CHAPTER V
CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Papain crystallization behavior depends on the method of nucleation generation and operating conditions. Within measurement uncertainty, solubility in acetate buffer (pH 5) was comparable to water, whereas solubility decreased with increasing methanol fraction and decreasing temperature.

Under the tested methanol addition protocol, antisolvent crystallization predominantly yielded amorphous aggregates and gel, while cooling crystallization at rate of 0.005 °C/min recovered papain crystals (needle-like) from concentrated solutions (saturated >0.77 g/mL, 100 rpm). Needle–like crystals at nucleation that evolved toward mixed needle/plate habits during growth; some plate-like appearance after drying may reflect preparation artifacts.

The SFO crystallization produced crystalline papain (also needle-like and mix plate-like after dryness) from dilute feeds (0.33–0.47 g/mL) at bulk −1.5 to 1 °C when the coil was held at −12.6 °C and −14 °C. The SFO technique achieved over 55% recovery with less than 30% solute loss into the ice phase at a freeze rate of 0.02 °C/min. PXRD indicated similar crystallinity for papain crystals grown in buffer (pH 5) versus water. Specific activity differences among SFO, cooling, and commercial references were not significant under the reported assay conditions, but SFO process uniquely capable crystallized papain from lower feed concentrations while maintaining crystallinity and specific activity, and making it particularly suitable for pharmaceutical applications requiring pure, functional protein crystals.

5.2 Recommendations

1) The pilot scale SFO crystallizer for papain should study with less solute loss than 20% into ice.

2) For separation papain by crystallization, if feed papain concentration is less than solubility, this SFO process is suitable. But for feeds near saturation, the cooling crystallization should be considered with slow cooling rate.

3) The enzyme activity determination should be analyzed in triplicate within the same batch and month of experimental operation to avoid the time dependent decay.