmful algae and associated metabolite BMAA toxin in aqueous suspension. *Plasma Processes and Polymers*, *16*(2), 1800137. https://doi.org/10.1002/ppap.201800137

Nowicka-Krawczyk, P., Mühlsteinová, R., & Hauer, T. (2019). Detailed characterization of the *Arthrospira* type species separating commercially grown taxa into the new genus *Limnospira* (Cyanobacteria). *Scientific Reports*, *9*(1), 694.

Nyabuto, D. K., Mariga, A. M., & Kibue, G. W. (2015). Growth Performance and Biochemical Analysis of the Genus *Spirulina* under different Physical and Chemical Environmental Factors, *Agricultural Research*. https://repository.must.ac.ke/handle/123456789/135

Ovando, C. A., Carvalho, J. C. D., Vinícius De Melo Pereira, G., Jacques, P., Soccol, V. T., & Soccol, C. R. (2018). Functional properties and health benefits of bioactive peptides derived from Spirulina: A review. *Food Reviews International*, *34*(1), 34–51. https://doi.org/10.1080/87559129.2016.1210632

Özer, T., Yalçın, D., Erkaya, I. A., & Udoh, A. U. (2016). Identification and characterization of some species of cyanobacteria, chlorophyta and bacillariophyta using fourier-transform infrared (FTIR) spectroscopy. *IOSR J. Pharm. Biol. Sci*, *11*, 20–27.

Payne, E. (2023). Purification of C-phycocyanin from Spirulina-adsorption pretreatment *options*.https://open.uct.ac.za/items/6aee71c3-2e04-42bf-b0a8-7e120230847d

Pfaller, M. A., Pappas, P. G., & Wingard, J. R. (2006). Invasive fungal pathogens: Current epidemiological trends. *Clinical Infectious Diseases*, *43*(Supplement_1), S3–S14.

Posten, C. (2009). Design principles of photo-bioreactors for cultivation of microalgae. *Engineering in Life Sciences*, *9*(3), 165–177. https://doi.org/10.1002/elsc.200900003

Priyadarshani, I., & Rath, B. (2012). Commercial and industrial applications of micro algae–A review. *Journal of Algal Biomass Utilization*, *3*(4), 89–100.

Ragusa, I., Nardone, G. N., Zanatta, S., Bertin, W., & Amadio, E. (2021). Spirulina for skin care: A bright blue future. *Cosmetics*, *8*(1), 7.

Rana, R., Herz, K., Bruelheide, H., Dietz, S., Haider, S., Jandt, U., & Pena, R. (2018). Leaf Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR)

biochemical profile of grassland plant species related to land-use intensity. *Ecological Indicators*, *84*, 803–810.

Rich, F. (1931). Notes on *Arthrospira platensis*. *Rev. Algol*, *6*, 75–79.

Richmond, A., & Thomas, H. G. (1986). Purification of melanoma growth stimulatory activity. *Journal of Cellular Physiology*, *129*(3), 375–384. https://doi.org/10.1002/jcp.1041290316

Rosales, N., Ortega, J., Mora, R., & Morales, E. (2005). Influence of salinity on the growth and biochemical composition of the cyanobacterium *Synechococcus* sp. *Ciencias Marinas*, *31*(2), Article 2. https://doi.org/10.7773/cm.v31i2.59

Sabat, S., Bej, S., Swain, S., Bishoyi, A. K., Sahoo, C. R., Sabat, G., & Padhy, R. N. (2025). Phycochemistry and pharmacological significance of filamentous cyanobacterium Spirulina sp. *Bioresources and Bioprocessing*, *12*(1), 27. https://doi.org/10.1186/s40643-025-00861-0

Shao, W., Ebaid, R., El-Sheekh, M., Abomohra, A., & Eladel, H. (2019). Pharmaceutical applications and consequent environmental impacts of Spirulina (*Arthrospira*): An overview. *Grasas y Aceites*, *70*(1), e292–e292.

Shapaval, V., Brandenburg, J., Blomqvist, J., Tafintseva, V., Passoth, V., Sandgren, M., & Kohler, A. (2019a). Biochemical profiling, prediction of total lipid content and fatty acid profile in oleaginous yeasts by FTIR spectroscopy. *Biotechnology for Biofuels*, *12*(1), 140. https://doi.org/10.1186/s13068-019-1481-0

Shapaval, V., Brandenburg, J., Blomqvist, J., Tafintseva, V., Passoth, V., Sandgren, M., & Kohler, A. (2019b). Biochemical profiling, prediction of total lipid content and fatty acid profile in oleaginous yeasts by FTIR spectroscopy. *Biotechnology for Biofuels*, *12*(1), 140. https://doi.org/10.1186/s13068-019-1481-0

Sharma, V., Yadav, J., Kumar, R., Tesarova, D., Ekielski, A., & Mishra, P. K. (2020). On the rapid and non-destructive approach for wood identification using ATR-FTIR spectroscopy and chemometric methods. *Vibrational Spectroscopy*, *110*, 103097.

