
CHAPTER 4 – RESULTS AND DISCUSSIONS

4.1 Kelakai Selection as Raw Material for Sprinkle

The selection of raw materials for this study was carefully considered. Kelakai (*Stenochlaena palustris*) samples were purposefully harvested from six sites across Palangkaraya, Central Kalimantan, each representing different soil types and land management practices. The sampling strategy for this study was informed by foundational research from Rahmawati, D. (2015), which investigated metal content in local soils and non-cultivated vegetables in Palangkaraya. The study established a critical principle: that soil type and land management practices significantly influence the accumulation of metals in vegetables, such as Kelakai (*S. palustris*). For instance, peat soils from locations such as the UPR campus and residential areas were found to contain exceptionally high concentrations of Aluminum (Al > 1200 ppm) and Zinc (Zn). Research showed that aluminum was the most accumulated metal in the vegetables. Chromium (Cr) and copper (Cu) showed the highest transfer factors from soil to plant tissue. Given the link between soil composition and elemental uptake, it was determined that kelakai from different locations would exhibit distinct mineral profiles (Rahmawati, D., 2015). Therefore, samples from diverse environments were essential to analyze the plant's natural variability.

4.1.1 Kelakai Metabolite Profiles from Six Different Geographical Locations

A comprehensive metabolic profile of Kelakai from six geographical locations was generated using Gas Chromatography-Mass Spectrometry (GC-MS). This technique is powerful for identifying volatile compounds produced by derivatization methods, due to reproducible fragmentation patterns that can be matched against robust spectral libraries (Putri, S. P., *et al.*, 2022). An initial Principal Component Analysis (PCA) was applied to the data to explore natural variations, revealing that samples clustered distinctly based on geographical origin. The resulting multivariate data were then visualized using multivariate analysis (Principal Component Analysis) to compare the overall metabolic compositions among the harvesting sites. Principal Component Analysis (PCA) results (Figure 4.1.1) showed that the ex-rice paddy field peat soil (KALP) sample was distinctly separated from all other samples in Principal

Component 1 (PC1), accounting for 26.7% of the total variance and effectively differentiating among the samples.

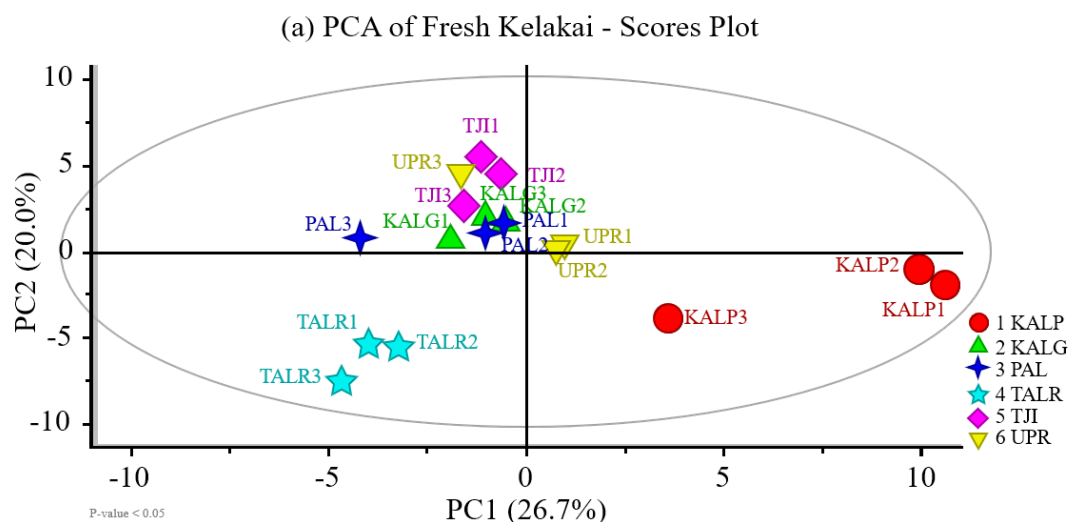


Figure 4.1.1.1 PCA score plot of kelakai metabolomic profiles from six different geographical locations

As shown in Figure 4.1.1, the samples were distinctly separated by the total variance explained by PC1. The KALP sample formed a distinct group on the far left of the scores plot. In contrast, the remaining five samples formed a larger cluster that was more closely related to each other. Subsequent nutritional evaluations, however, focused on the other five samples (KALG, TJI, UPR, PAL, and TALR) to ensure the selection of raw materials from diverse, reproducible, and sustainable sources for product development.

