COMPARATIVE STUDY OF SOLAR DRYING AND FREEZE DRYING METHODS IN AMINO ACID CONTENT OF CRUDE FICIN ENZYME FROM *FICUS AURATA (MIQ.)*

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ABSTRACT

Purpose: This study aimed to analyze the profiles of amino acids in the crude ficin enzyme isolated from *Ficus aurata (Miq.)* with different drying methods. **Methodology**: The presence of amino acids was determined by UPL chromatography. **Results**: The findings indicated that freeze drying techniques retained more amino acids in figs than was observed in the case of solar drying. In particular, the amount of L-glutamic acid in freeze drying was 2.39 mg/g, while that of solar drying was 1.43 mg/g. Similar results were observed in L-lysine and L-aspartic acid, which were relatively higher in freeze drying than solar drying, with concentrations of 2.43 mg/g and 2.55 mg/g, respectively, as opposed to 0.99 mg/g and 1.76 mg/g in solar drying. **Findings**: It was observed that freeze drying gave the best opportunity to reduce the loss of amino acid of the ficin enzyme. **Novelty**: This study focused on different types of solar energy and freeze energy economic systems in enzymatic activities in the ficin enzyme extracted from *Ficus aurata (Miq.)*. **Originality**: The perspective toward the development of drying parameters for the enhancement of important enzymatic characters from this particular variety. **Conclusion**: This research considers the changes in amino acid content during the drying of ficin enzyme from Ficus aurata through different drying methods. It is shown that freeze drying enhances the retention of critical amino acids and is thus one of the most efficient methods for the enzymatic processing of this plant species. **Type of Paper**: Empirical research paper.

Keywords: *ficus aurata (Miq.); ficin enzyme; amino acids; solar drying; freeze drying.*

INTRODUCTION

Ficus aurata (Miq.), a tree species within the genus Ficus, contains many biochemical properties. Ficin is a plant-based proteolytic enzyme that is one of the most active compounds in *Ficus aurata (Miq.)* and has been actively studied for potential use in the food, medicine, and biotechnology industries. Due to its proteolytic properties, Ficin is advantageous for meat tenderizers, protein hydrolysis, and treating several diseases (Shearer & Kniel, 2021; Zheng, Wang, Li, Li, & Liu, 2020). More recent studies have revealed the role of ficin in enhancing and possibly showing therapeutic effects on food products (Amani, Mohebodini, Khademvatan, & Jafari, 2020; Iwaniak, Hrynkiewicz, Minkiewicz, Bucholska, & Darewicz, 2020). The effectiveness of ficin in different applications is highly dependent on its stability,

which is equally determined by factors such as storage and processing methods applied at harvest time (Baidamshina, 2023; Liburdi, Boselli, Giangolini, Amatiste, & Esti, 2019).

The primary preservative used for the plant enzymes, including ficin, is drying, which is very commonly used. Drying causes the substrate's moisture level to drop, reducing the chances of microbial growth and enzyme activity and increasing the material's storage period (Karami, Akbari-adergani, & Duangmal, 2022). Solar and freeze drying are the two most used drying technologies, though they have pros and cons (Ariza, 2024; Kumar & Yao, 2022).

Solar drying of plant materials is a relatively simple, inexpensive, and economical method because it relies solely on solar radiation to raise temperatures and remove moisture from the plant materials. In most cases, the raw material, such as leaves of *Ficus aurata (Miq.)*, or the enzymes, is placed on a horizontal surface or in a solar collector to receive direct sunshine. Solar drying is based on the evaporative drying concept, where solar heat raises the temperature of the material and causes it to lose moisture to its surroundings (Marinescu, 2019). One of the main advantages of solar drying is its low energy requirement since it depends on a free and green energy resource - the sun. There is a limitation of this process whereby it can be prolonged and can cause uneven drying in places that experience intermittent sunshine (Shafiee, Goli, Khoshkhoo, & Hosseini, 2021). In addition, the high temperatures of sack solar driers can also lead to a loss of specific heat-sensitive molecules, such as some amino acids in enzymes like ficin (Сорокин et al., 2023).

Freeze drying, also known as lyophilization, is a complex dehydration technique that combines extreme cold and a vacuum to preserve the structure and content of delicate substances, such as enzymes (Uba, Manogaran, Gunasekaran, Effendi Halmi, & Shukor, 2020). It consists of three basic steps: freezing, primary drying (sublimation), and secondary drying (desorption). It is based on the hypothesis that by maintaining low temperatures and vacuum extraction of water, the enzyme position and its active organizations are achieved in the original form (Postiglione, Accorsi, Ganswindt, & Crossey, 2022). This method is also helpful for the heat-sensitive chemicals set up in conventional drying, causing thermal distortion (Hermosilla, Pastene, & Acevedo, 2021). Freeze drying is beneficial in the preservation of amino acid sequences of enzymes; however, it is more energy-consuming and expensive compared to solar drying.

