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# PROTEIN CONTENT ANALYSIS OF PROTEASE ENZYMES EXTRACTED FROM FICUS SPECIES AND PAPAYA USING QUBIT FLUOROMETER

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**ABSTRACT:** *This study aims to quantify the protein content in crude ficin enzymes obtained from the fruit and latex of various Ficus species and Papaya. This research has been encouraged by the considerable potential of these enzymes in different biotechnological applications, while data with regard to the protein content of these enzymes is limited. This study used a quantitative approach using qubit fluorometer to quantify the protein content in crude enzymes derived from the fruit and latex of these plants. Based on results, the protein content in crude ficin enzymes, two types of research obtained from Ficus aurata (Miq.) fruit was 0.92µg/ml and from papaya fruit as 3.16 µg/ml. For latex, it was observed that the protein content in crude ficin enzymes was determined as 6.14 µg/ml for Ficus aurata (Miq.), 2.58 µg/ml for Ficus racemosa L., and 5,27 µg/ml for Ficus padana Burm.f.. The protein content from papaya latex was 15,98 µg/ml. These findings show the differences in the protein content of the enzymes obtained from different species of Ficus and Papaya as a basis for further study on using these enzymes for biotechnological purposes.*
***Keywords:*** *protein, enzyme, ficin, papain, qubit fluorometer*

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## INTRODUCTION

Proteins and enzymes are two groups of biomolecules of primary importance in many biological activities. They are instrumental in accelerating biochemical processes, information transmission, and tissue construction in the living. Proteins are structural components of the cells, while enzymes are the components that speed up the biotransformation processes, contributing to the ordinary course of metabolic processes (Mótyán, Tóth, & Tőzsér, 2013). Very importantly, there are many enzymes, but special ones known as proteolytic enzymes or simply proteases are in the spotlight because of their unique hydrolase activity. This hydrolysis is significant during protein degradation, but it is also exploited in many industries, such as the food, pharmaceutical, and waste disposal industries (Toe et al., 2019; Tsado et al., 2021).

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Proteases are used in the food industry for meat tenderization, cheese manufacture, and flavor enhancement of fermented products (Lim, Foo, Loh, Mohamad, & Abdullah, 2019; Phupaboon, Hashim, Phumkhachorn, & Rattanachaikunsopon, 2023). They also resort to their therapeutic properties when considering the healthcare industry, for example, gastrointestinal tract diseases and inflammatory diseases (Fh et al., 2019). Furthermore, the findings indicate that proteolytic enzymes are also crucial for waste management because they augment the biodegradation of organic waste material (Klaude et al., 2011; Majdinasab, Aminlari, Aminlari, & Niakosari, 2010). However, the field of protein engineering and biotechnology understands much more utility of the proteolytic enzymes in the recent developments because it is now possible to create a novel enzyme with all the appropriate characteristics needed for a particular type of industry (Souza et al., 2015). In that context, such information also provides evidence for more research on proteolytic enzymes, including their modes of action and where they can be used, as such sources can enhance efficacy in various fields.

Accurate determination of protein concentrations in enzyme extracts is significant during enzyme use and protein evaluation, among other biochemical utilizations. In this study, we utilize the Qubit Fluorometer 4.0, a modern and advanced equipment well-known for its effectiveness and reliability concerning protein measurement. (Mótyán et al., 2013) suggest that the Quibits Fluorometer efficiently detects proteins at low levels while reducing interference by other substances in the crude sample. Such advantage is more applicable in the case of crude enzyme preparations, which contain a large population of proteins that cannot be easily analyzed with conventional tools like a UV spectrophotometer due to absorbance interference by raw sample soup and contaminants (Toe et al., 2019; Tsado et al., 2021). There has nearly been a simplified and accelerated determination of protein content using the Qubit Fluorometer 4.0, making it useful for laboratories conducting many analyses. From its design, the properties of proteins can be determined with high confidence, which is required for the successful outcome of the follow-up tests (Lim et al., 2019). It is also important to note that in the above range, the fluorometer can also be used with effectiveness on several different biological samples, including recombinant proteins derived from microbial and fungal sources, widening the use of this technology for several studies related to microbiology, biochemistry, biotechnology, etc (Fh et al., 2019; Phupaboon et al., 2023).

This study aims to objectively assess the amount of protein in detail by extracting and freeze-drying crude fiction enzymes from *Ficus racemosa L.*, *Ficus aurata (Miq.)*, and *Ficus padana Burm.f.* species latex and fruit and drawing comparisons. The protein concentration in the papain isolated from the fruit will also be determined. As much as it is universally acknowledged that proteolytic enzymes are essential, there are minimal studies that examine the observation of protein content diversification among various species of ficus and papaya, especially in crude enzyme extracts.

