
CHAPTER 2 - LITERATURE REVIEW

This chapter provides a comprehensive exploration of Kelakai, the core ingredient of this study. As a non-cultivated vegetable, *Stenochlaena palustris* (local name: kelakai) is notably more accessible than species like *Diplazium esculentum* (local name: bajei), and its consumption is deemed safe for human health in appropriate quantities (Rahmawati, D., 2015). Upon this, the chapter delves into local food innovation, characterizing the metabolite profiles of *S. palustris* under diverse soil conditions and environmental factors, as well as the nutritional shifts that occur during processing. It also examines the feasibility of utilizing Kelakai as a functional food substitute and a sustainable nori alternative in savory sprinkle products.

2.1 Kelakai (*Stenochlaena palustris*)

Stenochlaena palustris (Burm.f.) Bedd. (Blechnaceae), it is an edible species of fern found in countries such as Bangladesh, India, and Malaysia (Noor, F. A., and Jahan, S., 2017). Kelakai is also available in Indonesia, specifically in the Kalimantan and Sumatra regions. Kelakai has long been a staple in Kalimantan's cuisine and is featured in various traditional dishes that are popularly consumed. It is also recognized as a medicinal plant, with antipyretic, antianemic, and fever-reducing properties (Neamsuvan, O., *et al.*, 2015; Rahmawati, D., 2015). This plant grows in nutrient-dense peatlands, even in less fertile growth media (Pandiangan, F. I., *et al.*, 2022). As a pteridophyte, kelakai thrives particularly well during the rainy season. Due to its adaptability, kelakai is easy to cultivate and can be grown in a variety of environments. Its nutritional profile further enhances its value. Pandiangan, F. I., *et al.* (2022) highlighted its richness in dietary fiber, iron, and antioxidants.

2.1.1 Nutritional Potency of Kelakai

Based on research by Pandiangan, F., *et al.* (2022), kelakai is rich in dietary fiber and iron, which can enhance blood hemoglobin (Hb) levels. High levels of iron help combat anemia. Kelakai has a significant concentration of iron, as noted by Rahmawati (2015), which is likely due to the high iron concentrations in riverine soils, possibly derived from mineral-rich river sediments. The phenolic compound derivatives include flavonoids, anthocyanins, proanthocyanidins, and

hydroxycinnamic acids (Chai, T.-T., *et al.*, 2015). Additionally, the research by Adawiyah, R., and Rizki, M. (2018) demonstrated that kelakai exhibits significant antioxidant activity, which may provide further health benefits to consumers. Bioactive compounds in kelakai may enhance the product's appeal in an increasingly health-conscious market.

A foundational study by Rahmawati, D. (2015) explored the relationship between metal content in local soils and its accumulation in non-cultivated vegetables in Palangkaraya, including kelakai (*S. palustris*). The research revealed that soil type and land management practices have a significant impact on the final metal content in these vegetables. For example, peat soils from the UPR campus and residential areas were found to have exceptionally high concentrations of aluminum (Al > 1200 ppm) and zinc (Zn). The study concluded that aluminum was the most accumulated metal in the plant tissues. At the same time, chromium (Cr) and copper (Cu) exhibited the highest transfer factors from the soil to the plant. The research also indicated that consuming these vegetables is safe within reasonable limits.

2.1.2 Kelakai as a Functional Local Commodity

Kelakai (*Stenochlaena palustris*) has gained attention for its traditional use in Kalimantan's culinary heritage and potential as a functional food. Its health benefits position it as a strong candidate for inclusion in the functional foods category, products that provide benefits beyond basic nutrition (Khoerunisa, T., 2022). The versatility of kelakai has spurred its development into various processed forms, with crispy kelakai chips being the most popular. These chips are recognized as a distinctive Indonesian souvenir from Kalimantan. With its umami-rich taste, kelakai holds potential as a marketable sprinkle alternative.

2.2 Nori and Furikake Definition

Nori, a variety of dried seaweed usually from the genus *Porphyra*, is a food commonly roasted and/or seasoned before being transformed into various forms, such as thin sheets for sushi wrapping or flakes used as toppings. This classification is consistent with the Indonesian Food and Drug Authority Regulation (PerBPOM) No. 34 of 2019, categorizing nori under dried seaweed (04.2.2.2).