Although solar drying and freeze drying are standard methods of preserving plant-based enzymes, little is known about the effects of these drying methods on the amino acids in ficin obtained from *Ficus aurata (Miq.)*. Amino acids are the building blocks of proteins,

and their quantity could influence the catalytic activity, stability, and properties of the enzymes to a great extent (Trevisol, Henriques, Antunes Souza, & Fúrigo, 2021). This research aims to investigate the amino acid composition of the ficin enzyme extracted from *Ficus aurata (Miq.)* and dried using solar and freeze drying methods. We hypothesize that freeze drying will be superior to solar drying since the latter can lead to heat damage to the aminoterminal region of the ficin enzyme due to high temperature. At the same time, the former operates at low temperatures (Shearer & Kniel, 2021).

The outcomes of this study will be important in understanding the effect of solar and freeze drying methods on the storage of enzymes in enzymes containing any plant source, thus assisting in the improvement of the drying parameters of *Ficus aurata (Miq.)* for effective enzymatic function.

MATERIALS AND METHODS

This study utilizes sap-tapping equipment, centrifuges, and several laboratory instruments. The materials employed in this study included latex from the fig plant species *Ficus aurata (Miq.)*, aquades, filter paper, NaN_3, and additional chemical components utilized in amino acid analysis.

Latex collection

The main resources utilized in this study were sap or latex extracted from the stems of *Ficus aurata (Miq.)* tree cultivated in Limau Manis Padang, collected in a sterile tube containing 0.05% NaN_3. All latex samples utilized in this investigation were collected in the early morning. The latex fluid was subsequently transferred to the laboratory under appropriate conditions and maintained at -20°C until used.

Preparation of crude extract

The frozen latex was maintained at 4°C until thawed, subsequently diluted with a 1.0:0.5 water-to-latex ratio, properly mixed, and centrifuged at 5000 rpm for 15 minutes at 4°C to eliminate gum and other particulates. The residue was eliminated, and the supernatant was filtrated using Whatman paper No. 1. The transparent liquid known as "crude extract" was utilized (Gagaoua et al., 2014). The crude extract was evenly spread across a glass plate. After that, it was dehydrated in a solar drying room for 8 hours. The latex was dehydrated until it crystallized into a brownish-white powder. Freeze drying was performed using a freeze dryer at a temperature of -33°C and a pressure of 0.1 mb for 4.5 hours. During this process, the precipitate dried and formed powder ranging from white to brownish. The dried powder were then ground into a fine powder and properly packaged.

Analysis of Amino Acids

The amino acid component analysis was conducted at the PT. Saraswanti Indo Genetech Laboratory utilizing the UPLC (18-5-17/MU/SMM-SIG) method.

Statistical Analysis

Amino acid data obtained from the UPL Chromatography test was analyzed using MS Excel for descriptive statistics.

RESULTS AND DISCUSSION

The current research aimed to analyze the effects of solar drying and freeze drying on the amino acids that comprise the ficin enzyme obtained from *Ficus aurata (Miq.)*. The study indicated marked differences in concentrations of amino acids when a freeze drier was used, and freeze drying was frequently utilized in concentrating all the tested materials. The knowledge obtained would help understand how different drying methods affect the durability and effectiveness of plant-origin enzymes.

The freeze drying helps to achieve higher concentrations of critical amino acids like L-glutamic acid, L-lysine, L-aspartic acid, L-leucine, and L-tyrosine, which are necessary for the activity and stability of the enzymes. L-glutamic acid, which was the most important for enzyme stability, increased from 1.43 mg/g in solar drying to 2.39 mg/g in Freeze drying, which represents an increase of more or less 67%. L-lysine, another vital protein stabilizing and functioning amino acid, increased from 0.99 mg/g in solar drying to 2.43 mg/g in freeze drying. This significant difference in concentration demonstrates that freeze drying was better in preserving amino acids like L-lysine and L-glutamic acid, whose deterioration is joint during solar drying (Swargiary, Verma, & Daimari, 2020).

The fundamental assumption behind these findings, in any case, the two drying methods applied to be different in their approaches. Freeze drying is when frozen water is subtracted in a vacuum, allowing the structure and bioactive ingredients, including amino acids, to be less exposed to high temperatures (Quirós-Pozo et al., 2023). On the contrary, solar drying relies on heat and sunlight, which may lead to inconsistency in drying and the destruction of heat-labile substances. The high temperature used during the process of solar drying (Hira, Gülfraz, Naqvi, Qureshi, & Gül, 2021) may have aided the destruction of some of the amino acids, such as L-serine and L-phenylalanine, and therefore expecting higher amounts of the amino acids in the freeze drying technique. For example, L-serine up from 1.11 mg/g in solar drying to 1.71 mg/g in freeze drying, whereas L-phenylalanine from 0.93

mg/g in solar drying to 1.35 mg/g in freeze drying. The results indicate that freeze drying effectively preserves amino acids and other heat-sensitive enzymes by decreasing the extent of thermal damage.

