Trehalose: A Key Stabilizer for Pandan Leaf (*Pandanus amaryllifolius* Roxb) Extract Powder

Eni Rohaeni[1], Triana Ulfah[2*], Raden Duhita Diantiparamudita Utama[3], Mita Ramadiyanti[4]

[1, 2, 3, 4] **Department of Agricultural Product Technology, Faculty of Agriculture, Universitas Insan Cendekia Mandiri, Bandung, Indonesia.**

*Email: trianaulfah@gmail.com

Abstract. Fragrant pandan (*Pandanus amaryllifolius* Roxb) is recognized as an herbal plant with antimicrobial properties, and is frequently used as a natural green colorant and a distinct aroma enhancer in food. However, its fresh form has a limited shelf life. This study aimed to evaluate the effect of trehalose concentration on the physical and sensory characteristics of pandan leaf extract powder processed using a rotary vacuum dryer. Trehalose was employed to preserve volatile components. The optimal treatment was subsequently analyzed for its moisture content and antimicrobial activity against Pseudomonas aeruginosa. The research findings indicate that the addition of 5% trehalose (P1) was the most effective treatment, yielding a moisture content of 9.8% and an inhibition zone of 35.6 mm. This result demonstrates that trehalose is effective in stabilizing the extract while maintaining its antimicrobial activity.

Keywords: antimicrobial activity, natural food colorant, pandan, rotary vacuum drying, trehalose.

Introduction

The availability of safe, high-quality, and environmentally friendly food additives (FA) is one of the key factors supporting a sustainable food system, as mandated in the Sustainable Development Goals (SDGs) Goal 2 (Zero Hunger) and Goal 12 (Responsible Consumption and Production). The utilization of natural FA, such as fragrant pandan leaves (*Pandanus amaryllifolius* Roxb.), not only adds value to food products but also reduces dependence on synthetic dyes and flavorings. According to the Indonesian Ministry of Industry Regulation No. 21 of 2021, FA serves as supplementary substances in the production process that improve product quality. Traditionally, fragrant pandan is used as a natural green colorant and aroma enhancer in food (Nazhifah, 2019), but its processing still relies on conventional methods, such as crushing fresh leaves and adding water.

Indonesia has a diversity of pandan species, including ornamental pandan (*Pandanus australis*), twisted pandan (*Pandanus utilis*), white pandan (*Pandanus bapthisii*), African pandan (*Pandanus pygmeus*), and fragrant pandan, which is the only type with a distinct aroma. This plant is easy to cultivate and widely distributed across various regions. The leaves contain active compounds such as essential oils, flavonoids, and alkaloids, which have antibacterial properties (Fitrianingsih & Surwender, 2016) and 2-acetyl-1-pyrroline, the compound responsible for its characteristic scent (Ambarwati et al., 2016). In the food industry, color and aroma are sensory attributes that strongly influence consumer preferences (Mahfud, 2015).

Although beneficial, fresh pandan leaves are perishable and have a relatively short shelf life. Therefore, appropriate processing technologies are required to extend shelf life without compromising quality. One innovative approach is processing them into powder through drying technology, which results in a product that is easy to measure, non-sticky, and long-lasting due to low moisture content (Nurwanto & Suswantinah, 2021). Vacuum drying is considered advantageous as it removes water at low temperatures, thereby minimizing damage to color, aroma, and nutrients. However, the drying process still poses risks of quality degradation due to the loss of volatile compounds and pigment degradation during heating.

Conclusion

Trehalose concentration affected the color, taste, and texture of pandan leaf extract powder, with no significant impact on aroma. The 5% trehalose treatment yielded the most favorable overall results, achieving optimal moisture content (9.8%) and strong antimicrobial activity (35.6 mm inhibition zone). Trehalose thus proves to be a suitable stabilizing agent for producing high-quality, shelf-stable pandan extract powder for both industrial and household applications.