Shi, W., Li, S., Li, G., Wang, W., Chen, Q., Li, Y., & Ling, X. (2016). Investigation of main factors affecting the growth rate of Spirulina. *Optik*, *127*(16), 6688–6694. https://doi.org/10.1016/j.ijleo.2016.04.125

Siano, F., Moccia, S., Picariello, G., Russo, G. L., Sorrentino, G., Di Stasio, M., La Cara, F., & Volpe, M. G. (2018). Comparative study of chemical, biochemical characteristic and ATR-FTIR analysis of seeds, oil and flour of the edible Fedora cultivar hemp (*Cannabis sativa* L.). *Molecules*, *24*(1), 83.

Sili, C., Torzillo, G., & Vonshak, A. (2012). *Arthrospira (*Spirulina*).* In B. A. Whitton (Ed.), *Ecology of Cyanobacteria II* (pp. 677–705). *Springer Netherlands*. https://doi.org/10.1007/978-94-007-3855-3_25

Sinetova, M. A., Kupriyanova, E. V., & Los, D. A. (2024). *Spirulina /Arthrospira/Limnospira*—Three Names of the Single Organism. *Foods*, *13*(17), Article 17. https://doi.org/10.3390/foods13172762

Singh, R. N., & Sharma, S. (2012). Development of suitable photobioreactor for algae production–A review. *Renewable and Sustainable Energy Reviews*, *16*(4), 2347–2353.

Soeder, C. J., & Binsack, R. (1978). Microalgae for Food and Feed: A Status Analysis: Proceedings of the German-Israeli Workshop*, Held 17-18 October 1977 at Neuherberg* (Vol. 11). Schweizerbart. https://library.wur.nl/WebQuery/titel/115493

Soni, R. A., Sudhakar, K., & Rana, R. S. (2017a). Spirulina – From growth to nutritional product: A review. *Trends in Food Science & Technology*, *69*, 157–171. https://doi.org/10.1016/j.tifs.2017.09.010

Soni, R. A., Sudhakar, K., Rana, R. S., & Baredar, P. (2021). Food Supplements Formulated with Spirulina. In S. K. Mandotra, A. K. Upadhyay, & A. S. Ahluwalia (Eds.), *Algae* (pp. 201–226). *Springer Singapore*. https://doi.org/10.1007/978-981-15-7518-1_9

Tarighat, M. A., Abdi, G., & Tussi, F. (2022). Spectroscopic discrimination of Persian Gulf alga species using chemometrics approaches: A Comparative study using data fusion. *Research Square,* https://www.researchsquare.com/article/rs-2040897/latest

The Use of Microalgae and Cyanobacteria in the Improvement of Agricultural Practices: A Review on Their Biofertilising, *Biostimulating and Biopesticide Roles*. (n.d.). Retrieved October 29, 2024, from https://www.mdpi.com/2076-3417/11/2/871

Thevarajah, B., Nishshanka, G. K. S. H., Premaratne, M., Nimarshana, P. H. V., Nagarajan, D., Chang, J.-S., & Ariyadasa, T. U. (2022). Large-scale production of Spirulina-based proteins and c-phycocyanin: A biorefinery approach. *Biochemical Engineering Journal*, *185*, 108541.

Toplak, M., Read, S. T., Sandt, C., & Borondics, F. (2021). Quasar: Easy Machine Learning for Biospectroscopy. *Cells*, *10*(9), 2300. https://doi.org/10.3390/cells10092300

Ughetti, A., D'Eusanio, V., Strani, L., Russo, A. L., & Roncaglia, F. (2024). Influence of Drying and Storage Conditions on the Volatile Organic Compounds Profile of *Spirulina Platensis*. *Separations*, *11*(6), 180.

Van Eykelenburg, C., & Fuchs, A. (1980). Rapid reversible macromorphological changes in *Spirulina platensis*. *Naturwissenschaften*, *67*(4), 200–201. https://doi.org/10.1007/BF01086309

Vareschi, E., & Jacobs, J. (1985). The ecology of Lake Nakuru: VI. Synopsis of production and energy flow. *Oecologia*, *65*(3), 412–424. https://doi.org/10.1007/BF00378917

Vieira Costa, J. A., Colla, L. M., & Filho, P. D. (2003). *Spirulina platensis* Growth in Open Raceway Ponds Using Fresh Water Supplemented with Carbon, Nitrogen and Metal Ions. *Zeitschrift Für Naturforschung C*, *58*(1–2), 76–80. https://doi.org/10.1515/znc-2003-1-214

Vieira, V. V., Benemann, J., Vonshak, A., Belay, A., Ras, M., Unamunzaga, C., Cadoret, J.-P., & Rizzo, A. (2025). Spirulina in the 21st century: Five reasons for success in Europe. *Journal of Applied Phycology*. https://doi.org/10.1007/s10811-025-03536-7