The consumption of nori is rising in Indonesia, a trend noted among both younger and older populations. Nori is a standalone snack ingredient in various dishes, including sushi. The primary raw material for nori, *Porphyra*, flourishes only in cold water, a condition the local climate does not provide. As a result, Indonesia relies heavily on imports from countries such as Japan, South Korea, and China, underscoring a significant need for innovative approaches that utilize sustainable, locally sourced materials. This need has spurred research into local substitutes, such as the work by Innayatuhibbah, G., *et al.* (2017), who explored using cassava leaves to create a nori-like functional food.

One of the most sought-after value-added applications for nori is the Japanese seasoning known as Furikake. The term “furikake,” which translates to “to sprinkle,” describes this convenient condiment created from a mixture of nori, roasted sesame seeds, salt, and other ingredients (Bando, M., *et al.*, 2024). Regarding nutrition, furikake is typically high in protein, fiber, essential minerals, and vitamins (Siddik, M. A. B., *et al.*, 2023).

2.3 Kelakai Nori Sprinkle Regulation

Developing a similar, locally sourced sprinkle product in Indonesia must align with the country’s national regulatory framework. According to the Indonesian Food and Drug Authority Regulation (PerBPOM) No. 34 of 2019, a product like a savory sprinkle falls under the category of Seasoning and Condiment (12.2.2). The regulation further defines it as a Ready-to-Use Seasoning (Bumbu Siap Pakai, 12.2.2.2), which is a seasoning added to food before or at the time of consumption, including products made from a mixture of spices and other food ingredients intended to enhance flavor and aroma. A key characteristic of this dry sprinkle is its low moisture content. Specifically, drying reduces moisture to inhibit microbial growth, prolonging shelf life (Hafsari, A., *et al.*, 2015). While a specific moisture limit for this exact category is not specified, PerBPOM provides benchmarks for similar dry ingredients, stipulating a moisture content of $\leq 10\%$ for Sesame Seeds (04.2.2.2.7) and $\leq 8\%$ for Dried Salted Vegetables (04.2.2.2.4). Therefore, achieving a moisture content of less than 8% is a critical quality parameter for the sprinkle product.

2.4 Nori Sprinkle Processing Method

Drying and processing powdered products extend shelf life, enhance practicality, and increase commercial value (Kusuma, H. A. W., *et al.*, 2019). As demand for functional foods grows, local communities innovate with regional commodities in various applications. Preservation methods, such as drying, prevent food degradation and maintain quality and nutrients (Kimestri, A. B., 2015). Drying reduces moisture to inhibit microbial growth, prolonging shelf life (Hafsari, A., *et al.*, 2015). This demand for safe preservation has driven advances in drying technologies, especially ovens and food dehydrators.

2.4.1 Quenching

Liquid nitrogen quick-freezing rapidly preserves fruits and vegetables by forming micro ice crystals that reduce cellular damage. This method is optimal for long-term storage, as it retains key nutrients, including vitamins, minerals, sugars, and antioxidants. A study on lychee found that it preserved texture and nutritional quality for up to eight months (Cáihuān, H. (黄才欢), *et al.*, 2015). Quenching protects raw materials from oxidation.

2.4.2 Oven-Drying Principle

The oven drying method will be used to evaporate the moisture content of the raw material. The basic principle of oven drying is to evaporate water by heating it to a specific temperature for a specified time. Drying is also used for preservation. The moisture content in food determines the rate at which it spoils, as all food spoilage requires the presence of water. The higher the moisture content in food, the faster it will deteriorate. Drying removes water, producing a product with a low water content (Hafsari, A. *et al.*, 2015).

2.4.3 Dehydrator Principle

The growing demand for efficient and safe food preservation has driven advances in drying technology. Food dehydrators remove moisture from food by controlling temperature, airflow, and drying time, promoting gradual water evaporation without

compromising nutritional and sensory qualities (Sutrisni, T., and Susanto, E., 2021). Deep, warm air circulates evenly to remove moisture, while adequate ventilation prevents condensation and maintains low humidity levels. Optimizing drying parameters according to the food type and thickness ensures maximum moisture reduction while preserving product quality. At an industrial level, drying extends shelf life, improves handling, and increases product value. For example, using a dehydrator to dry bay leaves, which are rich in bioactive compounds, reduces microbial growth and preserves flavor and nutrients (Shodikin, M., *et al.*, 2024).