The evaluation of protein concentrations in crude extracts from various Ficus species and Carica papaya is essential for understanding their enzyme potential and applications. Ficus species, particularly *Ficus racemosa L., Ficus aurata (Miq.),*

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and *Ficus padana Burm.f.*, are known for their proteolytic enzymes, such as ficin, which have diverse applications in industries ranging from food processing to pharmaceuticals. Ficin, a cysteine endopeptidase, has been shown to possess significant proteolytic activity, It might be utilized for meat tenderization and other biotechnological applications (Pacifico, 2024). The study by (Shinde et al., 2019) emphasizes the importance of these enzymes in enhancing the efficiency of specific processes, highlighting their therapeutic functions and potential industrial applications.

In addition to ficin, Carica papaya contains papain, another cysteine protease with broad specificity for peptide bonds. Papain is particularly noted for its ability to hydrolyze proteins, making it valuable in meat processing and as a digestive assist (Pacifico, 2024). The comparative analysis of protein content in crude extracts from these plants can reveal variations in enzyme activity and potential therapeutic functions. For instance, the study by (Wahyuni, Susanti, & Iswari, 2017) on ficin isolation from *Ficus septica* demonstrated that enzyme activity varies significantly with temperature, indicating the need for detailed biochemical characterization to optimize its use in various applications.

Moreover, understanding the biochemical structures of these enzymes is essential for maximizing their industrial applications. The study of the protein content and enzyme activity in Ficus and papaya can provide insights into their functional properties, which can be beneficial for industries such as food, pharmaceuticals, and cosmetics. For example, the presence of specific phytochemicals in Ficus species has been linked to their enzyme inhibitory activities, which can be advantageous in managing conditions like diabetes (Olaokun, McGaw, Eloff, & Naidoo, 2013). This highlights the potential of Ficus species not only as sources of proteolytic enzymes but also as functional ingredients in health-related applications.

## METHOD

This study is an experimental research conducted to examine the protein content in crude ficin enzymes derived from the fruit parts and latex of several ficus species and papaya. The tools used in this study are sap tapping tools, centrifuges, a Qubit Fluorometer 4.0, and other laboratory instruments. The materials utilized in this research were latex and fruit of fig plants species of *Ficus padana Burm.f.*, *Ficus racemosa L.*, *Ficus aurata (Miq.)* and latex of Papaya (*Carica papaya L.*) obtained from the limau manis Padang area. Aquades, filter paper, $NaN_3$, sodium phosphate buffer, L-cysteine, and other chemical component used in Qubit™ Protein Assay.

### *Procedure of Enzyme Extraction*

The main resources utilized in this study were sap or latex and the fruits of *Ficus padana Burm.f.*, *Ficus racemosa L.*, *Ficus aurata (Miq.)* and *Papaya*. The latex extracted from the stems is subsequently placed in a tube containing 0.05% $NaN_3$ and preserved at -20°C. Frozen latex was thawed at 4°C and diluted with distilled water in a 1.0:0.5 ratio, mixed, then centrifuged at 5000 rpm for 15 minutes at 4°C to exclude grit and other insoluble substances. The supernatant was filtered using Whatman paper. Clarified juice is termed "crude extract" (Gagaoua et al.,

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2014). The raw material for fruit is chosen to be green and fresh, weighing up to 200 g, then washed and sliced to a thickness of around 2.5 mm. The fruit was subsequently chopped with a blender, utilizing 450 mL of 50 mM sodium phosphate buffer (pH 7.0) treated with 10 mM L-cysteine. The chopped and homogenized fruit was centrifuged at 150 rpm for 45 minutes at 4°C. The sample followed filtration with a filter cloth, insoluble impurities were eliminated, and the supernatant was then filtered through Whatman paper (Gagaoua, Hoggas, & Hafid, 2015).

***Procedure of Protein Assay***

The preparation and reading of the protein standards and samples using the Qubit™ 4 Fluorometer following the guidelines in the protein assay kit user guide.

## RESULT AND DISCUSSION

The protein analysis of the protease enzymes obtained from different sections of Ficus species and the Papaya fruit indicates a difference in the protein content. With regards to the findings of this study, it is evident from Table 1 that the fruit of the *Ficus aurata (Miq.)* contains 0.92 µg/ml of crude ficin enzymes, while the fruit of Papaya has a higher content of 3.16µg/ml. In terms of western blot analysis of latex protein, the protein content varied among the species of Ficus, where *Ficus aurata (Miq.)* latex had the highest protein content at 6.14 µg/ml, protein content of *Ficus padana Burm.f.* at 5.27 µg/ml and *Ficus racemosa L.* at 2.58 µg/ml. Compared to that, the protein amount in papaya latex is even more significant, as marked 15.98 µg/ml.

