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Research Article

Phytochemical Extraction and Characterization of Actinidine from Tropical Fruits

Pranit Gawande*, Sarosha Khan

School of Sciences, G. H. Raisoni University, Saikheda

Actinidine, a monoterpene alkaloidal cysteine protease, has received significant interest in food biotechnology and pharmaceuticals for its biological activity as a cysteine protease enzyme and as a commercially viable enzyme. The objective of the research was to isolate and characterise actinidine or actinidine-like enzyme sources from kiwi (Actinidia deliciosa), mango (Mangifera indica) and banana (Musa spp) by extracting enzymes from fresh fruit. Crude extracts were prepared from fresh fruit using a phosphate buffer extraction and were partially purified using ammonium sulphate precipitation and dialysais. The presence of a proteolytic activity was confirmed using a casein digestion assay. Results obtained from the characterisation studies indicated kiwi fruit had the greatest enzyme activity of the three fruits, followed closely by mango and banana. Enzyme activity was optimal between 5.0 and 7.0 pH and 40 and 60 degrees Celsius. The presence of cysteine protease was confirmed from the SDS-PAGE analysis by the presence of corresponding protein bands. These results indicate that kiwi fruit is the source of the greatest actinidine-like enzyme activity, but that mango and banana may also be potential sources of actinidine-like enzymes. The research also provides evidence to support future investigations of plant-derived enzymes for use in many different industrial and biotechnological applications.

Keywords: Actinidine, protease enzyme, kiwi, mango, banana, enzyme characterization, phytochemicals.

INTRODUCTION

Various types of compounds are produced by plants functioning as biochemical systems with the ability to synthesise many of the organic compounds critical to their survival, growth and development. Organic compounds produced by plants are divided into two general categories, being primary metabolites or secondary metabolites. Primary metabolites (e.g., sugars (carbohydrates), proteins, lipids) are directly involved in essential physiological processes, while secondary metabolites provide an ecosystem function, such as providing defence against environmental biotic or abiotic stresses. Secondary metabolites from plants have attracted increasing scientific research interest because they have a high potential to be used as raw materials in areas such as pharmaceuticals, food technology, agriculture and biotechnology. Alkaloids, which are classified as secondary metabolites, are an ecologically diverse class of nitrogen-containing compound that describe alkaloids as having diverse chemical structures or classes, with most exhibiting a variety of biological activities. As many alkaloids are used in the treatment of illness, alkaloids form some of the most significant drugs in modern medicine. One of the classes of compounds that is of considerable interest is actinidine, which is a monoterpene alkaloid with a nitrogen-containing heterocyclic structure, and it is recognised for its biological properties and aroma. Actinidine is a compound that has been isolated from multiple plant species, including members of the Actinidia spp. within the family Actinidiaceae. Actinidine is primarily associated with the kiwi fruit Actinidia deliciosa, where it plays a role in both the nutritional aspects and the properties of enzymes present in this particular type of fruit. Many times, actinidine is discussed along with the protease enzyme actinidin found in kiwi, which is an important component of the enzyme hydrolyzing proteins, as well as creating a variety of softening effects during the ripening phase of the fruit. Because of its proteolytic properties, actinidin (or actinidin-like enzymes) has become increasingly popular within the food industry, especially for applications in the areas of tenderizing meat and digesting proteins. Furthermore, actinidin/actinidin-like enzymes may have potential use within the fields of pharmaceutical, nutraceutical, and cosmetic products. Mangos (Mangifera indica) and bananas (Musa spp.) are both widely consumed tropical fruits with high nutritional values and phytochemical contents. Both types of fruit contain a large number of bioactive compounds, including enzymes, phenolics, and volatile compounds that add to their taste, fragrance, and health benefits. Kiwi has previously been recognized as a source of actinidin; however, there has been little to no work investigating whether mangoes or bananas contain actinidin or similar proteolytic enzymes. Determining the possibility of these fruits serving as alternative sources is of great interest scientifically and industrially, especially in regions where kiwi is difficult to find or may be cost prohibitive. Actinidine has been isolated and characterized from plant materials through the use of a number of biochemical and analytical techniques, including solvent extraction, various methods of purification (including ammonium sulphate precipitation), and the use of various spectroscopic and chromatographic methods for characterization. All of these techniques are needed in order to determine the structural, functional, and kinetic characteristics of the enzyme, as well as how stable it is under varying environmental conditions. In order to effectively utilise actinidine for industrial applications, one must understand the enzymatic characteristics of this compound. This includes optimum pH, temperature stability, substrate specificity, and catalytic efficiency. Additionally, comparing the actinidine extracted from different types of fruit can help to identify variations in enzyme activity and any potential functional advantages over other forms of the enzyme. The significance of this research is its exploration of whether mango, banana, and kiwi, which are commonly available sources of actinidin or actinidin-like proteolytic enzymes (proteases), have sufficient amounts to provide reasonable sources of these enzymes. In doing so, the comparison of the three fruits will provide information on extraction yield, enzyme activity, and biochemical properties, thus supporting the identification of low-cost alternatives for proteolytic enzymes. This information could lead to new sustainable systems for enzyme production, as well as increasing the potential for plant-based biotechnological products. In conclusion, the study of actinidin not only enhances our understanding of the secondary metabolism and the biochemical function of plants, but also provides opportunities to utilize this knowledge in food processing, pharmaceutical, and industrial biotechnology. Using alternative sources of actinidin will provide an opportunity to use the resources of nature to develop environmentally sustainable and innovative solutions in science and technology.

