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Chemical Analysis of *Carica papaya* L. Crude Latex

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ABSTRACT

Crude latex of *Carica papaya* L. has been known to offer a lot of benefits and potentials especially in the agricultural industry and human health. This study focuses on the latex coming from its fruits of Papaya CX variety. Seven to eight longitudinal incisions were made in order to allow latex to appear and drain in the collecting devices. 439.5 g dried latex was stored in plastic containers and freezed. Results showed that dried latex contained higher amount of crude protein (57.24 ± 0.69%), followed by moisture (17.76 ± 0.09%), ash (7.00 ± 0.01%), crude fat (5.21 ± 0.13%) and crude fiber (0.67 ± 0.09%) based on the complete proximate analysis. In the enzyme analysis, papain had protease activity of 2655 units·g^{-1} at pH 5.5 and 285 units·g^{-1} at pH 9.0. These results provided evidence that papain as a protease enzyme is found in the crude latex of papaya which is a major constituent in various proteolytic activities. Crude latex from *C. papaya* L. can be utilized to address the issues in agricultural farms to accelerate production and reduce environmental hazards.

Keywords: *Carica papaya*; Papaya CX Variety; Latex; Protease Activity; Papain; Complete Proximate Analysis

1. Introduction

Latex-bearing plants are believed to provide protection against the attack of herbivores. Latex is known to compose of various kinds of proteins including enzymes which interact with the cellular aspect of the host insects resulting in growth inhibition, physiological damages and mortality. This prompted much research endeavour aiming to provide exact information on the defense mechanisms offered by the constituting compounds among lateces. Many of these compounds provide resistance to herbivores via toxicity or antinutritive effects, whereas others are involved in the stickiness that can mire insect herbivores [1]. These defense-related components appearing in latex of distant phylogenetic groups are thought to have possible biological effects on herbivores. Among these compounds are the proteases which have shown toxicological effects on insects. Proteases from a variety of sources (viruses, bacteria, fungi, plants, and insects) have toxicity towards insects [2]. Some of these insecticidal proteases evolved as venom components, herbivore resistance factors, or microbial pathogenicity factors, while other proteases play roles in insect development or digestion, but exert an insecticidal effect when over-expressed from genetically engineered plants or microbial pathogens.

Many of these proteases are cysteine proteases, although insect-toxic metalloproteases and serine proteases have also been examined [2]. Cysteine proteases are reported from latex of plant families such as Caricaceae, Moraceae and Apocynaceae [3,4]. In addition, some latex proteins are confined to specific plant taxa and have been suggested to be involved in plant defense. These compounds include phosphatase in Euphorbiaceae [5]; lipase in Caricaceae, Euphorbiaceae, Apocynaceae [6-8]; and glutaminyl cyclase in Caricaceae (papaya) [9,10]. *Carica papaya Linn.* being a monoecious, dioecious or hermaphrodite tree is the most common species of the family Caricaceae [11,12]. *Carica papaya* preparations can be efficiently used in tissue burn and microbial/helmintic infection. It can be also used as insecticidal/molluscicidal activity against various pests [13]. This plant contains specialized cells (laticifers) dispersed throughout most plant tissues that secrete "latex" [14]. Papaya latex is a thixotropic fluid with a milky appearance that contains about 85% water. An insoluble particulate fraction whose composition is still practically unknown, makes up 25% of the dry matter. The soluble fraction, however, contains both the usual ingredients such as carbohydrates (~10%), salts (~10%) and lipids (~5%), and representative biomolecules such as glutathione, cysteine proteinases (~30%)

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and several other proteins. Consequently, the resulting non-water-soluble material is generally considered as waste, and in comparison to the water soluble fraction, little is known regarding its chemical composition [15]. Moreover, the levels of these enzymes vary in the fruit, latex, seeds, leaves and roots [16]. Besides, female trees have been found to differ in the amounts of the compounds produced. Moreover, *C. papaya* is among the few latex-bearing plants whose noxious chemical contents have not been reported [17].

The objective of this study is to characterize the chemical constituents of *C. papaya* L. crude latex in terms of proteins and proteases which are the plant defence against herbivorous insects negatively affecting agricultural production.

