



**THE INFLUENCE OF DRYING METHODS ON THE QUALITY
CHARACTERISTICS OF KAI (*Cladophora glomerata*) POWDER
AND ITS POTENTIAL APPLICATION IN
DYSPHAGIA FOOD FORMULATION**

YONN PWINT PHYU

**MASTER OF SCIENCE
IN
INNOVATIVE FOOD SCIENCE AND TECHNOLOGY**

**SCHOOL OF AGRO-INDUSTRY
MAE FAH LUANG UNIVERSITY**

2025

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**THIS THESIS IS A PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
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Thesis Title: The Influence of Drying Methods on the Quality Characteristics of Kai
(*Cladophora glomerata*) Powder and Its Potential Application in
Dysphagia Food Formulation

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Yonn Pwint Phyu

Thesis Title	The Influence of Drying Methods on the Quality Characteristics of Kai (<i>Cladophora glomerata</i>) Powder and Its Potential Application in Dysphagia Food Formulation
Author	Yonn Pwint Phyu
Degree	Master of Science (Innovative Food Science and Technology)
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ABSTRACT

People with dysphagia require foods that are safe to swallow, and 3D food printing offers a promising approach for developing such texture-modified products; however, its effectiveness depends on the availability of ingredients with suitable functional and rheological properties. Kai (*Cladophora glomerata*), a freshwater alga traditionally consumed in Northern Thailand, is rich in protein, minerals, and bioactive compounds. However, its functional properties are strongly influenced by drying methods. This study investigated the effects of freeze-drying (FD), sun drying (SuD), and tray drying at 50°C (TD) on Kai powder properties and its potential application in 3D-printable dysphagia-friendly food formulations.

In Phase I, fresh Kai was processed by FD, SuD, and TD, and the resulting powders were evaluated for proximate composition, mineral content, *in vitro* protein digestibility, chlorophyll and phytochemical content, antioxidant and anti-inflammatory activity, techno-functional properties, microstructure, and FTIR characteristics. TD retained the highest protein (13.95 %), chlorophyll (4.41 mg/g), flavonoid (6.60 mg QE/g), and antioxidant (FRAP: 20.86 mmol Fe²⁺/g) contents, along with the greatest absolute digestible protein yield (9.57 g/ 100 g) and exhibited potent anti-inflammatory activity at low concentrations (12.5–25 µg/mL), suppressing NF-κB-mediated inflammatory responses while strongly activating the Nrf2/HO-1 antioxidant defense pathway. Sun drying showed the highest phenolic content, DPPH activity, water holding capacity, and protein digestibility (82.55 %). FD preserved superior emulsifying properties and demonstrated anti-inflammatory activity at 100–200 µg/mL.

All drying methods produced mineral-rich powders with heavy metal contents below EU safety limits.

Due to its superior balance of nutritional quality, functional performance, and practical scalability, TD was selected for Phase II and formulated with xanthan gum (XG) and konjac gum (KG) using response surface methodology. Sixteen formulations were evaluated for rheological behavior, texture profile, print accuracy, syneresis, deformation, microstructure, and dysphagia-oriented texture characteristics. All formulations exhibited pseudoplastic shear-thinning behavior. Formulations with total gum between 6 % and 9 % and XG:KG ratios of approximately 1:2 to 3:1 gave the best print accuracy and passed IDDSI Level 4, Japanese Standard Regulation, and UDF Stage 4 criteria. The RSM models showed good predictive performance ($R^2 = 0.8456$ – 0.9750) and the predicted optimum formulation contained 5.265 % xanthan gum and 3.186 % konjac gum.

Overall, the findings demonstrate that drying-induced structural modifications strongly influence the functional performance of Kai powder and highlight the potential of tray dried Kai as a functional ingredient for personalized dysphagia-friendly 3D food printing applications. Validation of the predicted optimum formulation remains to be performed to confirm its practical printing performance.

Keywords: Freshwater Algae, Drying Techniques, Bioactive Compounds, Techno-functional Properties, 3D Food Printing, Hydrocolloids

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ABBREVIATIONS AND SYMBOLS

3D	Three-dimensional
AAA	Aromatic amino acids
ABTS	2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay
CCK-8	Cell Counting Kit-8
COX-2	Cyclooxygenase-2
DEX	Dexamethasone
DPPH	2,2-diphenyl-1-picrylhydrazyl assay
EC	Emulsifying capacity
FC	Foaming capacity
FD	Freeze drying
FRAP	Ferric reducing antioxidant power assay
FS	Foam stability
FTIR	Fourier transform infrared spectroscopy
HO-1	Heme oxygenase-1
IDDSI	International Dysphagia Diet Standardization Initiative
INFOGEST	International consensus method for <i>in vitro</i> digestion
iNOS	Inducible nitric oxide synthase
IVPD	<i>In vitro</i> protein digestibility
KG	Konjac gum
LPS	Lipopolysaccharide
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NO	Nitric oxide
Nrf2	Nuclear factor erythroid 2-related factor 2
OHC	Oil holding capacity
RSM	Response surface methodology
SAA	Sulfur-containing amino acids
SC	Swelling capacity
SDS-PAGE	Sodium dodecyl sulfate–polyacrylamide gel electrophoresis

ABBREVIATIONS AND SYMBOLS

SEM	Scanning electron microscopy
SGF	Simulated gastric fluid
SIF	Simulated intestinal fluid
SSF	Simulated salivary fluid
SuD	Sun drying
TD	Tray drying
TFC	Total flavonoid content
TPA	Texture profile analysis
TPC	Total phenolic content
UDF	Universal Design Food
WHC	Water holding capacity
XG	Xanthan gum
a^*	Redness–greenness coordinate
Adj. R^2	Adjusted coefficient of determination
a_w	Water activity
b^*	Yellowness–blueness coordinate
C^*	Chroma
ΔE^*	Total color difference
G'	Storage modulus
G''	Loss modulus
h°	Hue angle
K	Consistency index (Power Law)
L^*	Lightness
n	Flow behavior index
p	Probability value
Pred. R^2	Predicted coefficient of determination
QE	Quercetin equivalent
R^2	Coefficient of determination

ABBREVIATIONS AND SYMBOLS

TE	Trolox equivalent
x_1	Xanthan gum concentration
x_2	Konjac gum concentration
α	Significance level
τ	Yield stress
η	Viscosity
$\dot{\gamma}$	Shear rate



CHAPTER 1

INTRODUCTION

1.1 Background and Problem Statement

The global population is expected to reach 9.7 billion by 2050, which will greatly increase food demand worldwide (UN, 2021). At the same time, climate change, land degradation, and resource limitations are reducing the sustainability of conventional food production. These challenges have driven growing interest in alternative food sources that are nutritious, sustainable, and efficient to produce. Freshwater algae have emerged as potential alternatives due to their ability to grow rapidly, require relatively few resources, and provide a rich source of nutrients.

Cladophora glomerata Kützinger, locally known as “Kai” or river weed, is a freshwater filamentous alga commonly found in the Nan, Yao, and Mekong river systems in Northern Thailand (Khuantrairong & Traichaiyaporn, 2012). Kai has long been consumed by local communities and is recognized for its potential functional health benefits, including digestive support, appetite stimulation, and relief of constipation (Srimaroeng et al., 2015). It is also rich in proteins, carbohydrates, dietary fiber, essential amino acids, and minerals, and has been reported to exhibit antioxidant, anti-inflammatory, and anti-ulcer activities (Surayot et al., 2016). These characteristics highlight its potential as a functional ingredient for value-added food development.

However, fresh Kai contains high moisture content and deteriorates rapidly after harvest, making preservation essential for further utilization. Drying is a key processing step to improve shelf life and enable food applications. Different drying methods can significantly influence the physicochemical, functional, and microstructural properties of algal powders, including water holding capacity, oil holding capacity, color, and surface morphology (Mu et al., 2025). These characteristics determine the usability of dried Kai powder as a functional food ingredient and must therefore be carefully evaluated to identify the most suitable drying method for subsequent product development.

Beyond conventional food applications, dried Kai powder also shows potential for incorporation into emerging food processing technologies such as 3D food printing. Three-dimensional (3D) food printing has recently emerged as an innovative technology for producing customized foods with controlled structure, texture, and nutrition. This technology is particularly relevant for individuals with dysphagia, as it enables the design of texture-modified foods that are easier and safer to swallow (Xing et al., 2022). To ensure safety and consistency, printed foods must comply with standardized frameworks such as the International Dysphagia Diet Standardization Initiative (IDDSI), Universal Design Foods (UDF), and Japanese Standard Regulation systems.

However, dried Kai powder alone does not possess sufficient viscoelastic and structural properties to form stable printed structures. Therefore, formulation modification is required using hydrocolloids to improve printability and post-extrusion stability. Among commonly used hydrocolloids, xanthan gum (XG) and konjac gum (KG) are widely studied due to their strong thickening and gelling properties. XG exhibits a rigid helical molecular structure, while KG is a flexible polysaccharide chain. Their combination can generate synergistic interactions that improve viscosity, shear-thinning behavior, and structural recovery after extrusion (Qiao et al., 2023).

The performance of 3D food inks is strongly influenced by hydrocolloid concentration and interaction effects, which affect rheological behavior, print fidelity, structural stability, and syneresis. These factors subsequently determine important texture attributes, including hardness, adhesiveness, and cohesiveness, which are critical for safe swallowing in dysphagia patients. Therefore, precise formulation optimization is necessary to achieve both satisfactory printability and appropriate textural characteristics.

Response Surface Methodology (RSM) is an effective statistical tool for optimizing multi-variable food systems. It allows evaluation of interaction effects between formulation variables and enables prediction of optimal conditions with reduced experimental trials (Myers et al., 2016). In this study, RSM is applied to optimize XG and KG ratios in Kai-based 3D food inks to achieve desirable rheological behavior, texture profile, printability, deformation resistance, and syneresis control.

Although Kai (*Cladophora glomerata*) has traditionally been consumed in Northern Thailand, systematic studies on its processing and utilization for value-added food applications remain limited. In particular, information regarding the effects of different drying methods on the physicochemical, functional, and microstructural properties of Kai powder is still insufficient, despite the importance of these properties for food application development. Therefore, evaluating suitable drying methods is necessary to improve the stability, functionality, and utilization potential of Kai powder. In addition, the application of Kai in dysphagia-oriented 3D-printed food systems remains limited. The development of texture-modified foods using 3D food printing requires suitable rheological and structural properties, which can be achieved through hydrocolloid optimization. However, studies investigating the optimization of XG and KG formulations in Kai-based 3D food inks are still scarce. Therefore, this study also aimed to develop and optimize a dysphagia-friendly 3D-printed food product using the selected Kai powder.

1.2 Objectives

1.2.1 Main Objective

To evaluate the influence of drying methods on the quality characteristics of Kai powder and its potential application in dysphagia-oriented 3D-printed food formulation.

1.2.2 Specific Objectives

1.2.2.1 To evaluate the effects of freeze drying, sun drying, and tray drying on the nutritional composition, bioactive compound retention, antioxidant and anti-inflammatory activities as well as techno-functional properties, and microstructure characteristics of Kai powder.

1.2.2.2 To investigate the effects of xanthan gum and konjac gum on the rheological behavior, texture profile, printing performance and post-printing stability of Kai-based 3D food inks, and to determine the predicted optimal formulation using Response Surface Methodology.

1.2.2.3 To assess the suitability of the 3D-printed Kai formulations according to International Dysphagia Diet Standardization Initiative (IDDSI) Level 4 criteria, Japanese Standard Regulation, and Universal Design Food (UDF) Stage 4 classification.

1.3 Scope of Research

The Kai algae used in this research were collected from the Nan River watershed at Paka Subdistrict, Tha Wang Pha District, Nan Province, Thailand.

This research was conducted in two phases. In Phase I, fresh Kai samples were processed using three drying methods: sun drying (SuD), tray drying (TD), and freeze drying (FD). The dried Kai powders were evaluated for their nutritional, phytochemical, physical, techno-functional, and microstructural properties. Nutritional analyses included proximate composition, mineral content, chlorophyll content, and *in vitro* protein digestibility. Phytochemical properties included total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities determined by DPPH, ABTS, and FRAP assays as well as cellular protective activities in macrophage cells. Physical properties included color and water activity. Techno-functional properties included water holding capacity (WHC), oil holding capacity (OHC), swelling capacity, foaming capacity and foam stability, emulsifying capacity and emulsion stability, rheological characterization, Fourier Transform Infrared Spectroscopy (FTIR), and Scanning Electron Microscopy (SEM). The results obtained were used to determine the most suitable drying method for subsequent food applications.

In Phase II, the selected Kai powder was used to develop 3D-printable food inks for dysphagia-oriented applications. Formulation optimization was performed using Response Surface Methodology (RSM) with a Design-Expert mixture optimal (custom) design consisting of 16 formulations. Xanthan gum (XG) and konjac gum (KG) were used as independent variables to investigate their effects on the properties of the 3D food inks.

Texture Profile Analysis (TPA) parameters, including hardness, adhesiveness, and cohesiveness, together with print accuracy, post-print deformation, and syneresis,

were used as response variables for formulation optimization. Rheological behavior, SEM microstructure, and dysphagia-related assessments were also evaluated to further understand the flow behavior, structural stability, printability, and swallowing safety of the formulations. Dysphagia suitability was assessed using the IDDSI fork drip and spoon tilt tests, as well as Japanese dysphagia food standards and Universal Design Foods (UDF) classifications.

1.4 Expected Output/ Outcome/ Impact

1.4.1 Research Outputs

1.4.1.1 Comprehensive data on the nutritional quality, bioactive properties, techno-functional properties, and physical characteristics of Kai powders from freeze drying, sun drying, and tray drying.

1.4.1.2 Experimental data on the rheological behavior, texture profile, printing performance, and post-printing stability of Kai-based 3D food inks formulated with varying concentrations of xanthan gum and konjac gum.

1.4.1.3 Validated regression models and predicted optimal formulation specifying the concentrations of xanthan gum and konjac gum, together with predicted values for texture, print accuracy, and stability.

1.4.1.4 A comparative classification of the 3D-printed Kai formulations against IDDSI Level 4 criteria, Japanese Standard Regulation I–III, and Universal Design Food Stage 4 classification, supported by rheological and microstructural observations.

1.4.2 Anticipated Outcomes

1.4.2.1 An understanding of how drying-induced structural modifications in Kai influence its nutritional quality, bioactive compound retention, antioxidant and anti-inflammatory activities, and techno-functional properties, providing a scientific basis for drying method selection in algal-based food applications.

1.4.2.2 Increased value and utilization potential of local Kai algae through the development of value-added food products from dried Kai powder.

1.4.2.3 Support for the development of safe, nutritious, and visually appealing texture-modified foods for elderly individuals and patients with dysphagia.

1.4.3 Projected Impact

This research contributes to the following United Nations sustainable development goals:

1.4.3.1 SDG 2 (Zero Hunger): Utilization of underutilized freshwater algae as a sustainable and nutritious food source, contributing to food security and dietary diversification.

1.4.3.2 SDG 3 (Good Health and Well-being): Development of texture-modified foods for individuals with dysphagia, supporting swallowing safety and nutritional intake for elderly and patient populations.

1.4.3.3 SDG 9 (Industry, Innovation, and Infrastructure): Evaluation of drying methods for algal processing, combined with 3D food printing and RSM-based formulation optimization for innovative food applications.

1.4.3.4 SDG 12 (Responsible Consumption and Production): Valorization of locally available algal biomass, reducing waste and supporting sustainable production practices.

1.4.3.5 SDG 14 (Life Below Water): Sustainable use and management of freshwater algal resources, promoting responsible harvest and utilization.

CHAPTER 2

LITERATURE REVIEW

2.1 Food Security

Food security remains a key issue of concern worldwide, influenced by factors like increasing populations and rising food costs (Abdallah et al., 2021).

2.1.1 Concern for Food Security

Climate change, with higher temperatures, reduced water availability, and increased carbon dioxide, has reduced crop production. This will make things worse in areas where people are already struggling with hunger and malnutrition (Myers et al., 2017). The effects of climate change are particularly concerning in regions like Africa and Asia. In Sub-Saharan Africa, staple crop yields are projected to decline by 10–20 % by 2050. Similarly, rice and wheat production in South Asia could decline by 10–15 % by mid-century due to heat stress and changing monsoon patterns (Abebaw, 2025). Under high-emission scenarios, wheat yields are predicted to decline by 15% in African countries and 16% in Southern Asian countries by 2050 (Pequeno et al., 2021). The food crisis will become more severe unless countries find alternative solutions to satisfy food needs. Accordingly, the search for innovative and sustainable food resources has become increasingly important to ensure adequate nutrition.

2.1.2 Fresh Water Algae: Potential Food Source

Alternative food sources, such as microalgae, have been extensively developed. However, some countries focus primarily on producing microalgae from seawater, which can be a challenge for regions lacking access to these marine resources. Therefore, it's important to explore the potential of freshwater algae as a viable alternative (Selvaraj et al., 2023).

Freshwater algae consist of various photosynthetic species that grow in freshwater habitats, including rivers, lakes, ponds, and streams. They are seen as a potential sustainable food source because they are packed with nutrients, protein, and essential amino acids (Costa et al., 2021). Freshwater algae have been a part of various

cultures' diets for centuries, but their potential for human consumption has gained more focus recently because of their low environmental footprint and rich nutritional content. These organisms are highly adaptable and capable of thriving in diverse environments, which attracts scientific interest in their possible uses beyond just providing nutrition. They have a lot of genetic diversity, with many types that range from tiny to larger forms (Tan et al., 2020).

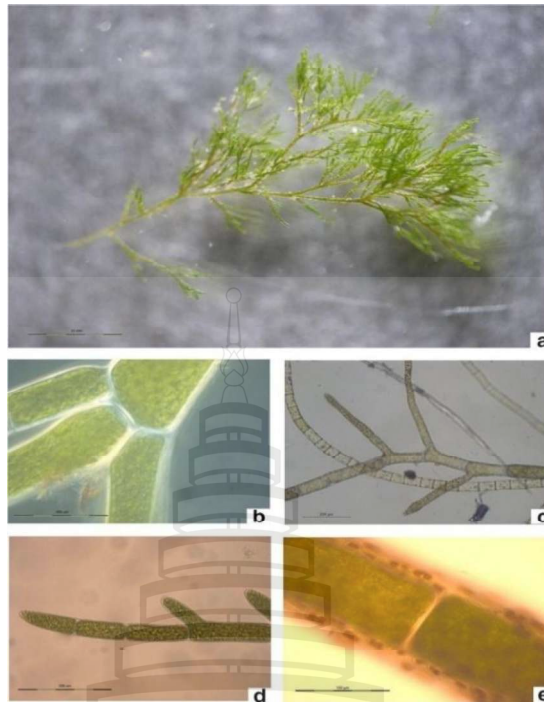
2.2 Kai Algae

Cladophora belongs to the Chlorophyta division and is commonly known as Mekong Weed in English and Kai in Thai (Laungsuwon & Chulalaksananukul, 2013). This genus includes 183 species that are taxonomically recognized (Alves et al., 2012; Leliaert et al., 2009).

Cladophora naturally adheres to substrate surfaces through a holdfast. However, it often detaches from these substrates and accumulates along shorelines, where it forms dense mats. *Cladophora* is filamentous green algae which can be profusely, sparsely, or densely branched. The cells of *Cladophora* are typically large, cylindrical, and multinucleated, possessing a network-like chloroplast and multiple pyrenoids (Figure 2.1). Its vivid green appearance is due to photosynthetic pigments, including chlorophyll a, chlorophyll b, β -carotene, and xanthophylls. In addition, the proportion of chlorophyll a relative to chlorophyll b is comparable to that reported for higher plant species (Laungsuwon, 2014).

For optimal growth, *Cladophora* typically requires high light intensity, hard water with high nutrients and a pH ranging from 7 to 10. Additionally, factors such as temperature, water flow rate, and presence of waves influence its metabolic processes and morphological development (Munir et al., 2019).

In Thailand, Kai is abundant in the upper Nan watershed, especially in Nan and Yao rivers at Amphur Pua and Amphur Tha Wang Pha, Nan province and also in the Mekong River at Amphur Chiang Saen and Amphur Chiang Khong, Chiang Rai province. Kai is abundant during November to March when water is clear with medium flowing rate and optimum temperature (Khuantrairong & Traichaiyaporn, 2012).



Source Michalak and Messyas (2021)

Figure 2.1 View of the Whole *Cladophora glomerata* Thalli Showing the Dense Branching: a. Overall View of Branched Thallus; b. Lateral Branch; c. Number of Branches in Apical Part of Thalli; d. Apical Cell with Primary Side Branch; e. Thallus with Reticulate Chloroplast and Epiphytic Diatoms on the Surface of Cell Wall

2.2.1 Nutritional Content of Kai

Four types of Kai algae samples are collected from Lithuanian rivers. The samples can be seen in Figure 2.2. As shown in Table 2.1, the levels of microelements, trace elements, and heavy metals are within permissible limits. Studies have shown that *C. glomerata* contains around 16 to 22 % crude protein on a dry weight basis. Its nutritional quality is further supported by a well-balanced essential amino acid composition, with total amino acids reaching around 140 g/kg. Moreover, polyunsaturated fatty acids accounted for up to 11.71 % of the lipid fraction, with a polyunsaturated-to-saturated fatty acid ratio of 0.22. Additionally, the lowest omega-6/omega-3 ratio is 1.30. *C. glomerata* has high content of proteins, amino acids, and fatty acids, so it is suggested as a beneficial alternative for animal feed (Nutaaitė et al., 2021).



Source Nutautaitė et al. (2021)

Figure 2.2 Kai Samples from Lithuanian River (Fresh and Dry)

Kai algae demonstrate significant potential as a high-quality protein source, particularly for individuals with specific dietary requirements such as patients with dysphagia. The amino acid profile of Kai algae shows that it meets or exceeds the World Health Organization's (WHO) recommended levels for several essential amino acids, including histidine, isoleucine, phenylalanine + tyrosine, threonine, and valine. While some amino acids, namely leucine, lysine, and methionine + cysteine, are present in slightly lower amounts, the overall amino acid composition of Kai algae remains favorable for human nutrition, can be seen in Table 2.2.

Dysphagia patients, who require high-protein foods to support recovery and preserve muscle mass, would benefit from the inclusion of Kai algae in their diet. Although some amino acids are slightly less abundant, the overall protein quality of Kai algae positions it as a promising food source. Furthermore, combining Kai algae with other protein-rich foods could complement its amino acid profile, ensuring that the dietary needs of dysphagia patients are fully met. Therefore, Kai algae represent a viable and nutritious option for enhancing the protein intake of individuals requiring specialized dietary interventions.

Table 2.1 Nutritional Content of Kai

Chemical Composition		%DM	
Crude Fat	0.18 to 0.35		
Crude Protein	16 to 21.52		
Crude Fiber	10.88 to 13.39		
Crude Ash	36.96 to 49.83		
Macro elements	g/kg DW	Micro elements	% DM
Na	1.46 to 4.15	Zn	7 to 23
P	0.16 to 0.49	Cu	4 to 7
K	3.25 to 6	Cr	7 to 15
Ca	26.34 to 27.16	Cd	<0.1
Mg	0.26 to 0.42	Pb	<1
Amino Acid Profile	g/kg DM		
Aspartic Acid	10.9 to 15.97		
Threonine*	3.49 to 6.77		
Serine	4.14 to 7.25		
Glutamic Acid	14.16 to 19.50		
Proline	5.98 to 7.22		
Glycine	8.06 to 11.46		
Alanine	8.49 to 10.73		
Valine*	8.39 to 10.42		
Methionine*	1.94 to 2.08		
Isoleucine*	5.94 to 7.69		
Leucine*	9.71 to 12.01		
Tyrosine	1.73 to 2.35		
Phenylalanine*	6.37 to 8.5		
Histidine*	3.4 to 3.68		
Lysine*	5.76 to 7.88		
Arginine	5.25 to 7.49		
Total	103.36 to 140.99		

Note * Indicates essential amino acids (EAAs).

Source Nutautaité et al. (2021)

Table 2.2 Adequacy of Kai Algae Essential Amino Acids Compared to WHO Adult Requirements

	Average Amino Acid Profile of Kai Algae		WHO Requirement	Meets WHO?
	g/kg DM	g/kg Protein		
Histidine	3.54	18.9	15	Yes
Isoleucine	6.82	36.3	30	Yes
Leucine	10.86	57.9	59	Slightly Low
Lysine	6.82	36.4	45	Low
Methionine + Cysteine (SAA)	2.01	11	22	Low
Phenylalanine + Tyrosine (AAA)	9.47	50.5	38	Yes
Threonine	5.13	27.3	23	Yes
Tryptophan	—	—	6	—
Valine	9.41	50.1	39	Yes

Note SAA represents sulfur-containing amino acids (methionine + cysteine), while AAA represents aromatic amino acids (phenylalanine + tyrosine). WHO amino acid requirement values are based on the adult amino acid scoring pattern.

Source Nutautaité et al. (2021), Organization and University (2007)

2.2.2 Kai as Potential Healthy Food Source

Kai is a freshwater alga with potential as food, animal-feed, and a source of bioactive compounds for pharmaceuticals and cosmetics. It also aids in environmental bioremediation by absorbing heavy metals, making it a versatile and sustainable resource. (Coelho et al., 2016; Kharkwal et al., 2012; Wilailak et al., 2017).

In Asia, the consumption of algae as food is relatively prevalent and is gaining traction in North America, South America, and Europe. In countries like Japan, China, and Korea, green algae constitute around 5 % of the human diet (Wetter & Dawes, 1982). It is an extensively consumed alimentary resource in France, Hawaii, and Thailand. (Khuantrairong & Traichaiyaporn, 2011).

Kai contains around 20 to 30 percent carbohydrates and 16 to 21 percent proteins (Nutautaité et al., 2021). Moreover, it is rich in minerals and amino acids (Surayot et al., 2016), so it is used as an ingredient for conventional bread to get

higher nutrition (Menezes et al., 2015). Compared with *Spirulina* and *Porphyra*, Kai contains a greater proportion of essential amino acids. In addition, its young filaments are frequently consumed as salad ingredients and have been reported to support digestive health by promoting regular bowel movements. It contains high amount of bioactive compounds and has therapeutic effects, promoting the healing of stomach ulcers and stimulating appetite (Srimaroeng et al., 2015). Moreover, Kai is rich in fatty acids, particularly saturated and polyunsaturated types, making it a promising natural source of these essential nutrients (Munir et al., 2019).

2.3 Drying Kai Algae

Because Kai algae have high moisture content (around 90 %), it is generally dried before commercialization to improve shelf life and makes it easier for storage and transportation (Nutaaitè et al., 2021). Drying is a fundamental post-harvest processing step that significantly influences the quality, nutritional value, and functional properties of algal and plant-based powders. Selecting an appropriate drying method is crucial to preserve sensitive bioactive compounds, maintain nutritional integrity, and ensure the powders' suitability for advanced food processing techniques such as 3D printing. This study will focus on three commonly employed drying techniques; sun drying, tray drying, and freeze drying with a focus on their mechanisms, advantages, limitations, and impacts on the nutritional and physicochemical characteristics of algal powders.

2.3.1 Overview of Drying

Drying is a thermodynamic process involving the removal of moisture from a biological substrate through simultaneous heat and mass transfer. The goal in algal biomass drying is to reduce moisture content typically from 85 % to below 10 %, which is necessary to inhibit microbial proliferation and oxidative reactions. However, due to the thermal sensitivity of major algal bio-actives such as pigments, unsaturated lipids, antioxidants, and proteins drying must be carefully optimized to prevent irreversible degradation (Aljabri et al., 2023; Min et al., 2022).

Critical factors affecting drying outcomes include temperature, air velocity, humidity, biomass thickness, and drying time. Inappropriate control of these variables

can result in pigment bleaching, protein denaturation, lipid oxidation, textural collapse, and compromised rehydration properties (Mu et al., 2025; Kim & Kim, 2022). Additionally, drying is one of the most energy-intensive steps in the downstream processing of algae, contributing to 60 to 80 % of total energy costs in commercial operations. Therefore, a trade-off exists between preserving functional quality and achieving process efficiency, which must be considered when selecting drying methods for Kai algae.

2.3.2 Methods of Drying

2.3.2.1 Sun Drying

Sun drying is a passive dehydration method that uses natural solar radiation and ambient airflow to evaporate water. While attractive for its low energy input and operational simplicity, sun drying suffers from significant variability due to environmental dependence and lack of process control. In the study by Aljabri et al. (2023), sun-dried *Tetraselmis subcordiformis* exhibited a 34 % reduction in chlorophyll and extensive cellular damage, as evidenced by SEM imaging which showed ruptured membranes and pigment leakage. Oxidative degradation of sensitive lipids led to significant PUFA loss ALA was reduced to 4.61 ppm and DHA was undetectable.

Mu et al. (2025) reported that drying of daylily (*Heemerocallis citrina*), an edible flower valued for its antioxidant properties, resulted in the highest concentration of volatile flavor compounds but also a significant decline in total phenolic content and antioxidant capacity. Sun drying also induced color darkening due to Maillard reactions and carotenoid transformation. Although effective in achieving low final moisture levels (~0.9 %), its inability to preserve nutritional quality and structural integrity limits its use in high-value food applications, particularly those requiring fine powder uniformity and functional rehydration.

2.3.2.2 Tray drying

Tray drying is a widely used method for the post-harvest processing of algae, due to its simplicity, scalability, and ability to deliver consistent drying kinetics. The process involves the circulation of heated air over thinly spread biomass, allowing for controlled convective heat transfer and efficient moisture removal. Compared to traditional sun drying, which is weather-dependent and less controllable, tray drying offers greater process uniformity and reliability. The typical operating temperature

range for tray drying is 40–70 °C, but many studies support the advantages of moderate temperatures (50 °C) in retaining the nutritional and structural integrity of seaweed.

At higher temperatures, significant degradation of sensitive compounds can occur. For example, Aljabri et al. (2023) demonstrated that tray drying *Tetraselmis subcordiformis* at 90 °C led to severe reductions in chlorophyll (62.6 mg/L), protein (20.8 % DW), and lipid content (21 % DW), with scanning electron microscopy (SEM) revealing notable microstructural collapse and vesicle deformation. This highlights the risks associated with high thermal exposure.

In contrast, several studies indicate that moderate drying temperatures offer better preservation of functional and nutritional qualities. Lee et al. (2014) found that drying *K. alvarezii* at 40 °C resulted in high retention of phenolic compounds, flavonoids, carotenoids, and antioxidant activity. Mu et al. (2025) found that tray drying at 55 °C preserved antioxidant activity and mineral content more effectively than sun drying, with SEM imaging showing reduced tissue collapse. (Nunes et al., 2024) also reported that oven-dried *K. alvarezii* samples showed higher flavonoid content and ferric reducing antioxidant power (FRAP) compared to freeze-dried counterparts, possibly due to better compound extractability or stability at mild heat levels.

Overall, the reviewed studies support the use of tray drying at approximately 50 °C is particularly relevant for producing high-quality algae biomass for food, cosmetic, and nutraceutical applications.

2.3.2.3 Freeze drying

Freeze drying is a sublimation-based dehydration technique that removes water from frozen algae under low pressure, bypassing the liquid phase and minimizing thermal degradation. It is considered the gold standard for preserving bioactive and structural integrity in sensitive biomasses. Aljabri et al. (2023) reported that freeze-dried *T. subcordiformis* retained the highest levels of chlorophyll (95.27 mg/L), protein (24.17 % DW), and lipids (26.51 % DW), along with the best PUFA preservation (ALA: 44.09 ppm).

Mu et al. (2025) confirmed similar trends in daylily, where freeze drying preserved the highest antioxidant activity (84.6 % ABTS scavenging) and polyphenol content. SEM images illustrated intact microstructures with high porosity, conducive to

excellent rehydration. Despite its advantages, freeze drying is energy-intensive (up to 45-50 kWh/kg) and costly in terms of both capital and operational expenses.

2.3.3 Comparative Evaluation of Drying Methods

This study focuses on the comparative impacts of sun drying, tray drying, and freeze drying on the nutritional quality, structural integrity, physicochemical properties, and functional performance of algae-based powders.

2.3.3.1 Nutritional and Bioactive Integrity

Drying techniques exert a substantial influence on the nutritional composition and retention of bioactive compounds in algae and other plant-based materials. A comparative analysis of sun drying, tray drying, and freeze drying reveals distinct trade-offs regarding chemical preservation, structural alterations, and operational efficiencies across different species.