This PCA loading plot identified the specific metabolites that contribute to the separation of the Kelakai samples seen in the scores plot. The plot reveals that metabolites such as 4-Aminobutyric acid, Isobutylamine, and Phosphate are located in the lower-right quadrant. This position on the loading plot corresponds directly to the position of the KALP samples on the PCA scores plot. Therefore, it can be concluded that these specific metabolites are characteristic markers of the KALP sample and are found in significantly higher concentrations in it compared to the Kelakai harvested from other locations. This unique chemical composition is the reason the KALP sample was identified as a distinct outlier.

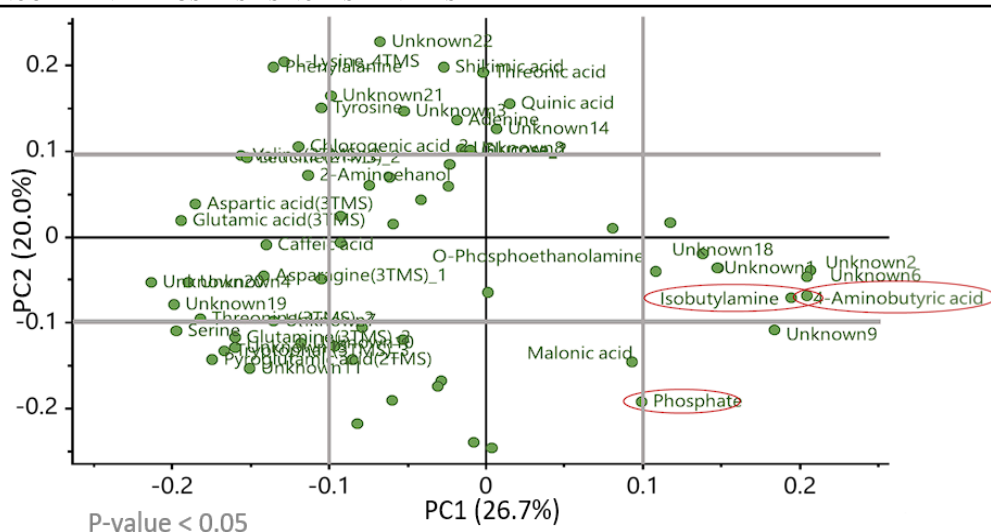


Figure 4.1.1.2 PCA Loading plot of kelakai metabolomic profiles from six different geographical locations

4.1.2 Kelakai Selection Based on Proximate Approaches

A previous study demonstrated that metal and mineral content in soil varies significantly depending on the location and soil type (Rahmawati, D., 2015). Therefore, the present study conducted a proximate analysis to compare and select the most nutritious Kelakai from five distinct locations representing these different conditions. To select the most nutritious raw material, a proximate analysis was conducted on three Kelakai samples from different growth substrates: peat soil (KALG), sandy soil (PAL), and sand (TJI). The analysis revealed that Kelakai grown on sand (TJI) was nutritionally inferior, showing significantly lower levels of iron, protein, and dietary fiber. In contrast, Kelakai from peat and sandy soils demonstrated superior nutritional profiles. These findings confirm that the growth substrate is a critical determinant of Kelakai nutritional quality, a principle that aligns with previous research on local soil analysis. Therefore, sourcing Kelakai from nutrient-rich peat or sandy soils is essential to maximize the health benefits of the final sprinkle product.