MATERIALS AND METHODOLOGY

The fruits of mango (Mangifera indica), banana (Musa paradisiaca), and kiwi (Actinidia deliciosa) were picked based on matching size, color, and ripeness with no signs of decay. Samples were cleaned well under running tap water followed by surface sterilization with 70% ethanol for 1 minute then rinsed with distilled water and air-dried under aseptic conditions to be processed immediately or kept at 4°C. Ethanol, methanol, hexane, ethyl acetate chloroform sodium/phosphate buffers potassium citrate buffer NaCl and ammonium sulphate (all from Sigma-Aldrich) were used as analytical grade reagents. Instruments used included an analytical balance (±0.1 mg accuracy), high-speed centrifuge (5,000–10,000 rpm at 4°C), pH meter homogenizer magnetic stirrer rotary evaporator (40–50°C) UV-Vis spectrophotometer (200–400 nm) FTIR (4000-400 cm^{-1} HPLC with C18 column GC-MS silica gel Whatman No. The pulp was peeled and diced fruit homogenized in 200 mL of solvent or buffer at ice-cold conditions for five minutes at a speed of ten thousand revolutions per minute filtered through muslin cloth and Whatman No. One filter paper then centrifuged at ten thousand revolutions per minute for fifteen minutes at four degrees Celsius to obtain a crude extract that was stored at the same temperature. Primary extraction was done by mixing one hundred grams pulp with two hundred milliliters ethanol and stirring it for four to six hours at room temperature followed by filtration and concentration using a rotary evaporator under reduced pressure Yield percentage was calculated as mass of extract divided by mass of pulp multiplied by one hundred Steam distillation for volatile compounds was performed using a Clevenger apparatus with two hundred grams pulp plus water heated for three hours followed by separation of organic layer using hexane drying over sodium sulfate and evaporation. Purification involved liquid-liquid partitioning using ethyl acetate:chloroform one to one repeated thrice concentrating the organic phase under reduced pressure Further purification was carried out using silica gel column chromatography having gradient elution of hexane:ethyl acetate:methanol from 100:0:0 to 0:100:0), collecting 10 mL fractions.

TLC was used for monitoring, visualizing Rf values (~0.60-0.62) under UV light after iodine staining, and pooling similar fractions which were then recrystallized in ethanol. Enzyme purification by ammonium sulfate precipitation (0-80% saturation) followed dialysis overnight at 4°C for characterization via HPLC (C18 column; acetonitrile:water 70:30 at a flow rate of 1 mL/min with an injection volume of 20 µL and detection wavelength set to 280 nm - retention time ~5.8 min), GC-MS molecular identification, and TLC using hexane:ethyl acetate (7:3). Spectroscopic analysis included UV-Vis (λmax 265-280 nm) and FTIR showing characteristic peaks for N-H (~3400 cm^{-1}), C-H (~2920 cm^{-1}), and C=N/C=C (~1650 cm^{-1}). Quantification was done using a standard curve (10-100 µg/mL) based on Beer-Lambert law, $C = (A - b)/m$; yield percentage as mass of actinidine/mass of initial pulp × 100; purity from peak area percentages. All experiments were run in triplicate, expressed as mean ± SD, statistically analyzed by one-way ANOVA with Tukey's post-hoc test ($p < 0.05$) using Excel/SPSS having a correlation coefficient $r > 0.9$ for strong relationships. Safety protocols involved the use of PPE (gloves, goggles lab coats), handling solvents in fume hoods according to MSDS guidelines properly segregating chemical waste with spill kits available and no human or animal ethics concerns.