Freeze drying is often used because it uses very cold temperatures, which protects heat-sensitive nutrients. For example, Mu et al. (2025) found that freeze-dried *Hemerocallis citrina* kept much higher levels of polyphenols (7.15 mg/g), flavonoids (2.37 mg/g), and carotenoids (54.70 µg) than hot-air dried samples (4.80 mg/g, 1.28 mg/g, and 11.62 µg) and sun-dried samples (3.10 mg/g, 0.49 mg/g, and 7.45 µg). This gave the freeze-dried samples a high antioxidant activity of 84.60 % in ABTS tests. Similarly, Zhao et al. (2022) showed that freeze drying kept the highest amounts of chlorophyll a and total phenolics in *Sargassum fusiforme*. However, freeze drying creates a highly porous, honeycomb-like structure. This open structure leaves a large surface area exposed to air, which can cause the powder to oxidize and degrade faster during storage

Convective tray drying, typically evaluated at temperatures between 50–60°C, serves as a functional intermediate processing method. Although thermal exposure can induce partial degradation of highly sensitive constituents such as ascorbic acid and certain phenolic fractions (Tagliapietra et al., 2024), the heat input simultaneously drives cellular shrinkage and tissue densification. This structural contraction minimizes internal exposure to atmospheric oxygen, which can stabilize specific powder attributes and sustain acceptable antioxidant retention without the high capital costs or vacuum-induced physical stressors associated with freeze drying. Additionally, research on tropical seaweeds shows that oven and tray drying can

actually help break down tough cell walls and release bound phenolic compounds, exceeding the antioxidant extraction levels of other methods (Charles et al., 2020).

Interestingly, research shows that open sun drying can sometimes protect specific nutrients in green seaweed better than other methods. A comparative study on the green seaweed *Ulva lactuca* found that sun-dried samples kept high levels of ortho-diphenols and strong antioxidant activity, closely matching the results of freeze drying (Uribe et al., 2019). While long exposure to the sun usually degrades nutrients due to shifting outdoor temperatures and air exposure (Zhao et al., 2022; Mu et al., 2025), its gentle, ambient process avoids the harsh vacuum stress of freeze drying and the intense heat of tray drying. Because it can preserve these specific antioxidants at zero energy cost, sun drying remains an important baseline method to evaluate in seaweed research.

In summary, the literature shows distinct trade-offs for each drying method regarding nutrient preservation, structure, and cost. Freeze drying excels at maintaining initial compound levels but leaves the material highly porous and vulnerable to air damage. Tray drying provides a practical compromise by balancing cost and stability, though heat can affect highly sensitive nutrients. Meanwhile, sun drying offers an economical baseline that can uniquely preserve certain antioxidants in green seaweeds. Because these outcomes depend heavily on the unique cell structure of the algae, a direct comparison is necessary to determine how these drying methods affect the quality, color, and nutrition of Kai samples.

2.3.3.2 Morphological and Physicochemical Characteristics

The structural and physicochemical attributes of dried seaweed powders, including microstructure, rehydration ratio, and color are highly dependent on the drying method used. SEM imaging provided by Zhao et al. (2022) revealed that freeze-dried *Sargassum fusiforme* retained a porous, honeycomb-like architecture, which supported enhanced rehydration and solubility. A similar porous matrix was observed in freeze-dried *Hemerocallis citrina*, contributing to improved color retention and reduced structural collapse (Mu et al., 2025).

However, freeze drying does not universally guarantee superior product attributes. Research on different macroalgae species has demonstrated that convective oven or tray drying can outperform freeze drying in preserving specific bioactive profiles. For instance, a comparative study on seaweeds demonstrated that moderate

convective air drying better retained total phenolic and flavonoid contents, as well as overall antioxidant performance, compared to freeze drying, which caused substantial pigment and antioxidant losses due to vacuum-induced dynamics (Uribe et al., 2019). Furthermore, controlled tray drying has been shown to offer equivalent color preservation and antioxidant retention to expensive freeze drying methods at a fraction of the operational cost (Charles et al., 2020).

Indeed, tray drying induces moderate shrinkage and densification due to convective heat exposure, which can stabilize certain powder properties. While Tagliapietra et al. (2024) noted that oven-dried *Sargassum filipendula* exhibited a slightly darker color and reduced foam capacity compared to freeze-dried samples, the tray-dried samples maintained structural integrity more effectively than those dried under natural sunlight. Conversely, sun drying was associated with the most pronounced morphological degradation due to extended exposure to environmental variables, causing extensive cellular collapse, tissue hardening, and severe browning (Mu et al., 2025; Zhao et al., 2022).

Overall, while freeze drying is traditionally considered an academic benchmark, alternative thermal methods like tray drying have demonstrated superior or highly comparable quality profiles for specific macroalgal biomass. Given these conflicting dynamics across different species and structural matrices, it is highly worthwhile investigating which specific drying method optimizes the physicochemical, color, and structural quality for Kai samples.

2.3.3.3 Suitability for 3D Printing in Dysphagia-Friendly Foods

The development of 3D printed dysphagia-friendly foods requires powders with appropriate rehydration capacity, gelation behavior, and extrusion stability. These parameters are largely influenced by drying-induced changes in structure and composition. Freeze drying, tray drying, and sun drying each offer distinct physical pathways for creating printable pastes, and each method alters the biomass matrix differently.

Mu et al. (2025) highlighted that freeze-dried plant tissues kept high levels of soluble protein, which naturally supports strong gel-forming capacity. The highly porous nature of freeze-dried powders allows for rapid water uptake, making them a common choice for quick reconstitution. However, an ultra-porous structure can

sometimes absorb water unevenly during paste preparation, which can create structural weak points or air pockets during layer-by-layer printing.

On the other hand, tray-dried materials demonstrate excellent hydration and structural stability when drying settings are properly optimized. Research by Tagliapietra et al. (2024) on marine macroalgae (*Sargassum*) showed that tray drying successfully preserves a significant amount of water absorption capacity and emulsion properties. Meanwhile, sun drying provides a completely different physical profile due to its slow, ambient drying mechanics. While studies like Zhao et al. (2022) observed lower rehydration ratios and weaker gelation behavior in sun-dried macroalgal samples due to a more compact, rigid cellular structure, this density can offer unique rheological behaviors. A more compact microstructure can alter the yield stress and viscosity of a paste, allowing it to behave differently under pressure compared to highly porous or thermally modified powders.

In short, the literature shows that freeze drying, tray drying, and sun drying all create unique physical and structural trade-offs that directly affect how a powder behaves in a printer. Therefore, a direct comparison is necessary to evaluate these specific structural, rehydration, and flow properties to determine how they affect kai powder in 3D printing applications.

2.4 Dysphagia

Across the world, populations are rapidly aging. The United Nations' 2022 report forecasts that the percentage of individuals aged 65 and above is projected to increase from 10 % in 2022 to 16 % by 2050. Additionally, by 2050, the population aged 65 years and older is expected to outnumber children under the age of five, reaching 1.5 billion. As a result, there is a higher necessity for elderly-oriented products and services for helping older populations (Sarkar, 2019).

Older people frequently encounter the risk of dysphagia, difficulty in swallowing and eating safely, as a result of age-related physiological changes. These changes can include lost dentures, reduced saliva production (xerostomia),

compromised coordination, muscle weakness, stroke, and other neurological disorders (Inui et al., 2017).

2.4.1 Dysphagia and Malnutrition

Eating difficulties like choking, food aspiration, and regurgitation can cause anxiety and frustration, making mealtime stressful. This can lead to lower appetite, reducing food intake and negatively affecting overall nutrition and health. Dysphagia in older adults is increasingly seen as a significant healthcare concern, as it puts them at risk for malnutrition, dehydration, sarcopenia (loss of muscle mass), and digestive problems. Therefore, alongside medical and surgical treatments, selecting appropriate foods and beverages plays a crucial role in ensuring safe swallowing, adequate nutrition, and comfort while eating for the elderly (Chao et al., 2024).

2.4.2 Aging and Nutritional Requirements

A well-balanced diet and adequate nutrition play a pivotal role in supporting the health, development, and longevity of elderly individuals, particularly those affected by age-related physiological changes and conditions like dysphagia. Nutritional interventions play a key role in addressing these challenges, offering an effective alternative to medication and physical activity, and helping to improve health outcomes in the elderly. The 2010 Dietary Guidelines for Americans state that women in the same age range should consume 1600–2000 kcal per day, while men over 65 should consume 2000–2600 kcal (Aslam & Vaezi, 2013). The fact that diet and nutritional status are linked to a number of diseases and complications that arise from the basic disease state should not be overlooked. In fact, poor food intake leads to lower immune and deterioration of body mass and health. Moreover, it can affect the memory of the elderly and causes undernutrition and frailty. Excessive food consumption, however, can often lead to other chronic conditions like cancer, diabetes, obesity, and high blood pressure. For that situation, it is important to understand the particular nutritional requirements that correspond to personal biological requirements and state of health, as well as the composition, functions, and suitable proportions of the three macronutrients that are necessary for the development of personalized diet that will fulfil the nutritional requirements for elderly (Baugreet et al., 2016).

2.4.2.1 Nutritional and Bioactive Integrity

1. Protein for Elderly

Protein is essential for maintaining body function, particularly for older adults. It plays a key role in muscle synthesis, nitrogen balance, and other biochemical processes. To support musculoskeletal health and prevent sarcopenia, older individuals are advised to consume 1 to 1.6 grams of protein per kilogram of body weight per day. Diet rich in essential amino acids (EEA) should be foundation of personalized nutrition for the elderly (Chao et al., 2024). While animal-based protein sources such as meat, fish, and eggs are commonly recommended, algae present a promising alternative.

Algae, including Kai algae, can provide a valuable source of protein and essential amino acids (EAAs). It is a nutritious, plant-based option that can substitute animal proteins in the diet. Algae are also low in fat and rich in vitamins, minerals, and antioxidants, making it a versatile and sustainable choice. For older adults, particularly those with dysphagia who may need easier-to-consume foods, algae can be an ideal addition to personalized nutrition plans, supporting overall health and well-being.

2. Carbohydrates for Elderly

Carbohydrates play a fundamental role in energy provision and are generally divided into two groups: simple carbohydrates, consisting of monosaccharides and disaccharides, and complex carbohydrates, which include oligosaccharides and polysaccharides. The way that carbohydrates are digested and absorbed affects blood sugar levels, energy metabolism, and overall health. Simple carbohydrates are readily absorbed and often lead to rapid fluctuations in blood glucose concentrations, while complex carbohydrates are broken down more slowly, contributing to a steady and prolonged release of glucose into the bloodstream. For people with conditions like Type II diabetes, it is important to consume carbohydrates that release glucose slowly, such as whole grains, vegetables, and legumes. Diets high in simple sugars and processed foods can lead to blood sugar imbalances and increase the risk of obesity, diabetes, and cardiovascular diseases. Fiber, a type of complex carbohydrate, also offers additional health benefits, such as improving digestive health and lowering cholesterol (Gallego et al., 2022). In this instance, senior citizens might avoid developing more serious health issues by choosing foods high in carbohydrates

that are appropriate for their bodily conditions. As a result, foods containing carbohydrates are categorized using the glycemic index (GI) based on how much glucose is released into the bloodstream 2 h after ingestion (Steele et al., 2014). High-GI foods, such as refined grains, processed potatoes, and sugar, quickly raise blood sugar levels, which can lead to long-term health issues like insulin resistance and type 2 diabetes. In contrast, low-GI foods, including whole grains, legumes, sweet potatoes, fruits, and innovative options like Kai algae, are digested more slowly, providing a gradual rise in blood sugar and offering several health benefits (Chao et al., 2024). Kai algae, with its rich nutritional profile, including protein and essential amino acids, can also contribute to stabilizing blood sugar levels. These foods help control blood sugar, reduce the risk of cardiovascular disease, and support weight management by promoting fullness and better digestion. Incorporating low-GI foods, including Kai algae, into the diet can improve overall health by stabilizing blood sugar and enhancing heart and digestive health.

3. Lipids for the Elderly

Lipids provide high-calorie content, essential nutrients, and improve the sensory qualities of food, enhancing both its nutritional value and appeal. Lipid molecules are built with fatty acids, which can have quite different effects on health depending on whether they are saturated or unsaturated. The effects of various fatty acids on health can vary substantially. The primary aim of dietary recommendations has been to reduce overall fat consumption; particularly saturated fats commonly found in animal products. Lower consumption of saturated fat can lower blood cholesterol and lower the risk of cardiovascular disease (Heggen et al., 2010). On the other hand, Omega-3 fatty acids, which are long-chain n-3 polyunsaturated fatty acids derived primarily from aquatic organisms, have been widely reported to provide various health benefits, such as regulating inflammatory responses and reducing the risk of cancer, and improving mental, cognitive, and visual health. Algae may be a good source of beneficial fatty acids offering a plant-based alternative for supporting better health

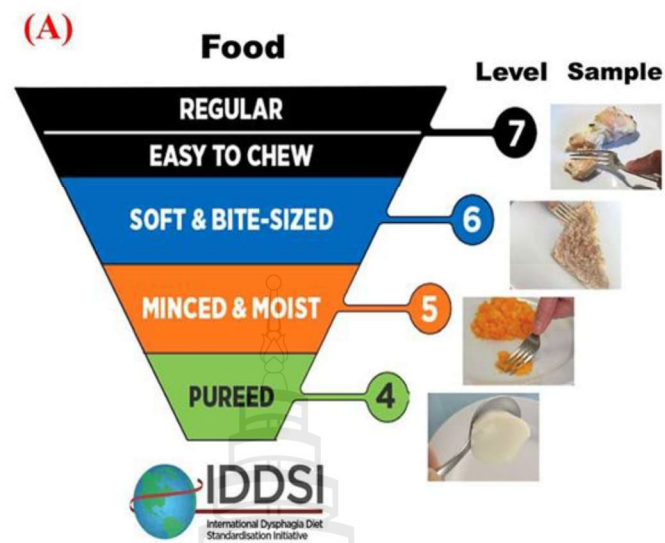
2.4.3 Guidelines for Ensuring Safe Food for Dysphagia Patients

Texture-modified solid foods are essentially divided into different categories according to their rheological and textural characteristics and are made using

standardized equipment, recipes, and guidelines (Ueland et al., 2020). This section takes readers on a thorough exploration, revealing the three essential frameworks, Universal Design Food (UDF), International Dysphagia Diet Standardization (IDDSI) and Japanese Standard Regulation, which classify the degree of dysphagia friendly food and make sure it is safe to swallow.

2.4.3.1 International Dysphagia Diet Standardization (IDDSI)

The IDDSI provides worldwide guidelines for texture-modified foods to ensure consistency and safety for individuals with swallowing difficulties (Figure 2.3). It includes eight levels (0–7). These levels serve as a framework for standardizing dysphagia-oriented food preparation, promoting swallowing safety and reducing the incidence of choking and aspiration. For levels 4 to 7 in the IDDSI framework, the fork pressure test is applied to evaluate the texture and consistency of food to ensure it is safe and manageable for individuals with swallowing difficulties. The fork pressure test ensures foods in levels 4 to 7 have the right consistency to be safely consumed. Foods in these levels should not require more pressure than 17 kPa, which simulates the tongue's force during swallowing (Sun et al., 2018). Level 4 (Pureed) foods are smooth, cohesive, and break apart easily under less than 17 kPa of fork pressure. These foods do not require chewing and are designed for safe swallowing without the risk of aspiration. Level 5 (Minced and Moist) foods are soft, moist, and may contain small lumps (2-4 mm) that require careful chewing. These foods remain moist without water separation and break apart under 17 kPa of pressure, making them suitable for individuals who can chew but need texture modifications. Level 6 (Soft and Bite-Sized) foods are soft, tender, and require moderate chewing. They can be broken with 17 kPa of fork pressure, making them safe for individuals who need soft, bite-sized pieces for easy swallowing. Finally, Level 7 (Regular) includes normal foods, such as firm, crunchy, or naturally soft, which require no modifications and are suitable for those with normal swallowing function.



Source Chao et al. (2024)

Figure 2.3 Standardized Classification of Texture-Modified Foods IDDSI

2.4.3.2 Universal Design Food (UDF)

The Universal Design Food (UDF) framework is a classification system developed to categorize food textures suitable for elderly individuals and patients with swallowing difficulties, particularly in aging societies such as Japan and South Korea (Figure 2.4). As the elderly population increases, nursing-care foods are being designed based on textural characteristics including hardness, adhesiveness, and cohesiveness, which are important factors affecting mastication and swallowing ability (Chao et al., 2022).

The UDF system classifies semi-solid foods into four stages primarily based on hardness values determined by texture profile analysis (TPA). Stage 1 (hardness $< 5 \times 10^5 \text{ N/m}^2$) includes softened foods that can be chewed using teeth. Stage 2 (hardness $< 5 \times 10^4 \text{ N/m}^2$) consists of foods that can be crushed using gums. Stage 3 (hardness $< 2 \times 10^4 \text{ N/m}^2$) represents soft foods that can be broken down using the tongue. Stage 4 (hardness $< 5 \times 10^3 \text{ N/m}^2$) refers to homogeneous and soft foods that can be swallowed without chewing and are intended for individuals with severe swallowing difficulty (Chao et al., 2023). Representative food examples and hardness classifications according to the UDF guidelines are illustrated in Figure 2.4.

 Universal Design Food (UDF) guidelines

Classification		Stage 1	Stage 2	Stage 3	Stage 4
Food characteristics		Easily chewed	Crushed by gums	Crushed by the tongue	Swallowed without chewing
		Harder and larger particle size	Larger particle size of	Soft solid with smaller particle size	Soft homogenous with invisible particle size
Food samples	Rice	Between regular rice and soft rice	Between soft rice and rice porridge	Rice porridge	Mashed rice porridge
					
	Egg	Thick omelet	Rolled omelet	Scrambled eggs	Soft steamed egg
					
	Mixed food	Meat and potato	Soft meat and potato with small ingredients	Softer meat and potato with smaller ingredient	Pasted meat and potato
					
Hardness (N/m ²)		5×10 ⁵	5×10 ⁴	2×10 ⁴	5×10 ³
Estimated swallowing power of the elderly		Swallowing food normally	Difficult to swallow solid food	Little difficult to swallow liquid foo	Little difficult to swallow liquid food

Source Chao et al. (2024)

Figure 2.4 Universal Design Food (UDF) guidelines

1. Japanese Standard Regulation for Foods for People with Swallowing Difficulty

Japan has established official standards for foods intended for individuals with swallowing difficulties to ensure safe consumption and reduce the risk of aspiration. These standards were established by the Ministry of Health, Labour and Welfare, Government of Japan in 2009 and later managed by the Consumer Affairs Agency, Government of Japan in 2010. The classification system evaluates dysphagia foods based on texture profile analysis (TPA) parameters, including hardness, adhesiveness, and cohesiveness, which are important factors affecting bolus formation, mastication, and swallowing safety.

The Japanese standard classifies foods into three categories according to the severity of swallowing difficulty. Standard Regulation I is intended for individuals with severe swallowing difficulty and require foods with relatively high structural stability. Standard Regulation II corresponds to foods suitable for individuals with moderate swallowing difficulty, while Standard Regulation III includes softer foods

intended for individuals with slight swallowing difficulty. The classification criteria for hardness, adhesiveness, and cohesiveness are presented in Table 2.3.

Table 2.3 Standard Criteria for Foods Authorized for People with Swallowing Difficulty Adapted from the Ministry of Health, Labour and Welfare, Government of Japan (2009) and Consumer Affairs Agency, Government of Japan (2010)

TPA Parameter	Standard Regulation I (Serious Swallowing Difficulty)	Standard Regulation II (Moderate Swallowing Difficulty)	Standard Regulation III (Slight Swallowing Difficulty)
Hardness (N/m ²)	2500-10000	1000 – 15000	300 – 20000
Adhesiveness (J/m ³)	< 400	< 1000	< 1500
Cohesiveness	0.2 – 0.6	0.2 – 0.9	Not specified

2.5 3D Printing Technology in Food Production

As an advanced food manufacturing technology, 3D food printing allows the precise customization of food structure and composition, enabling the creation of functional food products that address the growing preference for nutritious and health-promoting foods. Functional foods are developed to incorporate bioactive compounds, which can be obtained through advanced extraction techniques. Algae and food processing by-products are considered promising sources due to their high content of bioactive constituents. The use of 3D food printing technology enables the development and manufacture of foods with tailored functional properties, creating opportunities to enhance product value within the food processing sector. Furthermore, this technology supports the emergence of a specialized functional food printing industry by facilitating customized food production.

Table 2.4 provides an overview of various 3D printing technologies, including their underlying principles, material types, applications, associated machines, and the companies that supply them. By referencing this table, it is possible to formulate a desired functional food by considering all the relevant parameters presented (Donn et al., 2022)

Table 2.4 Characteristics of Major 3D Food Printing Technologies, Including Operating Mechanisms, Food Applications, Equipment, and Industrial Providers

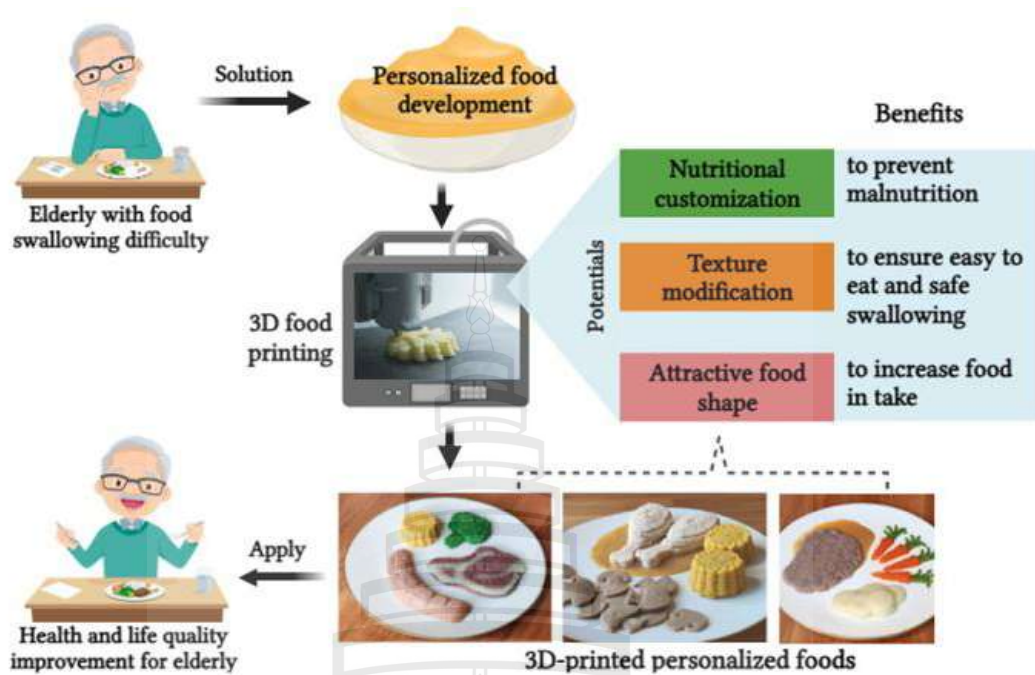
	Extrusion -based printing	Binder jetting	Inkjet printing
Operating Mechanism	Deposition of material through controlled extrusion	Selective binding of powder particles using liquid binders	Drop-on-demand deposition and Continuous jet printing
Printable Materials	Semi-solid and paste-like formulations	Powder substrates combined with liquid binders	Low-viscosity liquid formulations
Representative Food Applications	Functional chocolate enriched with flavonoids; confectionery products fortified with β -glucans and phenolic compounds	Functional chocolate products; omega-3-fortified pizza powders; model food structures	Functional chocolate; β -glucan-enriched dough systems; protein-fortified meat pastes and cheeses; reduced sugar fruit spreads; phenolic-rich gel products
Representative Equipment	Foodini, Choc Creator, AIBOULLY Chocolate Printer, Createbot Food Printer	Chefjet, Fujifilm Dimatix systems	Foodjet, Filament Sixhead 3D
Commercial Supplier	Choc Edge, AIBOULLY, Createbot	3D systems, Fujifilm Dimatix	De Grood Innovations, TNO

Source Donn et al. (2022)

2.5.1 3D Printing of Dysphagia Friendly Food

As people age, eating and swallowing can become difficult due to physical changes or conditions like stroke. For elderly individuals with dysphagia, food must be specially designed with safe, consistent textures. Semi-solid foods should avoid being fibrous, too hard, too viscous, or too watery. Texture-modified foods, like purees or puddings, are often recommended as they are soft, smooth, and easy to swallow, reducing the risk of choking and aspiration. These foods can help improve food intake and prevent malnutrition. However, their unappealing appearance can make them less appetizing for the elderly. 3D food printing, which allows for the customization of food inks, has been used to create foods with balanced nutrients and specific textures to meet individual health needs. Recently, there has been growing interest in using 3D-printed foods with modified textures, especially for elderly people with difficulty swallowing. Outline can be seen in Figure 2.5. The goal is to produce foods that are nutritious, safe, and visually appealing, improving the overall mealtime experience for this group (Chao et al., 2024).

The preparation of food ink is a crucial step in developing 3D-printed foods with modified textures. During this process, raw food materials often undergo physical treatments like size reduction and homogenization to a uniform ink (Ueland et al., 2020). Additionally, ingredients such as hydrocolloids (e.g., polysaccharides), starches, or binding agents are added to get the safe and consistent texture. Linking these macronutrients and hydrocolloids can significantly affect the texture and appearance of the product. To achieve successful printing, food ink must have viscoelastic properties, behaving like a liquid during extrusion but solidifying after deposition to maintain its shape and stability (Baugreet et al., 2016). Therefore, adding hydrocolloids modifies the texture and ensures stable structure during the 3D food printing process. Hydrocolloids are a group of polysaccharides or polymers that have the ability to absorb water and form gels, viscous solutions, or suspensions. They are widely used in the food industry for their thickening, gelling, and stabilizing properties. Examples include xanthan gum, agar, gelatin, pectin, carrageenan, and alginate, which help improve texture, consistency, and shelf life in various food products. These are frequently combined with food ingredients to create 3D-printed texture-modified foods that are given to elderly people who have dysphagia (Chao et al., 2024).



Source Chao et al. (2024)

Figure 2.5 Outline of Texture modified Dysphagia Friendly Food

2.5.2 The Role of Functional Ingredients in 3D Printing and Their Impact on Print Quality

2.5.2.1 Carbohydrates

1. Sweeteners

Sweeteners such as stevia, erythritol, maltitol, and xylitol are commonly used to balance flavor, texture, and nutritional needs. Stevia is calorie-free and provides intense sweetness without significantly altering the texture of printed foods. Erythritol is low-calorie, highly soluble, and maintains texture without affecting blood sugar levels. Maltitol, a sugar alcohol, mimics sugar's sweetness and helps retain moisture, while xylitol is also low in calories and offers oral health benefits, reducing the risk of cavities. The substitution of sucrose with maltitol and xylitol in chocolate formulation can significantly reduce the risk of obesity, as these sugar substitutes offer a lower energy density while maintaining sweetness and texture. By incorporating complex polysaccharides from Ganoderma, wolfberry, and liriop, a healthier sugar-free chocolate was developed with enhanced crystallization and solidification properties,

improving stability and structure during 3D printing (Li et al., 2014; Xiao et al., 2019; Li et al., 2019). This approach provides a promising solution for creating healthier chocolate options in 3D food printing applications.

2. Gums

Gums, such as xanthan gum (XG), locust bean gum (LBG), kappa-carrageenan (KG), and konjac gum (KGM), are commonly used in 3D food printing for their ability to modify the texture, viscosity, and mechanical properties of food inks, improving printability and food quality. Optimal ink formulations exhibit superior 3D printing capability, no water seepage, and dense microstructures with a minimal quantity of HCs. Increased starch content and decreased water percentage in vegetables result in reduced HC requirements in the ink composition (Pant et al., 2021).

In 3D food printing, the incorporation of XG has been shown to enhance the mechanical strength and rheological properties of printed products, especially when exposed to high temperatures during post-processing. Its ability to regulate ink viscosity and prevent particle expansion makes it effective in improving the uniformity of prints made with raw materials like carrot, spinach, and broccoli powders (Kim et al., 2018).

A study on *Hypsizygus marmoreus* with varying amounts of XG, locust bean gum, and kappa-carrageenan found that XG reduced viscosity and improved swallowing ease, while kappa-carrageenan increased hardness and chewing resistance. The samples with 0.3 % XG and 0.9 % LBG showed optimal 3D printability and were classified as a level 5 dysphagia diet, except for those with 0.9 % kappa-carrageenan. These findings suggest that 3D-printed foods with 0.3 % XG could be a suitable option for dysphagia diets, providing good texture and ease of swallowing (Liu et al., 2023).

In a study involving shiitake mushrooms, the combination of XG and KG enhanced mechanical strength of the inks (Liu et al., 2021). Additionally, a study using fish paste with different ratios of KGM and XG demonstrated significant texture improvement, with the optimal ratio being 1:1, suggesting potential for enhanced texture in 3D-printed seafood products (Yu et al., 2023).

3. Pectin

Pectin is a natural hydrocolloid from plants. It is widely used in 3D printing applications that require agents to form gel structure. It maintains its gelling

ability within the gastrointestinal tract, offering various health benefits such as managing diabetes, improving lipid and cholesterol metabolism, and alleviating conditions like dumping syndrome (Lawaetz et al., 1983). Moreover, pectin has been identified as having potential therapeutic properties for conditions such as obesity, cancer, atherosclerosis, and intestinal infections (Lattimer & Haub, 2010). In 3D food printing, studies by (Vancauwenberghe et al., 2018) have explored the use of low methoxylated pectin gels as inks, successfully demonstrating the encapsulation of air bubbles and plant cells. The incorporation of calcium ions (Ca^{2+}) helps form pectin gels by cross-linking free carboxyl groups, improving the printability and structure of the ink (Fraeye et al., 2010).

4. Apricot Polysaccharide

When apricot polysaccharide (0.5 %, m/v) and modified polysaccharide (0.1 %, m/v) were incorporated into soy protein isolate (SPI), the resulting gel exhibited the highest hypolipidemic activity, achieving binding rates of 75.33 % for sodium taurocholate and 72.86 % for sodium glycocholate. In addition, this gel demonstrated promising characteristics for 3D printing applications (Xu et al., 2023).

5. Dietary Fiber

Dietary fibers, primarily obtained from vegetables, fruits, and whole grains, are vital for human nutrition, supporting digestive health and reducing the risk of chronic diseases. Referred to as the "seventh nutrient," these fibers play a critical role in promoting gastrointestinal health by enhancing peristalsis, aiding in the movement of food through the digestive system, regulating nutrient absorption, and helping prevent or relieve constipation (Ströhle et al., 2018). The U.S. Food and Drug Administration recognize the health benefits of dietary fiber, stating that a diet high in vegetables, fruits, and whole grains, and low in cholesterol and saturated fats, can reduce the risk of coronary heart disease. Consequently, the integration of dietary fibers into 3D printing processes is not only essential for product innovation but also offers substantial health benefits.

Cellulose, the most abundant organic polymer on Earth and a key structural component of plant cell walls, is one of the most important insoluble fibers in diet. As humans lack the necessary enzymes to digest cellulose, it passes through the

digestive system largely undigested (Wüstenberk, 2014). Holland et al. (2018) successfully employed finely milled cellulose fibers in 3D printing applications, using glucomannan and XG.

Kim et al. (2018) investigated the effects of incorporating vegetable powders (carrot, spinach, and broccoli) into 3D printing formulations, using concentrations ranging from 10 % to 30 % (w/w) of vegetable powders combined with various hydrocolloids. Their findings revealed that increasing the concentration of vegetable powders significantly influenced the rheological properties and printability of the formulations. Moreover, the addition of XG helped to standardize printability across the different vegetable sources. Similarly, (Lee et al., 2019) examined the printing performance of spinach powders with varying particle sizes. They found that a particle size of 307 μm improved the mechanical strength of the printed objects while preserving their shape, structure, and surface smoothness.

Severini et al. (2018) explored the use of fruits and vegetables, such as pears, carrots, broccoli leaves, kiwi, and avocado, in the production of 3D printed smoothies. The resulting pyramid-shaped smoothies were visually more appealing than traditional smoothies, and the phenolic content, antioxidant capacity, and sensory characteristics (color, taste, and odor) remained largely unaffected by the 3D printing process. Huang et al. (2019) combined brown rice with guar and xanthan gums in a 3D printing offers superior nutritional value compared to white rice, with higher levels of protein, fat, vitamins, minerals, and dietary fiber.