**Table 1. Protein Content of Protease Enzymes Extracted from Ficus Species and Papaya**

| Sample | Protein Content (µg/ml) |
|---|---|
| **Latexs** | |
| *Ficus aurata (Miq.)* | 6.14 |
| *Ficus racemosa L.* | 2.58 |
| *Ficus padana Burm.f.* | 5.27 |
| Papaya (*Carica papaya L.*) | 15.98 |
| **Fruits** | |
| *Ficus aurata (Miq.)* | 0.92 |
| Papaya (*Carica papaya L.*) | 3.16 |

The proteolytic activity of the latex of papaya (*Carica papaya L.*) is higher than that of Ficus latex. This is the point of view presented by several sources that note the biochemical properties and uses of crude Papain. Multiple studies, however, indicate that papaya latex is a valuable source of proteases, with papain inclusion derived from the latex being one of the most popular in food and biotechnological industries across the globe. (Baidamshina et al., 2021) elaborate on the enzymatic characteristics of Papain, targeting the scope of this enzyme in the food industry, specifically meat preparation processes involving its tenderization and hydrolyzing of proteins into safer and more appetizing products, which in this case, refers to the end-products of meat enhancing papain activity (Baidamshina et al., 2021). (Marković, Milošević, Djuric, Lolić, & Polović, 2021), in the same vein, focus on the purification and characterization of Papain's activity, showing concern

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over the high proteolytic activity of Papain and, therefore, its possibility of being used as an active component in food and drug market (Volf et al., 2017), (Juwita, 2023) investigates the potential of papaya latex as a natural tenderizer of meat and explains how it improves the quality of meat products (Olaokun, McGaw, Eloff, & Naidoo, 2013).

The enzyme activities of proteases derived from several sources, including Papaya, are evaluated by (Meza-Espinoza et al., 2017), and their possible use in food processing is compared, therefore emphasizing the role of Papain in the protein hydrolysis (Baidamshina et al., 2021). (Giangrieco et al., 2023) cover the immune response towards papain-like cysteine proteases, which also calls for using Papain in food and medicine applications. (Macalood, Vicente, Boniao, Gorospe, & Roa, 2013) emphasize the significance of papaya latex as a highly effective source of proteolytic enzymes, its versatile possibilities in the food industry, and pharmaceutical and biotechnological practices.

*Ficus aurata (Miq.)* *Ficus racemosa L.* *Ficus padana Burm.f.* *Carica papaya L.*

**Figure 1. Ficus Species and Papaya (*Carica papaya L.*)**

The protein content of *Ficus racemosa L.* is lower than that of the other species; however, it is still used in industries that require moderate levels of proteolytic activity. This is supported by the success of three trials using *Ficus racemosa L.* , which has antimicrobial action, indicating further application in several pharmaceutical processes requiring moderate proteolytic action (Murthy & Rao, 2018). In addition, in this case, the protease isolated from *Ficus racemosa L.* can be used in the composition of certain medicines. This plant has a long tradition of being used in traditional medicine to treat many diseases (Murthy & Rao, 2018). In the results of this study, although the protein content of latex obtained from *Ficus racemosa L.* is low, additional research must be carried out to confirm the protease activity of *Ficus racemosa L.* and its possible application in industry.

*Ficus padana Burm.f.* showed a latex protein concentration of 5.27 µg/ml, setting it between *Ficus racemosa L.* and *Ficus aurata (Miq.)*. Future firms may find ficin, a cysteine protease, in *Ficus padana Burm.f.* due to its high protein concentration. (Aïder, 2021) identified that Ficus carica latex contains ficinin, a proteolytic enzyme with potential applications in meat tenderization and cheese production. Due to its ficin content, *Ficus padana Burm.f.* may have similar enzymatic properties, making it a potential industrial prospect. Extraction of ficin from *Ficus padana Burm.f.* might offer a sustainable, cost-effective alternative to synthetic enzymes in food processing (Aïder, 2021).

A low protein content in *Ficus padana Burm.f.* latex allows enzyme extraction without denatured proteins. For enzyme function, activity must be

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maintained. According to (Srisai et al., 2022), latex protease extraction can be modified to enhance activity while maintaining enzyme functionality. *Ficus padana Burm.f.*, as a source of ficin, may benefit industries requiring natural enzymes, particularly in meat and dairy processing where enzyme activity is important for product quality (Aïder, 2021).

Derardja, et al. (2019) noted that many other plants have protease properties, such as ficin, which is used in the production of tender meats and cheesecakes, as well as in beer brewing, among many others. Ficin, like all other plant proteases, has recently been the focus of immobilization studies that seek to increase the stability and reuse of such enzymes in industry (Sun, et al., 2010). In addition to the above, such an approach not only enhances the efficacy of the enzymes but also reduces the cost that would have been incurred in the re-invention of enzymes in continuous processes.