2. Materials and Methods

2.1. Study Area

Carica papaya L. plantation is located at Tiwi-Simanok, Linangkayan, Naawan, Misamis Oriental which is 1 km from the national high way of Naawan, Misamis Oriental (**Figure 1**) and can be reached in a 10 min drive from the poblacion. The plantation site is approximately 2 ha land partly planted with coconut, banana and bamboos along the mangrove swamp. This site was formerly utilized as a cornfield with coconuts planted in between (**Figure 2**). Linangkayan is one of the outlying areas among the 10 barangays covering the municipality of Naawan, Misamis Oriental. These barangays remain to be rural areas with 2370 residents in Linangkayan in 2007 and are characterized by dry and wet climate. Linangkayan, being coastal is composed partly of sandy loam soil. The plain areas of Linangkayan are mostly planted with coconut, bananas with some trees and bamboos.

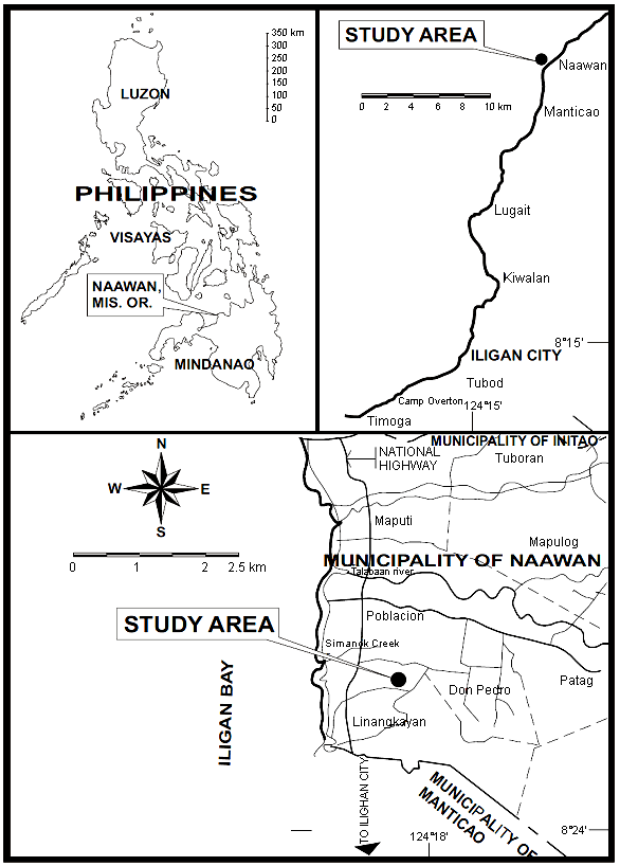

Figure 1. ***Carica papaya*** **L. plantation at Tiwi-Simanok, Linangkayan, Naawan, Misamis Oriental.**

2.2. Collection of Latex

Latex of *C. papaya* L. *was* collected from locally grown plants in Tiwi-Simanok, Linangkayan, Naawan, Misamis Oriental (**Figure 2**). Flowers, fruits and whole plant pictures of *C. papaya* L. species of papaya CX variety from Del Monte, Phils. were sent to the National Museum of the Phils., Manila for verification. The same plants were used as latex source throughout the study. Fresh latex was collected from locally grown *C. papaya*. Initially, 4 to 6 longitudinal incisions at 3 mm deep were made on the unripe mature fruit surface from fruit stalk end to the tip of the fruit by using a stainless steel knife between 0600 and 0800 h during bright sunshine [18,19]. The incisions were repeated 4 times at 3 da interval. The exuded latex was allowed to run down the fruit and drip into collecting devices (aluminum trays) raised in the trunk (**Figures 3-5**).