2.5.2.2 Functional Protein

1. Pea Protein

Pea protein contains a high concentration of essential amino acids, with the exception of methionine (Palander et al., 2006). Chuanxing et al. (2018) investigated the influence of pea protein on the printability of potato starch-based 3D printing ink. The study demonstrated that the textural, thermal, and structural properties (including granular, crystalline, and chemical structure) of starch-based ink significantly improved with an increase in pea protein content. The incorporation of pea protein at a concentration of 1 % was found to optimize the quality of the 3D-printed food matrix. This level of supplementation enhanced printability and structural stability, improved textural characteristics, and contributed to the nutritional value of

the final product. Additionally, pea protein aids in gel formation and contributes to desirable textural properties in food product (Lam et al., 2018). Consequently, it serves as a substitute for fat in low-calorie food products and aids in reducing dietary fat consumption. These factors decrease the prevalence of being obese, atherosclerosis, and malignant tumors.

2. Algae

Nostoc sphaeroides is an algal food that holds significant value in traditional medicine. *Nostoc sphaeroides* has been historically proposed as a remedy for various medical conditions. Research has shown that *Nostoc sphaeroides* possesses several health benefits, including anti-inflammatory and antiviral properties, reduce cancer, and lower cholesterol levels (Park et al., 2008). *Nostoc* algae has gelling properties which give unique rheological properties. An et al. (2019) investigated formulations containing fresh *nostoc*, powdered *nostoc*, and a combination of powdered *nostoc* and potato starch. Fresh *nostoc* demonstrated the capability to form a gel suitable for printing; however, the resulting product was neither even nor stable. The rehydrated one was determined to be more suitable for 3D printing compared to using fresh *nostoc*. Adding starch, along with extended heating and rehydration, significantly enhanced the 3D fabrication of *N. sphaeroides* powder.

2.5.3 The Role of Internal Structures and Their Impact on the Texture Properties of 3D Printed Food

Customized food can meet the specific nutritional needs of various consumers, such as pregnancy, infants, elderly, and athletes. The product is adaptable for various eating environments, including demanding conditions such as military battlefields, physical exertion, low oxygen and fatigue at high altitudes, microgravity during space travel, confined spaces, and low-pressure environments in aircraft. Under these conditions, the enjoyment associated with eating may decrease, and there is a potential for risks such as choking, coughing, or aspiration. Texture design is crucial for the customization of 3D-printed food, in addition to its nutritional value. Modifying structural elements such as infill pattern and density to alter the texture of 3D-printed food constitutes an innovative approach in the field of 3D food printing. (Zhao et al., 2021).

2.5.3.1 Infill Structure

The existing research is limited regarding the relationship between the quality of internal structures and the textural and overall quality of 3D-printed foods. The infill structure plays a crucial role in determining the mechanical strength and stability of these products, highlighting the significance of structural design in achieving both functional and sensory properties of food (Zhao et al., 2021). The commonly used infill structures include rectilinear, honeycomb and hilbert structure. This can be seen in Figure 2.6.

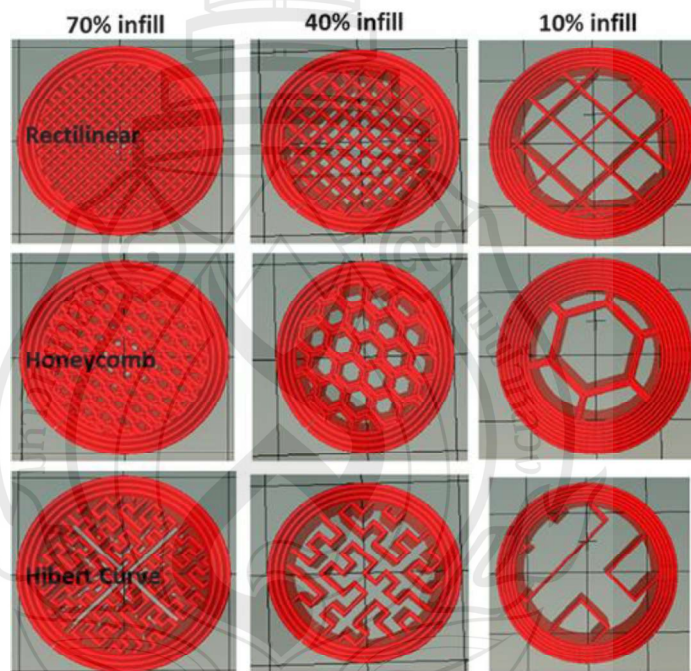
Mantihal et al. (2017) utilized dark chocolate beans to produce three hexagonal shapes: cross bracing, parallel bracing, and without bracing, subsequently measuring the force required to fracture them. The findings indicated that hexagonal structures generated through cross bracing exhibited greater stability, necessitating a higher breaking force compared to those formed by parallel bracing. Mantihal et al. (2019b, 2019a) utilized two types of chocolates, in conjunction with plant sterol powders and add magnesium stearate as food additives, creating infill structures: “honeycomb,” “Hilbert,” and “star.” The “honeycomb” and “star” designs showed greatly enhanced mechanical properties due to their vertical and horizontal structural integration. Selecting an appropriate infill structure prior to printing is essential for achieving a more stable 3D printed object. Moreover, infill structure has significant effect on the protection of probiotics within the print material (Zhang et al., 2018).

2.5.3.2 Infill Density

When analyzing the impact of internal structure on texture changes in 3D printed food, it is crucial to emphasize the importance of infill density as a key factor. Huang et al. (2019) found that the texture properties, specifically gumminess and hardness, of 3D printed brown rice products were directly correlated with infill density, with values increasing as infill density rose from 15 % to 75 %. To enhance the stability and mechanical strength of structures, it is essential to appropriately deposit mass and manage the void within the internal structure of 3D printed food. Differences in samples with different infill density can be seen in Figure 2.7.

Yang et al. (2019) found that at an infill density of 100 % for 3D printed lemon juice gel, the product exhibited bulging as it expands. Filling levels of 60 % to 80 % resulted in a higher pore density, whereas 90 % filling yielded the best product.

Liu et al. (2018) demonstrated that increasing the infill density from 10 % to 70 % resulted in higher values of Young's modulus and firmness in mashed potato printing. Mantihal et al. (2017, 2019b) demonstrated that 3D printing of chocolates gives softer texture than that of traditional ones, including the one with a 100 % filling. Mass and density of chocolate can be regulated by adjusting the infill density with 3D printing technology. In sensory analysis, for appearance, participants exhibited a significant preference on chocolate with 25 % and 50 % infill density compared to the 100 % infill density. Additionally, samples with 25 % infill density were favored for their hardness. 3D printed chocolate can result in the desired texture through variations in internal structure, while also enhancing appearance and overall preferences.



Source Liu et al. (2018)

Figure 2.6 Infill Structures and Percentages

2.6 Rheological Properties of Food Systems

Rheology is the study of the flow and deformation of matter under applied forces, providing a fundamental engineering framework for evaluating the behavior of food materials during unit operations such as pipeline transport, mixing, and material

extrusion (Rao, 2014). For semi-solid matrices and fluid food inks, viscosity and viscoelasticity represent the primary rheological dimensions governing structural stability, phase retention, and extrusion performance (Steffe, 1996).

2.6.1 Viscosity

Viscosity is a measure of a fluid's internal resistance to flow. A fluid with higher viscosity flows slower and appears thicker than a fluid with lower viscosity. Mathematically, viscosity is defined as the ratio of shear stress (τ) to shear rate ($\dot{\gamma}$):

$$\eta = \tau / \dot{\gamma}$$

where η is the viscosity (Pa·s), τ is the shear stress (Pa), and $\dot{\gamma}$ is the shear rate (s^{-1}).

Food materials can be classified as Newtonian or non-Newtonian fluids according to their flow behavior. Newtonian fluids exhibit constant viscosity regardless of the applied shear rate, whereas non-Newtonian fluids display viscosity changes when shear conditions vary (Steffe, 1996). Most food dispersions, gels, hydrocolloid solutions, and food printing inks exhibit non-Newtonian behavior due to the presence of macromolecules, suspended particles, and complex structural interactions.

Among non-Newtonian behaviors, shear-thinning (pseudoplastic) behavior is the most common in food systems. In shear-thinning materials, viscosity decreases as shear rate increases. This phenomenon occurs because polymer chains become aligned in the direction of flow and intermolecular interactions are partially disrupted under shear stress (Steffe, 1996). Shear-thinning behavior is advantageous for extrusion processes because it facilitates material flow through narrow nozzles while allowing viscosity recovery after deposition.

2.6.2 Power Law Model

The flow characteristics of many non-Newtonian food materials can be described using the Power Law (Ostwald–de Waele) model (Holdsworth, 1971):

$$\tau = K\dot{\gamma}^n$$

where K is the consistency index (Pa·sⁿ) and n is the flow behavior index (dimensionless).

The apparent viscosity can also be expressed as:

$$\eta = K\dot{\gamma}^{n-1}$$

The consistency index reflects the overall resistance of a material to flow. Higher K values generally indicate thicker and more viscous systems, whereas lower values indicate more fluid-like behavior. The flow behavior index describes the degree of deviation from Newtonian flow. A value of $n = 1$ represents Newtonian behavior, $n < 1$ indicates shear-thinning behavior, and $n > 1$ indicates shear-thickening behavior (Rao, 2014).

In extrusion-based food printing, materials with relatively high consistency and pronounced shear-thinning characteristics are desirable because they can flow under applied pressure while retaining their shape after extrusion (Liu et al., 2020).

2.6.3 Yield Stress

Yield stress is defined as the minimum stress required to initiate flow in a material. Below the yield stress, the material behaves as an elastic solid, whereas above this threshold it begins to flow as a viscous fluid (Rao, 2014). Yield stress is particularly important in semi-solid foods and printing inks because it contributes to shape retention and resistance to structural collapse after extrusion. Materials possessing appropriate yield stress can support successive deposited layers and reduce spreading during printing. Consequently, yield stress is commonly used as an indicator of printability and structural stability in extrusion-based food printing applications (Outrequin et al., 2023).

2.6.4 Viscoelastic Properties

Many food materials exhibit both elastic and viscous characteristics and are therefore classified as viscoelastic materials. Small-amplitude oscillatory shear (SAOS) measurements are commonly used to evaluate viscoelastic behavior through the storage modulus (G') and loss modulus (G'').

The storage modulus (G') represents the elastic component and indicates the amount of energy stored and recovered during deformation. In contrast, the loss modulus (G'') represents the viscous component and reflects the amount of energy dissipated as heat (Steffe, 1996).

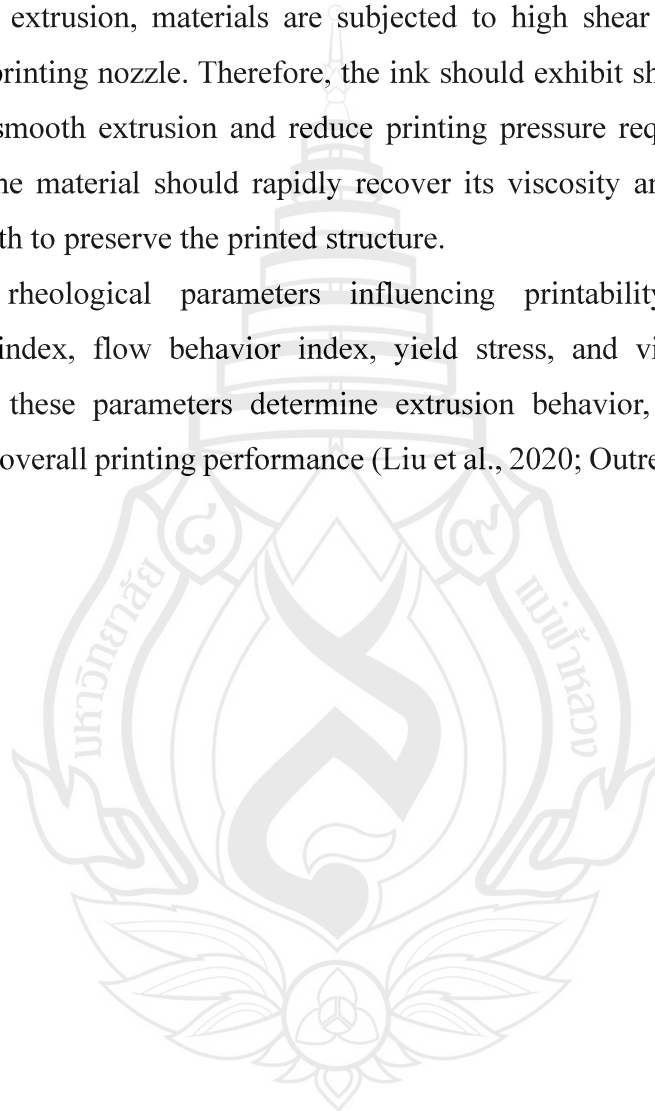
When G' exceeds G'' , the material exhibits predominantly solid-like behavior and possesses a stronger internal network structure. Conversely, when G'' is greater than G' , the material behaves more like a viscous liquid. A dominant G' is generally

associated with improved shape retention and structural stability in 3D-printed food products (Outrequin et al., 2023).

2.6.5 Importance of Rheology in 3D Food Printing

Rheological properties play a crucial role in determining the printability of food inks. During extrusion, materials are subjected to high shear forces while passing through the printing nozzle. Therefore, the ink should exhibit shear-thinning behavior to facilitate smooth extrusion and reduce printing pressure requirements. Following deposition, the material should rapidly recover its viscosity and maintain sufficient elastic strength to preserve the printed structure.

Key rheological parameters influencing printability include viscosity, consistency index, flow behavior index, yield stress, and viscoelastic properties. Collectively, these parameters determine extrusion behavior, shape fidelity, layer stability, and overall printing performance (Liu et al., 2020; Outrequin et al., 2023; Seol et al., 2025).



CHAPTER 3

MATERIALS AND METHODS

3.1 Materials and Equipment

3.1.1 Raw Material

Kai algae sample was collected from the local kai algae collectors near Nan River at Paka Subdistrict, Tha Wang Pha District, Nan Province, Thailand, in March 2025. A photograph of Kai algae under Nan River is shown in Figure 3.1.



Figure 3.1 Kai Algae from the River in Nan Province

3.1.2 Chemicals and Equipment

The chemicals used for each analysis are listed in Table 3.1, and the main instruments are summarized in Table 3.2.

Table 3.1 List of Chemicals

Parameters	Chemicals
Protein	Boric acid (H_3BO_3), Bromocresol green, Ethanol (95 %), Hydrochloric acid (HCl), Methyl red, Sodium hydroxide, Sulphuric acid, Kjeldahl digestion catalyst: Copper sulfate: Potassium sulfate 1:10, Tris (hydroxymethyl) aminomethane (THAM)
Fat content	Petroleum ether
Crude Fiber	n-Octanol, Sodium hydroxide, Sulfuric acid
Amino acid	Hydrochloric Acid (HCl), Sodium hydroxide (NaOH)
<i>In Vitro</i> Digestion	Potassium chloride (KCl), potassium phosphate monobasic (KH_2PO_4), sodium bicarbonate (NaHCO_3), sodium chloride (NaCl), magnesium chloride hexahydrate ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$), ammonium carbonate ($(\text{NH}_4)_2\text{CO}_3$), hydrochloric acid (HCl), sodium hydroxide (NaOH), calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$), α -amylase (salivary amylase), pepsin (porcine gastric pepsin), pancreatin (porcine pancreas extract), bile salts, hydrochloric acid (HCl), sodium hydroxide (NaOH)
SDS-PAGE	FastCast Acrylamide Kit, ammonium persulfate (APS), 10 $\times$ Tris/Glycine/SDS buffer, sodium dodecyl sulfate (SDS), Tris base, glycine, β -mercaptoethanol, glycerol, bromophenol blue, protein molecular weight standard, Coomassie Brilliant Blue R-250, methanol, glacial acetic acid
Minerals	Nitric acid (HNO_3), hydrogen peroxide (H_2O_2)
Chlorophyll Content	Acetone
TPC	Gallic acid, Sodium carbonate (Na_2CO_3), Folin-Ciocalteu reagent
DPPH	2,2-diphenyl-1-picrylhydrazyl (DPPH), Methanol, trolox
TFC	Ethanol, NaNO_2 , AlCl_3 , NaOH, Quercetin (standard)

Table 3.1 (continued)

Parameters	Chemicals
FRAP	Acetate buffer, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, Ferrous sulfate, HCl, TPTZ (2,4,6-tripyridyl-S-triazine)
ABTS	ABTS (2,2-azino-bis-3-ethyl-benzothiazoline-6-sulfonic acid), Ethanol, potassium persulfate, Trolox
Anti-inflammatory	Dimethyl sulfoxide (DMSO), RAW 264.7 cells, high-glucose Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), phosphate-buffered saline (PBS), lipopolysaccharide (LPS) from <i>E. coli</i> , dexamethasone (DEX), Cell Counting Kit-8 (CCK-8) reagent, Griess reagent Solution A and Solution B, sodium nitrite (NaNO_2), RIPA lysis buffer, protease inhibitors, phosphatase inhibitors, a BCA protein assay kit, acrylamide/bis-acrylamide solution, tris base, glycine, sodium dodecyl sulfate (SDS), ammonium persulfate (APS), TEMED, methanol, polyvinylidene difluoride (PVDF) membranes, a pre-stained protein ladder, non-fat dry milk, tween-20, anti-iNOS primary antibody, anti-COX-2 primary antibody, anti-NF- κ B p65 primary antibody, anti-p-NF- κ B p65 primary antibody, anti-Nrf2 primary antibody, anti-HO-1 primary antibody, anti- β -actin primary antibody, HRP-conjugated secondary antibodies, and enhanced chemiluminescence (ECL) detection reagent

Table 3.2 List of Equipment

Equipment	Model
Digital balance 4 digits	ML 204/01, Mettler Toledo, Switzerland
Blender	HR 2011, Philips electronics, Suhou, China
Hot air oven	UNB 500, Bec Thai Bangkok Equipment and Chemical.
Muffle Furnace	Carbolite/CWF 1300, England
Vortex	G560E
Centrifuge	ThermoFisher scientific, D-37520, Osterode, Germany
Refrigerator	Mitsubishi Tiara, Bangkok, Thailand
Crude Fiber Analyzer	Fibertec analyzer, Foss tecator, Denmark
Protein digestion unit	Foss/2006 digesto, Foss tecator, Sweden
Soxhlet extraction	Foss/Soxtec TM -2055, Foss Analytical, Slangerupgade, Denmark
Colorimeter	Hunter Lab/Color Quest XE, Hunter Associates Laboratory, Virginia, USA
A dew point water activity meter	Aqualab, Decagon, USA
Homogenizer	RW 20 digital, IKA, Bec Thai Bangkok, Thailand
Water bath	Memmert/ WB 14, Schwabach, Germany
Sonicator	M2800, Branson Ultrasonics, Mexico
pH meter	Mettler-Toledo, LE438, Greifensee, Switzerland
Microplate	Hunter Lab/ Color Quest XE, Laboratory, Virginia, USA
Spectrophotometer	USA
Freeze dryer	Alpha 3-4 LSC basic, Osterode am Harz, An der Unteren Söse 50, Germany
Tray dryer	KluayNamThaiTowop, Thailand
Vacuum packaging machine	S220L BV/ STAR, Kerzers, Switzerland
Scanning electron microscopy	Tescan, MIRA4, Czech

Table 3.2 (continued)

Equipment	Model
Texture analyzer	TEX-100N; Japan Instrumentation System Co., Ltd., Nara, Japan
Electrophoresis equipment	Mini Protean tetra cell units (Bio-Rad Laboratories, Inc., Richmond, CA, USA
Rheometer	a HAAKE MARS III rheometer, Thermo Scientific, Germany
3D Printer	Foodini, Natural Machines, Spain

3.2 Methods

The study was divided into two principal phases. Phase I examined the influence of different drying processes on the properties of Kai algae. Phase II focused on the development of dysphagia-friendly 3D-printed products derived from the algal material.

3.2.1 Phase I: Impact of Drying Methods on Properties of Kai Algae

3.2.1.1 Overall Methodology

In order to determine how drying techniques affect the physicochemical and nutritional properties of Kai algae, sun drying, tray drying and freeze drying were applied. After every treatment, the samples were stored at -18 °C. These samples were further analyzed to determine how these treatments affect nutritional properties, bioactive properties, antioxidant properties and also physicochemical properties. The general framework can be seen in Figures 3.2 and 3.3.

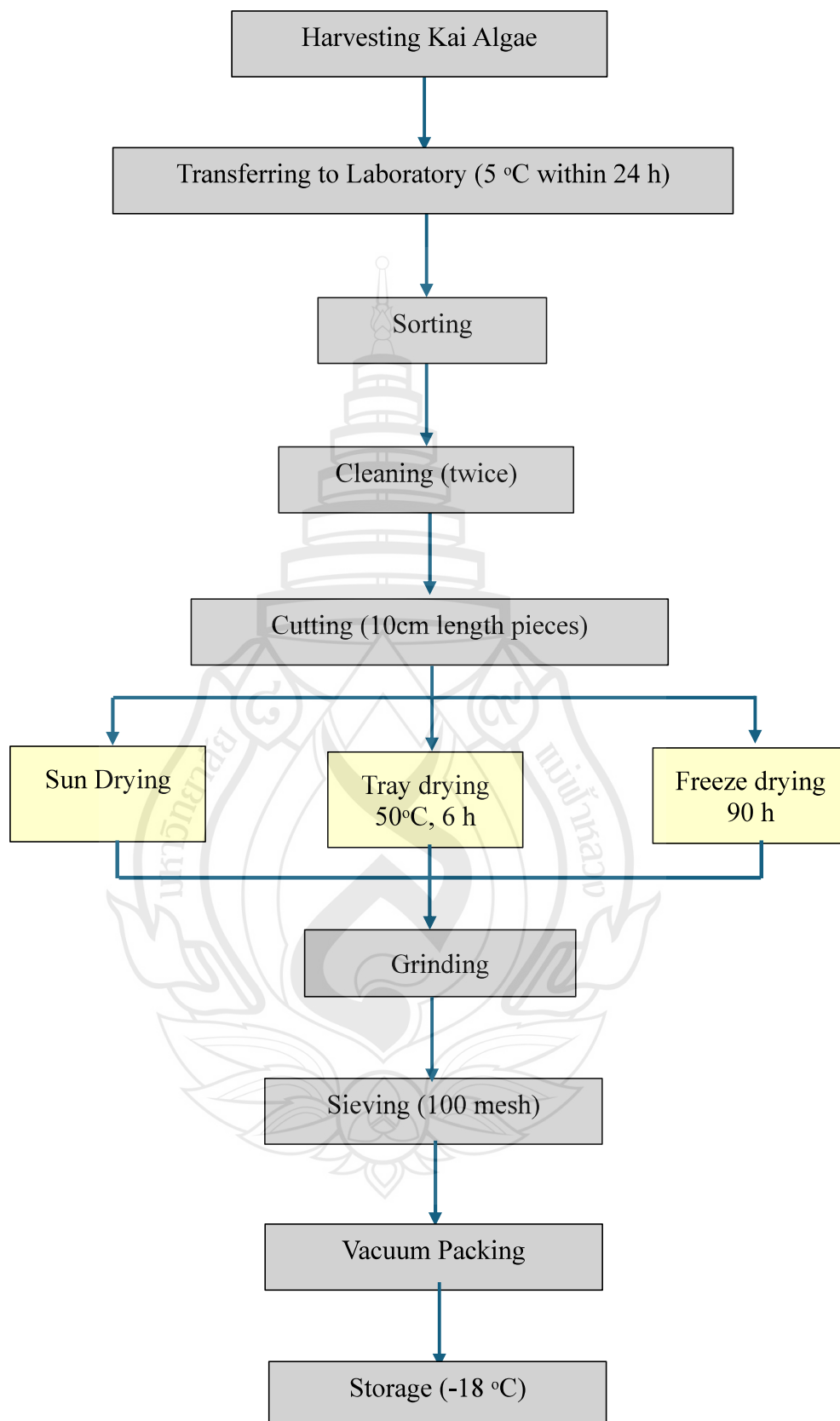


Figure 3.2 General Framework for Different Drying Process

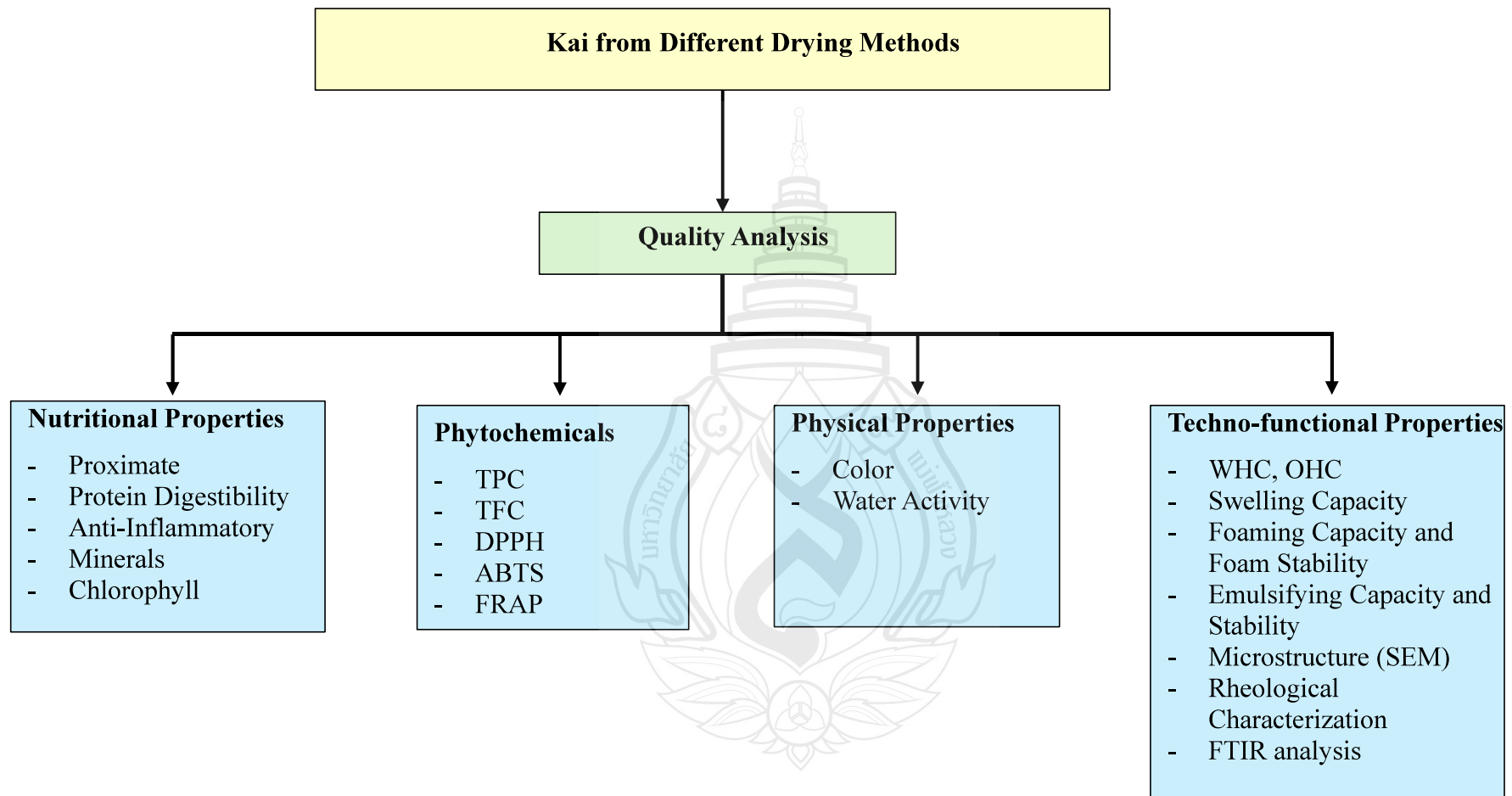


Figure 3.3 Study of Effect of Processing on Nutritional, Physical and Techo-Functional Properties of Kai from Different Drying Methods

3.2.1.2 Sample Preparation and Treatments

1. Sample Preparation

Fresh Kai algae were collected from the river and transported to the laboratory within 24 h under refrigerated conditions (5 °C). Upon arrival, the samples were sorted manually to remove impurities. After washing twice using tap water for removing adhering debris and foreign materials, the cleaned algae were subsequently cut into approximately 10 cm pieces prior to drying treatment.



Figure 3.4 Fresh Kai Algae Sample

2. Drying Techniques

After cleaning and size standardization, the Kai samples were dried by different methods: sun drying, tray drying, and freeze drying. Following dehydration, all dried samples were milled into powder and sieved through a 100-mesh size to get uniform particle size. It was then vacuum packaged and stored at -18°C until further analysis. All experimental measurements were performed in triplicate using independently prepared samples.

1) Sun Drying

For sun drying, Kai algae were spread evenly in a single layer on mesh trays and exposed to natural sunlight. It was dried until there was no change in weight to ensure complete moisture removal (Figure 3.5).



Figure 3.5 Sun Drying Under Natural Sunlight

2) Tray drying

For tray drying, Kai was evenly distributed on stainless-steel trays (1 cm height) and dried at 50 °C for 6 h using a tray dryer (Figure 3.6). Moisture content was measured and controlled to be less than 15 %. Moisture levels below 15 % have been accepted for improving the stability of dried seaweed by limiting microbial growth and extending shelf life (Setiowati et al., 2026).



Figure 3.6 Tray drying at 50 °C

3) Freeze drying

For freeze drying, clean and uniform-sized Kai algae was packed and frozen at -18 °C and lyophilized under 0.1 mbar pressure for 90 h for further analysis. The temperature of the cold condenser was maintained at approximately -70 °C. (Figure 3.7).



Figure 3.7 Freeze drying

3. Sample Extraction

A 1 g portion of Kai powder was combined with 10 mL of 80 % (v/v) methanol. The mixture was then subjected to sonication for 30 min at ambient temperature, followed by centrifugation at 14,000 rpm for 15 min at 4°C. The residual pellet was reextracted once under the same conditions. The supernatants from both extraction cycles were combined, filtered through Whatman No. 1 filter paper, and the volume was adjusted to 40 mL with 80 % methanol (Konsue et al., 2023). This extract was used for TPC, TFC, DPPH, ABTS and FRAP assays.