The papaya fruit and, therefore, latex were found to have the highest protein content among the samples collected; the fruit had 3.16 µg/ml of protein, while the latex was even richer at 15.98 µg/ml. Similarly, the papaya plant's latex contains a significant amount of protein due to the presence of papain, a cysteine protease with excellent activity that has a wide range of applications in the food, cosmetic, and pharmaceutical industries, as shown by (Prabha & Modgil, 2018). Papaya latex is a good source for enzyme extraction on a large scale due to its high protein content, which means that it can be used for various industrial applications without excessive focused processing.

Papain plays a significant role in food science, especially in tenderizing meat and improving other products. Papain plays a significant role in food science, especially in tenderizing meat and improving other products (Damgaard, Otte, Meinert, Jensen, & Lametsch, 2014). Previous studies have shown that proteases from papaya latex papain are widely used due to their protein-degrading ability, which is crucial in meat tenderization and the production of protein hydrolysates (Soares et al., 2017). The use of papain as a biocatalyst in meat enzymatic processes not only improves texture but also contributes to the creation of active peptides with antioxidant and antihypertensive properties (Annaházi, Schröder, & Schemann, 2021; Bian et al., 2019).

Furthermore, papain is useful for purposes other than consumption. It is a useful ingredient in medications and cosmetics due to its high proteolytic activity. The capacity of papain to debride external tissue without causing injury is demonstrated by its extracorporeal application in topical wound care and tissue regeneration products (Elder et al., 2021). According to (Ghanbari et al., 2015), its significance in popular cosmetics is further highlighted by its ability to improve transdermal therapy.

Papain has been purified more effectively without decreasing effectiveness due to recent advancements in enzyme isolation and purification. (Fatmawati, Zulfiana, & Handayani, 2023). This increases the need for plant enzymes across several industries and makes papaya latex a more economical and ecologically friendly source of proteases. The difference in protein content between papaya fruit and its latex is consistent with previous investigations. In general, latex has a higher

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concentration of bioactive enzymes than fruit. The protein content in papaya latex is 15.98 µg/ml. However, protein content in fruit is 3.16 µg/ml.

*Ficus aurata (Miq.)* and *Ficus padana Burm.f.* have been considered acceptable choices for enzyme extraction; however, their latex shows a lower protein concentration than papaya. However, there are still niche applications for Ficus species, particularly in areas where papaya is scarce or where specific characteristics of ficin, such as substrate specificity, are preferred. This suggests that while papaya latex is most useful for mass consumption, Ficus species may have specific roles in the enzyme workplace (Dafoe, Huang, & Yang, 2017; Serio-Silva, Rico-Gray, Salazar, & Espinosa-Gómez, 2002).

Studies have shown that fruit generally contains lower levels of protease enzymes than latex. This supports the idea that most plants produce a protective substance called latex, which, while remaining pure at its source, acts on protective proteins to increase activity. These enzymes are found in latex for various reasons, including resistance against invasive herbivores and a range of other internal metabolic processes (Kumar, Sharma, Souza, Ramos, & Carvalho, 2014).

Further research should be conducted to enhance latex extraction methods, considering the results obtained to increase the quantity of enzymes produced for industrial utilization. Techniques, including enzyme immobilization and purification, can increase the stability and activity of the purified enzymes, improving their applicability in a variety of settings (Rusmadi, Shahari, Amri, Tajudin, & Mispan, 2020). Furthermore, producing specific enzyme preparations for biotechnology applications could become feasible if specific characteristics of enzymes from various Ficus species were examined.

## CONCLUSION

Based on the research results, it can be concluded that (1) The protein content in crude ficin enzymes varies significantly among different species of Ficus and Papaya, with *Ficus aurata (Miq.)* showing the lowest protein content in fruit and latex and Papaya latex exhibiting the highest protein content; (2) The protein content in the crude ficin enzymes extracted from Ficus species latex is relatively lower than the papain enzyme from Papaya latex, indicating that papaya latex is a more potent proteolytic source; (3) The study highlights the potential of Ficus species, particularly *Ficus aurata (Miq.)*, *Ficus racemosa L* and *Ficus padana Burm.f.*, as promising sources of ficin enzymes, which could be further explored for biotechnological applications; (4) These findings provide valuable insights for future studies aimed at utilizing plant-derived protease enzymes for applications in the food industry and pharmaceuticals and potentially developing treatments for inflammatory diseases.

## RECOMMENDATION

This study provides the following suggestions: Further research should be conducted to optimize the extraction process, stability, and activity of crude fiction enzymes from Ficus species and Papaya latex to enhance protein yield and enzymatic activity for potential industrial applications.

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