2.4.4 Size Reduction Process

Particle size reduction is crucial in production, particularly to enhance the quality and effectiveness of end products. Particle size reduction aims to increase the product's surface area, directly affecting the dissolution rate and bioavailability of the compounds contained therein. Reducing the particle size of pharmaceutical compounds increases their surface area, leading to higher dissolution rates and improved bioavailability (Kumar, R., *et al.*, 2021).

2.4.5 Nori Production Method

The production process begins with harvesting the seaweed and thoroughly washing it to remove impurities. Next, the seaweed is chopped into smaller pieces and turned into a slurry. The slurry is then spread into thin layers and dried carefully to preserve the flavor and texture of the nori. Additives may also enhance nori's taste, texture, and stability, making it a versatile and popular ingredient worldwide.

2.4.5.1 Wet Method

This method begins with soaking the seaweed in water, grinding it, and cooking it. This step enhances the natural flavor and increases the seaweed's softness, facilitating its transformation into nori sprinkles (Tianasari, E., *et al.*, 2018). First, the preparation of nori involves washing the seaweed to eliminate dirt and contaminants. Once cleaned, the seaweed is soaked for a specified period to break down its cell structure and enhance the quality of the final product. This soaking is crucial for softening the seaweed, making it easier to process and work with. After soaking, the seaweed is

rinsed to remove any remaining dirt and impurities. The next phase involves grinding the seaweed into a fine paste, which ensures an even distribution during molding. The seaweed slurry is then cooked with seasonings to enhance its flavor while preserving the texture of the nori. After cooking, the seaweed paste is formed into thin sheets and dried. This drying process is vital, as it influences the final texture of the nori.

2.4.5.2 Dry-based Method

According to Orilda, R. *et al.* (2021), the nori drying process begins with washing and cleaning to remove dirt and other contaminants. After that, the nori is cut randomly to ensure that all portions can be dried evenly. Drying occurs in an oven set to a predetermined temperature, such as 70°C, for a specific duration ranging from 285 to 360 minutes. The drying process aims to achieve the desired moisture content, which should not exceed 10.69% (Orilda *et al.*, 2021). This oven drying method provides better temperature control compared to natural sun drying. The moisture content and water activity (A_w) were measured following the drying process to ensure the nori met the required quality standards.

2.5 Nutritional Analysis

2.5.1 Metabolomics

Metabolomics is a tool for quantitatively assessing metabolites that has been widely applied in food science for quality assessment. Recently, metabolomics-based gas chromatography-mass spectrometry (GC-MS) has become a standard tool for analyzing not only volatile compounds but also non-volatile compounds, utilizing various derivatization methods (Putri, S. P., *et al.*, 2022). Metabolomics can help evaluate stress responses, optimize post-harvest processes based on metabolic changes, monitor changes during food production and processing, and assess and predict food quality.

2.5.1.1 GC-MS Instrumentations and Conditions

Gas chromatography-mass spectrometry (GC-MS) combines separation via gas chromatography (GC) and detection via mass spectrometry (MS) to analyze

metabolites with high specificity. Separation of metabolites based on their volatility partitioning between a stationary phase (column) and a mobile phase (inert gas). Non-volatile compounds require derivatization to increase volatility (Putri, S. P., *et al.*, 2022). High retention time reproducibility is achieved by calculating the index of the detected peaks for each metabolite and comparing it to existing online databases. Additionally, identifying the mass spectra is possible due to the standardized method of electron ionization at 70 eV (Putri, S. P., *et al.*, 2022). Furthermore, the online library can be utilized freely if the analytical settings are similar. With the aid of a derivatization process that enables the analysis of non-volatile compounds in addition to volatile ones, and the robust and reproducible advantages of GC-MS, it is not surprising that GC-MS is rapidly emerging as the instrument of choice for researchers (Putri, S. P., *et al.*, 2022).