3.2.1.3 Determination of Nutritional Properties

1. Proximate Analysis

1) Moisture Content

Moisture content was determined by oven drying. A 3 g sample of Kai algae was placed in a dish that had been previously dried and weighed. The dish was heated at 105 °C for 24 h, after which no further change in mass was observed. The sample was then cooled inside a desiccator before reweighing. Moisture percentage was calculated from the difference in mass before and after drying (AOAC, 2000).

$$\text{Moisture (\%)} = \frac{\text{Initial weight before drying} - \text{Final weight after drying}}{\text{Initial weight before drying}} \times 100$$

2) Protein Content

The crude protein content of Kai algae was determined according to the standard Kjeldahl method (AOAC 954.01). Approximately 1.0 g of Kai algae was mixed with 5.0 g of catalyst mixture ($\text{Na}_2\text{SO}_4:\text{CuSO}_4$, 10:1 ratio) and 5 mL concentrated sulfuric acid. It was digested at 350°C for 2 h until complete clarification is achieved. After dilution and alkalization with 40 % NaOH, ammonia was distilled into 4 % boric acid and titrated with 0.1 M HCl. The nitrogen content was determined from the titration results and converted to protein by conversion factor 6.25.

$$N = \frac{V(\text{HCl}) \times N(\text{HCl}) \times 14}{m \times 1000} \times 100$$

$$\text{Protein} = F \times N$$

$$\text{Protein (\%)} = 6.25 \times N (\%)$$

Where, V(HCl): volume of HCl used for titration

N(HCl): normality of HCl (0.1N)

m: sample weight on dry matter basis

N: Nitrogen (%) F: conversion factor (6.25)

3) Crude Fiber

Crude fiber content was analyzed in accordance with AOAC method 960.39. Approximately 1 g of dried Kai algae (recorded as W_1) was placed in a pre-dried porcelain crucible. The crucible was placed in a Fibertec™ cold extraction unit and subjected to three solvent extractions with 25 mL of analytical grade acetone (10 min each), followed by vacuum filtration and a final rinse with distilled water. For the digestion phase, the crucible was transferred to a Fibertec hot extraction unit. The sample was treated sequentially with 1.25 % (v/v) sulfuric acid and 1.25 % (w/v) sodium hydroxide; 2–3 drops of n-octanol were added as an anti-foaming agent. After digestion, three additional acetone extractions were performed to remove residual lipids. The solvent was completely evaporated, and the crucible was oven-dried overnight at $105 \pm 2^\circ\text{C}$, then cooled in a desiccator containing silica gel and reweighed (W_2). Subsequently, the crucible was incinerated in a muffle furnace at $525 \pm 5^\circ\text{C}$ for 4 h to determine the ash content, cooled again in a desiccator, and the final weight (W_3) was recorded. Crude fibre content was calculated as

$$\% \text{ Crude fiber} = [(W_2 - W_3)/W_1] \times 100$$

4) Ash

Ash content was determined in accordance with AOAC method 923.03. Prior to the commencement of sample analysis, all crucibles underwent overnight heating in a muffle furnace set at 550 °C to remove any residual surface contaminants. After the crucibles had cooled inside a desiccator for 30 min, they were weighed with a precision of three decimal places. Approximately 3 g of each sample was then transferred into the prepared crucibles. The loaded crucibles were carefully heated on a hot plate until the sample was completely charred and visible smoke emission stopped. Subsequently, the samples were placed into the muffle furnace and incinerated at 550 °C for a period of 12 h. Following combustion, complete ashing was confirmed by visual inspection: a color change from black to light grey indicated that the process was finished. After the crucibles had cooled to room temperature in a desiccator, the ash-containing crucibles were reweighed using the same level of precision. The ash content, expressed as a percentage, was calculated according to the equation below:

$$\% \text{ Ash} = (\text{Weight of ash} / \text{Weight of sample}) \times 100$$

5) Fat

The crude fat content of Kai was determined using petroleum ether-based extraction method (AOAC 960.39). Empty extraction bottles were dried overnight at 105 °C until constant weight. Kai powder (2 g) was weighed, sealed within a lipid-free filter paper wrapper, and transferred into the Soxhlet extraction thimble. The boiling flask received 75 mL of petroleum ether (40-60 °C boiling fraction), and the condenser was attached to a recirculating cooling water system. The heating rate was regulated to sustain a solvent distillation speed of 150 droplets per min. After the extraction step was completed, the organic solvent was completely evaporated from the flask. The flask was subsequently dried at 105 °C, placed in a desiccator until it reached room temperature, and then reweighed. The crude fat percentage was derived using the following equation:

$$\% \text{ Fat} = (\text{Weight of fat extracted} / \text{Weight of sample}) \times 100$$

6) Carbohydrate

Total carbohydrate content was determined by difference using the equation:

$$\% \text{ Carbohydrate} = 100\% - (\% \text{ Moisture} + \% \text{ Ash} + \% \text{ Protein} + \% \text{ Fat} + \% \text{ Crude Fiber})$$

2. Protein Digestibility

1) *In vitro* Digestion

Simulated salivary fluid (SSF), simulated gastric fluid (SGF), and simulated intestinal fluid (SIF) were prepared according to the electrolyte compositions specified in the standardized static INFOGEST protocol (Brodkorb et al., 2019). The composition of each simulated fluid is presented in Table 3.3.

A 1 g portion of dried Kai algae powder was placed directly into a 50 mL centrifuge tube and combined with 4 mL of distilled water to form a bolus. To this mixture, 3.5 mL of SSF, 0.9 mL of α -amylase solution (845 U/mL), and 25 μ L of 0.3 M CaCl_2 were added, resulting in a final α -amylase activity of 75 U/mL and a final Ca^{2+} concentration of 1.5 mM. Distilled water was added to bring the total volume to 10 mL, and the pH was adjusted to 7.0 if necessary. The tube was vortexed for 10 s and incubated at 37 °C for 2 min under continuous shaking in a water bath.

Following the oral phase, 7.5 mL of SGF, 1.8 mL of pepsin solution (22,462 U/mL), and 5 μ L of 0.3 M CaCl_2 were added to the same tube, yielding a final pepsin activity of 2000 U/mL and a final Ca^{2+} concentration of 0.15 mM. The pH was lowered to 3.0 using 1 M HCl. Distilled water was added to achieve a total volume of approximately 20 mL. The sample was incubated at 37 °C for 2 h under continuous shaking. The pH was checked at 10 min and 60 min and maintained at 3.0 ± 0.1 throughout the incubation.

After gastric digestion, 11 mL of SIF, 0.9 mL of pancreatin solution (4500 U/mL), 2 mL of bile salt solution (100 mg/mL), and 40 μ L of 0.3 M CaCl_2 were introduced. This resulted in a final trypsin activity of 100 U/mL, a final bile salt concentration of approximately 10 mM, and a final Ca^{2+} concentration of 0.6 mM. The pH was raised to 7.0 using 1 M NaOH, and distilled water was added to bring the final volume to about 40 mL. The mixture was incubated at 37 °C for 2 h under continuous shaking, with pH monitored every 30 min and maintained at 7.0 ± 0.1 .

To terminate the digestion process, the tubes were cooled down in an ice bath for 10 min. The digested samples were immediately centrifuged at 10,000 rpm for 5 min at 4 °C to stop all the enzymatic activity. The supernatant was collected, and its protein content was measured using the Biuret method (Anyiam et al., 2025). The percentage of *in vitro* protein digestibility (IVPD) was determined by the following equation:

$$\text{IVPD}(\%) = \frac{P_1 - P_2}{P_1} \times 100$$

Where P_1 is the initial protein content and P_2 is the protein content after *in vitro* digestion



Table 3.3 Electrolyte Composition of Simulated Digestive Fluids Used in the INFOGEST Static *In Vitro* Digestion Method

Constituent	Stock Concentration	SSF (pH 7)		SGF (pH 3)		SIF (pH 7)	
		Vol of Stock (ml)	Final Conc. (mmol/L)	Vol of Stock (ml)	Final Conc. (mmol/L)	Vol of Stock (ml)	Final Conc. (mmol/L)
KCl	0.5	18.9	15.1	8.6	6.9	8.5	6.8
KH ₂ PO ₄	0.5	4.6	3.7	1.13	0.9	1	0.8
NaHCO ₃	1	8.5	13.6	15.6	25	53.1	85
NaCl	2	0	0	14.8	47.2	12	38.4
MgCl ₂ ·6H ₂ O	0.15	0.63	0.15	0.5	0.1	1.38	0.33
(NH ₄) ₂ CO ₃	0.5	0.075	0.06	0.63	0.5	0	0
HCl	6	0.11	1.1	1.63	15.6	0.88	8.4

Note The final volume of each simulated digestive fluid was made up to 500 mL using distilled water. CaCl₂·2H₂O was added separately during digestion to obtain final concentrations of 1.5 mM, 0.15 mM, and 0.6 mM in SSF, SGF, and SIF, respectively.

Source Brodkorb et al. (2019)

2) Electrophoresis

Protein molecular weight profiles were analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) based on the procedure described by Laemmli (1970). Separation was carried out using a polyacrylamide gel consisting of a 12 % resolving layer and a 4 % stacking layer. Each protein sample was mixed with an equal volume (1:1 v/v) of loading buffer to achieve a final protein concentration of 2 mg/mL. The composition of the loading buffer was as follows: 0.5 mol/L Tris-HCl (pH 6.8), 0.5 % bromophenol blue, 10 % glycerol, 2 % sodium dodecyl sulfate (SDS), and β -mercaptoethanol, which served as a reducing agent. The resulting mixtures were then heated at 95°C for 5 minutes and subsequently allowed to return to ambient temperature.

Subsequently, 20 μ L of each prepared sample was loaded onto the gel and separated at 15 mA per gel using a Mini-PROTEAN Tetra Cell system (Bio-Rad Laboratories, Inc., Richmond, CA, USA). Following electrophoresis, the gels were stained overnight with Coomassie Brilliant Blue R-250 under gentle agitation (50 rpm). Excess stains were removed using methanol-acetic acid-water de-staining solution until a clear background was obtained. After electrophoresis, the gels were dehydrated and imaged under white light. A protein ladder covering a molecular weight range of 2 to 250 kDa was employed as the reference standard for estimating the apparent molecular masses of the separated protein bands.

3. Minerals

Mineral composition was determined following the method of Nandang et al. (2025) with minor adjustments. Briefly, 0.3 g of Kai sample was treated with concentrated nitric acid and hydrogen peroxide, after which the digested solution was made up to 50 mL with double-distilled water. Blank samples prepared under identical conditions were analyzed simultaneously. The concentrations of major minerals, trace elements, and selected heavy metals were quantified using ICP-MS/MS and reported as mg kg⁻¹ dry matter.

4. Chlorophyll Content

Chlorophyll a and b contents were measured according to Nandang et al. (2025) with modifications. To extract chlorophyll, a 100 mg portion of the sample

was combined with 10 mL of acetone and kept at 4°C for 30 min. After this incubation period, the mixture was centrifuged at 3000 rpm for 10 min at 4°C to remove insoluble material. The clear supernatant obtained was then transferred to a spectrophotometer, and its absorbance was recorded at two wavelengths: 663 nm and 646 nm. The concentrations of chlorophyll a and chlorophyll b were derived from the equations given below. Final values were normalized to milligrams of chlorophyll per gram of sample, considering both the extraction solvent volume and the initial sample mass.

$$\text{Chl a (mg} \cdot \text{L}^{-1}) = (12.25 \times \text{Abs}_{663}) - (2.79 \times \text{Abs}_{646})$$

$$\text{Chl b (mg} \cdot \text{L}^{-1}) = (21.5 \times \text{Abs}_{646}) - (5.10 \times \text{Abs}_{663})$$

$$\text{Chl a (mg/g)} = \text{Chl a (mg/l)} \times \frac{V \text{ (ml)}}{1000} \times \frac{1}{\text{Weight (g)}}$$

$$\text{Chl b (mg/g)} = \text{Chl b (mg/l)} \times \frac{V \text{ (ml)}}{1000} \times \frac{1}{\text{Weight (g)}}$$

3.2.1.4 Determination of Phytochemicals

1. Determination of Total Phenolic Compound

Total phenolic content was measured using a spectrophotometric assay with Folin-Ciocalteu reagent, following ISO standard 14502-1 (2005). An aliquot of 25 μL of sample extract was dispensed into a 96-well microplate, followed by the addition of 125 μL of 10 % (v/v) Folin–Ciocalteu reagent and 100 μL of 7.5 % (w/v) sodium carbonate solution. Distilled water was used as the blank. The reaction mixture was mixed thoroughly and allowed to stand at room temperature for 60 min prior to absorbance measurement at 765 nm using a microplate reader. A calibration curve was prepared using gallic acid as the reference standard. The total phenolic content was then calculated and reported as milligrams of gallic acid equivalent per gram of dry sample (mg GAE/g).

2. Determination of Total Flavonoid Content

Total flavonoid content was determined using a modified version of the Christ-Müller method (Hu et al., 2022; Korzeniowska et al., 2020). A 0.5 mL portion of the extract was initially combined with 70 μL of 5 % (w/v) sodium nitrite solution. After allowing the reaction to proceed for 5 min, 150 μL of 10 % (w/v) aluminum chloride solution was added, and the mixture was kept in the dark for an additional 5 min. Neutralization of the reaction mixture was then carried out by adding 0.5 mL of 1

M sodium hydroxide, followed by dilution with 1.3 mL of distilled water. The mixture was allowed to equilibrate for 5 min, and its absorbance was measured at 510 nm. Quercetin was used as the calibration standard, and the total flavonoid content was expressed as milligrams of quercetin equivalent per gram of dry sample (mg QE/g).

3. Determination of DPPH Radical Scavenging Activity

DPPH radical scavenging capacity was assessed according to a modified ISO 14502-1:2005 procedure. An aliquot of 50 μ L extract was reacted with 1.95 mL of methanolic DPPH solution (0.06 mM). Following incubation under dark conditions for 30 min, the absorbance of the mixture was recorded at 517 nm using a UV-Vis spectrophotometer. Methanol was used as the control blank. Antioxidant activity was quantified using a Trolox calibration curve and reported as mg TE/g dry weight.

4. Determination of ABTS Radical Scavenging Activity

The ABTS radical scavenging activity was assessed following a modified protocol originally described by Re et al. (1999). To generate the ABTS⁺ radical cation, a 7 mM ABTS stock solution was mixed with 2.45 mM potassium persulfate, and the reaction mixture was left to incubate in darkness at ambient temperature for no less than 16 h. Before conducting the assay, the resulting ABTS⁺ solution was diluted with ethanol until its absorbance reached 0.70 ± 0.02 at 734 nm. In the actual measurement, 60 μ L of the sample extract was combined with 240 μ L of the diluted ABTS⁺ solution and allowed to react for 6 min. The absorbance of the reaction mixture was then recorded at 734 nm. Trolox served as the reference standard, and the antioxidant activity was reported as milligrams of Trolox equivalent per gram of dry sample (mg TE/g).

5. Determination of Ferric Reducing Antioxidant Potential

The ferric reducing antioxidant potential (FRAP) was quantified according to the method developed by Benzie and Strain (1996). A 400 μ L of aqueous sample extract was mixed with 2.6 mL of freshly prepared FRAP reagent. The reagent was prepared by combining 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl, and 20 mM ferric chloride solution at a ratio of 10:1:1. The reaction mixture was incubated at 37 °C for 30 min in the dark prior to absorbance measurement at 595 nm. Distilled water was used as the blank. A standard curve was constructed

using ferrous sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), and the results were expressed as mmol Fe^{2+} equivalents per gram dry weight.

3.2.1.5 Determination of Anti-inflammatory Properties

1. Cell Culture and Maintenance

RAW 264.7 murine macrophage cells were cultured in high-glucose Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % fetal bovine serum (FBS). The cells were maintained at 37 °C in a humidified incubator with a 5 % CO_2 atmosphere and subculture upon reaching approximately 80 to 90 % confluence. Only cells in the logarithmic growth phase were used for all experiments (Thai et al., 2024).

2. Preparation of Kai Extracts

Stock solutions (20 mg/mL) were prepared by dissolving freeze-dried and tray-dried Kai powders in cell-culture-grade dimethyl sulfoxide (DMSO). The mixtures were vortexed and then sonicated for 30 min at 37 °C, followed by centrifugation at 12,000 rpm for 10 min. The resulting supernatants were sterilized by filtration through 0.22 μm membrane filters and stored at -20 °C until further use. Before treating cells, the stock solutions were diluted with DMEM to the required working concentrations, ensuring that the final DMSO concentration remained below 0.1 %.

3. Cell Viability Assay

A Cell Counting Kit-8 (CCK-8) was used to evaluate cell viability. RAW 264.7 cells were seeded into 96-well plates at a density of 1×10^4 cells per well and incubated for 24 h. The cells were then exposed to Kai extracts at concentrations of 6.25, 12.5, 25, 50, 100, and 200 $\mu\text{g/mL}$ for an additional 24 h. After this treatment, 10 μL of CCK-8 reagent was added to each well, and the plates were incubated for 2 h at 37°C. Absorbance was measured at 450 nm using a microplate reader. Cell viability was calculated using the following formula:

$$\text{Cell viability (\%)} = (\text{Absorbance of sample} / \text{Absorbance of control}) \times 100$$

Concentrations exhibiting cell viability greater than 90 % were considered non-cytotoxic and selected for subsequent anti-inflammatory experiments.

4. Determination of Nitric Oxide Production

The inhibition of nitric oxide (NO) release in activated macrophages was evaluated using the colorimetric Griess assay with slight modifications from the method described by Thai et al. (2024). RAW 264.7 murine macrophages were plated into 96-well plates at a concentration of 1×10^5 cells per well and maintained for 24 h at 37°C under a humidified 5 % CO₂ environment. Subsequently, the cells were exposed to the Kai extract (using concentrations previously determined as non-cytotoxic from the viability assay) together with lipopolysaccharide (LPS, 1 µg/mL) to induce inflammation, followed by a further 24 h incubation period. Untreated cells served as the blank control, while cells treated with LPS alone served as the inflammation model group. Dexamethasone (10 µM) was used as a positive control, anti-inflammatory drug. After the incubation period, 50 µL of culture supernatant was withdrawn from each well and transferred into a fresh 96-well plate. An equal volume (50 µL) of Griess reagent (prepared as a 1:1 mixture of Solution A and Solution B) was then added to each well and mixed thoroughly. The resulting reaction mixture was kept in darkness at ambient temperature for 15 min, after which the absorbance was recorded at 540 nm using a microplate reader. Nitrite concentration was quantified using a sodium nitrite (NaNO₂) standard curve and expressed as NO production.

5. Western Blot Analysis for Anti-inflammatory Property

Protein expressions of inflammatory and cytoprotective markers were evaluated using Western blotting. After 24 h of treatment under the conditions described above, RAW 264.7 cells were collected. The cells were rinsed twice with ice-cold phosphate-buffered saline (PBS) and then lysed using RIPA lysis buffer supplemented with protease and phosphatase inhibitors to extract the cellular protein fraction. The protein concentration of each lysate was quantified using a bicinchoninic acid (BCA) assay kit. Equal amounts of protein from each sample were loaded onto an SDS-polyacrylamide gel, separated by electrophoresis, and subsequently transferred to polyvinylidene difluoride (PVDF) membranes.

After the membranes were blocked with 5 % non-fat milk in TBST for 1 h at room temperature, they were incubated overnight at 4 °C with primary antibodies against inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), nuclear

factor kappa B (NF-κB) p65, phosphorylated NF-κB p65 (p-NF-κB p65), nuclear factor erythroid 2-related factor 2 (Nrf2), heme oxygenase-1 (HO-1), and β-actin. Following the washing step with TBST, the membranes were treated with horseradish peroxidase (HRP)-conjugated secondary antibodies for 1 h at room temperature. Protein bands were visualized using an enhanced chemiluminescence (ECL) detection system and quantified using densitometric analysis, with values normalized to β-actin (Thai et al., 2024).

3.2.1.6 Determination of Physical Properties

1. Color

The surface color of the dried *Cladophora glomerata* powder was measured using a HunterLab CIE Lab colorimeter (ColorQuest XE, Hunter Associates Laboratory, Reston, VA, USA). The instrument was calibrated against a standard white reference tile with values of $L^* = 93.80$, $a^* = -0.82$, and $b^* = 0.91$. Three replicate measurements were performed on homogenized samples under a D65 illuminant with a 10° observer. The following color parameters were recorded: L^* (lightness, ranging from 0 = black to 100 = white; a^* (green [negative] to red [positive] chromaticity); b^* (blue [negative] to yellow [positive] chromaticity)

The total color difference (ΔE_{AB}^*) was computed using the formula described by (Armesto et al., 2016).

$$\Delta E_{AB}^* = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2}$$

where L_1^* , a_1^* , and b_1^* are control values. L_2^* , a_2^* , and b_2^* are the values of the samples subjected to different treatments.

2. Water Activity

Water activity was measured determined using a water activity meter (2108018, Novasina AG, Lachen, Switzerland). Following calibration, an appropriate quantity of each sample was placed into the measurement cup, inserted into the sealed chamber, and the reading was recorded once stabilized.

3.2.1.7 Determination of Techno-Functional Properties

1. Water Holding Capacity (WHC) and Oil Holding Capacity (OHC)

A total of 0.1 g of freeze-dried sample was mixed with either 10 ml of distilled water or 5 g of corn oil in a centrifuge tube and vortexed at high speed for 30

seconds. The resulting mixture was allowed to stand for 1 h at room temperature, followed by centrifugation at 10,000 rpm for 10 min in a high-speed centrifuge. WHC and OHC were calculated as g/g (Zhao et al., 2024). All samples were analyzed in triplicate.

2. Swelling Capacity

Swelling capacity (SC) was measured following the procedure of Zhao et al. (2024). A 0.2 g sample was placed into a 15 mL graduated cylinder, and 10 mL of distilled water was added. The suspension was stirred gently and then left to stand at ambient temperature for 24 h. The final volume occupied by the swollen sample was recorded. SC was calculated as:

$$SC \left(\frac{\text{mL}}{\text{gDW}} \right) = \frac{V_1 - V_0}{m_0}$$

where V_0 and V_1 represent the sample volume before and after swelling (ml), respectively, and m_0 is the dry weight of the sample (g).

3. Foaming Capacity (FC) and Foam Stability (FS)

Foaming properties were evaluated based on the procedure described by Lamsal et al. (2007) with minor modifications. A 100 mg portion of Kai powder was suspended in 10 mL of distilled water. The suspension was homogenized at maximum speed for 5 min using an IKA RW 20 digital homogenizer. Immediately after homogenization, the entire dispersion (including both liquid and foam) was transferred into a 100 mL graduated cylinder. The total volume was recorded, and foaming capacity was calculated as:

$$FC (\%) = \frac{\text{Volume After Whipping}}{\text{Volume Before Whipping}} \times 100$$

Foam stability (FS) was evaluated by recording the foam volume at 10, 20, 30, 40, 50, 60, 70, 80, and 90 min after homogenization. The remaining foam was expressed as a percentage of the initial foam volume and calculated using equation:

$$FS (\%) = \frac{\text{Volume After Standing} - \text{Volume Before Whipping}}{\text{Volume Before Whipping}} \times 100$$

4. Emulsifying Capacity (EC) and Stability (ES)

Emulsifying properties were assessed following the protocol of Lamsal et al. (2007) with minor modifications. A 200 mg sample of Kai powder was suspended

in a mixture of 10 mL distilled water and 10 mL soybean oil. The suspension was homogenized at 10,000 rpm for 1 min, after which the initial height of the formed emulsion layer was recorded. The homogenized mixture was then centrifuged at 1300 relative centrifugal force (rcf) for 5 min. Emulsifying capacity was calculated using the equation below:

$$EC (\%) = \frac{\text{Height of Emulsified Layer after Centrifugation(cm)}}{\text{Initial Emulsion Height}} \times 100$$

Emulsion stability (ES) was determined by heating the emulsion at 80 °C for 30 min in a water bath, followed by cooling under running water for 30 min and centrifugation under the same conditions as described above. ES was calculated using equation:

$$ES (\%) = \frac{\text{Height of Emulsified Layer after Heating}}{\text{Height of Emulsified Layer before Heating}} \times 100$$

5. Determination of Microstructure (SEM)

The microstructure of the samples was examined using a scanning electron microscope (SEM; TESCAN MIRA, TESCAN ORSAY HOLDING, Brno, Czech Republic). Specimens were trimmed into small fragments of approximately 5 mm². These fragments were attached to aluminum mounting stubs using double-sided conductive carbon tape. To enhance electrical conductivity, the mounted specimens were coated with a thin layer of gold via sputter deposition for 45 s using a Quorum SC7620 sputter coater (Quorum Technologies Ltd., East Sussex, UK). Imaging was carried out at accelerating voltage of 5 kV, with micrographs captured at three different magnifications: 150×, 300×, and 1000×.

6. Fourier Transform Infrared Spectroscopy

FTIR analysis was performed on the dried Kai powders using a Fourier transform infrared spectrometer. Each powder sample was thoroughly blended with spectroscopic-grade potassium bromide (KBr) at a weight ratio of 1:100 (sample: KBr). The resulting mixture was compressed into transparent pellets with a thickness of approximately 1 mm. Spectral measurements were recorded at ambient temperature across a wavenumber range of 4000 to 400 cm⁻¹.

3.2.1.8 Statistical Analysis

Each experiment was performed in triplicate using independently prepared samples. Data for general comparisons were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test in SPSS software. For anti-inflammatory assays, statistical analysis was performed using GraphPad Prism with one-way ANOVA followed by Tukey's multiple comparison test. Statistical significance was defined at $p < 0.05$.

3.2.2 Phase II: Developing 3D Printed Dysphagia Friendly Algal Food

3.2.2.1 Overall Methodology

Formulation of food ink where the optimum formula was obtained by using RSM optimal design.

Design Expert: RSM [Optimal (Custom) Design, N=16]

Factors: XG, KG

Response: TPA (Hardness, Adhesiveness, Cohesiveness), Printability (Print Accuracy, Deformation), Syneresis

Observe dysphagia suitability, Rheological behavior, SEM

3.2.2.2 Formulation of Food Ink

Prior to formulation development, Kai powders produced by freeze drying, sun drying, and tray drying were comparatively evaluated in Phase I. The selection of the powder for Phase II was based on its overall nutritional quality, bioactive properties, techno-functional characteristics, and suitability for dysphagia-friendly 3D food printing applications. The powder demonstrating the most favorable overall performance according to these criteria was selected for subsequent formulation development. Tray dried Kai algae samples were ground and sieved by a 100-mesh sieve to get uniform size particles. Konjac gum (KG) plus xanthan gum (XG) of a total of 3 to 9 % of the algae amount was used as hydrocolloids. Deionized water ingredient (7 times the mixture weight) was the last ingredient. Design expert software was used in which RSM mixture optimum design was chosen to optimize concentrations of Kai, water and two types gum. This design helped to evaluate their individual and combined effects on texture properties while minimizing the number of experimental trials. The mixture was mixed manually for 3 min to ensure homogeneity while minimizing foam

and air bubble formation. It was then covered with plastic wrap and stored at room temperature for 20 min before utilization.

3.2.2.3 Design of Experiment

Design-Expert software was used to develop an optimal custom experimental design, which created dysphagia friendly optimum food ink with maximal printability and minimal syneresis and deformation. XG and KG were set as continuous variables, ranging from 0 to 9 %, with constraints applied to ensure that their total concentration remains between 3 % and 9 %. A total of 16 experimental runs were selected using the software's optimization algorithm as seen in Table 3.4. TPA (hardness, adhesiveness and cohesiveness), printability (print accuracy and stability) and syneresis were used as the responses for the optimization of protein ink formulation. The results were interpreted to identify the optimal combination of XG and KG based on statistical significance and practical feasibility.

Table 3.4 Experimental Conditions (RSM)

Run	Factor 1 (A: XG %)	Factor 2 (B: KG %)
1	2.07	4.32
2	0	9
3	0.60936	2.39064
4	6.90978	2.09022
5	5.265	0
6	5.265	0
7	0.225	5.67
8	2.07	4.32
9	9	0
10	0.225	5.67
11	4.365	4.14806
12	0.60936	2.39064
13	3	0
14	4.365	4.14806
15	4.14	1.935
16	2.43003	6.56997

3.2.2.4 Optimization Criteria and Desirability Function

To determine the optimal formulation of XG and KG for 3D printable Kai-based food inks, a multi-response optimization approach was employed using the desirability function method (Table 3.5). The independent variables (XG and KG concentrations) were constrained within the experimental design range (0–9 %, w/w). The optimization criteria were defined based on the functional requirements for extrusion-based 3D printing and dysphagia-oriented food applications. Hardness, adhesiveness, and cohesiveness were set within target ranges to ensure appropriate textural properties, while print accuracy was maximized to achieve high shape fidelity. Syneresis and deformation were minimized to enhance structural stability and water retention. Each response was assigned an equal level of importance to achieve a balanced optimization outcome. The overall desirability function was then used to identify the optimal combination of XG and KG that simultaneously satisfied all response criteria.

Table 3.5 Optimization Criteria and Constraints Applied in RSM Desirability Analysis

Variable	Type	Goal	Range	Importance
XG (%)	Factor	In range	0–9	3
KG (%)	Factor	In range	0–9	3
Hardness	Response	Target range	1000–15000	3
Adhesiveness	Response	Target range	0–400	3
Cohesiveness	Response	Target range	0.2–0.6	3
Print Accuracy (%)	Response	Maximize	91.87–96.10	3
Syneresis (%)	Response	Minimize	1.54–2.84	3
Deformation (%)	Response	Minimize	0.07–2.39	3

3.2.2.5 3D Printing Setup and Fabrication Parameters

1. 3D Printing Equipment and Conditions

Samples were fabricated using an extrusion-based food 3D printer (Foodini®, Natural Machines, Spain). A 1.5 mm nozzle was used for all printing experiments. The line thickness and layer height were both set at 1.4 mm. Printing was

performed at a printing speed of 3500 mm/min with an ingredient flow speed of 1.7. The fill factor was set to 1 %, and the turning speed factor was maintained at 0.

To improve first-layer deposition, the first ingredient flow was set to 6, with a first ingredient hold of 4.2 mm. The first-layer nozzle height was fixed at 1.4 mm, and the first-layer speed was maintained at 100 %. During non-printing movements, a jump height of 3 mm was applied. The ingredient hold and minimum hold distance were set to 3 mm and 10 mm, respectively, while the resume ingredient flow was set to 0.

All printing experiments were conducted at room temperature (25 ± 2 °C) without any pre-heating or active thermal control.

2. Printing Geometry

The printing geometry was designed using the internal software of the Foodini® printer. Samples were printed with a rectangular geometry of 30×40 mm (width $\times$ length) and a total height of 10 mm, corresponding to 7 printed layers.

3. Toolpath Generation

The toolpath was automatically generated by the Foodini® software using default settings, without manual modification of the deposition strategy. The resulting toolpath produced a continuous rectilinear spiral-like deposition pattern, allowing uniform material extrusion with minimal start–stop events which can be seen in Figure 3.8. Concentric infill was not applied, and advanced functions including smart fill, outlining, inversion, and stylization were disabled. Smoothing was enabled to improve surface uniformity of the printed samples.

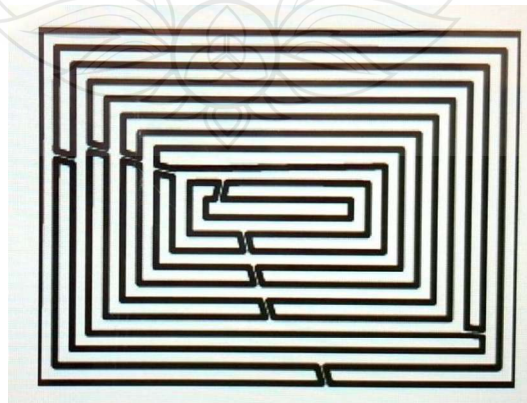


Figure 3.8 Printing Pattern: Rectilinear Spiral-like Deposition Pattern

3.2.2.6 Physical and Mechanical Performance Responses

1. Print Accuracy and Stability

As for printing accuracy: the length, width and height of the printed samples were measured using vernier calipers. According to previous report with little modification (Ji et al., 2022), the printing accuracy was calculated by equation:

$$\text{Print Accuracy}(\%) = \frac{\left(1 - \frac{|H_e - H_s|}{H_s}\right) + \left(1 - \frac{|L_e - L_s|}{L_s}\right) + \left(1 - \frac{|W_e - W_s|}{W_s}\right) + \left(1 - \frac{|P_e - P_s|}{P_s}\right)}{4} \times 100$$

Where H_e represents the height of the 3D-printed product and H_s is the height of the reference model. L_e and L_s denote the length of the printed product and model, respectively, while W_e and W_s refer to the width of the printed product and model. In addition, P_e indicates the number of complete layers in the printed product, whereas P_s corresponds to the number of layers in the model.

Printing stability was evaluated by measuring the length, width, and height of the printed cuboids using vernier calipers. The samples were then stored at 4 °C for 24 h and remeasured, and stability was assessed based on the deformation rate.

$$\text{Deformation rate}(\%) = \frac{|H_b - H_a|}{H_b} \times 100$$

2. Syneresis of Food Inks

Syneresis was studied using the filter-paper blotting method that was previously reported Xing et al. (2022). The experiment was conducted by placing 1 g of protein ink at the center of a Whatman filter paper No. 4 within a 1 cm radius circle. The food inks were left undisturbed for 30 min to allow liquid spreading, after which they were photographed and the diameter of the liquid spread zone was measured. The spread area was calculated from the measured diameter, assuming a circular spread pattern. All measurements were performed in triplicate.

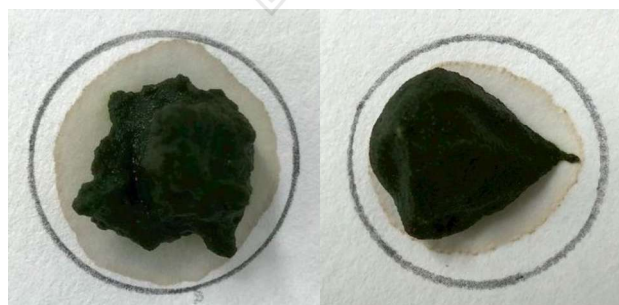


Figure 3.9 Syneresis score

3. Texture Properties

Texture was analyzed using texture analyzer (TEX-100N; Japan Instrumentation System Co., Ltd., Nara, Japan). Texture profile analysis was selected as testing method. Cell load was set at 100 N and the cylindrical probe with a diameter of approximately 13 mm was used. The samples were pressed twice with 50 % strain. The move speed and test speed of probe were set to 600 and 120 mm/min, respectively. Hardness, adhesiveness and cohesiveness were calculated using TEX-PCI software. The calculation setting was set at 5 % fragility and 314.15 mm² of Jig area.