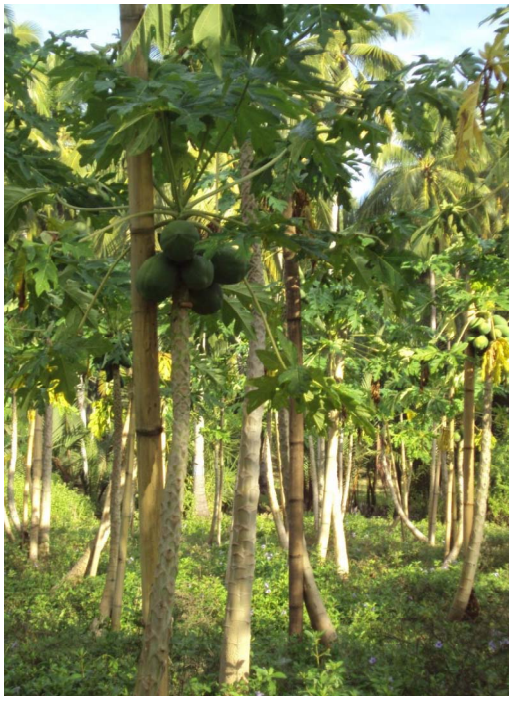

Figure 2. ***Carica papaya*** **L. plantation at Tiwi-Simanok, Linangkayan, Naawan, Misamis Oriental (Actual site).**

Figure 3. *Carica papaya* L. latex collection by incision using stainless steel knife.

Figure 4. *Carica papaya* L. latex allowed to drip in the aluminum tray raised in the trunk.

2.3. Isolation of Latex from *C. papaya*

The collected latex was spread on trays and left for drying through solar at 40˚C for 14 h (**Figures 6** and **7**). With the aid of laboratory mortal and pestle, the latex was ground producing a greenish or grey powder known as papain [20] which is known to have a proteolytic activity slightly higher than that of the fresh latex [21].

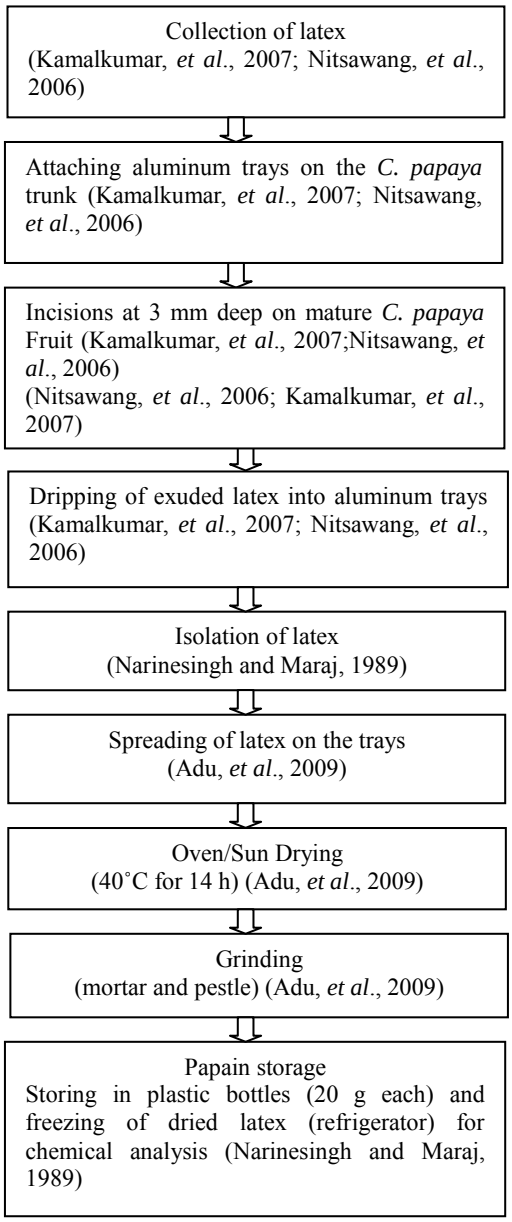

Figure 5. Flow chart of the methods involved in the study.

2.4. Analysis of Dried Latex Sample

A 100 g of *C. papaya* L. crude latex was taken for complete proximate analysis and an additional of 10 g for papain-enzyme. The variety of papaya used in the study is papaya CX from Del Monte Philippines, Cagayan de Oro City and its verified scientific name is *Carica papaya* L. of the Caricaceae family. Dried latex sample of 100 g and 10 g were transferred separately to plastic containers and brought to Biotech Phils., UPLB, Laguna, for complete proximate analysis in order to determine the

Figure 6. Spreading of *C. papaya* crude latex on aluminium tray.