Table 4.1.2 Proximate composition and iron (Fe) content of selected Kelakai samples from different soil types

Parameters (Unit)*	peat soil (KALG)	sandy soil (PAL)	sand (TJI)
Iron (Fe) (mg/100g)	1.25 ± 0.01 ^a	1.43 ± 0.00 ^b	1.05 ± 0.00 ^c
Ash Content (%)	1.20 ± 0.01 ^a	1.35 ± 0.01 ^b	0.97 ± 0.02 ^c
Calorie From Fat (Kcal/100g)	3.83 ± 0.00 ^a	0.00 ± 0.00 ^a	12.02 ± 0.19 ^a
Total Fat (%)	0.43 ± 0.01 ^a	0.02 ± 0.00 ^b	1.34 ± 0.21 ^c
Moisture Content (%)	87.58 ± 0.10 ^a	87.51 ± 0.21 ^a	91.85 ± 0.06 ^b
Total Calories (Kcal/100g)	47.01 ± 0.30 ^a	44.6 ± 0.85 ^a	35.44 ± 0.06 ^b
Carbohydrate (By Difference) (%)	7.55 ± 0.16 ^a	7.66 ± 0.18 ^a	3.27 ± 0.01 ^b
Protein Content (%)	3.25 ± 0.07 ^a	3.49 ± 0.03 ^a	2.59 ± 0.07 ^b
Dietary Fiber (%)	6.53 ± 0.01 ^a	7.02 ± 0.08 ^b	2.55 ± 0.04 ^c

The selection of the optimal raw material for the sprinkle formulation was conducted through a multi-criteria evaluation that scored potential candidates based on three key parameters: metabolite profile consistency, nutritional value from proximate analysis, and logistical accessibility. First, metabolomic analysis was used to ensure a reproducible source, which led to the exclusion of the KALP sample as a distinct outlier. Next, proximate analysis confirmed that soil-grown Kelakai was nutritionally superior to sand-grown samples. Finally, logistical viability was assessed based on proximity to urban centers to ensure a sustainable supply chain.

Table 4.1.3 Justification Matrix for the Selection of Kelakai Raw Material

Parameters			Metabolite Profiles	Proximate profiles	Proximity; easy to access	Points earned
Conclusions			The KALP sample was a distinct outlier and deemed unsuitable for product development.	Sand-grown Kelakai had significantly lower iron, protein, and dietary fiber than soil-grown Kelakai.	Close proximity to urban markets, facilitating transport and distribution	
Kalampangan 1	KALP	ex-rice paddy field peat soil	0	0	0	0
Kalampangan 2	KALG	peat soil	1	1	0	2
PAL20	PAL	sandy soil	1	1	0	2

Talawang Raya	TALR	peat soil	1	1	1	3
Tjilik Riwut	TJI	sand	1	0	0	1
Palangkaraya University	UPR	sand	1	0	0	1

Based on this evaluation, the Kelakai from Talawang Raya (TALR) emerged as the optimal choice. It was the only candidate to successfully meet all three criteria, demonstrating a consistent metabolite profile, a high nutritional value from its peat soil environment, and close proximity to urban markets, making it the most suitable raw material for production.

4.2 Dried Kelakai Profiles

This section presents the analysis of the dried Kelakai samples, including their metabolite profiles, moisture content, and iron content, which informed the selection of the optimal candidates for the final product.

4.2.1 Selection of Dried Kelakai for Kelakai Nori Sprinkle Raw Material

The process was designed to determine how various drying techniques for Kelakai affected moisture, taste, and processing efficiency, which would aid in the subsequent product formulation stage. The selection of the optimal dried Kelakai for the final consumer study was guided by two primary factors: production practicality and visual appearance. In the initial oven-drying trials, the most practical and efficient pre-treatment was determined to be the non-soaked, non-ground method, as used for samples KND and KQD, because it saved significant time and labor. In terms of appearance, quenching proved crucial for preserving a desirable dark green color that resembles the seaweed in traditional nori, as described by Bando, M., *et al.* (2024). Therefore, the KQD sample (quenched, non-soaked, non-ground) was identified as the best overall oven-dried sample, as it combines both an efficient process and a visually appealing product. However, the oven-drying process itself demonstrated limitations in operational efficiency, with a very low loading density of 0.00605 g/cm³. To address this scalability issue, the superior pre-treatment from KQD was applied using a dehydrator to create sample KDE. This method proved far more suitable for potential mass production, with the dehydrator exhibiting a loading density of 0.06475 g/cm³, which is ten times higher than that of the oven. Given their

shared practical pre-treatment and superior visual characteristics, both the KQD sample and the KDE sample were chosen as the optimal candidates to proceed to the final consumer acceptance study.