3.2.2.7 Dysphagia Safety Suitability Assessment

1. IDDSI Test

In 2016, the International Dysphagia Diet Standardization Initiative (IDDSI) introduced a framework that classifies liquids and foods appropriate for individuals with swallowing difficulties into eight distinct levels. Among these, five levels apply to beverages, five to solid foods, and two categories are shared between both groups (IDDSI, 2016). Based on preliminary observations, the prepared samples are expected to fall within Level 4 of this classification system.

To assign each sample to an IDDSI category, two qualitative tests were performed following the IDDSI guidelines. For the fork drip test, a portion of the ink sample was lifted with a standard fork, and the extent to which the material passed through the prongs was observed. This flow behavior was then matched against the descriptions provided for each food level. The spoon tilt test was carried out by taking a teaspoonful of the sample, holding the spoon motionless over a plate, then slowly inclining it to one side and delivering a gentle flick. The response of the sample during this procedure was compared with the IDDSI criteria. During this procedure, the behavior of the ink sample was monitored and compared with IDDSI descriptions (Xing et al., 2022).

2. Japanese Standard and UDF Classification

The texture profile analysis (TPA) results, including hardness, adhesiveness, and cohesiveness, were compared with the Japanese standards for foods for people with swallowing difficulty presented in Table 2.2. In addition, hardness

values were compared with the Universal Design Food (UDF) classification criteria shown in Figure 2.5 to determine the corresponding dysphagia food stage.

3.2.2.8 Rheology Characterization

A HAAKE MARS III rheometer (Thermo Scientific, Germany) fitted with a 35 mm parallel plate system was used to characterize the rheological behavior of the samples. The Kai algae ink (1 g) was loaded onto the lower plate, and the parallel plate gap was set to 1 mm. To prevent moisture loss and maintain a stable temperature, a solvent trap was used during testing. Prior to data acquisition, each sample was held at 25°C for 5 min to achieve thermal equilibrium. Steady-state flow curves were recorded across a shear rate sweep from 0.1 to 100 s⁻¹. The yield characteristics were evaluated under oscillatory conditions at a fixed frequency of 1 Hz, with the applied shear stress varying between 1 and 2000 Pa (Xing et al., 2022).

3.2.2.9 Determination of Microstructure (SEM)

Microstructural analysis was performed using a scanning electron microscope. Freeze-dried printed Kai samples were cut into small cubes (less than 1 cm size), placed on aluminum stubs with double-sided conductive carbon tape, and coated with gold for 45 s using a Quorum SC7620 sputter coater (Quorum Technologies Ltd., UK) to improve conductivity. Samples were then observed at 300× magnification under an accelerating voltage of 5 kV (Nandang et al., 2025).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Impact of Drying Methods on Properties of Kai Algae

This section describes how different drying methods (freeze drying, sun drying, and tray drying) influenced the nutritional, physical and techno-functional characteristics of Kai powder, which subsequently determine its suitability for food formulation.

4.1.1 Nutritional Properties

4.1.1.1 Proximate Composition

Drying method significantly affected the proximate composition of Kai (Table 4.1). Moisture was lowest in FD and TD and highest in SuD, indicating less efficient dehydration under ambient conditions, while ash was the dominant component in all samples and was slightly higher in FD and TD than in SuD, consistent with reports for *Cladophora* from other regions (Michalak & Messyas, 2021). This is likely because Kai naturally contains high levels of minerals such as calcium, potassium, magnesium, and phosphorus, which are relatively stable during drying. Therefore, mineral content remained the predominant fraction regardless of the drying method employed.

Protein content was highest in TD samples (13.95 %), while FD and SuD showed comparable levels (11.21–11.46 %). This trend is consistent with reports on *Kappaphycus alvarezii* and *Sargassum polycystum*, in which convective drying resulted in higher apparent protein levels due to solid-phase concentration and enhanced nitrogen accessibility after thermal denaturation (Nurshahida et al., 2018).

FD retained the highest crude fiber content (25.95 %), whereas SuD and TD showed significantly lower values, suggesting better preservation of structural carbohydrates during freeze drying. Since crude fiber is mainly composed of cellulose and a portion of hemicellulose, the low-temperature sublimation process may have minimized thermal modification of these cell wall components, contributing to the

higher crude fiber content observed in FD compared with TD and SuD (Guo et al., 2024; Tagliapietra et al., 2024).

Fat and carbohydrate contents were relatively low in all samples. SuD exhibited the highest fat (2.95 %) and carbohydrate (18.58 %) contents, whereas TD showed the lowest carbohydrate level. This reduction may reflect partial thermal and oxidative degradation of labile carbohydrates during tray drying. Additionally, because carbohydrate content was determined by difference, the relatively higher protein and ash contents in TD may have further contributed to the lower calculated carbohydrate value (Tagliapietra et al., 2024; Nurshahida et al., 2018).

Table 4.1 Proximate Composition of Freeze Dried (FD), Sun Dried (SuD) and Tray Dried (TD) Kai Samples

	FD	SuD	TD
Moisture (%)	5.20 ± 0.08 ^b	6.65 ± 0.17 ^a	5.40 ± 0.13 ^b
Ash (%)	38.26 ± 0.06 ^a	35.75 ± 0.05 ^b	38.34 ± 0.16 ^a
Protein (%)	11.21 ± 0.20 ^b	11.46 ± 0.08 ^b	13.95 ± 0.27 ^a
Fiber (%)	25.95 ± 0.89 ^a	24.61 ± 0.07 ^b	24.76 ± 0.02 ^b
Fat (%)	2.23 ± 0.09 ^b	2.95 ± 0.15 ^a	2.30 ± 0.11 ^b
Carbohydrate (%)	17.15 ± 0.73 ^b	18.58 ± 0.24 ^a	15.26 ± 0.46 ^c

Note Values are presented as mean ± standard deviation (SD). Different superscript letters within the same row indicate statistically significant differences between treatments ($p < 0.05$).

4.1.1.2 *In vitro* Protein Digestibility of Kai Algae

The digestibility of Kai algae samples was evaluated under simulated gastrointestinal conditions using SDS-PAGE and quantitative protein analysis.

SDS-PAGE analysis (Figure 4.1) showed that all undigested samples exhibited protein bands at approximately 25–60, and 5–17 kDa across all drying treatments. After enzymatic digestion, the 10–15 kDa and 20–25 kDa bands were no longer detectable, indicating efficient hydrolysis of low-molecular-weight protein fractions. In contrast, bands in the higher molecular weight region (25–60 kDa) were still observed in digested samples; however, a strong signal was also present in the

enzyme-only blank. This suggests that part of the remaining protein-like signal may originate from enzyme residues or digestion media proteins rather than undigested Kai proteins. Therefore, SDS-PAGE provides reliable evidence of digestion for proteins below 25 kDa, while interpretation of higher molecular weight regions is limited by enzyme-related background interference (Dhamaratana et al., 2025; Egodaunya et al., 2020).

Quantitative protein analysis further supported differences in digestibility among drying methods (Figure 4.2). Sun-dried samples exhibited the highest digestibility (82.55 %), followed by freeze-dried samples (76.54 %), whereas tray-dried samples showed the lowest digestibility (68.61 %). Although tray-dried samples contained the highest total protein content (13.95 % dry mass) and consequently the highest absolute digested protein fraction (9.57 % dry mass), they also retained a larger undigested fraction (4.38 % dry mass), resulting in reduced overall digestibility efficiency.

The observed differences may be attributed to structural modifications induced by drying conditions. Sun drying likely applies mild thermal and oxidative stress, promoting partial protein denaturation that enhances enzyme accessibility without extensive aggregation. In contrast, tray drying involves higher thermal exposure, potentially promoting protein aggregation, which can reduce enzymatic accessibility and lower digestibility (Tagliapietra et al., 2024; Stévant et al., 2018). Freeze drying preserves native protein structure, which may limit initial enzyme accessibility but avoids thermal damage, resulting in intermediate digestibility.

In summary, sun drying optimized protein digestibility efficiency, whereas tray drying maximized total protein retention but reduced digestibility performance. SDS-PAGE confirmed extensive hydrolysis of low-molecular-weight proteins (<25 kDa), while interpretation of higher molecular weight regions should be considered cautiously due to enzyme-derived background signals.

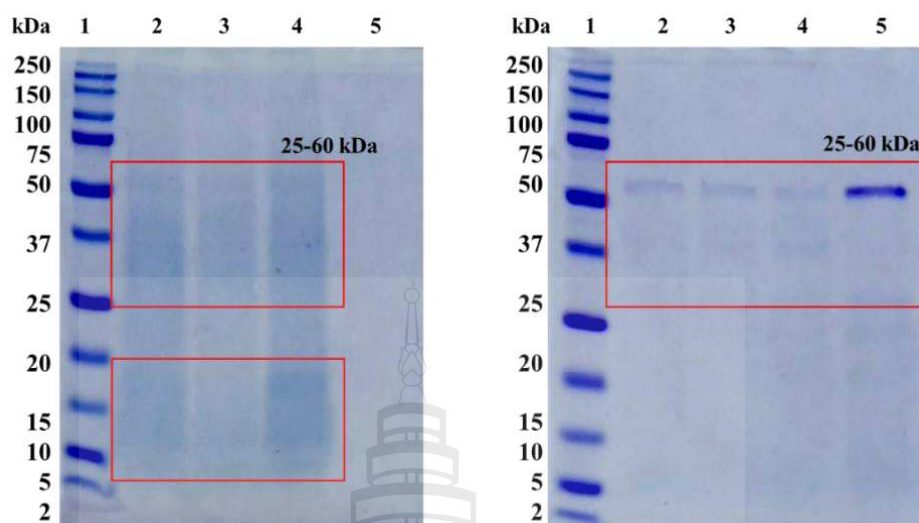


Figure 4.1 SDS-PAGE Analysis of the Kai protein. Left panel: Before Digestion; Right Panel: After Digestion. Lane 1-5 Protein Marker, FD, SuD, TD and Blank Respectively

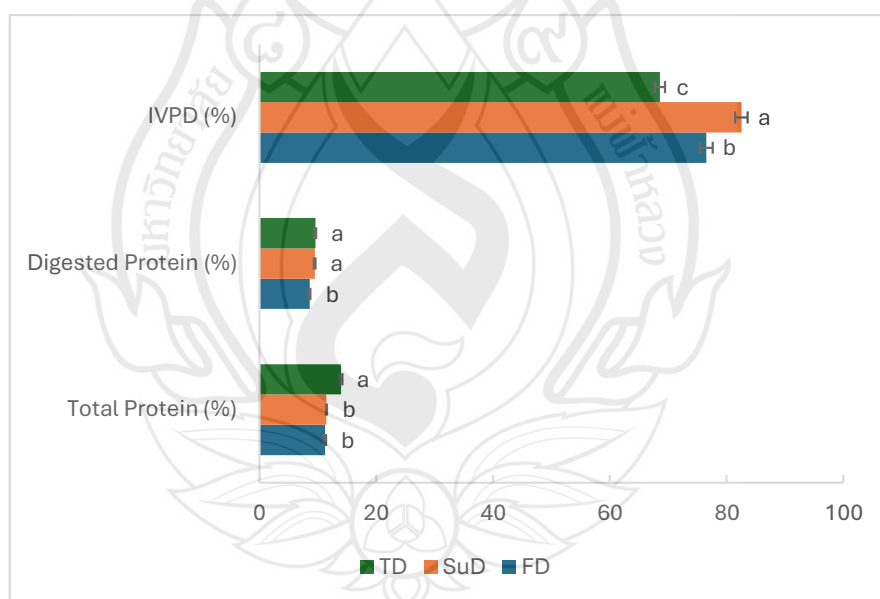


Figure 4.2 *In vitro* Protein Digestibility (IVPD) of Kai Samples. The bar graph shows total protein content (%), digested protein fraction measured in the supernatant after simulated gastrointestinal digestion (%), and IVPD (%) calculated as $\text{digested protein} \div \text{total protein} \times 100$. Different superscript letters on the bar graph indicate statistically significant differences between treatments ($p < 0.05$)

4.1.1.3 Mineral Composition

Minerals were abundant across all drying methods (Table 4.2). SuD exhibited significantly higher Na and Fe contents, likely due to concentration effects associated with prolonged moisture loss and possible deposition of environmental particulates during open-air drying. In contrast, FD retained significantly higher Mg, Ca, K, Mn, and Zn concentrations, suggesting improved mineral preservation under low-temperature vacuum dehydration conditions. K, Ca, and Mg were the predominant macro-minerals across all treatments, consistent with mineral profiles previously reported for *Cladophora* species from other geographical regions (Prazukin et al., 2024). For micro-minerals, FD preserved the highest Zn, TD the highest Cu, while SuD generally has high Co, Ni, Cr and V, indicating that each drying method slightly shifts the trace-element pattern while maintaining overall abundance (Mu et al., 2025) (Nurshahida et al., 2018).

Heavy metal concentrations remained below the maximum permissible limits established by the European Union, with As ≤ 1.68 , Cd ≤ 0.027 , Pb ≤ 0.339 , and Hg $\leq 0.021 \text{ mg} \cdot \text{kg}^{-1}$, confirming the safety of the processed Kai algae for food applications (Vitor Verdelho, 2023).

Table 4.2 Mineral Composition of Freeze Dried (FD), Sun Dried (SuD) and Tray Dried (TD) Kai Samples

Element	FD (mg/kg)	SuD (mg/kg)	TD (mg/kg)
Macro-minerals			
Na	35,976.28 $\pm$ 111.15 ^b	37,851.83 $\pm$ 386.14 ^a	34,668.44 $\pm$ 236.25 ^c
Mg	36,925.00 $\pm$ 125.41 ^a	36,824.94 $\pm$ 389.53 ^b	36,311.28 $\pm$ 245.20 ^b
Ca	71,647.00 $\pm$ 16.42 ^a	42,770.67 $\pm$ 1006.78 ^b	19,309.00 $\pm$ 3,775.39 ^c
K	101,033.50 $\pm$ 18,097.22 ^a	35,026.61 $\pm$ 10960.10 ^b	53,532.83 $\pm$ 20,811.20 ^b
Mn	1,976.87 $\pm$ 23.52 ^a	1,817.09 $\pm$ 69.03 ^b	1,237.14 $\pm$ 52.65 ^c
Fe	1,370.85 $\pm$ 24.04 ^b	1,559.99 $\pm$ 83.31 ^a	1,017.56 $\pm$ 31.84 ^c
Micro-minerals			
Zn	13.97 $\pm$ 1.30 ^a	8.75 $\pm$ 0.49 ^c	11.66 $\pm$ 0.55 ^b
Cu	1.60 $\pm$ 0.16 ^b	1.47 $\pm$ 0.05 ^b	2.21 $\pm$ 0.09 ^a
Co	0.59 $\pm$ 0.05 ^b	0.87 $\pm$ 0.01 ^a	0.59 $\pm$ 0.02 ^b
Cr	0.269 $\pm$ 0.034 ^c	0.797 $\pm$ 0.044 ^a	0.431 $\pm$ 0.044 ^b
V	1.438 $\pm$ 0.037 ^b	3.469 $\pm$ 0.345 ^a	1.339 $\pm$ 0.047 ^b

Table 4.2 (continued)

Element	FD (mg/kg)	SuD (mg/kg)	TD (mg/kg)
Heavy Metals			
As	0.868 ± 0.082 ^b	1.676 ± 0.597 ^a	0.651 ± 0.020 ^b
Cd	0.027 ± 0.003 ^a	0.026 ± 0.001 ^a	0.023 ± 0.001 ^a
Pb	0.149 ± 0.013 ^b	0.339 ± 0.005 ^a	0.157 ± 0.007 ^b
Hg	0.021 ± 0.003 ^b	0.016 ± 0.002 ^b	0.011 ± 0.003 ^a

Note Values are presented as mean ± standard deviation (SD). Different superscript letters within the same row indicate statistically significant differences between treatments ($p < 0.05$).

4.1.1.4 Phytochemical Composition and Antioxidant Properties

The drying method significantly affected the phytochemical composition and antioxidant activity of Kai powder ($p < 0.05$). TPC was highest in SuD (1.13 mg GAE/g) (Figure 4.3 A), followed by FD, while TD exhibited the lowest value. The higher TPC observed in SuD may be associated with prolonged mild drying conditions that facilitated the release of bound phenolic compounds from the cellular matrix. In contrast, the lower TPC in TD suggests partial degradation of heat-labile phenolic compounds during convective drying (Mu et al., 2025; Babaei Rad et al., 2025).

Total flavonoid content showed a different trend, with TD exhibiting the highest value (6.60 mg QE/g) (Figure 4.3 B), followed by SuD and FD. This finding suggests that flavonoid compounds were relatively more stable under controlled thermal drying conditions and that convective drying improved flavonoid extractability through partial disruption of cellular structures and enhanced solvent accessibility (Babaei Rad et al., 2025).

These compositional differences were reflected in the antioxidant assays. SuD exhibited the highest DPPH radical scavenging activity (3.12 mg TE/g) (Figure 4.3 C), consistent with its elevated TPC, suggesting that phenolic compounds contributed substantially to free radical scavenging activity. In contrast, TD showed the highest FRAP and ABTS values (20.86 mmol Fe²⁺/g and 1.56 mg TE/g, respectively) (Figure 4.3 D and Figure 4.3 E), indicating that the higher flavonoid content and other thermally

modified antioxidant constituents may have contributed to enhanced reducing power and electron transfer-based antioxidant activity (Nunes et al., 2024).

FD generally produced intermediate values across assays, representing a more conservatively processed baseline with minimal modification of the native antioxidant profile. Overall, the results highlight a trade-off between preserving total phenolics and enhancing flavonoid extractability, with SuD favoring phenolic-rich, DPPH-active powders, and TD promoting flavonoid-driven reducing capacity.

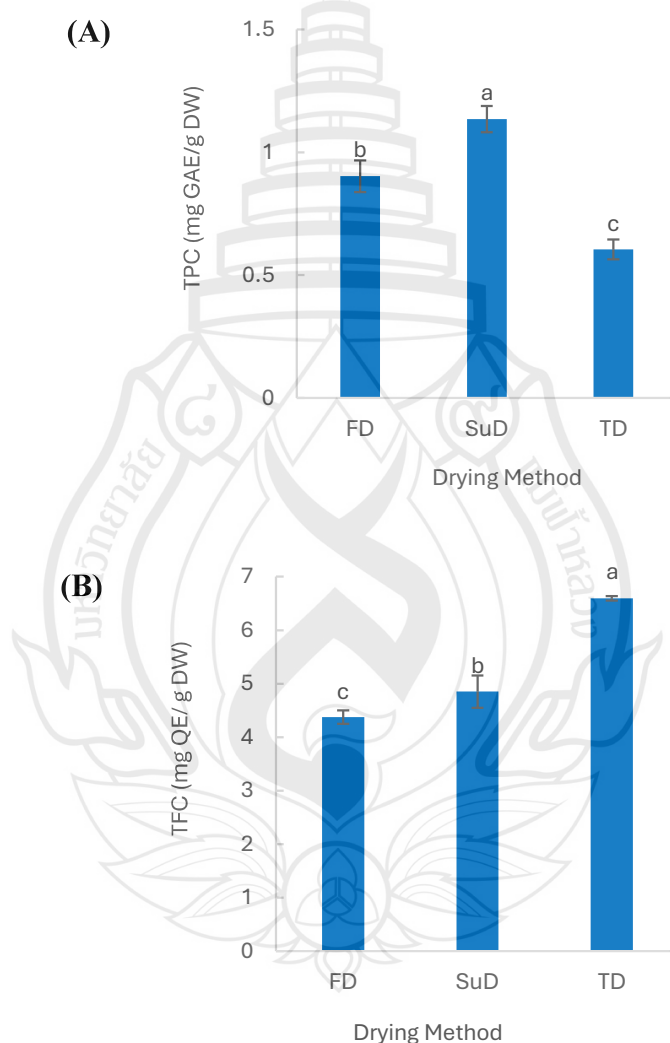


Figure 4.3 Phytochemical Composition and Antioxidant Properties of Kai from different Drying Methods. (A) TPC; (B) TFC; (C) FRAP; (D) ABTS; (E) DPPH. Different superscript letters above the bar indicate statistically significant differences between treatments ($p < 0.05$)

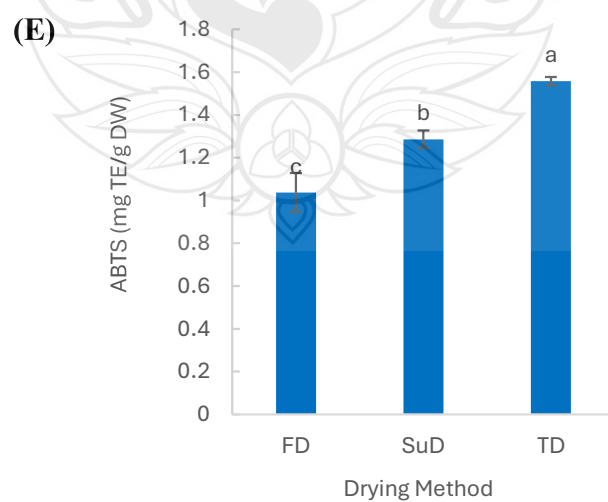
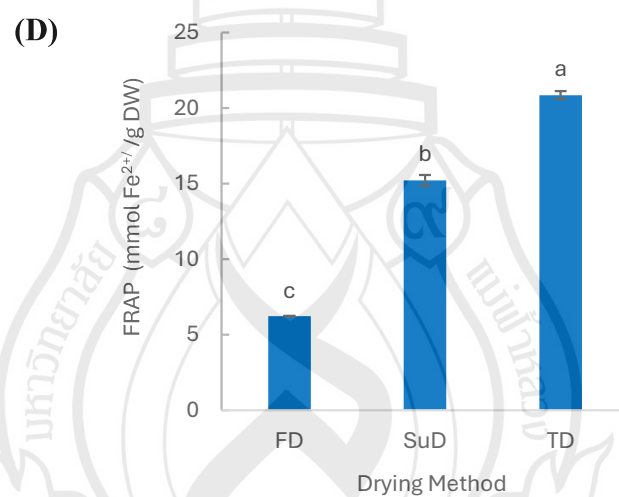
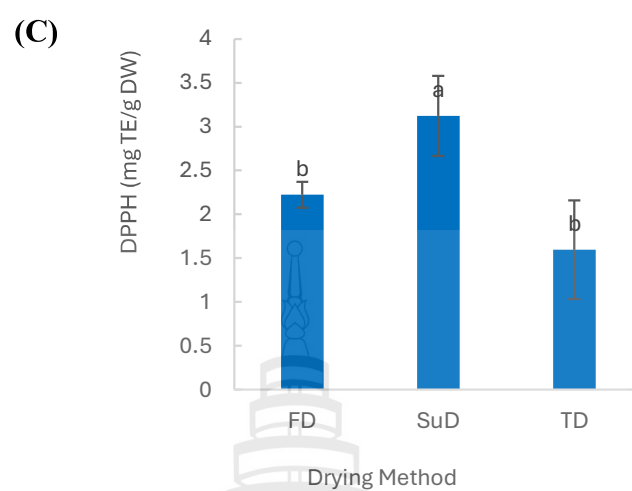


Figure 4.3 (continued)

4.1.1.5 Chlorophyll Content

Drying method significantly affected chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents of Kai samples ($p < 0.05$) (Figure 4.4). TD exhibited the highest chlorophyll *a* (2.57 mg/g), chlorophyll *b* (1.84 mg/g), and total chlorophyll contents (4.41 mg/g), followed by FD, whereas SuD showed the lowest pigment levels. The greater chlorophyll retention in TD suggests that controlled convective drying at moderate temperature and shorter drying duration effectively limited pigment degradation while improving pigment extractability through partial cellular disruption (Stévant et al., 2018). In contrast, the lower chlorophyll contents observed in SuD were likely associated with prolonged exposure to light and oxygen during open-air drying, which promoted photooxidation and chlorophyllase-mediated degradation. Similar reductions in chlorophyll stability under ambient drying conditions have been reported in dehydrated plant materials subjected to sun drying (Mu et al., 2025).

The chlorophyll *a/b* ratio ranged from 1.05 to 1.49 and followed the order $FD > TD > SuD$, indicating greater degradation of chlorophyll *a* relative to chlorophyll *b* during drying. This trend is consistent with the higher susceptibility of chlorophyll *a* to heat-, light-, and oxidation-induced degradation.

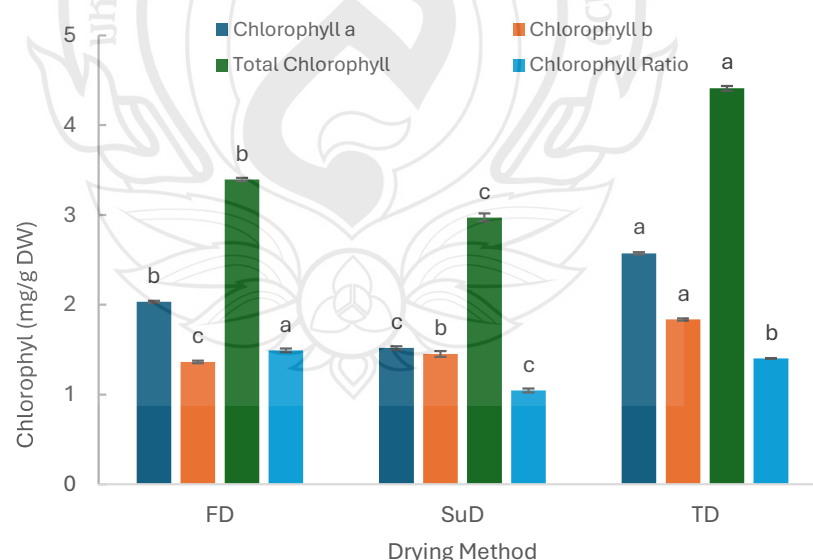


Figure 4.4 Chlorophyll content and ratio of Kai powder processed by different drying methods. Different superscript letters above the bar indicate statistically significant differences between treatments ($p < 0.05$)

4.1.1.6 Anti-inflammatory Properties

1. Cell Viability

The cytotoxic effects of FD and TD Kai extracts on RAW 264.7 cells were evaluated using the CCK-8 assay at concentrations of 0, 6.25, 12.5, 25, 50, 100, and 200 $\mu\text{g/mL}$. As shown in Figure 4.5, FD extracts maintained cell viability above 90 % at all tested concentrations, whereas TD exhibited signs of cytotoxicity beginning at 50 $\mu\text{g/mL}$. Therefore, the maximum non-cytotoxic concentration and the concentration immediately below it was selected as the high- and low-dose treatment levels, respectively, for subsequent anti-inflammatory experiments. Accordingly, FD extracts were tested at 200 and 100 $\mu\text{g/mL}$, while TD extracts were tested at 25 and 12.5 $\mu\text{g/mL}$. The increased cytotoxicity of the TD extract may be associated with enhanced extractability and thermal modification of intracellular bioactive compounds, including possible chlorophyll derivatives, which could contribute to reduced cell viability at higher concentrations (Sáez-González et al., 2024; Zubia et al., 2009).

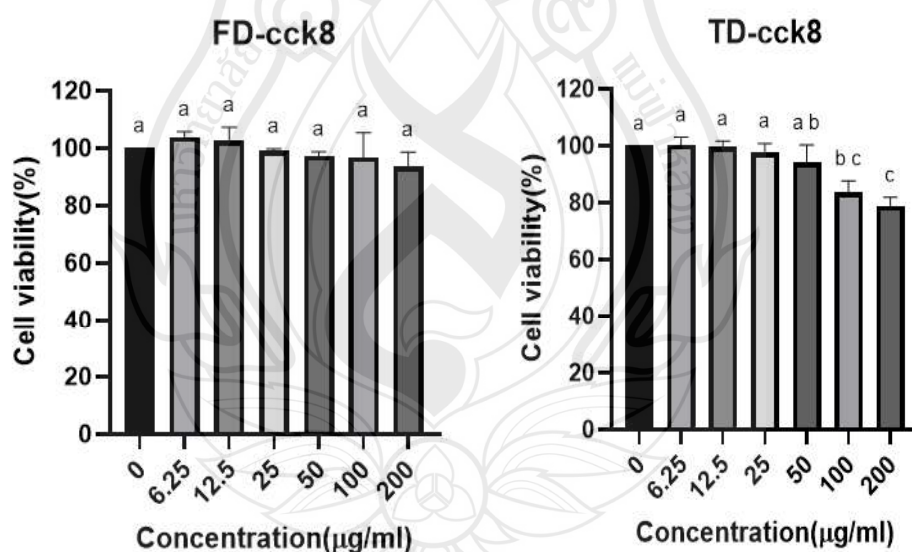


Figure 4.5 Effects of FD and TD Kai extracts on the viability of RAW 264.7 cells.

Different superscript letters above the bar indicate statistically significant differences between treatments ($p < 0.05$)

2. NO Assay

As shown in Figure 4.6, LPS treatment significantly induced nitric oxide (NO) production in RAW 264.7 cells compared to the untreated control group, confirming the successful establishment of the macrophage inflammation model. Conversely, treatment with both dexamethasone (DEX) and the Kai extracts significantly suppressed this LPS-induced NO elevation.

Between the two processing methods, the freeze-dried extract produced a greater absolute reduction in NO levels than the tray-dried extract. This higher efficacy is consistent with its superior preservation of total phenolic content (TPC) and DPPH radical scavenging activity, suggesting better retention of heat-sensitive phenolic compounds. This finding agrees with previous reports that freeze drying better preserves phenolic antioxidants, thereby enhancing anti-inflammatory and anti-nitrosative activities at higher concentrations (Ontawong et al., 2024).

However, these effects were evaluated at each extract's maximum non-cytotoxic concentration, meaning the freeze-dried extract benefited from a substantially higher usable dose range (100-200 $\mu\text{g/mL}$) compared with the tray-dried extract (12.5-25 $\mu\text{g/mL}$). Despite this limitation, the tray-dried extract still significantly suppressed NO production even at 25 $\mu\text{g/mL}$, indicating relatively strong potency at low concentrations. This activity is consistent with its phytochemical profile, where tray-dried samples exhibited higher total flavonoid content (TFC) as well as markedly greater FRAP and ABTS antioxidant capacities. This suggests that although thermal drying reduces the maximum safe dose due to increased cytotoxicity, it may enhance the formation or release of certain flavonoids and reducing agents that contribute to strong anti-inflammatory effects even at low exposure levels.

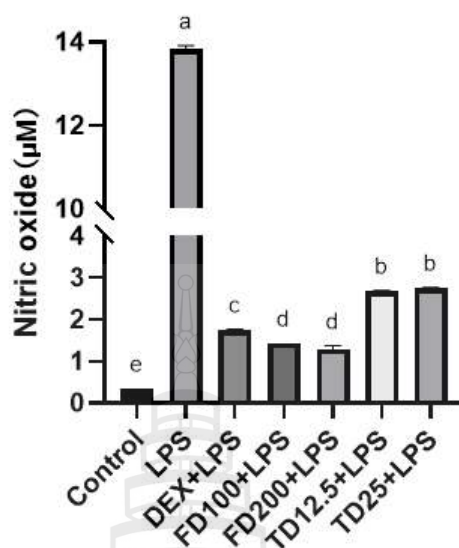


Figure 4.6 Effects of FD and TD Kai Extracts on NO Production in LPS-Induced RAW 264.7 Cells. Different superscript letters above the bar indicate statistically significant differences between treatments ($p < 0.05$)

3. Effects on Key Proteins in the NF- κ B Signaling Pathway in RAW 264.7 Cells

Western blot analysis showed that LPS stimulation markedly increased the expression of iNOS and COX-2 compared with the control group, indicating successful induction of the inflammatory response (Figure 4.7). DEX treatment reduced the expression of these inflammatory proteins, confirming the validity of the experimental model.

Both FD and TD extracts attenuated the LPS-induced expression of iNOS and COX-2 (Figure 4.7 B-C). For iNOS, FD100, FD200, and TD 25 reduced protein expression to levels close to the control group, whereas TD 12.5 exhibited a weaker inhibitory effect. A similar trend was observed for COX-2 expression, where all extract-treated groups showed substantially lower levels than the LPS group. Among the treatments, TD25 produced the lowest COX-2 expression, followed by FD100 and FD200. Analysis of NF- κ B signaling further demonstrated that LPS stimulation increased the phosphorylation of NF- κ B p65, as reflected by the elevated p-NF- κ B p65/NF- κ B p65 ratio. Treatment with FD and TD extracts reduced NF- κ B p65 phosphorylation to varying degrees. FD200 exhibited the greatest reduction in the

phosphorylation ratio, followed by TD25, indicating stronger suppression of NF- κ B activation than the other treatments (Figure 4.7 D).