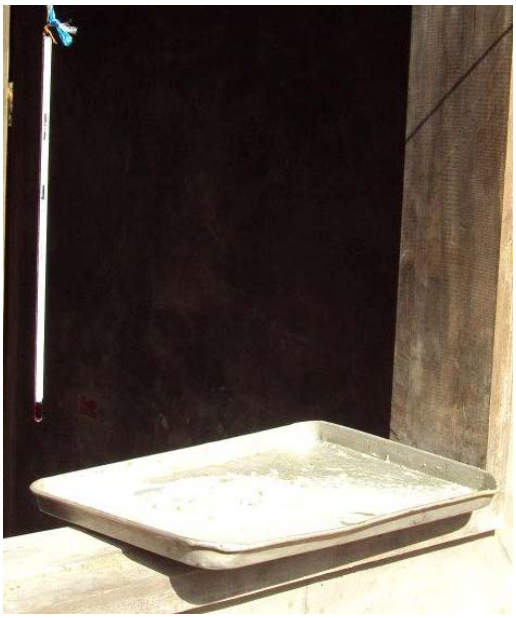

Figure 7. Solar and air drying of *C. papaya* crude latex at 30˚C - 40˚C.

components and protease activity, respectively. Protease activity was employed utilizing the Hammersten casein as substrate. The sample was passed unto 60 mesh sieve to obtain uniform sample size. Approximately 0.12 g sample was weighed and 10 mL of each buffer was added. The mixture was stirred for 30 min and then centrifuged for 5 min at 12,000 rpm to obtain a clear supernatant and then diluted with the same buffer. The diluted enzyme solution was allowed to react with the substrate of desired pH for 10 min at 55˚C. The reaction was stopped by addition of trichloroacetic acetic acid and the amount of tyrosine released was determined spectrophotometrically using a standard curve at 280 nm. Analysis was based on one unit of protease activity which releases 1.0 micromole of tyrosine min^{-1} from 0.5% Hammersten casein in 0.2 M acetate buffer pH 5.5 and 0.2 M glycine-NaOH buffer pH 9.0 at 55˚C.

2.5. Papain Storage

The dried products were packed in air-tight plastic containers and stored in a cool, dry place. Four plastic containers at 100 g capacity were used to pack crude papain flakes or powder since metal containers would result in loss of enzyme activity (**Figure 8**). These were kept and stored in freezer at −20˚C [18] in order to avoid reduction of its shelf life (**Figure 9**). Native and modified papain preparations were stored at 25˚C and 45˚C, and enzymatic activity was measured at scheduled times. It is generally accepted that a month's stability of an enzyme at 45˚C is roughly equal to that of one year at room temperature [22]. The latex from these unripe fruits presented a high activity compared with the fruit skin. Under the temperature evaluated conditions does not exist a significant statistic difference for the specific enzymatic activity for the selected drying processes. The only main difference presented was obtained according to the latex source [23].

Figure 8. Flakes-formed crude latex of *C. papaya* in plastic container.

Figure 9. *C. papaya* dried crude latex stored-freeze in plastic containers.

2.6. Disposal

All trays and other materials used in the latex collection and drying were washed thoroughly with water and detergent soap and kept dried. Waste water was allowed to run to the sink.

2.7. Documentation

The whole process in the latex characterization was documented by using a DSC-S950 Sony digital camera.

3. Results

Complete proximate analysis of *C. papaya* L. dried latex showed that it contained higher amount of crude protein at approximately (57.24 ± 0.69%) over other components such as moisture (17.76 ± 0.09%), ash (7.00 ± 0.01%), crude fat (5.21 ±0.13%) and crude fiber (0.67 ± 0.09%) (**Table 1**).