To address these challenges, the addition of a stabilizing agent such as trehalose is relevant. Trehalose is a non-reducing disaccharide that is stable against temperature and pH changes and is resistant to acid hydrolysis. It can preserve volatile components, mask off-notes, enhance product stability, and extend shelf life (Zlatic, 2017). Recent studies also indicate that trehalose plays a crucial role in maintaining bioactive compounds such as anthocyanins during drying, supporting the development of functional foods aligned with sustainability principles (Castagnini et al., 2021).

Previous studies have shown that drying methods affect the physical and sensory characteristics of the product. Spray drying of pandan juice tends to produce paler colors than vacuum drying due to encapsulation of pigments by maltodextrin (Mufarida, 2016). The resulting aroma is also weaker due to the loss of some volatile components (Nurwanto & Suswantinah, 2021). Moreover, the antioxidant potential of pandan leaves is also influenced by drying duration (Angraiyati & Hamzah, 2017).

Despite existing research on drying and stabilizing agents, specific studies comparing the effects of various trehalose concentrations on the characteristics of pandan leaf extract powder produced by vacuum drying are still limited. This research gap is significant, considering that innovations in natural food additive processing contribute to reducing food waste and improving production efficiency, aligning with the core objectives of SDG 12 (Responsible Consumption and Production) (Sadiq et al., 2024).

The urgency of this research lies in its contribution to the development of natural FA processing technologies that support the SDGs, particularly in producing value-added food products that are safe, high-quality, and have an extended shelf life. Consequently, the resulting pandan leaf powder can be widely applied in the food industry as a stable, practical, and eco-friendly natural colorant and flavoring.

Based on this background, this study aimed to evaluate the effect of varying trehalose concentrations (5%, 10%, 15%, and 20%) on the physical, chemical, and sensory characteristics of pandan leaf extract powder produced by vacuum drying. The results are expected to provide recommendations for optimal formulations suitable for both industrial and household-scale production of pandan leaf powder.

Methods

This study was conducted from July to October 2023 at the Laboratory and Workshop, Jl. Cihanjuang No.138, the Laboratory of Universitas Insan Cendekia Mandiri, the Food Analysis Laboratory of Universitas Pasundan, and the West Java Provincial Health Laboratory. The objective was to evaluate the effect of varying trehalose concentrations on the physical, chemical, microbiological, and organoleptic characteristics of fragrant pandan (Pandanus amaryllifolius Roxb.) extract powder produced using rotary vacuum drying (Nurwanto & Suswantinah, 2021; Zlatic, 2017).

Fresh pandan leaves were sourced from Cimahi Selatan District, and trehalose was applied at four concentrations: 5%, 10%, 15%, and 20%. For antimicrobial activity tests, Pseudomonas aeruginosa, amoxicillin, agar media, and the pandan extract powder from each treatment were used. The main equipment included a rotary vacuum dryer, digital scale, knife, spoon, 60-mesh sieve, grinder, containers, and analytical tools such as Petri dishes, spectrophotometer, oven, desiccator, paper discs, tweezers, sterile cotton, and an incubator.

A Completely Randomized Design (CRD) with one factor—trehalose concentration—was applied, comprising four treatments and three replications, resulting in 12 experimental units: P1: Pandan + 5% trehalose, P2: Pandan + 10% trehalose, P3: Pandan + 15% trehalose, and P4: Pandan + 20% trehalose.

The procedure began with weighing 5.494 g of fresh pandan leaves per treatment. Leaves were washed, drained for 30 minutes, cut into 1–1.5 cm pieces, and weighed again. Drying was performed using a rotary vacuum dryer at 70°C for 8 hours with trehalose added according to the treatment. The dried leaves were weighed, ground, and sieved to obtain fine powder for analysis.

Organoleptic testing was conducted using a hedonic scale with 20 semi-trained panelists assessing color, aroma, taste, and texture on a 1–5 scale (1 = strongly dislike, 5 = strongly like). Samples were randomly coded and presented under standardized conditions.