Tabel 1. Amino Acid Concentrations in Different Drying Methods

Amino Acid Type	Solar drying	Freeze drying
L-Serine (mg/g)	1.11±0.00	1.71±0.01
L-Glutamic acid (mg/g)	1.43±0.01	2.39±0.04
L-Phenylalanine (mg/g)	0.93±0.00	1.35±0.00
L-Isoleucine (mg/g)	1.01±0.00	1.45±0.01
L-Valine (mg/g)	0.70±0.00	1.09±0.01
L-Alanine (mg/g)	0.79±0.00	1.16±0.00
L-Arginine (mg/g)	1.12±0.00	1.65±0.00
Glycine (mg/g)	0.95±0.00	1.34±0.00
L-Lysine (mg/g)	0.99±0.71	2.43±0.00
L-Aspartic Acid (mg/g)	1.76±0.01	2.55±0.01
L-Leucine (mg/g)	1.44±0.00	2.14±0.00
L-Tyrosine (mg/g)	0.78±0.01	1.46±0.00
L-Proline (mg/g)	0.93±0.00	1.42±0.00
L-Threonine (mg/g)	1.13±0.00	1.74±0.00
L-Histidine (mg/g)	0.43±0.01	0.70±0.00

The non-essential amino acids were present in increased amounts within freeze drying. This implies that even structural amino acids, which make the enzyme more stable and active, were more effectively preserved (Mule & Naikwade, 2022) since L-proline, L-alanine, and L-glycine were found in greater concentrations when freeze drying was employed. L-proline contained increased levels of 0.93 mg/g in solar drying and 1.42 mg/g in freeze drying, illustrating that freeze drying is superior for maintaining catalytic activity and structural integrity of the enzymes. The conservation of these amino acids is essential for prolonged storage and industrial utilization of enzymes, where timescale stability is important (Ghribi, 2023).

Figure 1. ***Ficus aurata (Miq.)***

On the other hand, L-lysine and L-aspartic acid amino acids, in particular, show the most significant difference relative to other drying techniques, respectively. Lysine is essential in maintaining the enzyme's catalytic activity and protects it from denaturation. The increase from 0.99 mg/g in solar drying to 2.43 mg/g in freeze drying signifies that in this drying, more of this amino acid, which is crucial for enzyme functionality, is retained (Nova et al., 2022). L-aspartic acid, which is involved in enzyme catalytic activity, moved from 1.76 mg/g during solar drying to 2.55 mg/g during freeze drying, indicating the higher retention of bioactive amino acids in the freeze drying method (Ajeigbe, 2023).

Considering the significant differences in amino acid contents between solar drying and freeze drying, it is evident that an adequate drying method should be selected regarding the intended application of the enzyme. Enveloping a deep concern regarding the activity and stability of the enzyme, Freeze drying is, therefore, the most suitable strategy as it significantly maintains the content of essential and non-essential amino acids in the enzyme (Ozcan, 2022). Free-drying should be preferred despite the challenges of high operating costs and energy requirements because it can retain a high concentration of amino acids and, thus, the bioactivity of heat-labile enzymes like ficin (Farooq et al., 2019).

On the other hand, although solar drying is cost-effective and more energy efficient, certain amino acids, significantly heat-damaged ones, might be reduced. The findings suggest

that although solar drying enables the economical preservation of enzymes, there are better methods than this when the stability of the enzyme is more critical. In such cases, where a lack of resources or cost is more important than the stability of the enzyme, solar drying could be an appropriate drying method but with a clear understanding of its limitations (Xia et al., 2022).

To complement this research shows that freeze drying causes less damage to amino acids than solar drying. The results of the study can be used to develop more advanced approaches to the evaluation of the efficiency characteristics of enzymes for further development in industry.

CONCLUSION

This study demonstrates that freeze drying is more effective than sun drying in preserving amino acids, including L-glutamic acid, L-lysine, L-aspartic acid, and L-leucine, which are necessary for the catalytic active site of ficin enzymes derived from Ficus aurata (Miq.). Freeze drying significantly enhances the concentrations of lysine and aspartic acid, which are crucial for enzymatic function and synthesis. The augmentation of amino acids resulting from the freeze-drying of ficin was statistically significant. Simultaneously, sun drying exhibited reduced amino acid concentrations, with heat-sensitive molecules such L-serine measuring 1.11 mg/g in sun drying against 1.71 mg/g in freeze drying. L-phenylalanine had a reduced concentration of 0.93 mg/g in sun-drying compared to 1.35 mg/g in freeze-drying. Freeze-drying is optimal for processes necessitating a diverse array of enzymes, as it preserves a greater quantity of the amino acids essential for ficin activity. Solar drying is suitable for applications requiring minimal or no enzyme activity, it should be executed cautiously due to the potential denaturation of amino acids.

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