The enzyme-crude papain activity of the dried crude latex of *C. papaya* L. showed that at pH 5.5 protease activity yielded 2655 units·g^{-1} and at pH 9.0 protease activity yielded 285 units·g^{-1} only (**Table 2**). The remaining brownish-white flakes formed of *C. papaya* dried crude latex were packed and stored freeze in 4 plastic containers with a capacity of approximately 100 g container^{-1} (**Figures 8** and **9**).

4. Discussion

Proximate analysis of dried crude latex of *Carica papaya Linn* of papaya CX variety revealed high amount of crude protein (57.24 ± 0.69%) with moisture (17.76 ± 0.09%), ash (7.00 ± 0.01%), crude fat (5.21 ± 0.13%) and crude fiber (0.67 ± 0.09%) (**Table 1**). Relatively, latex is a milky fluid with a complex mixture of constituents, like proteins, vitamins, carbohydrates, lipids, terpenes, alkaloids, and free amino acids [24]. The presence of certain enzymes like chitinases and proteases in latex vacuoles suggests that they may help plants for defense against pathogens, parasites, and herbivores by attacking the invader once the plant cell is lysed [25]. Proteases are enzymes that catalyze the degradation of peptides and proteins. Proteases have significant role in numerous physiologic processes in the living organisms, as well as in different industrial processes. It was verified that proteases that are direct specific and selective modifications of proteins, such as the activation of proenzymes, sanguineous coagulation, digestion of fibrin clots, secretory protein processing and transport through membranes, germination, senescense, defense against plant pathogens (especially fungi and insects), and acquisition of nutrients and apoptosis [26-32]. Other previous papaya latex researches reported that the plants are rich in cysteine proteinases enzymes. These enzymes are used widely for protein digestion functions in the food and pharmaceutical industries [33]. Furthermore, cysteine proteases have traditionally been viewed as lysosomal mediators of terminal protein degradation and enzymes that catalyze hydrolysis of amide bonds [34]. Basically, cysteine proteases are classified into various kinds and one of which is papain, a plant proteolytic enzyme for the cysteine proteinase family. Cysteine protease enzyme is found naturally in papaya (*C. papaya* L.) manufactured from the latex of raw papaya fruits. The enzyme is able to break down organic molecules made of amino acids, known as polypeptides and thus plays a crucial role in diverse biological processes in physiological and pathological states, drug designs and industrial uses [35] and the enzyme is the most thoroughly characterized of the thiol proteinases [36]. Papain has revealed to be an enzymatic protein of significant biological and economic importance, since the unique structure of papain provides functionality and helps explain how this proteolytic enzyme works and makes it valuable for a variety of purposes [35]. This proteolytic enzyme usually consists of two well-defined domains which provide an excellent system for studies in understanding the folding-unfolding behavior of proteins [37]. The protein is stabilized by three disulfide bridges in which the molecule is folded along these bridges creating a strong interaction among the side chains which contributes to the stability of the enzyme [38,39]. Its three-dimensional structure consists of two distinct structural domains with a cleft between them. This cleft contains the active site, which contains a catalytic diad that has been likened to the catalytic triad of chymotrypsin [35]. Papain occurs in all parts of the tree except the root [40]. A well managed papaya production has re-

Table 1. Complete proximate analysis of dried crude latex of *Carica papaya* L.

Sample Code	Crude Protein (%)	Moisture (%)	Ash (%)	Crude Fat (%)	Crude Fiber (%)
Dried latex *Carica papaya*	57.24 ± 0.69	17.76 ± 0.09	7.00 ± 0.01	5.21 ± 0.13	0.67 ± 0.09

Table 2. Protease activity analysis of dried crude latex of *Carica papaya* L.