2.5.1.2 Metabolomics Sample Preparation Techniques

The metabolomics workflow for food samples must be homogeneous to reduce experimental errors. Liquid samples such as coffee, milk, and tea can be used directly. However, samples in solid form must be dried and then ground into powder. Lyophilization, known as freeze-drying, removes water from samples under vacuum conditions to prevent degradation, enhance derivatization efficiency, and preserve thermolabile metabolites. The extraction solvent is then added to the sample to extract non-volatile, low-molecular-weight hydrophilic compounds, which include amino acids, sugars, and organic acids. Liquid-liquid extraction using methanol and chloroform is a standard method in some studies. Methanol dissolves polar metabolites. Water enhances the extraction phase of hydrophilic compounds, while chloroform partitions lipids and non-polar metabolites. According to Putri, S. P., *et al.* (2022), derivatization converts non-volatile or thermally labile metabolites into volatile, thermally stable derivatives for GC-MS. Derivatization is performed by a combination of oxidation with methoxyamine hydrochloride and silylation with N, O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA), N-methyl-N-trimethylsilyl acetamide (MSTFA), and/or trimethylchlorosilane (TMCS). After derivatization, the samples were transferred to GC vials and analyzed by GC/MS. The carrier gas must

be inert to provide optimal flow rates for chromatographic separation and minimize fragmentation during MS ionization.

2.5.1.3 GC/MS data analysis

Analyzing GC-MS data includes deconvolution, metabolite identification, and statistical assessment. Deconvolution separates overlapping peaks and reduces noise using software such as MS-DIAL, which aligns peaks and removes background noise. Metabolites are then identified by matching mass spectra and retention indices using databases such as FiehnLib or MassBank. Retention index calibration using n-alkanes ensures consistent identification throughout the process. Confidence levels ranged from level 1 (RI and MS matching using standards) to level 3 (an unknown compound classified by chemical class). Statistical analysis revealed significant metabolites and biological pathways, including univariate (Tukey HSD, ANOVA) and multivariate tests (PCA). Quality control measures, such as pooled samples and internal standards, ensured the reliability of the data across all workflows. The metabolic profiles are characterised using Gas Chromatography-Mass Spectrometry (GC-MS). An initial Principal Component Analysis (PCA) of the data revealed distinct sample clustering based on origin (Phillips, K. M., *et al.*, 2019).

2.5.2 Proximate Analysis

Besides metabolomics, proximate tests play a crucial role in determining the overall quality and reliability of products by assessing key compositional attributes. Proximate analysis systematically evaluates essential nutritional parameters: moisture, ash, fat, protein, and dietary fiber. It provides insight into the nutritional profile and stability of food ingredients (AOAC, 2005). The following sections elaborate on the impact of proximate tests on product quality and reliability. Proximate analysis ensures that products meet specified quality standards for consumer safety and satisfaction. For instance, in wheat-based products, moisture and fat content are critical for shelf life and texture, while ash content can indicate the presence of impurities (Boyapati, T., 2020). Moisture content is a crucial compositional data point in food ingredients and products because it determines the content of other components (Andarwulan, N., *et al.*, 2014). Fat is measured as triglycerides and

extracted using non-polar solvents, typically via the Soxhlet method. Protein content, a vital measure for assessing nutritional quality, is commonly determined using the Kjeldahl method, which quantifies total nitrogen. In addition, total dietary fiber (TDF) refers to the amount of indigestible carbohydrates in plants and functional fibers added to processed foods. It is assessed using enzymatic-gravimetric methods (AOAC 985.29), which simulate human digestion to determine the fiber content resistant to enzymatic breakdown.

2.5.3 Iron (Fe) Content Analysis

Iron is essential in forming hemoglobin, a protein found in red blood cells that delivers oxygen from the lungs to the brain and other tissues. In our body, iron is conjugated in two forms: an active form of ferrous (Fe^{2+}) and an inactive form of ferric (Fe^{3+}) (Emawati, E., *et al.*, 2017). Sample preparation involves deconstructing plant samples with strong acids to separate the metals in the samples for analysis (Khoirunnisa, A., *et al.*, 2023). Destruction dissolves or converts the sample into a measurable form, allowing its elemental content to be analyzed. Determining iron content is also essential, as it may contribute to adequate micronutrient intake. Iron is typically quantified using wet digestion with nitric acid and spectrophotometric analysis at specific wavelengths.