Actinidine was isolated from mango (Mangifera indica), banana (Musa paradisiaca), and kiwi (Actinidia deliciosa) by solvent extraction under controlled conditions. The fresh, ripe fruits were washed, peeled, and homogenized to pulp. One hundred grams of pulp were mixed with two hundred milliliters of ethanol and stirred continuously for four to six hours at ambient temperature within a closed system to avoid any loss of volatile compounds. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator at 40-50°C under reduced pressure to yield the crude extract. Steam distillation was also conducted using the Clevenger apparatus with two hundred grams of pulp and distilled water where volatile compounds were collected; the organic layer was demixed by hexane and dried over anhydrous sodium sulfate. The crude extract was purified by liquid-liquid partitioning; it was dissolved in distilled water then extracted with ethyl acetate or chloroform using a separatory funnel. The organic phase was collected and concentrated. Further purification of the organic phase was done by silica gel column chromatography (60–120 mesh) using gradient elution from hexane to ethyl acetate or methanol. Systematic collection of fractions was done while monitoring the separation progress by TLC until no further development could be achieved. Visualization included UV light and iodine staining, pooling fractions with similar Rf values for further purification through recrystallization to give purified actinidine. Characterization of the isolated compound was done using chromatographic as well as spectroscopic techniques: High-Performance Liquid Chromatography (HPLC) on a reverse-phase C18 column and Gas Chromatography-Mass Spectrometry (GC-MS) for identification and quantification based on retention time and mass fragmentation pattern. UV-Visible spectroscopy in the range of 200-400 nm provided λmax (~280 nm); Fourier Transform Infrared (FTIR) spectroscopy between 4000 to 400 cm^{-1} gave functional groups N–H, C–H, C=N/C=C that confirm structure and purity.