These findings are consistent with the observed reduction in NO production and suggest that modulation of NF- κ B signaling contributes to the anti-inflammatory activity of Kai extracts. NF- κ B plays a central role in regulating the expression of inflammatory mediators, including iNOS and COX-2; therefore, suppression of NF- κ B activation can attenuate inflammatory responses and reduce NO production. The lower phosphorylation of NF- κ B p65 observed in FD-treated cells is consistent with the stronger inhibition of NO production observed in the present study. Similar anti-inflammatory mechanisms have been reported for algal extracts, which suppress the expression of iNOS and COX-2 through modulation of NF- κ B signaling pathways in activated macrophages (Jayawardena et al., 2019; Matin et al., 2024).

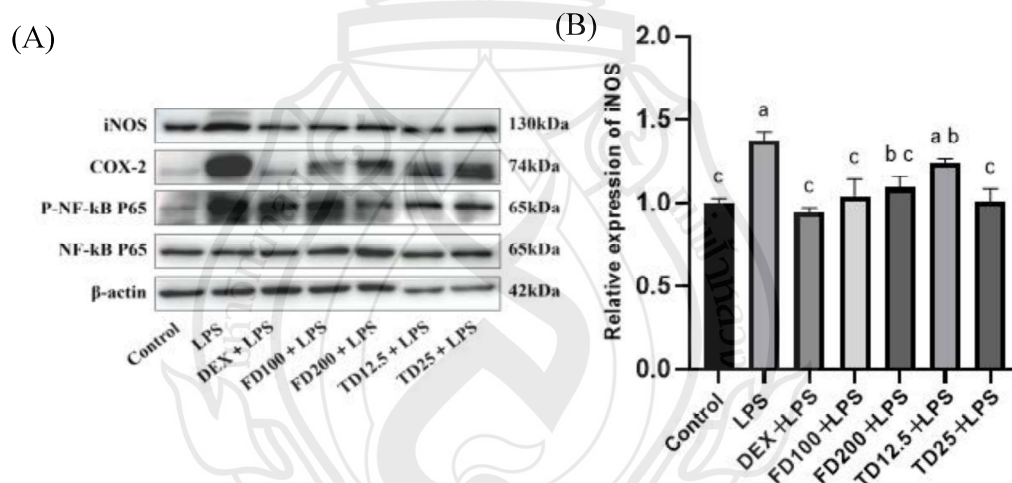


Figure 4.7 Effects of FD and TD Kai Extracts on NF- κ B Signaling Pathway-Related Protein Expression in LPS-Induced RAW 264.7 Cells. (A) Representative Western Blot Images of iNOS, COX-2, NF- κ B p65, and Phosphorylated NF- κ B p65 (p-NF- κ B p65). (B) Relative Expression of iNOS Protein. (C) Relative Expression of COX-2 Protein. (D) Ratio of p-NF- κ B p65/NF- κ B p65. Different superscript letters above the bar indicate statistically significant differences between treatments ($p < 0.05$)

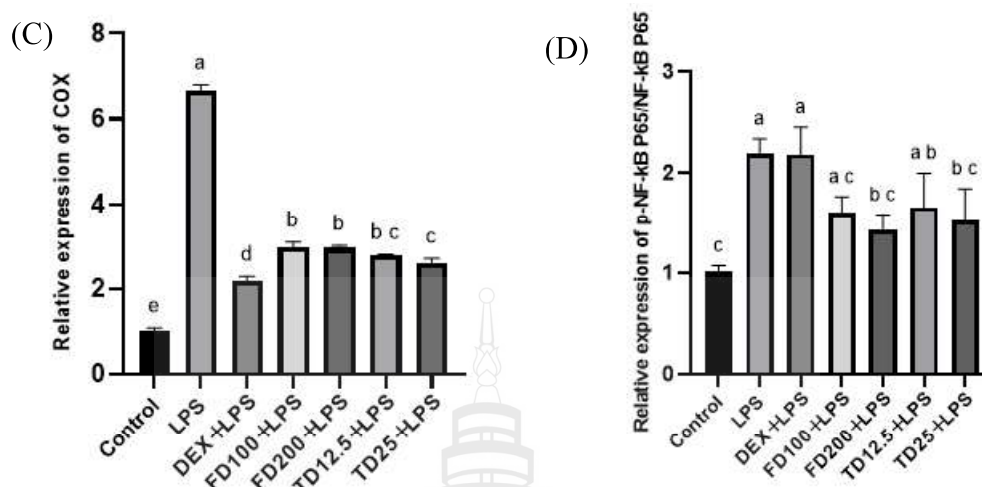


Figure 4.7 (continued)

4. Effects on Key Proteins in the Nrf2 Signaling Pathway in RAW 264.7 Cells

LPS stimulation reduced the expression of both Nrf2 and HO-1 compared with the control group, indicating suppression of the cellular antioxidant defense system (Figure 4.8). DEX treatment partially restored the expression of these proteins. Both FD and TD extracts modulated Nrf2 and HO-1 expression to varying degrees (Figure 4.8 A-C). Among the tested treatments, TD25 exhibited the highest expression of both Nrf2 and HO-1 and showed a significant increase compared with the LPS group. FD100, FD200, TD12.5, and DEX showed intermediate expression levels between those of the LPS and TD25 groups. These findings suggest that TD25 showed the strongest upregulation of Nrf2 and HO-1 expression among the tested treatments. Overall, the results indicate that the extracts may contribute to the regulation of cellular antioxidant defense mechanisms, although the extent of upregulation varied among treatments.

The increased Nrf2 and HO-1 expression observed in the TD extract at 25 µg/mL corresponds well with its higher levels of chlorophyll a, chlorophyll b, total chlorophyll, total flavonoid content, as well as its stronger FRAP and ABTS antioxidant activities compared to the freeze-dried extract. Tray drying at 50 °C likely disrupted the Kai cell structure, enhancing the release of heat-stable flavonoids and chlorophyll pigments. These compounds contribute to antioxidant activity and are known to

influence cellular signaling pathways, thereby promoting Nrf2 activation and subsequent HO-1 expression. Previous studies have similarly reported that algal bioactive compounds and photosynthetic pigments can activate the Nrf2/HO-1 pathway, supporting cellular defense against oxidative stress (Jayawardena et al., 2018; Ontawong et al., 2024). Therefore, the stronger activation of the Nrf2/HO-1 pathway by TD extracts may be related to the retention of antioxidant phytochemicals and photosynthetic pigments during processing.

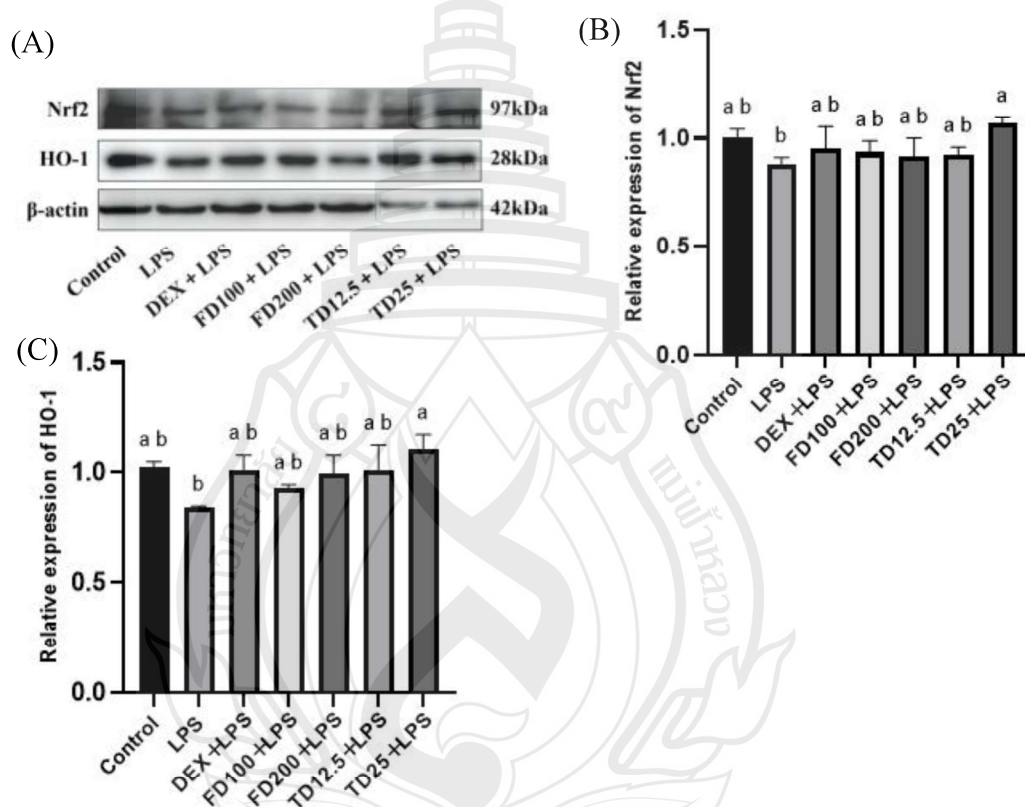


Figure 4.8 Effects of FD and TD Kai Extracts on Nrf2 Signaling Pathway-related Protein Expression in LPS-induced RAW 264.7 cells. (A) Representative Western Blot Images of Nrf2 and HO-1. (B) Relative Expression of Nrf2 Protein. (C) Relative Expression of HO-1 Protein. Different superscript letters above the bar indicate statistically significant differences between treatments ($p < 0.05$)

4.1.2 Physical Properties

4.1.2.1 Color Attributes

All Kai samples were located within the green–yellow region of the CIELAB color space ($h^\circ = 103\text{--}111^\circ$), indicating overall retention of algal pigmentation after drying (Table 4.3). TD exhibited a darker and more saturated green color, with lower L^* (58.88), more negative a^* (−7.45), and higher chroma values ($C^* = 21.38$), consistent with its superior chlorophyll retention. The stronger green intensity observed in TD suggests improved preservation of chlorophyll pigments under controlled convective drying conditions.

FD exhibited an intermediate color profile ($L^* = 60.95$, $a^* = -5.73$), indicating moderate preservation of green pigmentation with limited thermal degradation. In contrast, SuD produced the lightest and least green powder ($L^* = 61.11$, $a^* = -3.87$) and showed a greater shift toward yellow hues ($h^\circ = 103.4^\circ$). This color shift likely resulted from chlorophyll degradation, pheophytin formation, and photooxidative reactions during prolonged exposure to light and oxygen under ambient drying conditions (Mu et al., 2025).

Despite all the differences, the overall color difference remained within an acceptable visual range, indicating that all three methods produced visually acceptable powders, with TD showing the highest green color preservation.

4.1.2.2 Water activity

Water activity was significantly affected by drying method ($p < 0.05$) (Table 4.3). The water activity values followed the order FD (0.42) < TD (0.46) < SuD (0.54), reflecting differences in drying efficiency among the applied methods. The lowest water activity observed in FD was attributed to efficient moisture removal through sublimation under low-pressure conditions, which enabled more complete dehydration of the algal matrix (Bhatta et al., 2020). TD exhibited intermediate water activity values, likely due to the combined effects of moderate drying temperature and forced air circulation, which enhanced moisture transfer during convective drying (Bin Hameed et al., 2016).

In contrast, the higher water activity observed in SuD indicated less effective moisture removal under uncontrolled ambient drying conditions. Fluctuations in temperature, relative humidity, and airflow during sun drying may have limited

dehydration efficiency and contributed to greater residual water retention (Bhatta et al., 2020).

The low water activity values of FD and TD powders indicate improved microbial and physicochemical stability during storage, whereas the comparatively higher aw of SuD may increase susceptibility to quality deterioration and therefore require more controlled packaging and storage conditions.

Table 4.3 Color Attributes of Freeze Dried (FD), Sun Dried (SuD) and Tray Dried (TD) Kai Samples

Parameter	FD	SuD	TD
Appearance			
L^*	60.95 ± 0.25^a	61.11 ± 0.19^a	58.88 ± 0.06^b
a^*	-5.73 ± 0.24^b	-3.87 ± 0.10^a	-7.45 ± 0.04^c
b^*	15.02 ± 0.08^c	16.26 ± 0.19^b	20.04 ± 0.01^a
C^*	16.08 ± 0.16^c	16.71 ± 0.19^b	21.38 ± 0.00^a
h°	110.88 ± 0.69^a	103.39 ± 0.36^b	110.38 ± 0.11^a
ΔE^*		2.25 ± 0.13^b	5.70 ± 0.02^a
Water Activity	0.42 ± 0.01^c	0.54 ± 0.01^a	0.46 ± 0.00^b

Note Values are presented as mean $\pm$ standard deviation (SD). Different superscript letters within the same row indicate statistically significant differences between treatments ($p < 0.05$)

4.1.3 Techno-functional properties

4.1.3.1 Water holding, oil holding and Swelling capacity

Drying method significantly affected the hydration-related functional properties of Kai powder ($p < 0.05$) (Figure 4.9). SuD exhibited the highest water holding capacity (WHC) (14.95 g/g), followed by FD and TD. The higher WHC observed in SuD may be attributed to partial structural collapse and cellular disruption during prolonged ambient drying, which increased exposure of hydrophilic functional groups and enhanced water binding capacity (Tagliapietra et al., 2024; Stévant et al., 2018).

Oil holding capacity (OHC) showed minimal variation among drying treatments, indicating that hydrophobic binding sites within the algal matrix were relatively preserved irrespective of drying conditions. This suggests that nonpolar structural components and lipid-associated interactions were minimally affected by the dehydration process.

Swelling capacity (SC) was highest in TD (26.41 mL/g), followed by SuD and FD. The enhanced SC observed in TD suggests that moderate thermal treatment promoted matrix relaxation and improved hydration-induced expansion of structural polysaccharides. In contrast, the comparatively rigid microstructure preserved during freeze drying may have limited volumetric expansion despite comparable OHC values (Stévant et al., 2018). Overall, the results indicate that drying conditions differentially modified the structural and hydration properties of Kai powder, thereby influencing its techno-functional behavior.

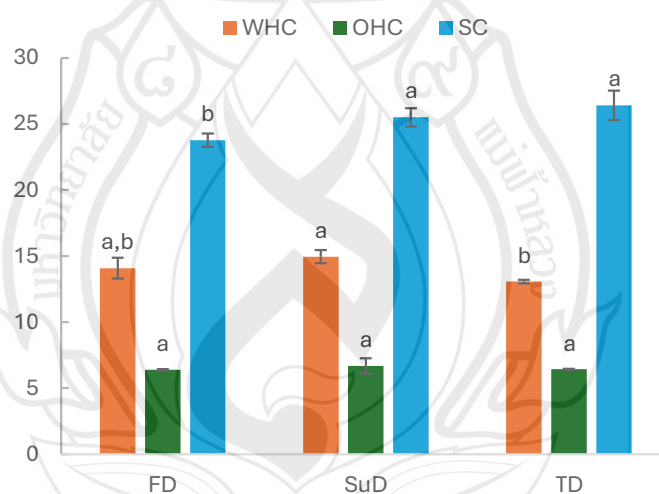


Figure 4.9 Water Holding, Oil Holding and Swelling Capacity of Freeze Dried (FD), Sun Dried (SuD) and Tray Dried (TD) Kai Samples. Different superscript letters above the bar indicate statistically significant differences between treatments ($p < 0.05$)

4.1.3.2 Foam formation and stability

Foam formation and stability were clearly decoupled by the different drying methods (Figure 4.10). TD generated the highest initial foaming capacity 44.55 %, FD showed slightly lower values, and the lowest observed in SD 27.39 %, indicating that

moderate heating may enhance air incorporation through partial unfolding of proteins and structural modification of polysaccharides (Li et al., 2023). However, TD foam collapsed rapidly during storage while FD and SD retained substantially higher foam volumes over 90 min. These patterns suggest that the structural changes induced by TD favor foam formation but yield less stable interfacial films coalescence (Tagliapietra et al., 2024; Stévant et al., 2018). FD and SD better preserve interactions that support cohesive, more stable foams. Meanwhile, FD preserves intermolecular interactions, such as hydrogen bonds and hydrophobic interactions, while SD induces mild oxidative cross-linking, both of which favor cohesive and viscoelastic interfacial layers that slow liquid drainage and maintain foam volume (Tagliapietra et al., 2024). FD and SD powders are more suitable for applications requiring stable foams, whereas TD is better suited for systems where rapid foam collapse is desirable.

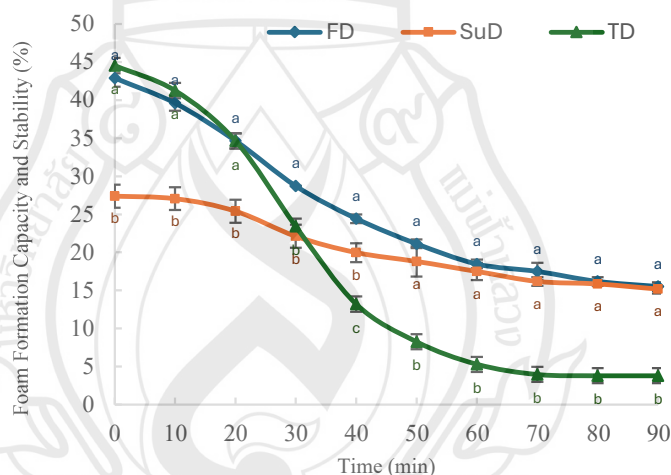


Figure 4.10 Foam Formation Capacity and Stability of Freeze Dried (FD), Sun Dried (SuD) and Tray Dried (TD) Kai Samples. Different superscript letters above the line graph indicate statistically significant differences between treatments ($p < 0.05$)

4.1.3.3 Flowability and Emulsifying Properties

Drying method had limited influence on powder flow properties (Table 4.4). Loose bulk density and tapped bulk density were slightly higher in TD; however, all samples exhibited Carr's index values above 30 % and Hausner ratios around 1.5,

corresponding to poor to very poor flowability according to European Pharmacopoeia classifications (Pugliese et al., 2017).

In contrast, emulsifying properties were more sensitive to drying conditions. FD exhibited the highest emulsifying capacity (63.33 %) and emulsifying stability (86.61 %), followed by SuD and TD (Table 4.4). The superior emulsifying performance of FD may be attributed to preservation of the native microstructure and interfacial functionality of proteins and polysaccharides during low-temperature dehydration. Conversely, thermal and oxidative stresses associated with TD and SuD may have partially altered surface-active components, thereby reducing their ability to form and stabilize oil–water interfacial films. Overall, the results indicate that freeze drying more effectively preserved the functional characteristics required for emulsion stabilization.

Table 4.4 Flowability and Emulsifying Properties of Freeze Dried (FD), Sun Dried (SuD) and Tray Dried (TD) Kai Samples

Analysis	FD	SuD	TD
Loose Density (g/cm ³)	0.112 ± 0.003 ^{ab}	0.108 ± 0.002 ^b	0.116 ± 0.002 ^a
Tapped Density (g/cm ³)	0.170 ± 0.007 ^{ab}	0.161 ± 0.004 ^b	0.179 ± 0.002 ^a
Compressibility	34.33 ± 4.51 ^a	32.67 ± 1.15 ^a	35.33 ± 0.58 ^a
Hausner Ratio	1.53 ± 0.11 ^a	1.49 ± 0.03 ^a	1.55 ± 0.01 ^a
Emulsifying Capacity (EC)	63.33 ± 0.42 ^a	61.81 ± 1.05 ^b	60.97 ± 0.24 ^b
Emulsifying Stability (ES)	86.61 ± 2.74 ^a	77.51 ± 1.71 ^b	76.99 ± 2.17 ^b

Note Values are presented as mean ± standard deviation (SD). Different superscript letters within the same row indicate statistically significant differences between treatments ($p < 0.05$).

4.1.3.4 Microstructure (SEM)

Scanning electron microscopy (SEM) analysis (Figure 4.11) revealed distinct morphological alterations in Kai filaments that provide structural validation for the techno-functional properties reported in Section 3.4. FD samples exhibited elongated and relatively smooth filaments with well-defined structural contours and minimal cellular disruption, indicating effective preservation of the native polysaccharide-protein matrix. This preserved microstructure was consistent with the superior emulsifying capacity and stability observed in FD, suggesting that low-

temperature dehydration helped maintain the structural integrity of proteins and polysaccharides associated with emulsifying functionality.

In contrast, SuD produced highly convoluted and shrunken filament structures, indicative of cellular collapse under prolonged ambient drying conditions. These structural modifications may have increased the exposure of hydrophilic binding sites, corresponding with the higher water holding capacity observed in SuD samples.

TD displayed uniformly twisted filaments reflecting controlled thermal deformation of the matrix, validating the enhanced swelling capacity measured experimentally. These SEM observations provide direct microstructural evidence supporting the structure-function relationships established through physicochemical analyses.

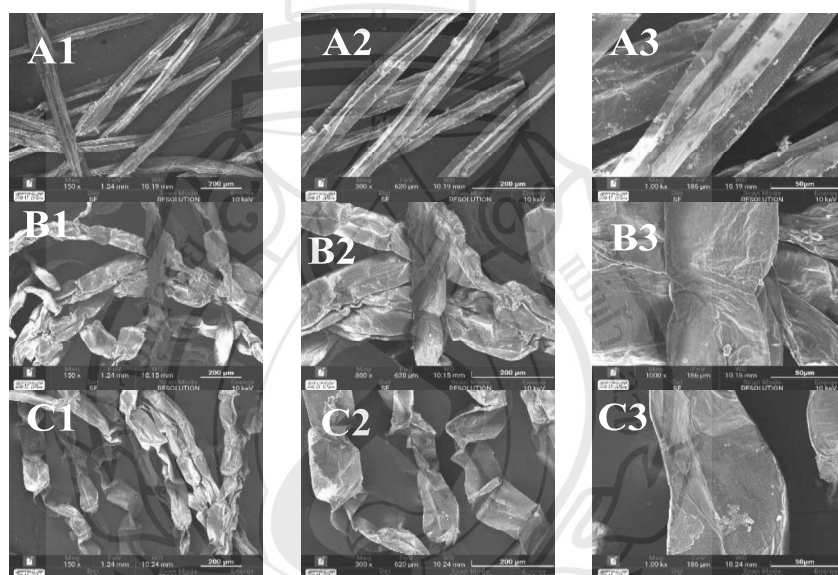


Figure 4.11 Microstructural Characteristics of Kai Samples Subjected to Different Drying Methods. A–C show representative images of FD, SD, and TD samples, respectively: (A1–C1) 150×, (A2–C2) 300×, and (A3–C3) 1000× micrographs (SEM) of Kai powders

4.1.3.5 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra of Kai samples showed the typical bands of polysaccharides and proteins, indicating that the main chemical groups were preserved across all drying methods (Figure. 12). All samples exhibited characteristic bands near 1640 cm^{-1} and 1540 cm^{-1} , assigned to amide I and amide II vibrations of proteins, confirming

proteinaceous components in the powders (Kačuráková & Wilson, 2001; Fleury & Lahaye, 1991). The absence of marked peak shifts among drying methods suggests that protein secondary structures were largely preserved, with only slight broadening of the amide I band in TD indicating minor thermally induced rearrangement. In the carbohydrate dominated region (approximately 1200–900 cm^{-1}), FD powders generally exhibited slightly sharper or more defined peaks, suggesting a more ordered polysaccharide structure, whereas TD and SD samples showed broader, less resolved bands consistent with partial structural rearrangement under thermal or environmental stress (Tagliapietra et al., 2024; Kačuráková & Wilson, 2000; Zhang et al., 2024).

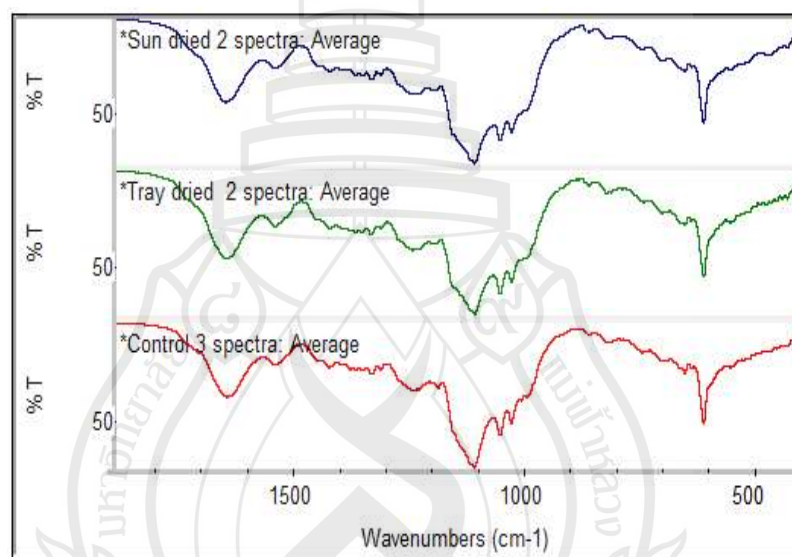


Figure 4.12 FTIR Spectra of Kai Samples Subjected to Different Drying Methods

In summary, each drying method imparted distinct physicochemical and functional characteristics to Kai powder. FD preserved native structural integrity, resulting in superior emulsifying properties, lower water activity, and enhanced anti-inflammatory activity at higher non-cytotoxic concentrations. SD exhibited the highest total phenolic content, DPPH radical scavenging activity, water holding capacity, and *in vitro* protein digestibility; however, its dependence on environmental conditions and prolonged drying time limited its practical scalability. In contrast, tray drying at 50°C provided the most favorable balance of nutritional quality, bioactive retention, and techno-functional properties, including the highest protein (13.95%), chlorophyll (4.41 mg/g), flavonoid (6.60 mg QE/g), and antioxidant contents (FRAP: 20.86 mmol Fe^{2+} /g;

ABTS: 1.56 mg TE/g), together with the greatest absolute digestible protein yield (9.57 g/100g). The moderate thermal treatment during TD induced controlled structural modifications that enhanced swelling capacity (26.41 mL/g) while maintaining adequate water holding behavior and rapid foam collapse characteristics particularly suitable for extrusion-based food printing applications. Additionally, TD demonstrated favorable low-dose anti-inflammatory potency through Nrf2/HO-1 pathway modulation and offered practical advantages including shorter drying time (6 hours), moderate energy requirements, and scalable processing conditions suitable for industrial application. Collectively, these findings indicate that TD provided the most suitable balance of nutritional quality, functional performance, and processing characteristics for subsequent 3D-printing applications. Accordingly, TD Kai powder was selected as the base material for developing 3D-printable dysphagia-friendly food formulations. The following section 4.2 describes the formulation optimization of 3D-printed dysphagia-friendly foods using TD Kai powder with XG and konjac gum KG as hydrocolloid modifiers.

4.2 Developing 3D Printed Dysphagia Friendly Algal Food

4.2.1 Response Surface Methodology (RSM) Optimization of Kai Food Ink

After preparation and printing of the 16 formulations generated from the mixture optimal design, the printed samples were analyzed for their textural and structural properties. RSM is an effective statistical tool for optimizing multi-variable food systems (Bezerra et al., 2008) and 3D-printable food inks (Zhang et al., 2022). TPA was performed to determine hardness, adhesiveness, and cohesiveness. Syneresis was also evaluated, while the dimensions of the printed samples were measured to determine print accuracy and deformation. These measured responses were then used to evaluate the effects of XG and KG concentrations and RSM optimization. The measured responses of all formulations are presented in Table 4.5.

4.2.1.1 Variation in Measured Responses Across Formulations

The experimental data showed considerable variation among the investigated formulations, indicating that the structural, physical, and handling

properties of the Kai algae food matrix were strongly influenced by the hydrocolloid composition.

As presented in Table 4.6, hardness exhibited the highest variability within the design space, ranging from 604.23 N/m² (Run 2: 0 % XG, 9 % KG) to 3310.52 N/m² (Run 4: ~6.91 % XG, ~2.09 % KG). This wide distribution (SD = 706.12 N/m²) demonstrates that the mechanical strength of the printed samples could be adjusted by modifying the concentrations and ratios of XG and KG. Formulations containing higher concentrations of both gums, such as Runs 11 and 14, produced stronger and more self-supporting structures, whereas formulations with lower total gum concentrations exhibited lower resistance to compression. (Mallesham et al., 2024; Xing et al., 2022).

In contrast, print accuracy remained relatively consistent across all formulations, ranging from 91.87 % to 96.10 %, with a low standard deviation of 1.13 %. The high print accuracy indicates that the printed structures closely matched the dimensions of the designed model, reflecting good shape retention after extrusion. This suggests that the combination of finely sieved tray-dried Kai powder and hydrocolloid hydration provided stable flow behavior and sufficient structural support during printing, enabling uniform deposition through the 1.5 mm nozzle while minimizing defects such as spreading, filament collapse, or nozzle blockage (Liu et al., 2018).

Syneresis values remained within a narrow range from 1.539 % to 2.835 %, with a standard deviation of 0.35 %, indicating good water retention stability among the formulations. This suggests that the hydrocolloid network effectively immobilized water within the matrix and minimizes phase separation during and after printing.

Deformation values ranged from 0.07 % to 2.39 %, showing clear differences in structural stability after printing. Binary formulations containing balanced proportions of XG and KG, particularly Runs 11 and 14, exhibited very low deformation values (<0.20 %), indicating improved shape retention and structural stability. This behavior suggests synergistic viscoelastic interactions between the two hydrocolloids, where XG contributed shear-thinning behavior and KG enhanced elastic recovery and structural reinforcement (Yu et al., 2023).

Table 4.5 Experimental Design and Measured Responses of Kai Inks

Factor		Response						
XG	KG	Hardness (N/m ²)	Adhesiveness (J/m ³)	Cohesiveness	Print Accuracy (%)	Syneresis	Deformation (%)	
2.07	4.32	1082.01	168.012	0.5753	94.07	2.27	0.92	
0	9	604.23	188.328	0.5699	94.08	2.27	1.89	
0.60936	2.39064	954.46	153.142	0.4122	92.66	2.717	2.39	
6.90978	2.09022	3310.52	187.014	0.4894	94.65	2.27	0.49	
5.265	0	986.15	165.061	0.4469	93.02	2.27	1.64	
5.265	0	954.96	165.253	0.4458	93.16	2.27	1.06	
0.225	5.67	1050.78	175.071	0.6281	94.19	2.835	1.22	
2.07	4.32	1079.18	172.172	0.5781	94.19	2.27	0.83	
9	0	1050.45	162.439	0.4894	94.45	1.767	0.22	
0.225	5.67	1114.12	172.545	0.6256	94.25	2.545	1.31	
4.365	4.14806	2132.74	183.094	0.5742	96.10	1.767	0.07	
0.60936	2.39064	954.16	140.068	0.4998	93.02	2.717	2.03	
3	0	954.96	126.445	0.4263	91.87	2.27	2.19	
4.365	4.14806	2355.56	180.993	0.5788	96.01	1.539	0.19	
4.14	1.935	1909.92	165.195	0.4933	93.15	2.27	0.94	
2.43003	6.56997	1273.28	189.129	0.6647	94.50	2.27	0.39	

Table 4.6 Summary Statistics of Measured Responses

Response	Min	Max	Mean	Standard Deviation
Hardness (N/m ²)	604.23	3310.52	1360.47	706.12
Adhesiveness (J/m ³)	126.45	189.13	168.37	17.29
Cohesiveness	0.412	0.665	0.531	0.078
Print Accuracy (%)	91.87	96.1	93.96	1.13
Syneresis (%)	1.54	2.84	2.27	0.35
Deformation (%)	0.07	2.39	1.15	0.76

4.2.1.2 Model Fitting and Statistical Adequacy

To evaluate the relationship between xanthan gum (XG, x_1), konjac gum (KG, x_2), and the measured responses of the Kai algae food ink, regression models were developed using Response Surface Methodology (RSM). The regression coefficients, determination coefficients (R^2 , Adjusted R^2 , and Predicted R^2), and Adequate Precision values are presented in Table 4.7.

The developed models showed good fitting and predictive capability for all measured responses. The coefficients of determination (R^2) for hardness (0.9750), syneresis (0.9628), cohesiveness (0.9472), and deformation (0.9314) indicated that more than 93 % of the observed variations were explained by the hydrocolloid composition. Even the lowest model fitting, observed for print accuracy ($R^2 = 0.8456$), remained within the acceptable range for biological and food-based systems (Liu et al., 2018).