Chemical analysis included moisture content determination, while microbiological analysis focused on antimicrobial activity against P. aeruginosa. The best treatment was determined using the effectiveness index method with weighting based on parameter importance (Cengistitama et al., 2022). Data were analyzed using ANOVA at a 5% significance level, followed by Duncan's multiple range test when significant differences were observed (Parwiyanti et al., 2019).

Result and Discussion

Color

Color is the first sensory attribute observed by panelists and serves as an indicator of food product quality. A color consistent with the inherent characteristics of the raw material will create a positive impression, whereas a deviating color may reduce product acceptance (Negara et al., 2016).

The organoleptic evaluation indicated that trehalose addition influenced panelists' preference for the color of pandan leaf extract powder. The highest mean score was recorded for treatment P4 (20% trehalose) at 3.90, followed by P1 (5%) at 3.85, P2 (10%) at 3.65, and P3 (15%) at 3.63.

Analysis of variance (ANOVA) revealed statistically significant differences among treatments ($p > 0.05$). Duncan's multiple range test showed that P1, P2, and P3 did not differ significantly from one another, but all differed significantly from P4. This finding indicates that a trehalose concentration of 20% provides optimal color stability, likely by protecting chlorophyll pigments from degradation during the drying process (Zlatic, 2017; Nurwanto & Suswantinah, 2021).

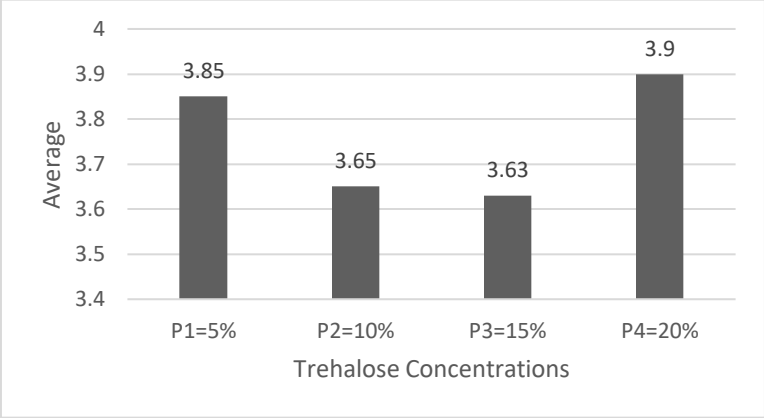

Figure 1. Panelists' preference scores for the color of pandan leaf extract powder

The bar chart illustrates a tendency for color scores to increase with higher trehalose concentrations, with P4 (20%) achieving the highest score. Panelists tended to prefer a bright green color that was not overly intense, making P4 the most visually appealing treatment.

Aroma

Aroma is the olfactory sensation produced by volatile compounds that stimulate the receptors in the nasal cavity. The distinctive aroma of pandan leaf is primarily attributed to the presence of 2-acetyl-1-pyrroline (2-AP) (Wongpornchai, 2006).

The sensory test showed variations in aroma preference scores across treatments. The highest mean score was obtained for P1 (5% trehalose) at 3.55, followed by P2 (10%) at 3.43, P4 (20%) at 3.42, and the lowest in P3 (15%) at 3.18. However, ANOVA revealed that these differences were not statistically significant ($p < 0.05$), indicating that trehalose concentration did not have a substantial effect on panelists' aroma preference.

This phenomenon may be due to the loss of volatile compounds during the drying process. According to Fellow (1988), volatile components in food are highly sensitive to elevated temperatures, and excessive heat can cause degradation of aromatic compounds. Although trehalose is known to act as a flavor protector, its effectiveness may be diminished if the drying process is not optimally controlled (Zlatic, 2017; Nurwanto & Suswantinah, 2021).