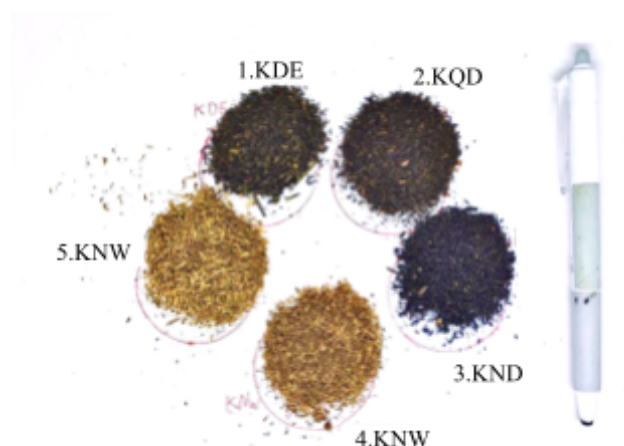


Figure 4.2.1 Dried Kelakai Samples

4.2.2 Principal Component Analysis (PCA) Data of Dried Kelakai in different pre-treatment processes

Following the various drying and pre-treatment processes, the metabolite profiles of the dried Kelakai samples were assessed by Principal Component Analysis (PCA) to understand the impact of different processing methods on their biochemical composition. The samples analyzed included those subjected to oven drying (KQW, KQD, KNW, KND) and dehydrator drying (KDE) (Table 3.1).

The PCA score plot (Figure 4.4.1) primarily explained that non-soaked and non-ground samples (KDE, KQD, and KND) clustered distinctly from soaked and ground samples (KQW and KNW). Representing 63.7% of the total variance, this factor emerged as the dominant one differentiating the samples based on their soaking and grinding pre-treatments. The physical pre-treatments are the major drivers of the observed metabolic shifts. PC2, which accounted for 9.4% of the variance, provided further distinction, primarily based on the quenching pre-treatment. Samples that underwent quenching (KDE, KQD, KQW) consistently clustered on the positive side of PC2, whereas non-quenched samples (KNW, KND) were grouped on the opposing side. Quenching significantly influences the final metabolite profile in dried Kelakai, serving as a distinct factor that contributes to its chemical composition.

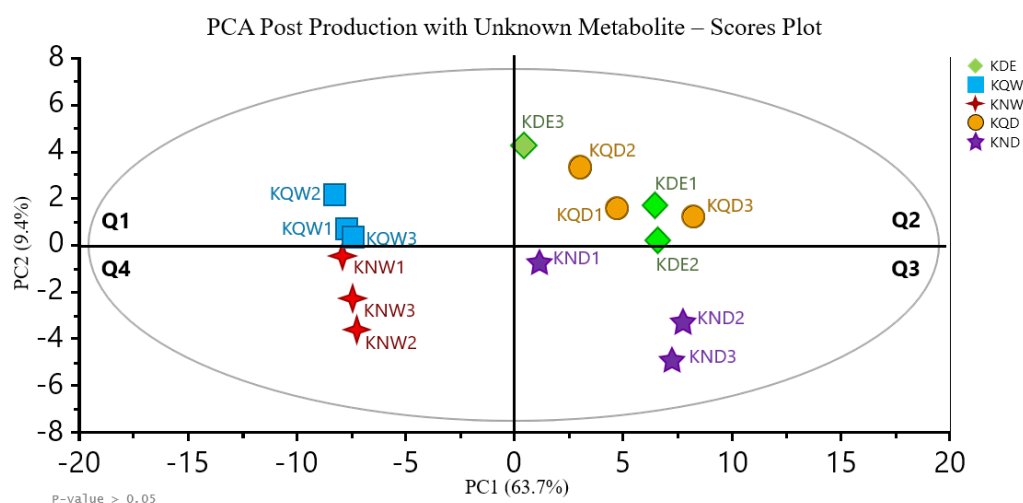


Figure 4.4.1 PCA Post Production with Unknown Metabolites - Scores Plot

Analysis of the PCA loading plot (Figure 4.4.2) revealed 72 compounds tentatively annotated in the dried Kelakai samples, including several amino acids, organic acids, sugars, and other compounds. The specific metabolites driving the separation along PC1 provide critical insights into the impact of processing methods on the separation.