The reliability of the models was further supported by the close agreement between Adjusted R^2 and Predicted R^2 values, where all differences remained below 0.20. This suggests that the models had good predictive stability with minimal overfitting. In addition, Adequate Precision values for all responses were higher than the recommended minimum value of 4.00, ranging from 12.6276 for syneresis to 27.0875 for hardness. These results indicate that the generated models had sufficient signal-to-noise ratios and could be used to predict and navigate the experimental design space effectively (Čukelj Mustač et al., 2023; Xing et al., 2022).

Based on the regression coefficients shown in Table 4.3, the predictive equations for the measured responses were generated as follows:

$$\text{Hardness (N/m}^2\text{)} = 582.11 + 133.32x_1 + 237.71x_2 - 86.79x_1x_2 - 8.97x_1^2 - 25.53x_2^2 + 30.35x_1^2x_2 \text{ (Eq. 4.1)}$$

$$\text{Adhesiveness (J/m}^3\text{)} = 126.26 - 5.39x_1 + 10.32x_2 + 3.82x_1x_2 - 0.35x_1^2 - 0.31x_1^3 \text{ (Eq 4.2)}$$

$$\text{Cohesiveness} = 0.41 + 0.01x_1 - 0.02x_2 + 0.02x_2^2 - 0.31x_1^3 - 0.002x_2^3 \text{ (Eq. 4.3)}$$

$$\text{Print Accuracy (\%)} = 90.68 + 0.41x_1 + 0.93x_2 - 0.06x_2^2 \text{ (Eq. 4.4)}$$

$$\text{Syneresis (\%)} = -15.35 + 11.13x_1 + 10.98x_2 - 3.94x_1x_2 - 2.12x_1^2 - 1.99x_2^2 + 0.34x_1^2x_2 + 0.32x_1x_2^2 \text{ (Eq. 4.5)}$$

$$\text{Deformation (\%)} = 0.03 - 0.003x_1 - 0.01x_2 - 3.94x_1x_2 + 0.0005x_2^2 + 0.12x_1^3 + 0.11x_2^3 \text{ (Eq 4.6)}$$

The regression equations (Eq. 4.1-4.6) demonstrate that the concentrations of XG and KG significantly influenced the structural and physical properties of the Kai algae food ink. Positive linear coefficients for hardness and print accuracy indicate that increasing hydrocolloid concentration generally improved structural strength and printing performance. In contrast, the opposite linear effects observed for adhesiveness suggest that KG increased surface stickiness, while XG helped reduce excessive adhesiveness.

The presence of quadratic and cubic terms in several models indicates that the responses were affected by non-linear interactions between the two hydrocolloids. These interactions suggest that balanced combinations of XG and KG contributed to improved structural stability, controlled adhesiveness, and enhanced printability of the Kai algae food ink.

Table 4.7 Regression Coefficients and Model Statistics for RSM Models

	Hardness	Adhesiveness	Cohesiveness	Print Accuracy	Syneresis	Deformation
Intercept	582.10615	126.25808	0.406238	90.68451	-15.34678	0.034057
x ₁	133.31718	-5.39286	0.008279	0.412844	11.13468	-0.003379
x ₂	237.71268	10.32262	-0.015666	0.925064	10.97804	-0.006094
x ₁ x ₂	-86.78666	3.82066			-3.94137	
x ₁ ²	-8.97086	-0.348821			-2.1184	
x ₂ ²	-25.5254		0.019085	-0.061359	-1.99333	0.000465
x ₁ ² x ₂	30.35336				0.337902	
x ₁ x ₂ ²					0.322513	
x ₁ ³		-0.308352			0.121387	
x ₂ ³			-0.001701		0.110116	
R ²	0.975	0.9147	0.9472	0.8456	0.9628	0.9314
Adjusted R ²	0.9583	0.8721	0.9279	0.807	0.907	0.9143
Predicted R ²	0.9074	0.7156	0.8712	0.7679	0.8443	0.7366
Adequate Precision	27.0875	14.4688	19.9519	13.4858	12.6276	21.575

4.2.1.3 Evaluation of Individual Responses & Response Surface Analysis

To evaluate the interactive effects of XG and KG on the structural properties of the Kai algae food ink, two-dimensional contour plots were analyzed for each measured response. These plots illustrate how changes in hydrocolloid composition influenced the textural and structural characteristics of the printed formulations.

1. Interactive Effects on Textural Attributes

The texture profile of the printed formulations is important for both structural stability and dysphagia suitability. The contour plots for hardness, adhesiveness, and cohesiveness are discussed below to explain the effects of XG and KG interactions within the formulation system.

1) Hardness

The hardness of the printed Kai algae formulations was significantly influenced by the proportions of XG and KG and was adequately described by a special cubic model, ranging from 604.23 to 3310.52 (Figure 4.13). The contour plot demonstrated that hardness was strongly influenced by the proportions of XG and KG in the formulation. Higher hardness values were mainly observed at elevated XG concentrations (6–7 %) combined with low-to-moderate KG concentrations (~2 %). This suggests that formulations containing higher proportions of XG and moderate proportions of KG exhibited greater mechanical strength. The observed response indicates that hardness was influenced by the combined effects of both hydrocolloids, with the XG ratio playing an important role in determining the structural properties of the printed matrix (Chao et al., 2024; Tako, 1992).

In contrast, formulations containing high KG concentrations and low XG levels exhibited lower hardness values. The curved contour lines indicate a non-linear relationship between hydrocolloid composition and hardness, suggesting that the effect of each component depended on the proportion of the other component. Furthermore, hardness tended to decrease at higher KG concentrations, indicating that increasing KG beyond a certain level was not beneficial for hardness development. Similar non-linear responses have been reported in hydrocolloid systems, where the functional properties depend on the balance and interactions between polymers rather than their individual concentrations alone (Qiao et al., 2023; Yu et al., 2023).

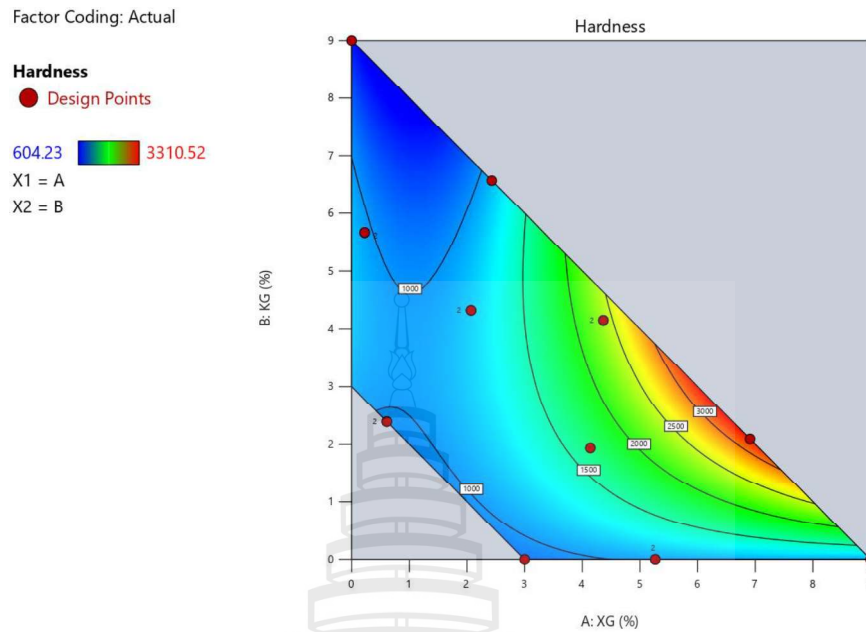


Figure 4.13 Contour Plot Showing the Interactive Effects of Xanthan Gum (XG) and Konjac Gum (KG) Concentrations on Hardness of 3D-printed Kai Algae Food Inks

2) Cohesiveness

Cohesiveness, which represents the internal bonding strength and resistance to structural breakdown during chewing, showed a third-order cubic relationship with values ranging from 0.4122 to 0.6647 (Figure 4.14). The contour plot indicated that cohesiveness was strongly influenced by the relative proportions of XG and KG in the formulation. Higher cohesiveness values were observed in formulations containing higher proportions of KG, suggesting that KG played an important role in enhancing the internal structural integrity of the printed matrix. This observation is consistent with previous studies reporting the gel-forming ability of KG and its contribution to the development of cohesive food structures (Cheng et al., 2024).

In comparison, changes in XG concentration produced relatively smaller effects on cohesiveness, suggesting that XG mainly contributed to viscosity enhancement and flow resistance rather than internal gel strength (Wei et al., 2021). The contour lines also showed that formulations with low KG concentrations exhibited the lowest cohesiveness values, particularly in regions containing high XG and minimal KG.

Balanced combinations of XG and KG at intermediate concentrations produced moderate cohesiveness values without excessively high stickiness. This suggests that combining the two hydrocolloids contributed to improved structural balance and cohesive bolus formation, which are desirable characteristics for dysphagia-oriented food applications (Xing et al., 2022).

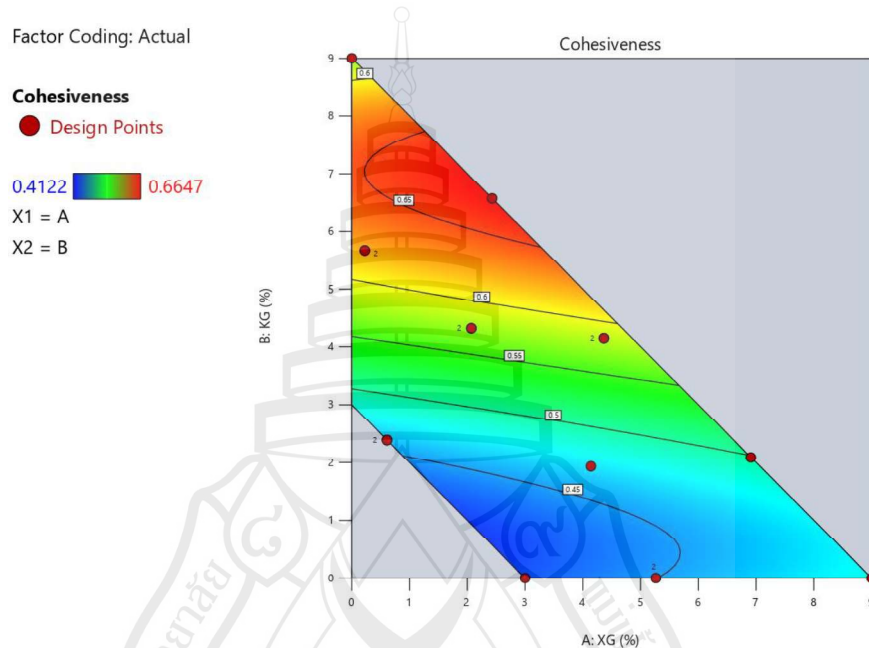


Figure 4.14 Contour Plot Showing the Interactive Effects of Xanthan Gum (XG) and Konjac Gum (KG) Concentration on Cohesiveness of 3D-printed Kai Algae Food Inks

3) Adhesiveness

Adhesiveness, which represents the work required to detach the food surface from oral tissues, ranged from 126.26 to 189.13 J/m³ and showed a curved response pattern across the design space (Figure 4.15). The contour plot demonstrated that adhesiveness was strongly influenced by the relative proportions of XG and KG in the formulation. Higher adhesiveness values were generally observed in formulations with total gum (>7 %) containing high and moderate proportions of KG, suggesting that KG contributed substantially to the development of surface stickiness. This observation may be associated with the water-binding capacity and hydrophilic nature

of KG, which can increase the adhesion of hydrocolloid-based systems (Chao et al., 2024).

In comparison, changes in the proportion of XG resulted in relatively smaller variations in adhesiveness. The contour lines indicated that formulations containing moderate-to-high proportions of KG exhibited the highest adhesiveness values, whereas formulations with lower KG proportions showed reduced surface stickiness. Furthermore, formulations containing balanced proportions of XG and KG exhibited moderate adhesiveness values, indicating that the response depended on the relative proportions of the two hydrocolloids rather than on either component alone (Wei et al., 2021).

This reduction may occur due to intermolecular interactions between XG and KG, which promote the formation of a more integrated gel network and reduce the exposure of hydrophilic groups on the surface of the printed formulations (Qiao et al., 2023). Controlled adhesiveness is important for dysphagia-oriented foods because excessive stickiness may increase the risk of food residues remaining in the oral or pharyngeal cavity after swallowing (Xing et al., 2022).

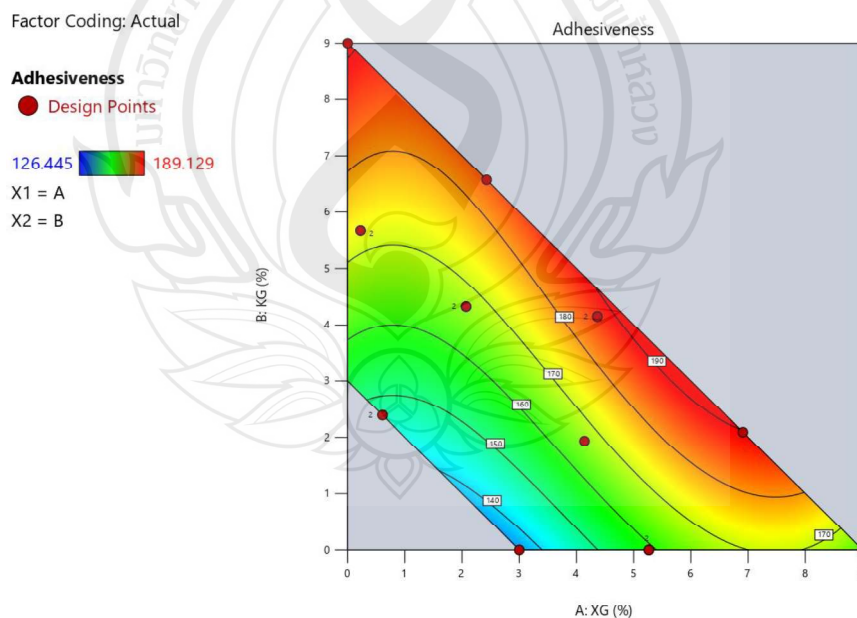


Figure 4.15 Contour Plot Showing the Interactive Effects of Xanthan Gum (XG) and Konjac Gum (KG) Concentrations on Adhesiveness of 3D-Printed Kai Algae Food Inks

2. Interactive Effects on Printability

The structural retention and processing fidelity of printed Kai constructs are governed by the balance between the ink's shear-thinning behavior and its mechanical yield stress after extrusion. Print accuracy and deformation displayed highly significant quadratic relationships, demonstrating sharp sensitivity to the XG-KG blending ratio.

1) Print Accuracy

Print accuracy ranged from 91.87 % to 96.10 % and was influenced by the combined concentrations of XG and KG (Figure 4.16). These values indicate a high degree of agreement between the printed structures and the intended design geometry. Although no universal print accuracy percentage has been established, dimensional quality criteria summarized by Čukelj Mustač et al. (2023) suggest that satisfactory printing performance can be achieved when width consistency is maintained within ± 5 % of the designed width, printed line diameter does not exceed 130 % of the nozzle diameter, and die swell remains below 70 %.

The contour plot showed that higher print accuracy values were achieved at intermediate-to-high concentrations of both hydrocolloids, particularly in balanced mixed-gum regions. These formulations produced more defined and stable printed structures, indicating improved extrusion behavior and shape retention after deposition. Extrusion-based 3D printing requires a pseudoplastic fluid that undergoes rapid shear-thinning inside the nozzle for smooth flow, followed by an instantaneous recovery of viscosity and yield stress upon deposition to support successive layers (Voon et al., 2019). A comprehensive review on 3D-printed dysphagia foods highlighted that XG and KG are among the most effective hydrocolloids for enhancing rheological properties and enabling non-printable nutrient-rich foods to achieve IDDSI compliance (Molimi et al., 2025). Tracing the contour lines down to low total concentration regimes shows a drastic drop in accuracy. When total biopolymer levels are insufficient, the lack of an interconnected network causes the extruded ink strands to spread and bleed, losing geometric fidelity.

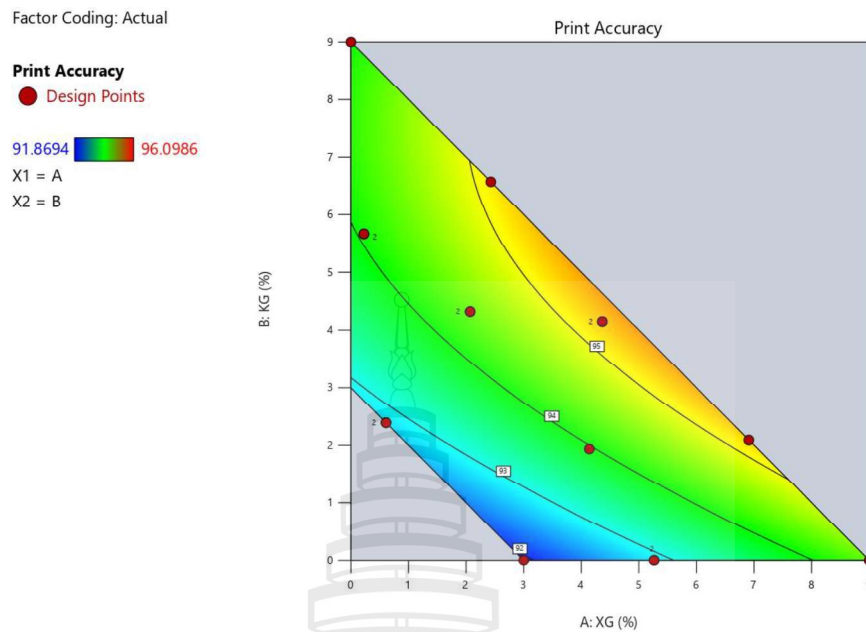


Figure 4.16 Contour Plot Showing the Interactive Effects of Xanthan Gum (XG) and Konjac Gum (KG) Concentrations on Print Accuracy of 3D-Printed Kai Algae Food Inks

2) Deformation

Deformation ranged from 0.07 % to 2.39 %, indicating differences in the structural stability of the printed formulations after deposition (Figure 4.17). The contour plot showed that the lowest deformation values were observed at high XG concentrations combined with low-to-moderate KG concentrations, particularly in the region around 5–8 % XG and 1–3 % KG. These formulations exhibited better shape retention and structural stability after printing.

In contrast, higher deformation values were mainly observed in formulations containing low XG concentrations with either very low or very high KG concentrations. This suggests that insufficient XG concentration weakened the structural framework of the printed formulations, resulting in greater collapse and deformation after deposition.

The reduced deformation observed in balanced XG–KG systems may be associated with the combined effects of the two hydrocolloids, where XG contributes shear-thinning behavior during extrusion while KG provides elastic recovery and structural reinforcement after printing (Cheng et al., 2024). Extrusion-

based 3D printing requires sufficient yield stress and viscoelastic properties to maintain shape fidelity after deposition (Outrequin et al., 2023). These findings were generally consistent with the rheological results, where formulations with lower flow behavior index (n) values together with higher consistency index (K) and yield stress values demonstrated improved printability and shape retention (Yu et al., 2023).

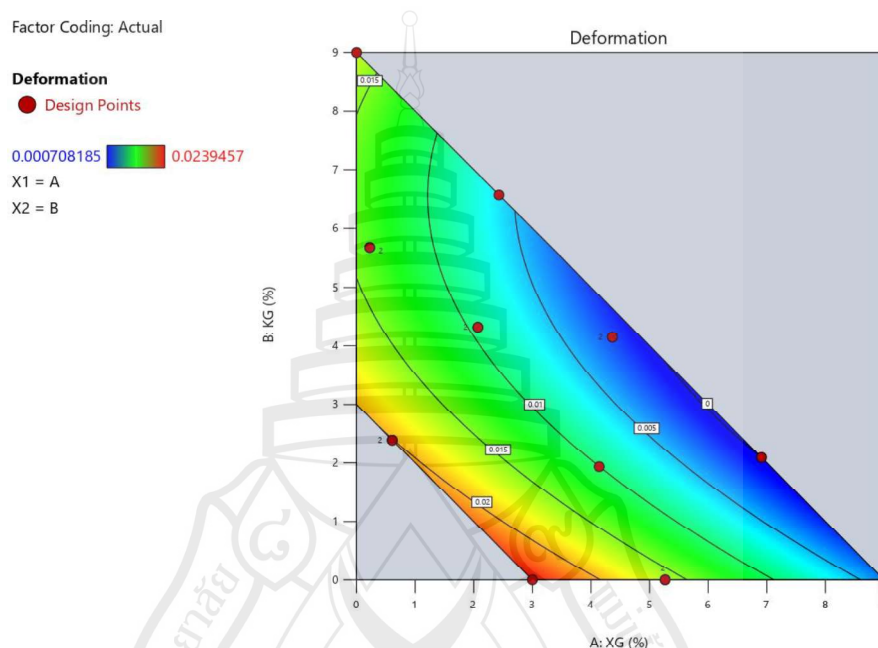


Figure 4.17 Contour Plot Showing the Interactive Effects of Xanthan Gum (XG) and Konjac Gum (KG) Concentrations on Deformation of 3D-Printed Kai Algae Food Inks

3. Interactive Effects on Syneresis

Syneresis, which represents water-holding capacity and phase stability during storage, ranged from 1.54 % to 2.84 % after 24 h storage at 4°C (Figure 4.18). Overall, all formulations exhibited relatively low syneresis values, indicating good water retention and phase stability within the Kai algae food ink system.

The contour plot showed that lower syneresis values were mainly observed at moderate-to-high concentrations of both XG and KG, particularly in balanced mixed-gum regions. In contrast, formulations containing low XG concentrations with intermediate KG levels exhibited relatively higher syneresis values.

These findings suggest that the interaction between XG and KG improved water immobilization within the hydrocolloid matrix.

The improved stability may be associated with the ability of XG to increase viscosity and reduce water mobility, while KG contributes to the formation of a three-dimensional gel network capable of retaining water through hydrogen bonding interactions (Qiao et al., 2023).

The contour plot also indicated that the reduction in syneresis became less pronounced at very high hydrocolloid concentrations, suggesting limited additional improvement in water retention at elevated gum levels. This behavior may be related to competition for water binding and localized polymer aggregation within the system (Yu et al., 2023). Overall, the low syneresis values observed across the formulations indicate that the XG–KG hydrocolloid system provided sufficient phase stability and minimized water separation during storage.

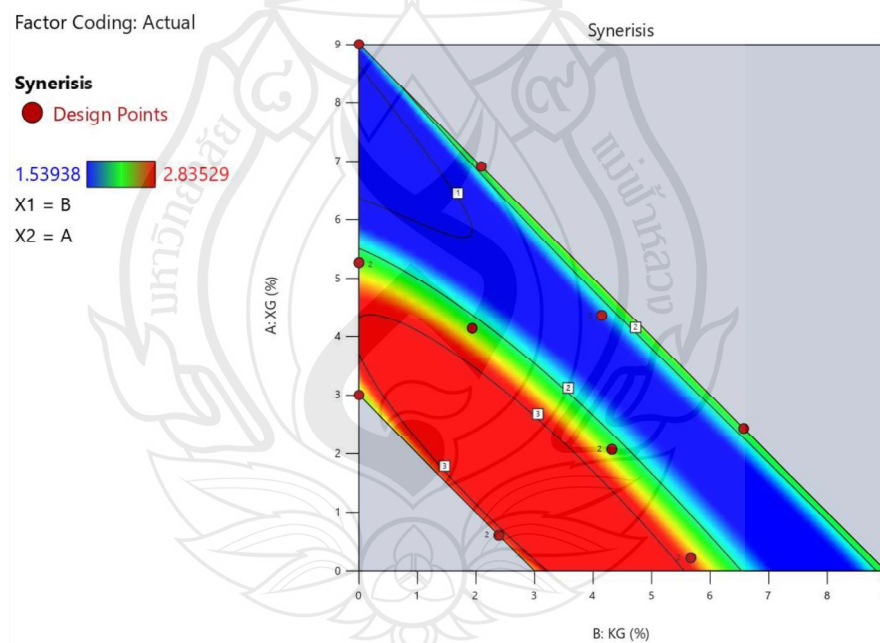


Figure 4.18 Contour Plot Showing the Interactive Effects of Xanthan Gum (XG) and Konjac Gum (KG) Concentrations on Syneresis of 3D-Printed Kai Algae Food Inks

4.2.1.4 Numerical Multi-Response Optimization

Multi-response optimization was conducted to determine the optimum blending ratio of XG (x_1) and KG (x_2). The optimization aimed to develop a formulation that balanced printability, structural stability, and texture properties suitable for dysphagia-oriented food applications. By simultaneously evaluating all measured responses through the generated mathematical models, the optimization process identified a formulation region capable of maximizing structural integrity while minimizing deformation and excessive adhesiveness after printing.

The optimization identified an optimum formulation region with a maximum desirability value ($D = 0.91$) at a combination of 5.265 % XG and 3.186 % KG (Table 4.8, Figure 4.19). This optimized formulation provided the best balance between printability, structural stability, and dysphagia-oriented texture properties among the developed formulations. The formulation exhibited high predicted print accuracy (95.2 %) together with minimal deformation (0.002 %), indicating good shape retention and post-print structural stability.

Under the optimized conditions, the predicted hardness, adhesiveness, and cohesiveness values were 2758.87 N/m², 188.11 J/m³, and 0.539, respectively. These predicted values satisfied the Japanese Dysphagia Diet 2021 Criteria I and UDF Stage 4 requirements, indicating potential suitability for individuals with severe swallowing difficulty. Similarly, Zhu et al. (2024) reported that pea protein-based dysphagia diets containing 0.5% xanthan gum exhibited optimal 3D printing behavior and successfully passed IDDSI Level 4 classification, supporting the effectiveness of hydrocolloid addition for texture-modified food development. The optimized formulation also demonstrated favorable predicted stability characteristics, including low syneresis (1.539 %) and minimal deformation, suggesting that the XG–KG hydrocolloid system provided suitable structural and textural properties for dysphagia-oriented food applications.

Table 4.8 Optimized Formulation and Predicted Responses

Solution	XG	KG	Hardness	Adhesiveness	Cohesive	Print Accuracy	Syneresis	Deformation	Desirability
1	5.265	3.186	2758.871	188.107	0.539	0.952	1.539	0.002	0.91

All Responses

● Design Points

0.000 1.000

X1 = A

X2 = B

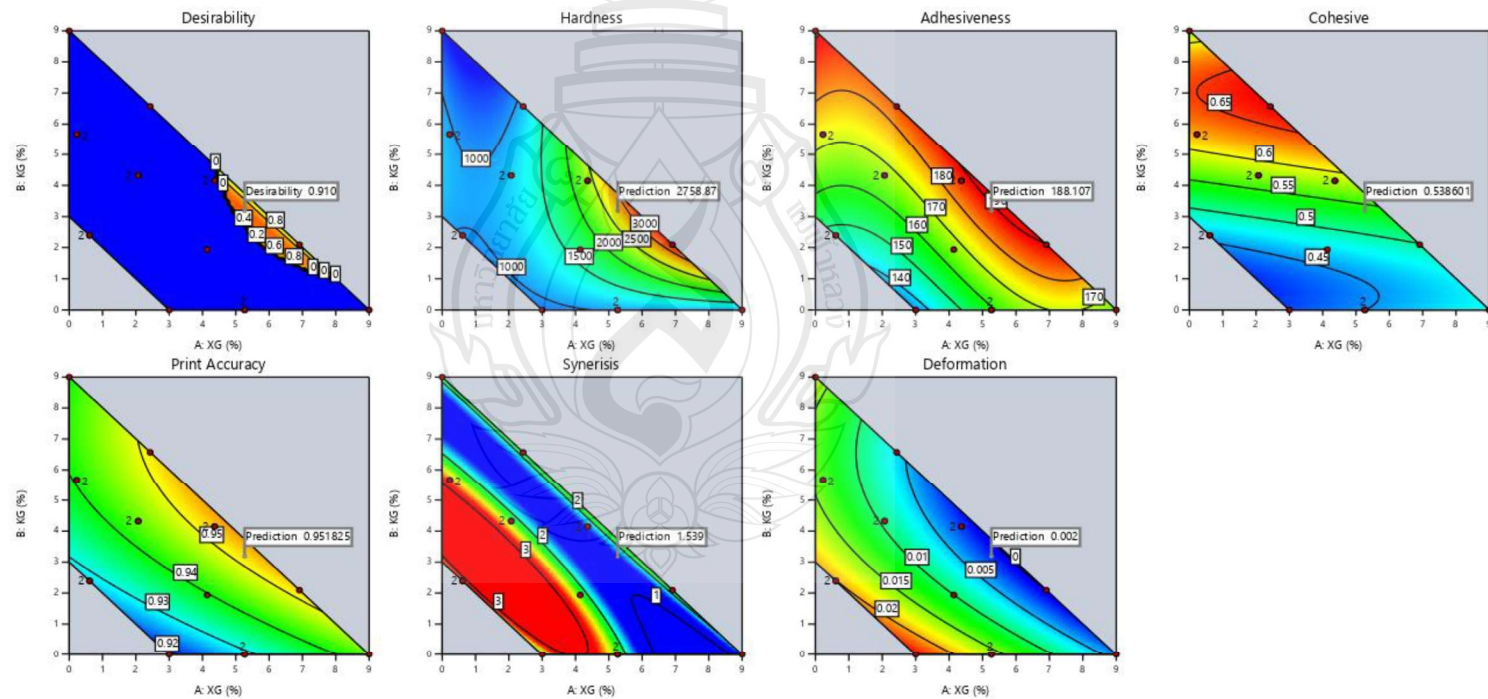


Figure 4.19 Overlay Contour Plots and Desirability Function for Simultaneous Optimization of XG and KG on Multiple Responses

4.2.2 Visual Appearance of Printed Constructs

Representative images of the printed Kai formulations are presented in Figure 4.20 to evaluate the post-print structural integrity of samples prepared with different XG and KG combinations. All formulations retained their overall printed structure immediately after extrusion, indicating that the hydrocolloid systems provided sufficient viscosity and internal network strength to support layer deposition without complete collapse.

Minor variations in dimensional retention and structural stability were observed among formulations, suggesting that hydrocolloid concentration influenced post-print behavior. Certain formulations exhibited slight spreading or deformation after deposition, whereas others maintained their printed dimensions more effectively. These visual observations were generally consistent with the measured print accuracy and deformation values obtained during optimization, where balanced XG-KG systems demonstrated improved structural retention after printing.

No visible liquid separation or surface moisture release was observed in any formulation, indicating low syneresis and effective water immobilization within the hydrocolloid matrix. Furthermore, all printed samples remained self-supporting under ambient conditions without major structural failure. Overall, the results demonstrated that the combined XG-KG hydrocolloid system generated sufficient structural stability and dimensional retention for extrusion-based 3D printing of Kai-based dysphagia-oriented foods.

4.2.3 Visual Appearance of Printed Constructs

4.2.4 Integrated Dysphagia Evaluation Based on Japanese Texture Standards and IDDSI-Based Utensil Assessment

The 3D-printed Kai formulations were evaluated for dysphagia suitability by integrating texture profile analysis (TPA) with the Japanese Dysphagia Diet 2021 standards, the Universal Design Food (UDF) framework, and IDDSI Level 4 criteria (Table 4.9). Hardness, cohesiveness, and adhesiveness were used as the main textural parameters.

Run 4 showed the highest hardness (3310.52 N/m²) and was classified under Japanese Standard I, suitable for individuals with severe swallowing difficulty. Most other formulations fell within Standards II and III. However, hardness alone did not

fully represent swallowing behavior, as cohesiveness and adhesiveness also influenced bolus formation and utensil handling.

All formulations met the IDDSI Level 4 fork drip test requirement by maintaining structure without flowing through the tines. Spoon test performance varied depending on internal gel structure. Low-strength formulations (Runs 2, 3, 5, 6, 12, 13) spread on the spoon due to insufficient structural integrity. High-cohesiveness formulations (Runs 7, 9, 16) showed strong adhesion to the spoon, resulting in poor release behavior.

Run 15 also passed both fork and spoon tests; however, its application was limited by poor surface uniformity and reduced print accuracy, indicating that its limitation was related to printing performance rather than swallowing functionality.

Based on UDF classification, all formulations were categorized as Stage 4, as hardness values were below $5 \times 10^3 \text{ N/m}^2$. This was consistent with both Japanese standards and IDDSI Level 4 classification.