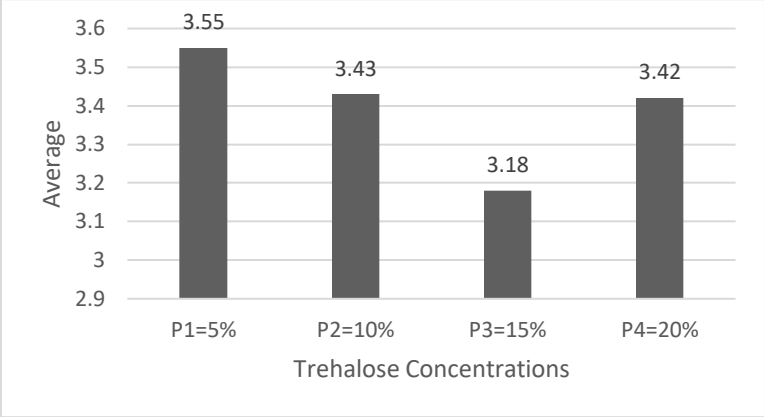

Figure 2. *Panelists' preference scores for the aroma of pandan leaf extract powder.*

The bar chart shows that the highest aroma score was obtained in P1 (5% trehalose) and the lowest in P3 (15% trehalose). Differences in scores among treatments were relatively small, suggesting that panelists' aroma preferences were generally consistent.

Texture

Texture is a visual and physical attribute that can be directly observed and significantly influences consumer perception of product quality. In pandan leaf extract powder, texture affects ease of mixing, product stability, and consumer acceptance.

The sensory evaluation showed that trehalose addition resulted in differences in texture preference scores among treatments. The highest mean score was recorded in P3 (15% trehalose) at 3.72, followed by P1 (5%) at 3.63, P4 (20%) at 3.43, and the lowest in P2 (10%) at 3.28.

ANOVA indicated significant differences among treatments ($p > 0.05$). Duncan's test revealed that P1 was significantly different from P2, P3, and P4; P2 did not differ significantly from P4 but differed from P1 and P3; and P3 did not differ significantly from P1, P2, or P4. These findings align with Parwiyanti et al. (2019), who reported that higher texture scores indicate firmer textures. Additionally, Sze-Yin and Lai-Hoong (2013) found that increasing trehalose concentration can reduce product hardness due to its water-binding capacity, which affects matrix structure.

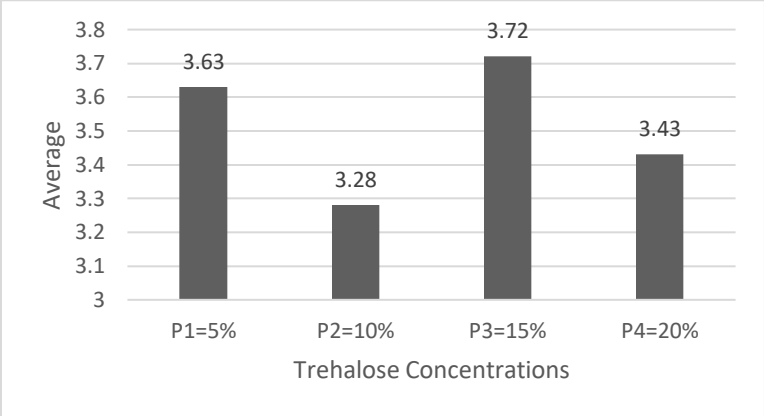

Figure 3. *Panelists' preference scores for the texture of pandan leaf extract powder.*

The bar chart illustrates variations in texture preference scores, with P3 (15% trehalose) achieving the highest score. This difference may be associated with trehalose's effect on the powder's microstructure, which influences the perceived texture during sensory evaluation.

Taste

Taste is a sensory attribute perceived through the gustatory system and is a key determinant of consumer acceptance. Taste evaluation is typically categorized into five levels: strongly dislike, dislike, neutral, like, and strongly like. In pandan leaf extract powder, taste is influenced by ingredient composition, sweetness level, and interactions between volatile compounds and the base product (Negara et al., 2016).

The sensory evaluation revealed that trehalose addition affected taste preference scores. The highest mean score was recorded for P4 (20% trehalose) at 3.65, followed by P3 (15%) at 3.55, P1 (5%) at 3.47, and the lowest in P2 (10%) at 3.25.