Principal Component Analysis (PCA) revealed a difference in the metabolic profiles of the dried Kelakai, separating the samples into two distinct groups based on their processing method. In contrast, the metabolic richness of the group of non-soaked and non-ground samples (KDE, KQD, KND) was associated with a majority of distinct compounds, containing 56 out of 72 annotated metabolites. Non-soaked and non-ground pre-treatment methods were more effective at preserving the overall biochemical profile of Kelakai. In contrast, the group that underwent soaking and grinding (KQW, KNW) was characterized by a much smaller set of only four metabolites, indicating that this intensive pre-treatment leads to a significant loss of metabolic diversity. To understand the biochemical basis for this separation, the corresponding loading plot was analyzed to identify the specific metabolites driving these patterns.

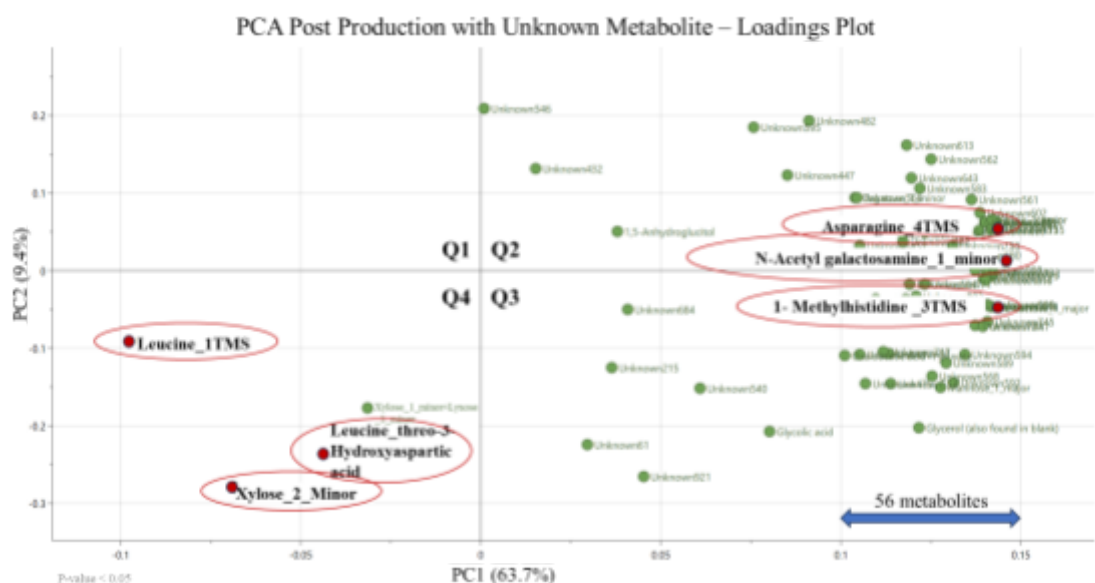


Figure 4.4.2 PCA Post Production with Unknown Metabolites - Loadings Plot

The non-soaked and non-ground samples were primarily characterized by high loadings of metabolites (Figure 4.4.2) related to nitrogen metabolism, such as 1-Methylhistidine and Asparagine, as well as the amino sugar N-Acetylgalactosamine. These soluble compounds suggested that minimal processing maintained a profile closer to the plant's natural physiological state. In contrast, the samples subjected to intensive soaking and grinding were defined by different compounds, including the amino acid Leucine and the structural sugar Xylose.

4.2.3 Moisture and Iron (Fe) Content of Dried Kelakai

The moisture content and the iron content were two essential quality parameters of the dried Kelakai. Shelf-stable products require adequate drying (Hafsari, A., *et al.*, 2015), and all five drying methods successfully reduced the moisture content to a level between 2.44% and 7.39%. The moisture content in the dried kelakai falls within the maximum allowed by Indonesian regulation, which is 8% (PerBPOM No. 34/2019), supporting the good stability of the product.