Overall, suitable dysphagia textures require a balance between hardness, cohesiveness, and adhesiveness to ensure proper bolus formation and spoon release. Run 4 showed the most balanced performance for severe dysphagia applications. Runs 1, 8, 10, 11, and 14 also demonstrated acceptable functional behavior. However, run 15 was excluded from final application due to poor print accuracy despite acceptable IDDSI performance.

Table 4.9 Cross-Framework Classification Matrix Combining Japanese Dysphagia Diet 2021 (Code 2) Criteria with UDI and IDDSI Level 4 Requirements

Formulation Group	Japanese Regulation	UDI	Observation Fork and Spoon Tests and Printed Sample	IDDSI Level 4 Status	Suitable for Application
Run 4	I	4	Fork: The sample remained cohesive on the fork without flowing through the tines. Spoon: The sample maintained its shape and slid off the spoon cleanly with minimal residue.	PASS	YES
Runs 1, 8, 10, 11, and 14	II	4	Fork: Samples retained their structure on the fork without dripping through the slots. Spoon: Samples formed a cohesive bolus and detached cleanly from the spoon surface.	PASS	YES
Run 15	II	4	Fork: The sample remained stable on the fork without dripping. Spoon: The sample detached cleanly from the spoon surface Note: Not smooth surface and poor 3D-printing accuracy (93.15 %) limited its suitability for final application.	PASS	NO (Printing limitation)
Runs 7, 9, and 16	II	4	Fork: Samples remained stable on the fork without dripping through the tines. Spoon: Samples failed the spoon tilt test due to excessive elasticity, resulting in stretching and strong adhesion to the spoon surface.	FAIL	NO
Runs 2, 3, 5, 6, 12, and 13	III	4	Fork: Samples initially retained their mass on the fork. Spoon: Samples failed the spoon tilt test because the weak structure collapsed and spread across the spoon surface instead of remaining cohesive.	FAIL	NO



Figure 4.20 Representative images of 3D-printed Kai-based samples (Run 1–16) with varying concentrations of XG and KG, along with IDDSI fork drip and spoon tilt test and syneresis test results



Figure 4.20 (continued)

4.2.5 Rheological Properties of Food Inks

To further explain the differences observed in printability performance and structural stability of the Kai-based dysphagia formulations, rheological characterization was performed using steady shear and oscillatory stress sweep analyses. The rheological behaviors of formulations containing XG and KG are presented in Figures 4.21-4.23.

As shown in Figure 4.21, all formulations exhibited decreasing viscosity with increasing shear rate, demonstrating typical pseudoplastic or shear-thinning behavior. Such behavior is desirable for extrusion-based dysphagia foods because the formulations maintain sufficient viscosity at rest while flowing more readily under applied shear during extrusion and swallowing (Yu et al., 2023). Differences in viscosity profiles among formulations suggested that the XG and KG influenced flow behavior of the food inks.

Stress sweep results are presented in Figure 4.22. In all formulations, the storage modulus (G') remained higher than the loss modulus (G'') within the linear viscoelastic region, demonstrating predominantly elastic behavior. With increasing applied stress, G' gradually decreased until the crossover point between G' and G'' was reached, representing structural breakdown and transition to viscous-dominant behavior. Mixed-gum systems generally maintained elastic behavior over a wider stress range than single-gum formulations, suggesting improved viscoelastic stability (Fan et al., 2026; Yu et al., 2023).

To further quantify the rheological properties, the steady shear data were fitted using the Power Law model. The apparent viscosity (η) as a function of shear rate ($\dot{\gamma}$) can be described by the following relationship: $\eta = K \cdot \dot{\gamma}^{(n-1)}$, where η is the apparent viscosity ($\text{Pa}\cdot\text{s}$), $\dot{\gamma}$ is the shear rate (s^{-1}), K is the consistency index ($\text{Pa}\cdot\text{s}^n$), and n is the flow behavior index. This form directly describes the shear-thinning behavior observed in Figure 4.21, where viscosity decreases with increasing shear rate when $n < 1$. The Power Law fitting parameters (K , n , and R^2) and yield stress for all formulations are presented in Table 4.10, while the visual trends are shown in Figure 4.23.

Table 4.10 Power Law Rheological Parameters and Yield Stress of Kai-Based Food Inks

XG	KG	n	K	R ²	Yield Stress
2.07	4.32	0.2932	151844.80	0.9810	179
0	9	0.2862	227771.80	0.9892	170
0.60936	2.39064	0.1933	145915.00	0.9817	180
6.90978	2.09022	0.3271	130106.80	0.9836	118
5.265	0	0.3189	115637.80	0.9784	79
5.265	0	0.3184	115744.40	0.9783	80
0.225	5.67	0.3228	120143.40	0.9693	162
2.07	4.32	0.2938	151705.00	0.9814	178
9	0	0.3675	109345.30	0.9680	79
0.225	5.67	0.3215	120005.20	0.9702	125
4.365	4.14806	0.2587	188408.30	0.9802	232
0.60936	2.39064	0.1944	145680.00	0.9826	231
3	0	0.3193	93260.99	0.9791	65
4.365	4.14806	0.2581	188191.50	0.9806	232
4.14	1.935	0.2340	179225.60	0.9853	267
2.43003	6.56997	0.3386	174020.30	0.9840	176

Note K = consistency index; n = flow behavior index; R² = coefficient of determination from Power Law fitting ($\eta = K \cdot \dot{\gamma}^{(n-1)}$). Yield stress determined from crossover point of G' and G'' during stress sweep analysis.

All formulations exhibited n values lower than 1 (0.1933–0.3675), confirming typical shear-thinning behavior expected for extrudable food gels. The R² values ranged from 0.9680 to 0.9892, indicating that the Power Law model provided excellent fit to the experimental data across all formulations. Mixed XG-KG formulations generally exhibited higher K and yield stress values than single-gum systems, reflecting stronger gel network formation. Formulations with moderate-to-high yield stress values (180–232 Pa) generally showed higher print accuracy (0.945–0.961) and lower post-print deformation (<0.9 %), whereas formulations with very low yield stress values (65–80

Pa) exhibited lower print accuracy (0.918–0.931) and greater deformation (up to 2.4 %). This observation agrees with previous studies reporting that sufficient yield stress is important for maintaining shape fidelity after extrusion (Outrequin et al., 2023). However, the formulation with the highest yield stress value (267 Pa) did not exhibit the highest print accuracy, suggesting that excessive rigidity may impair extrusion stability. Similarly, the formulations with the lowest n values (0.193–0.194) showed comparatively higher deformation, indicating excessive structural breakdown under shear and reduced post-print stability.

Overall, balanced rheological behavior characterized by moderate-to-high yield stress and consistency together with adequate shear-thinning behavior generally provided improved print accuracy, lower post-print deformation, and firmer texture characteristics. In contrast, formulations with very low yield stress showed insufficient resistance to deformation, whereas excessively high yield stress reduced extrusion stability. Relationships between rheological behavior and cohesiveness, adhesiveness, or syneresis were less consistent, suggesting that these properties were additionally influenced by water distribution and formulation interactions within the matrix. These findings suggest that optimization of dysphagia-oriented food inks requires careful adjustment of the XG ratio to achieve sufficient viscosity and structural resistance for shape fidelity without excessively restricting flow behavior.

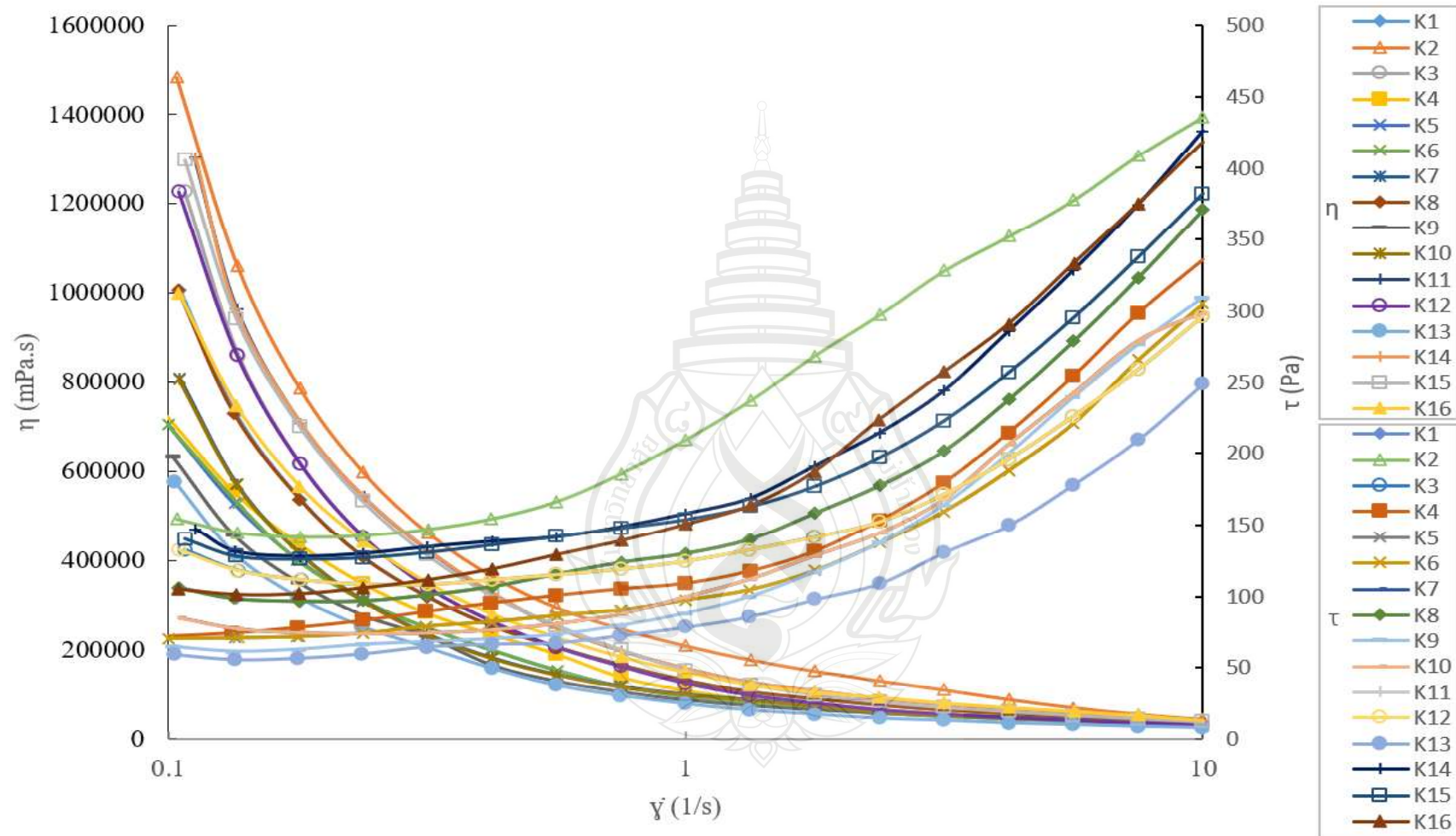


Figure 4.21 Apparent Viscosity and Shear Stress as a Function of Shear Rate of Kai-Based Food Inks Containing Different Ratios of XG and KG

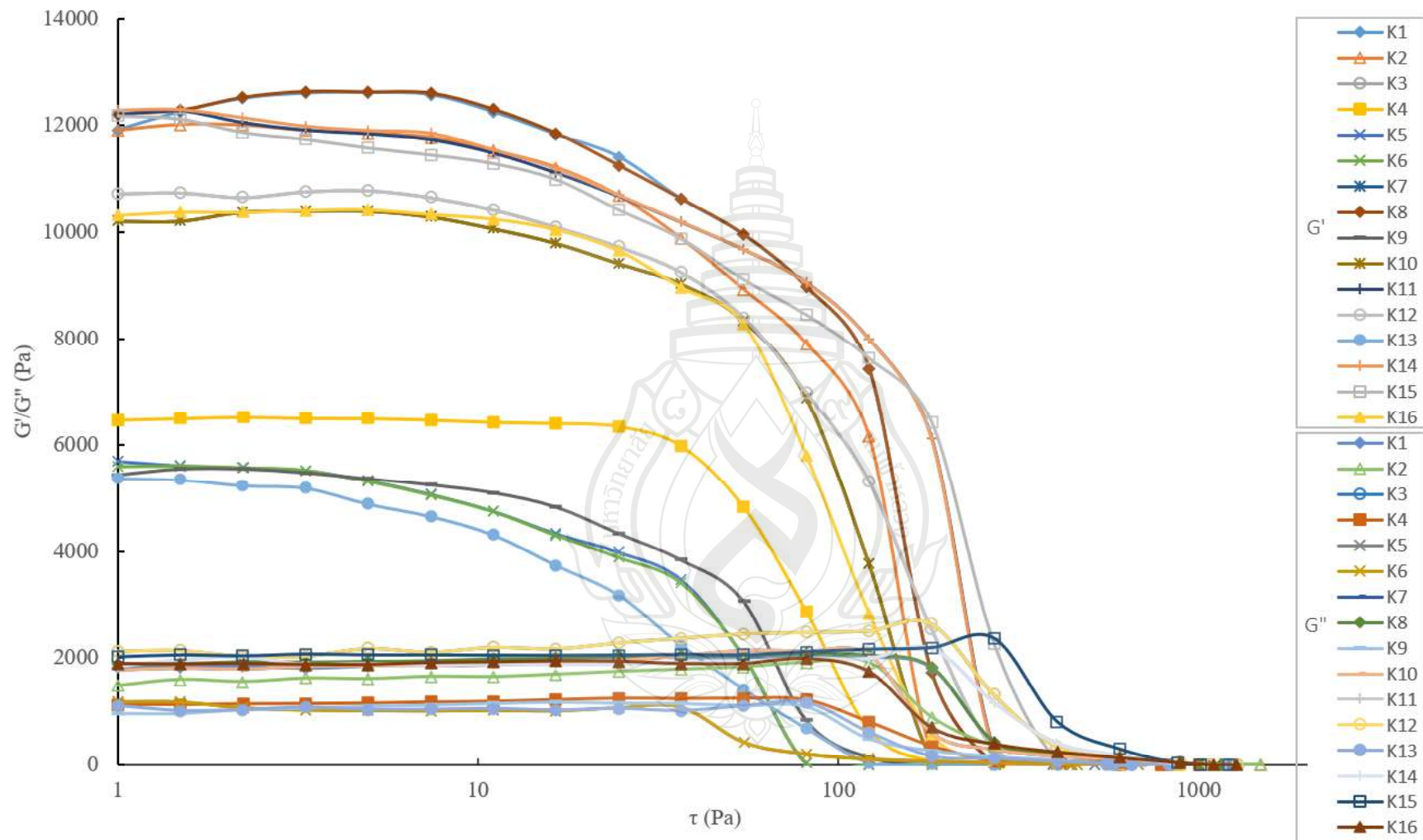


Figure 4.22 Stress Sweep Analysis of Kai-Food Inks with Different XG and KG Concentrations

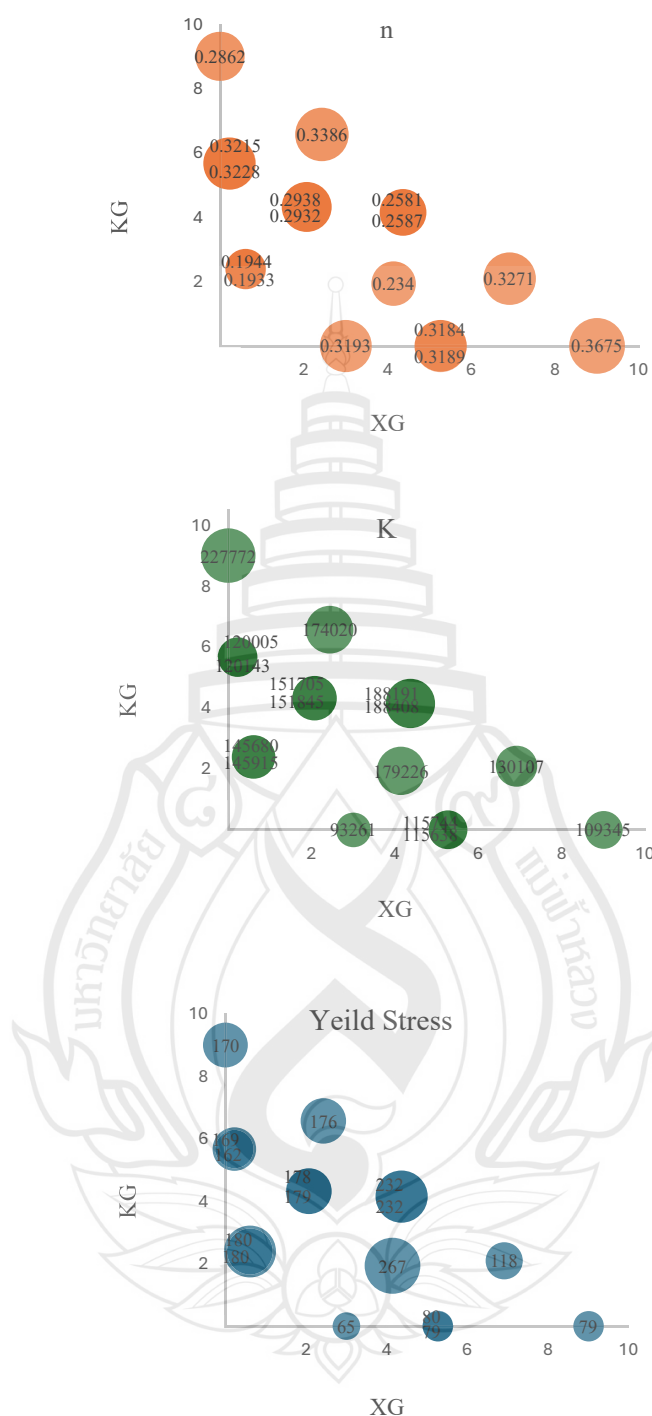


Figure 4.23 Effect of Xanthan Gum (XG) and Konjac Gum (KG) Concentration on the Rheological Parameters of Kai-Based Food Inks. Bubble size corresponds to the magnitude of the measured rheological parameter. Color intensity is used to differentiate replicated and non-replicated formulations in the optimal mixture design, with darker bubbles indicating replicate runs

4.2.6 Microstructure SEM

The microstructure of the 16 printed formulations was examined by SEM at 300× magnification for the top surface (Figure 4.24) and the cross-section (Figure 4.25). Both views were analyzed because surface topography reflects extrusion quality and printing fidelity, while the cross-section reveals the internal polymer network that governs mechanical strength, water immobilization and shape stability (Qiao et al., 2023; Yu et al., 2023).

The samples which contain only XG (Runs 13, 5, 6, 9) showed a progressive trend: as XG concentration increased, the top surface became smoother, and the cross-section evolved from a dense matrix to one containing visible pores. XG alone does not form true gels, but its rigid helical backbone can create a three-dimensional network when synergistically combined with mannan-based gums (Schreiber et al., 2020). In the absence of KG, however, pore structures remained incomplete. The KG-only sample (Run 2) had a smooth but fibrous surface and a dense, non-porous cross-section. Under neutral pH, KG does not fully deacetylate, which prevents stable junction zone formation and results in a weak, disorganized network (Penroj et al., 2005).

Low-gum formulations (Runs 3 and 12) exhibited rough surfaces and dense cross-sections with visible gaps, indicating that insufficient polymer concentration cannot sustain a continuous network. KG-dominant systems (Runs 7, 10 and 16) displayed surfaces ranging from medium-smooth to very rough, and cross-sections with incomplete pore development, large irregular voids or phase separation. KG-rich blends tend to form isolated, elastic domains that cause excessive adhesiveness (Yu et al., 2023; Yu et al., 2022). The XG-dominant formulation (Run 15) had a rough surface with holes but a dense cross-section; without sufficient KG to bridge the XG helices, an open pore architecture does not develop, and print accuracy remains limited.

Formulations with balanced XG:KG ratios produced clearly different microstructures. Runs 1 and 8 ($\approx 1:2$) showed relatively smooth surfaces with holes and thin-walled, interconnected pores in cross-section. Runs 11 and 14 ($\approx 1:1$) displayed small, uniform surface pores and a regular, thick-walled porous architecture. Run 4 ($\approx 3:1$) had a smooth surface with minor pores and the thickest pore walls. This transition from dense to uniform interconnected porous networks is characteristic of

XG-KG synergistic gelation, where a 1:1 ratio has been reported to maximize gel strength and water-holding capacity (Li et al., 2025; Yu et al., 2023).

Only formulations with total gum $\geq 6\%$ and XG:KG between 1:2 and 3:1 developed a uniform, thick-walled interconnected porous network. These microstructures corresponded to the highest print accuracy, lowest deformation, low syneresis and successful IDDSI testing. Formulations outside this compositional window showed dense, collapsed or phase-separated structures and failed swallowing assessments. The results confirm that microstructure is the physical link between hydrocolloid composition and functional performance in 3D-printed dysphagia foods.

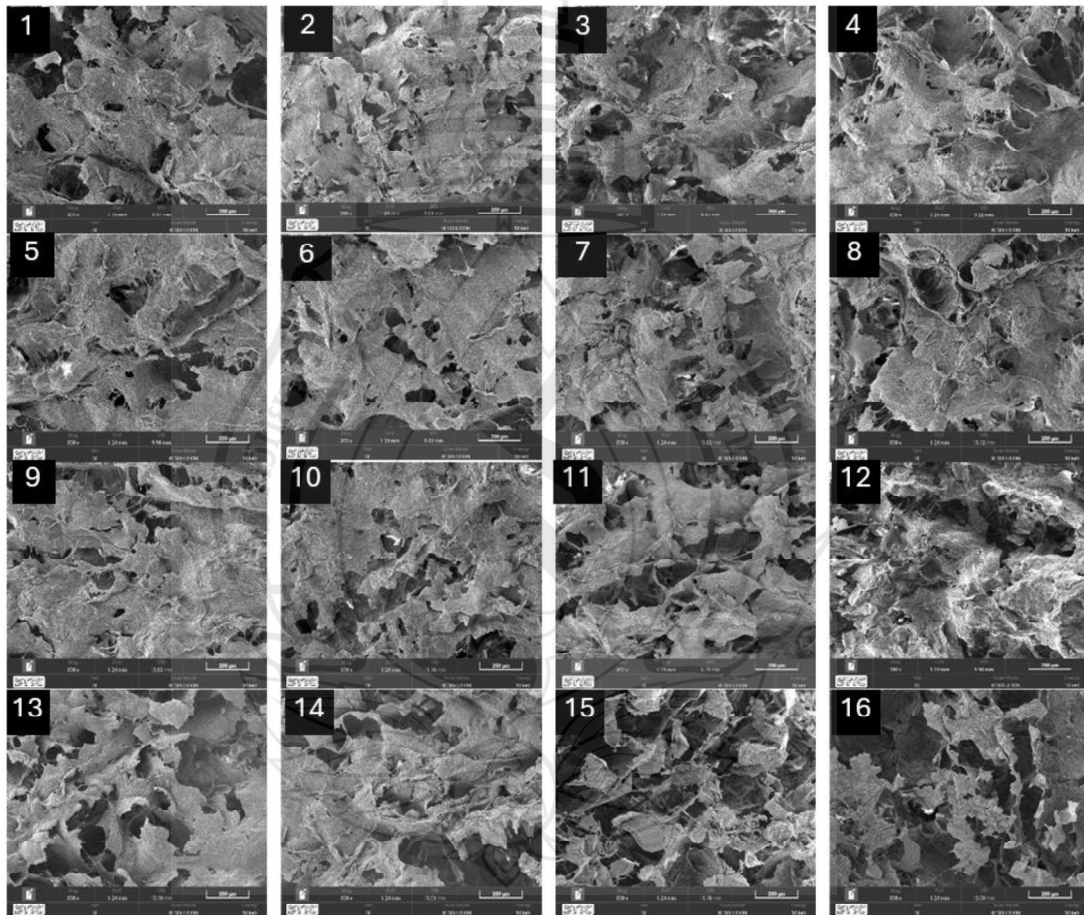


Figure 4.24 Scanning Electron Micrographs of the Top Surface of 3D-Printed Kai-Based Formulations (Runs 1–16). Magnification: 300×

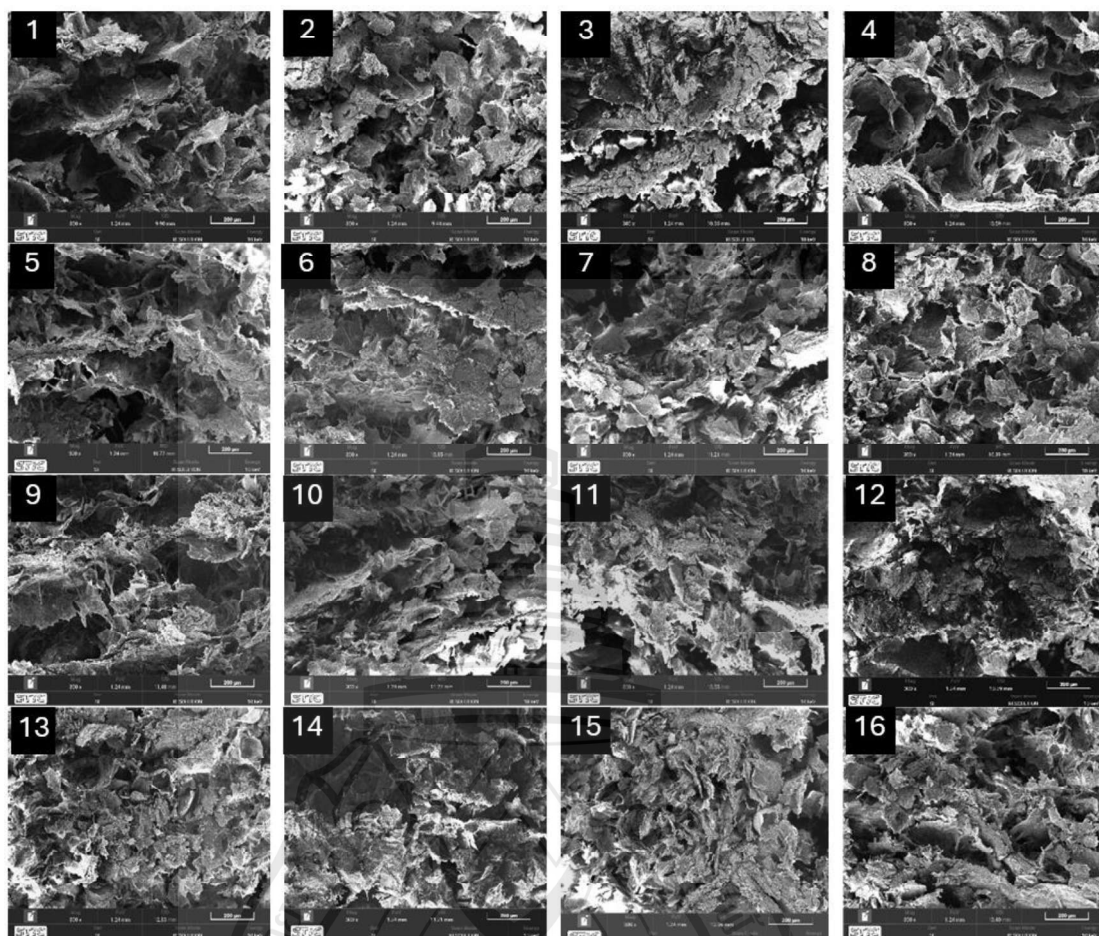


Figure 4.25 Scanning Electron Micrographs of the Cross Section of 3D-Printed Kai-Based Formulations (Runs 1–16). Magnification: 300×

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This study investigated the influence of drying methods on the quality characteristics of Kai powder and its potential application in dysphagia-oriented 3D-printed food formulation.

Among the three drying methods evaluated, TD at 50°C provided the most favorable balance of nutritional quality, bioactive compound retention, and functional performance. It exhibited the highest protein (13.95 %), chlorophyll (4.41 mg/g), flavonoid (6.60 mg QE/g), antioxidant contents, and swelling capacity (26.41 mL/g). Although SuD showed the highest protein digestibility (82.55%), TD produced the greatest absolute digestible protein yield (9.57 g/ 100 g) due to its higher protein retention. In terms of anti-inflammatory activity, TD demonstrated strong low-dose potency through NF- κ B/Nrf2 pathway modulation, while FD showed greater efficacy at higher doses. All drying methods produced mineral-rich powders with heavy metal levels below EU safety limits.

Each drying method results in distinct functional characteristics suitable for different food applications. FD preserves superior emulsifying properties and smoother filamentous structures, making it suitable for emulsion-based food systems. SuD exhibited the highest total phenolic and flavonoid contents, water holding capacity, and protein digestibility, making it suitable for hydration-focused applications such as soups, sauces, or rehydratable products. TD demonstrated the best overall balance of nutritional quality, functional performance, especially high swelling capacity, rapid foam collapse, moderate water holding behavior, and practical scalability, making it the most suitable choice for extrusion-based 3D food printing applications.

Based on these findings, TD was selected for Phase II formulation development. TD powder was formulated with XG and KG to develop 3D-printable dysphagia-friendly food inks using response surface methodology. All formulations exhibited

pseudoplastic shear-thinning behavior suitable for extrusion-based printing. The RSM models showed good predictive performance, with R^2 values ranging from 0.8456 to 0.9750 for hardness, cohesiveness, adhesiveness, print accuracy, syneresis, and deformation. RSM predicted an optimum formulation of 5.265 % xanthan gum and 3.186 % konjac gum with favorable predicted texture, printability, and stability characteristics. Formulations with total gum between 6 % and 9 % and XG:KG ratios of approximately 1:2 to 3:1 demonstrated the best print accuracy and met IDDSI Level 4, Japanese Dysphagia Diet 2021, and Universal Design Food Stage 4 criteria.

Overall, this study demonstrates that drying-induced structural modifications significantly influence the nutritional, bioactive, and techno-functional properties of Kai powder, and that TD Kai shows potential as a functional ingredient for dysphagia-oriented 3D printed food applications.

5.2 Recommendations

5.2.1 Recommendations for Future Research

Future work should prioritize experimental validation of the predicted optimum formulation using a functional extrusion-based 3D food printer. Validation studies should include assessment of actual print accuracy, structural stability, rheological recovery, syneresis, deformation behavior, and dysphagia compliance according to IDDSI and Japanese dysphagia food standards.

Sensory evaluation and flavor optimization are also necessary. The formulations developed in this study contained only Kai powder, xanthan gum, konjac gum, and water; no flavoring agents, salt, sugar, or taste-masking ingredients were added. Future research should assess the palatability of the 3D-printed products and explore the addition of natural flavorings (e.g., vegetable powders, herb extracts, mild seasonings, or fruit purees) to improve consumer acceptance, particularly for elderly and dysphagic populations who may have reduced appetite or altered taste sensitivity.

Further studies involving storage stability, nutritional bioavailability (*in vivo* protein digestibility), and clinical swallowing assessment (video fluoroscopy or fiberoptic endoscopic evaluation) are recommended to strengthen the practical

applicability of Kai-based dysphagia foods. Additional research on alternative hydrocolloid combinations and printing parameters may further improve formulation performance and personalization potential.

5.2.2 Recommendations for Practical Applications

Based on the findings of this study, tray drying at 50 °C is recommended as the most suitable drying method for producing Kai powder intended for extrusion-based 3D food printing applications because it provided the best balance between nutritional quality, structural behavior, functional performance, and processing practicality.

The hydrocolloid ranges identified in this study particularly total gum concentrations between 6 and 9 % with XG:KG mass ratios of approximately 1:2 to 3:1 may serve as useful formulation guidelines for future development of dysphagia-oriented 3D-printed foods. However, because the predicted optimum formulation was not experimentally validated, these ranges should be considered as starting points for further optimization rather than fixed specifications.

5.2.3 Limitations of the Study

This study was conducted using Kai collected from a single geographical source and harvest period, which may limit direct extrapolation of the findings to other environmental conditions or seasonal variations. Protein digestibility was evaluated only through *in vitro* methods, and clinical swallowing performance was not assessed in human subjects.

In addition, although the response surface models demonstrated strong statistical performance, the predicted optimum formulation was not experimentally validated because the 3D printer became unavailable during the final stage of experimentation. Consequently, the optimized formulation remains a model-based prediction requiring future experimental confirmation before definitive conclusions regarding its practical performance can be established. Sensory properties and long-term storage stability were also not evaluated.

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