Sample Code	Biotech Code	Protease activity*	
		pH 5.5	pH 9.0
Dried crude latex from *Carica papaya* (crude papain)	P-1301	2655 units·g^{-1}	285 units·g^{-1}

*One unit of protease activity is defined as the amount of enzyme that releases 1.0 micromole of tyrosine per minute from 0.5% Hammersten casein in 0.2 M acetate buffer pH 5.5 and 0.2 M glycine-NaOH buffer pH 9.0 at 55˚.

corded higher papain yield of 8.17 g·fruit^{-1} and highest papain of 686.29 g·plant^{-1} in a period of 6 mo [35]. As to the analysis of the protease activity, results showed that papain at pH 5.5 had a protease activity of 2655 units·g^{-1} and 285 units·g^{-1} only at pH 9.0 (**Table 2**). The results imply that papain is more active in its activity in slightly acidic medium than in a basic. Several studies supported the idea as papain exhibits its greatest activity at an acidity equal to the concentration of the hydrogen ion of 10^{-5} N; *i.e.*, slightly more acid than is necessary to cause methyl red to change from yellow to red [41]. It is the definite hydrogen ion concentration at which papain was most active proteolytically. The conditions of acidity for the optimum action of papain are found to be pH = 5 [41]. Plant-based enzymes, such as bromelain from pineapple and papain from papaya, have proteolytic activity [42]. Papain as a cysteine hydrolase is stable and active under a wide range of conditions. It is very stable even at elevated temperatures [43]. Papain is unusually defiant to high concentrations of denaturing agents, such as, 8 M urea or organic solvent like 70% EtOH [38,44]. The enzyme has been reported to be generally more stable in hydrophobic solvents and at lower water contents and can catalyze reactions under a variety of conditions in organic solvents with its substrate specificity little changed from that in aqueous media [45]. However, most cysteine proteases are unstable and weakly active at neutral pH and thus are optimized to function in acidic intracellular vesicles [34]. Optimum pH for activity of papain is in the range of 3.0 - 9.0 which varies with different substrate [39,44]. Under the temperature evaluated conditions does not exist a significant statistic difference for the specific enzymatic activity for the selected drying processes. The only main difference presented was obtained according to the latex source [23]. Papain being solubilized in water showed greater enzymatic activity [46]. Besides, the hydrolytic activity of the latex depended upon the state of development of the fruit [47]. Almost ripe fruits yielded latex which split only proteins and was without effect on peptones, whereas latex from unripe fruits showed activity towards both proteins and peptones, and latex from very young fruits showed "full activity". The greener the fruit, more active is the papain [35].

5. Summary and Conclusion

1. The collection and the isolation of crude dried latex of *Carica papaya* L. were done for chemical analysis characterizing its protein and proteases as the constituents for plant defence against herbivores insects.

2. Complete proximate analysis of dried crude latex of *Carica papaya* L. showed that it contained higher concentration of crude protein (57.24 ± 0.69%) followed by moisture (17.76 ± 0.09%), ash (7.00 ± 0.01%), crude fat (5.21 ± 0.13%) and crude fiber (0.67 ± 0.09%).

3. A protease activity of 2655 units·g^{-1} was obtained at pH 5.5 and 285 units·g^{-1} only at pH 9.0.

4. Crude latex of *C. papaya* could be highly considered a potential source for proteolysis especially when applied in slightly acidic medium where its protease activity is much higher.

5. The protease activity analysis was especially focussed on the crude papain content of the *carica papaya* latex.

6. Evidently, papain is one of the protease enzymes being noted to have been significantly employed or actively participated in many proteolytic activities which could be beneficial when applied in farms addressing the many hazardous environmental issues like pollution, degradation, pest control, health problem and many others.

6. Implications and Recommendations

Chemical analysis of *Carica papaya* L. crude latex revealed the presence of crude proteins in higher amount as compared to other chemical constituents. Protease enzyme being protein in nature showed a higher protease activity of 2655 units·g^{-1} at pH 5.5 and 285 units·g^{-1} only at pH 9.0. These results implied that proteolysis of crude latex from *C. papaya* L. could be more effective when applied in slightly acidic medium than in a basic. This study suggests the potential of *C. papaya* L. crude latex in the control of pest population that ensues declining farm production. There shall be more studies involving crude latex of *C. papaya* on its action to other plants and also to the insects' morphological formations. Furthermore, this study can serve as a reference among researchers to continue investigating more valuable information on the potentials offered by *C. papaya* L. to improve farm production and resolved issues on environmental degradation and health related problems. Moreover, this study will boost agricultural production using crude latex of *C. papaya* as pest control knowing its protein contents and its protease activity.

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