# Evaluating the Enzymatic Activity of Papain under Different Forms of Papaya at Optimal pH

K.M.J.P.S. Gunathilake[1]*, R.D. Harini[1], N.C. Wickramasinghe[1], M.N.F. Rizla[1], R.A.M. Dilshani[1], D.D.Y. Rasanjanee[1], R.G.L. Rathnayake[2]

[1]*KIU University, Colombo, 10120, Sri Lanka.*
[2]*University of Uva Wellassa, Badulla, 90000, Sri Lanka.*
**b7b8058@kiu.ac.lk*

Papaya (*Carica papaya*) contains papain, a natural digestive enzyme. This study examined papain activity in five forms unripe, ripe, fermented, dried, and cooked under conditions mimicking the pH and the temperature of small intestine (pH 6.5, 37°C), where protein digestion occurs. Casein hydrolysis measured enzyme activity to identify which form retains the most activity, offering insights into health promotion and dietary use of papaya. Each form (1g) was homogenized in phosphate buffer (1:10), centrifuged, and the extracted enzyme was tested using a casein hydrolysis assay. The reaction mixture, containing 1.1 ml casein solution and 0.1 ml enzyme extract, was incubated for 10 minutes, terminated the reaction by using trichloroacetic acid, and centrifuged. The absorbance of the resulting supernatant was measured at 280 nm to quantify tyrosine release, indicating enzyme activity. The results were expressed as Casein Digestion Units per gram (CDU/g). The in vitro study produced major differences in papain activity for the five forms of papaya. The highest activity was observed in unripe preparation (26.08 CDU/g), followed by ripe (15.56 CDU/g), fermented (7.96 CDU/g), and dried (1.32 CDU/g) preparations. It was also noted that there was no quantifiable enzyme activity detected in cooked papaya. The one-way ANOVA confirmed that the differences in mean papain activity across the five forms of papaya were statistically significant ($p < 0.001$). The results showed the loss of papain activity through both heat and drying, with both unripe and ripe forms providing the majority enzyme activity potential in digestive conditions. This study contributes to understanding the level of papain activity in each form of papaya to understand which one provides the most enzyme benefits when consumed and supports the dietary benefits.

**Keywords:** Papain, papaya, pH, enzyme activity