The analysis of iron (Fe) content, an essential micronutrient for human health, yielded more complex results. The fresh Kelakai started with an iron content of 2.93 ± 0.12 mg/100g (on a dry basis). While all processing methods resulted in a decrease from this baseline, the final iron content was significantly influenced by the pre-treatment

process. The data revealed a distinct trend: samples that underwent soaking and grinding (KQW: 1.22 ± 0.03 mg/100g and KNW: 0.90 ± 0.04 mg/100g) had substantially higher iron content than the samples with minimal, non-ground pre-treatment (KQD: 0.21 ± 0.02 mg/100g and KDE: 0.16 ± 0.04 mg/100g).

Table 4.2.3 Moisture content and Iron (Fe) content of Dried Kelakai

Sample Code	Iron (Fe) content (mg/100g)			Moisture Content (%)		
TALR	2.93	±	0.12	86.96	±	1.08
KDE	0.16	±	0.04 ^a	4.61	±	1.01
KQD	0.21	±	0.02 ^a	7.39	±	0.18
KQW	1.22	±	0.03 ^b	2.44	±	0.35
KNW	0.90	±	0.04 ^{bc}	5.95	±	1.03
KND	0.29	±	0.04 ^{bed}	5.08	±	0.97

Although it may be tempting to think that soaking and grinding increase iron retention, a closer examination of this aspect indicates otherwise. The extreme and noticeable rise is evident in the samples that were mechanically ground. The fact that the samples of the soaked and ground kelakai were characterized by a higher iron content powerfully confirms the assumption that the excess iron content proved to be an artifact of processing, possibly due to the leaching of the iron of the grinding apparatus, which is also reported in the literature (Sharma, S., *et al.*, 2021). A conclusion is therefore drawn that the minimum pre-treatment method provides a better representation of the naturally retained iron by Kelakai; it is possible that the grinding may contribute more iron to the process.

4.3 Results of Kelakai Nori Sprinkle Sensory Evaluation

Based on the extensive evaluation of drying methods and initial sensory analysis assessments (as elaborated in sections 4.2.1 and 4.2.2), the most suitable forms of dried Kelakai were identified and selected for product development. Specifically, three distinct Kelakai Nori Sprinkle formulations were prepared: one incorporating dried Kelakai from the dehydrator (KDE-based sprinkle), another utilizing the best

oven-dried sample (KQD-based sprinkle), and a negative control formulation without Kelakai, serving as a baseline for comparison.

Sprinkle products were evaluated by 43 naive panelists using a 9-point hedonic scale (1 = Extremely Dislike, 9 = Extremely Like). The comprehensive sensory evaluation results are presented in Table 4.3.1.

Table 4.3.1 Sensory Evaluation Result of Sprinkle Products

Parameters	*ORI	**KDE	***KQD
Overall	6.56 ± 1.08 ^b	6.05 ± 1.45 ^b	7.37 ± 1.41 ^a
Visual	7.56 ± 1.53 ^a	7.21 ± 1.71 ^a	7.21 ± 1.92 ^a
Aroma	7.72 ± 1.18 ^a	6.70 ± 1.70 ^b	7.23 ± 1.56 ^a
Taste	6.58 ± 1.45 ^b	6.05 ± 1.51 ^b	7.47 ± 1.40 ^a

*ORI: negative control of sprinkle, ** KDE: dehydrator-dried kelakai nori sprinkle,
***KQD: oven-dried kelakai nori sprinkle, $p < 0.05$, $n = 43$

Table 4.3.1 revealed that the oven-dried Kelakai sample (KQD) was preferred and tasted significantly better than the nori sprinkle (ORI) and the dehydrator-dried Kelakai (KDE). According to the flavor characteristics, the ORI and KQD were similar and more liked than the KDE. Dehydrator treatment preserved less desirable volatile flavor compounds from the Kelakai, which could be further explored through metabolomics data. Visual appearance, however, was not significantly different across all three samples, confirming that the addition of Kelakai and the tested drying methods did not adversely affect the product's color or physical appearance. The good performance of the oven-dried sample in key sensory attributes, which KQD was rated as "Like moderately" (7.37) on overall score, suggests that such treatment retains or enhances the flavour attributes of interest more efficiently while minimizing the potential adverse aroma effects of the kelakai addition. After identifying the KQD-based sprinkle as the most preferred formulation through this sensory evaluation, its proximate composition was further analyzed.