Virgin, I., Diaz-Chavez, R., Morris, E. J., Haileselassie, T., Tesfaye, K., De Cliff, S., Njau, K., Munganyinka, E., Muyambi, F., & Otim, M. O. (2022). The state of the bioeconomy in eastern Africa: 2022. *Stockholm Environment Institute, The East African Science and Technology Commission and BioInnovate Africa*. https://bioinnovate-africa.org/wp-content/uploads/2024/10/The-State-of-the-Bioeconomy-in-Eastern-Africa-2024.pdf

Vonshak, A., Laorawat, S., Bunnag, B., & Tanticharoen, M. (2014). The effect of light availability on the photosynthetic activity and productivity of outdoor cultures of *Arthrospira platensis* (Spirulina). *Journal of Applied Phycology*, *26*(3), 1309–1315. https://doi.org/10.1007/s10811-013-0133-1

Wang, C.-Y., Fu, C.-C., & Liu, Y.-C. (2007). Effects of using light-emitting diodes on the cultivation of *Spirulina platensis*. *Biochemical Engineering Journal*, *37*(1), 21–25.

Wang, Z. P., & Zhao, Y. (2005). Morphological Reversion of *Spirulina (arthrospira) Platensis* (cyanophyta): From Linear to Helical. *Journal of Phycology*, *41*(3), 622–628. https://doi.org/10.1111/j.1529-8817.2005.00087.x

Wu, T., Zhong, N., & Yang, L. (2018). Identification of Adulterated and Non-adulterated Norwegian Salmon Using FTIR and an Improved PLS-DA Method. *Food Analytical Methods*, *11*(5), 1501–1509. https://doi.org/10.1007/s12161-017-1135-4

Wurmann, C., & Routledge, E. A. B. (2016). Aquaculture diversification in South America: General views and facts and case studies of the Republic of Chile and the federative republic of Brazil. *In FAO Fisheries and Aquaculture Proceedings*, 51.

Yan, B., Gremlich, H.-U., Moss, S., Coppola, G. M., Sun, Q., & Liu, L. (1999). A Comparison of Various FTIR and FT Raman Methods: Applications in the Reaction Optimization Stage of Combinatorial Chemistry. *Journal of Combinatorial Chemistry*, *1*(1), 46–54. https://doi.org/10.1021/cc980003w

Zhang, Y., Li, X., Li, Y., Liu, S., Chen, Y., Jia, M., Wang, X., Zhang, L., Gao, Q., Zhang, L., Yu, D., & Ge, B. (2024a). Regulation of different light conditions for efficient biomass production and protein accumulation of *Spirulina platensis*. *Journal of Oceanology and Limnology*, *42*(1), 174–186. https://doi.org/10.1007/s00343-023-2360-x

Zhang, Y., Li, X., Li, Y., Liu, S., Chen, Y., Jia, M., Wang, X., Zhang, L., Gao, Q., Zhang, L., Yu, D., & Ge, B. (2024b). Regulation of different light conditions for efficient biomass production and protein accumulation of *Spirulina platensis*. *Journal of Oceanology and Limnology*, *42*(1), 174–186. https://doi.org/10.1007/s00343-023-2360-x

Zhuang, D., Abbas, J., Al-Sulaiti, K., Fahlevi, M., Aljuaid, M., & Saniuk, S. (2022). Land-use and food security in energy transition: Role of food supply. *Frontiers in Sustainable Food Systems*, *6*, 1053031.

APPENDIX

SOLUTION PREPARATION

Zarrouk's Medium Composition (g·L^{-1})

- $NaHCO_3$	16.8	g
- K_2HPO_4	0.5	g
- $NaNO_3$	2.5	g
- K_2SO_4	1.0	g
- NaCl	1.0	g
- $MgSO_4$·4H2O	0.2	g
- EDTA-Na_2·2H2O	0.1	g
- $CaCl_2$·2H2O	0.04	g
- $FeSO_4$·2H2O	0.01	g

BIOGRAPHY

Mr. Sinaphon Chairat was born in Phitsanulok, Thailand, on July 25, 1996. He received his bachelor's degree in science from Chiang Mai University's Department of Biology in Chiang Mai. Thailand in 2021

He started a master's program at Suranaree University of Technology, Nakhon Ratchasima's School of Biotechnology, Institute of Agricultural Technology. Where he was received the "SUT-OROG scholarship."

At the 35th Annual Meeting of the Thai Society for Biotechnology and International Conference (TSB 2023), held in Nakhon Ratchasima, Thailand. He gave a poster presentation, “An Implementation of Focal Plane Array (FPA) Fourier Transform Infrared Spectroscopy (FT-IR) in Assessing Biochemical Profiles of Spirulina (*Arthrospira platensis*) Cultured at Different Photoperiods and with Different Water Sources.”