Trehalose has approximately 45% of the sweetness intensity of sucrose and is capable of maintaining sweetness perception for a longer period (Research, 2020; Richards et al., 2002). Panelists tended to prefer the taste of P4 due to its mild sweetness and stability, which matched well with the neutral taste of rice as the application medium.

ANOVA indicated a significant effect ($p > 0.05$) on taste preference scores among treatments. Duncan's test showed that P1 and P2 did not differ significantly from P3 and P4. P3 did not differ significantly from all treatments, while P4 also did not differ significantly from P1, P2, and P3.

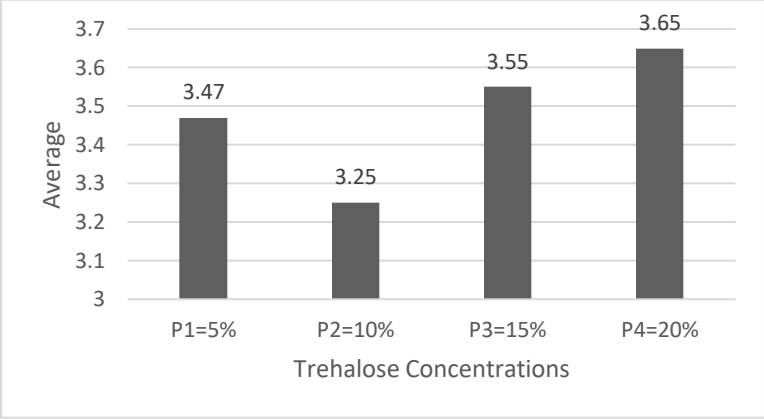

Figure 4. *Panelists' preference scores for the taste of pandan leaf extract powder.*

The bar chart shows that P4 (20% trehalose) had the highest taste score, while P2 (10% trehalose) had the lowest. This trend suggests that higher trehalose concentrations may enhance taste acceptance, although overall differences among treatments were relatively small.

Moisture Content

Moisture content analysis of pandan leaf extract powder was performed using the gravimetric method, which is based on drying the sample until a constant weight is achieved. This method was selected due to its simplicity and the absence of expensive reagent requirements (Sugiarti & Setyawati, 2017).

The results showed that the pandan leaf extract powder had a moisture content of 9.8%, which is below the maximum allowable limit for dried simplicia, namely ≤10% (Krisyanella, 2013). Low moisture content is essential for maintaining product stability and shelf life, as water can serve as a medium for the growth of microorganisms such as bacteria and fungi. Excessive moisture content accelerates microbial growth, leading to faster spoilage.

These findings are consistent with Sugiarti & Setyawati (2017), who stated that drying to a low moisture content can slow material degradation and preserve the quality of active compounds. Therefore, the pandan leaf extract powder produced in this study meets the moisture content requirement for dried products and has the potential for good storage stability.

Antimicrobial Activity

The antimicrobial activity test showed that pandan leaf extract powder at a 5% concentration was able to inhibit the growth of *Pseudomonas aeruginosa*, as indicated by the formation of a clear zone around the test disc. The inhibition zone had a diameter of 35.6 mm, which, based on the antibacterial activity classification by Susanto et al. (2012), falls into the "very strong" category (≥ 21 mm).

Pseudomonas aeruginosa is a Gram-negative bacterium whose cell wall structure differs from that of Gram-positive bacteria. Gram-positive bacteria have a thick and rigid peptidoglycan layer containing teichoic acid, whereas Gram-negative bacteria, such as *P. aeruginosa*, possess a thinner cell wall that is more susceptible to physical damage, including from antibacterial agents (Putra, 2018). The active compounds in pandan leaf extract are presumed to target the bacterial cell wall, facilitating the penetration of these compounds into the cell membrane (Khasanah et al., 2014).

These results support previous studies reporting that pandan leaf extract contains bioactive compounds such as flavonoids, alkaloids, and tannins, which have antibacterial potential (Ambarwati et al., 2016;

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