4.4 Product Description and Nutritional Profile of Kelakai Nori Sprinkle

Kelakai nori sprinkle is a dry product, a free-flowing granular sprinkle that has a savory taste due to the main ingredients. The critical quality attribute is a low moisture content, which is necessary to ensure shelf stability and prevent microbial growth. Hence, the product's formulation was such that the ending moisture level must be below 8%. According to Indonesian FDA Regulation (PerBPOM) No. 34 of 2019, the product falls within Food Category 12.2.2 (Seasoning and Condiment). More specifically, it falls under subcategory 12.2.2.2 (ready-to-use seasoning). The final product composition is as follows: sesame seeds (29%), vegetable oil, spices (containing fried shallots), kelakai (5%), seaweed (5%), sugar, savory salt, flavor enhancers (MSG), and an antioxidant (alpha-tocopherol). To keep consumers safe, the label displays allergen information. "Contains possible traces of cereal, egg, fish, crustacean, peanut, soybean, milk, and tree nuts."

Table 4.4.1 Nutritional Profile of Selected Kelakai Nori Sprinkle

Parameters (Unit)*	Kelakai Nori Sprinkle		Amount per Serving of 5g		%DV**
Iron (Fe) (mg/100g)	5.15	± 0.00	0.26	mg	3.31%
Ash Content (%)	5.97	± 0.05	0.30	%	
Calorie From Fat (Kcal/100g)	380.43	± 3.44	19.02	Kcal	
Total Fat (%)	42.27	± 0.38	2.11	%	3.52%
Moisture Content (%)	2.27	± 0.05			
Total Calories (Kcal/100g)	578.43	± 2.31	28.92	Kcal	1.35%
Carbohydrate (By Difference) (%)	34.08	± 0.04	1.70	%	0.50%
Protein Content (%)	15.42	± 0.33	0.77	%	1.19%
Dietary Fiber (%)	29.69	± 0.83	1.48	%	4.95%
* $p < 0.05$					
** Percent daily values are based on a 2150 kcal diet. Your daily values may be higher or lower depending on your calorie needs.					

The nutritional profile of the chosen Kelakai Nori Sprinkle (KQD-based) is provided in Table 4.5.1 on a 5-gram normal serving size basis. The total energy per serving is 29 kcal, of which the source is fat (19 Kcal). The final moisture content of the sprinkle was 2.27%, a critically important parameter, which is significantly lower than the 8% limit of dry sprinkle products.

4.5 Industrial Point of View on Kelakai Nori Sprinkle

A strategic practice that balances the price, the quality, and logistics is mandatory in industrial-scale production. To obtain a steady and high-quality raw material, a shift from wild harvesting to controlled cultivation is required, ideally on a soil medium to ensure better nutritional value than sand growth medium and in close proximity to urban markets to facilitate efficient transport and distribution (Chapter 4.1 Kelakai Selection as Raw Material for Sprinkle). The recommended method of production would involve minimal pre-treatment and drying using a dehydrator with a far greater capacity and lower cost than an oven (Chapter 4.2: Dried Kelakai Profiles).

A key advantage for commercialization is that the Kelakai nori sprinkle can be locally produced, which significantly lowers production costs compared to relying on imported nori. However, realizing this potential requires addressing critical logistical and stability challenges. A robust supply chain must be developed to transport fresh Kelakai from its growth regions, like Central Kalimantan, to production facilities while maintaining its quality.

Furthermore, ensuring product stability is essential for a viable shelf life. The primary concerns are preventing moisture absorption to maintain texture and inhibiting lipid oxidation, which leads to rancidity. These challenges are directly relevant to the Kelakai sprinkle due to its formulation containing vegetable oils and sesame seeds. To mitigate the risk of rancidity, an antioxidant (alpha-tocopherol) was included in the formulation (Chapter 4.4: Product Description and Nutritional Profile of Kelakai Nori Sprinkle).