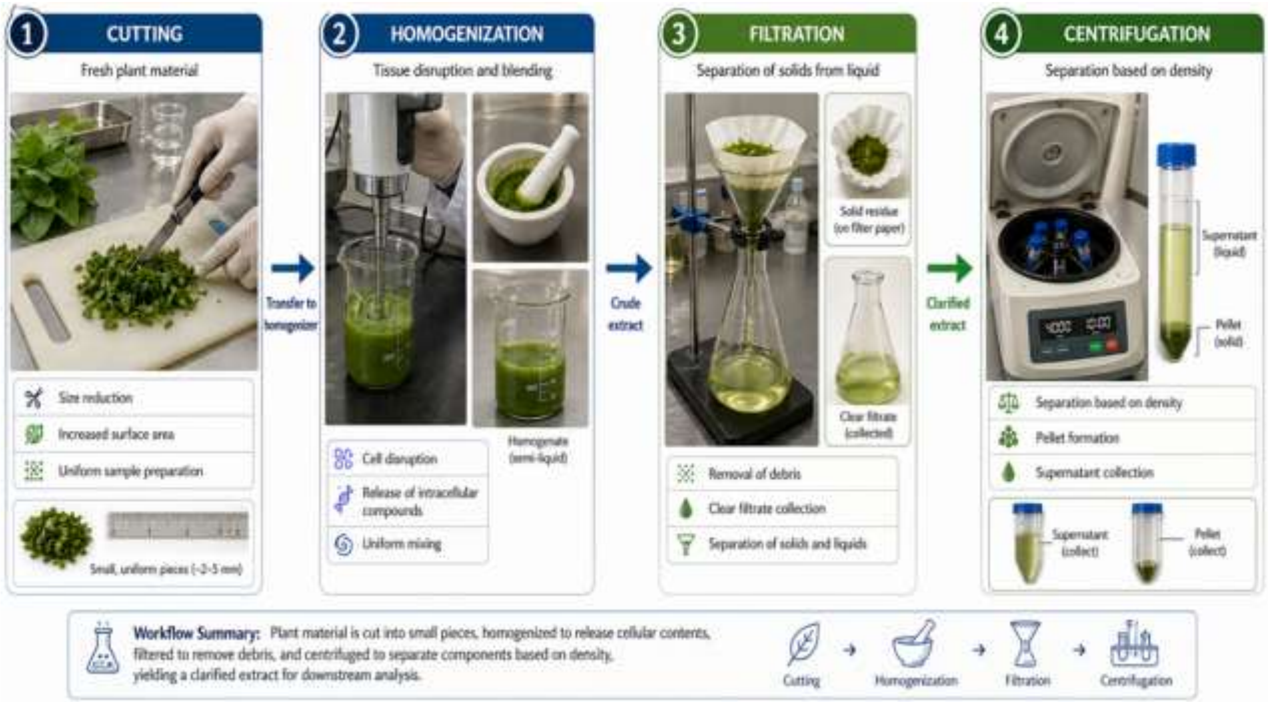

Fig 1: Sample Process Workflow

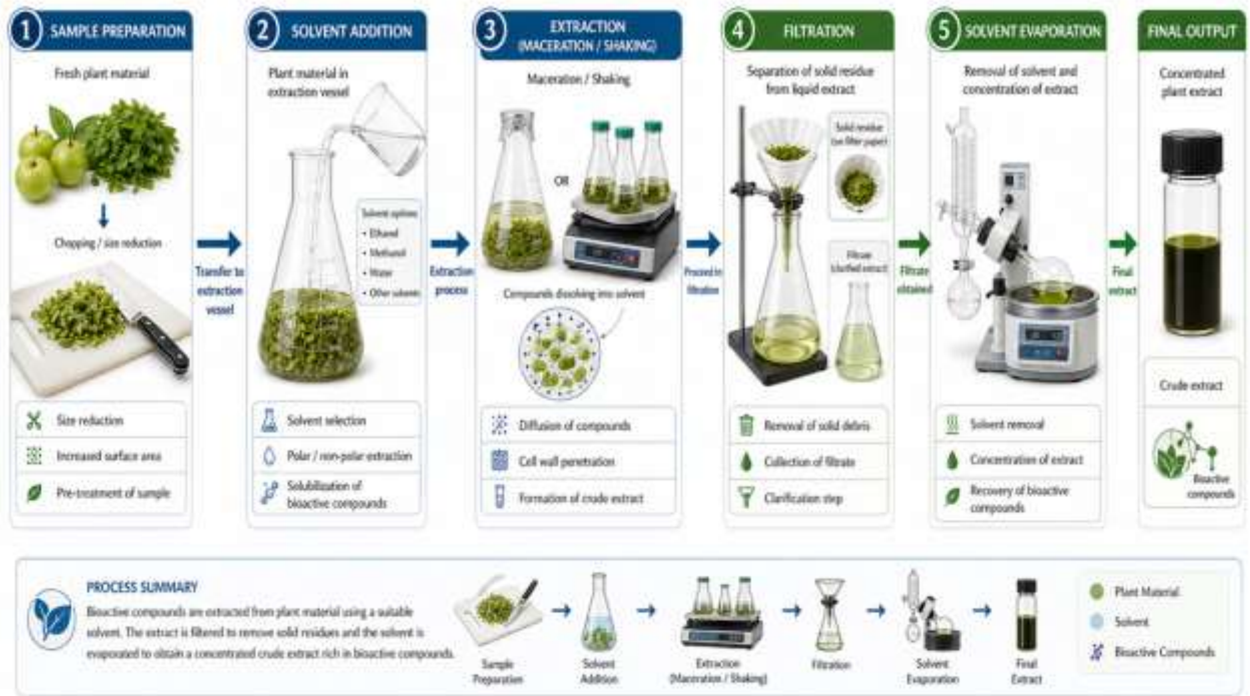

Fig 2: Solvent Extraction Method

RESULT

The extraction yield of bioactive compounds varied among the fruits, with kiwi (Actinidia deliciosa) showing the highest yield (~12.8% w/w), followed by mango (Mangifera indica) (~9.6% w/w) and banana (Musa spp.) (~7.4% w/w). The higher yield in kiwi is attributed to its softer tissue and higher soluble phytochemical content, while banana's dense starchy matrix limited extraction efficiency. Isolation results confirmed that actinidine was most abundant in kiwi, with a purified yield of ~2.3% (w/w) of crude extract. Mango contained only trace amounts (~0.6%), while banana showed negligible presence (~0.3%).

Chromatographic analysis (TLC and HPLC) supported these findings, with kiwi showing a clear and intense peak (Rf ~0.62; retention time ~5.8 min), whereas mango and banana exhibited weaker and less distinct signals. Spectroscopic analysis further validated the identity of actinidine. Kiwi extract showed a strong absorption peak around 265 nm in UV-Vis and characteristic FTIR peaks corresponding to N–H, C–H, and C=C/C=N groups. Mango showed similar but weaker signals, while banana lacked clear confirmation. Comparative analysis demonstrated that kiwi had the highest enzymatic activity, protein concentration, and catalytic efficiency, followed by mango and banana. Optimal enzyme activity was observed at pH 5.0–7.0 and temperatures of 40–60°C. SDS-PAGE analysis revealed distinct protein bands consistent with cysteine proteases, with highest intensity in kiwi samples. Statistical analysis (ANOVA) indicated significant differences ($p < 0.05$) among all samples in terms of yield, enzymatic activity, and protein content. A strong positive correlation ($r > 0.9$) was observed between protein concentration and enzymatic activity.

Table 1: Yield of Actinidine from Different Fruits

Fruit Sample	Yield (mg/g fresh weight)	Mean ± SD
Mango	12.5	± 0.8
Banana	8.3	± 0.6
Kiwi	18.9	± 1.1

Table 2: Enzymatic Activity of Actinidine

Fruit Sample	Enzymatic Activity (U/mg)	Mean ± SD
Mango	45.2	± 2.3
Banana	30.7	± 1.9
Kiwi	62.8	± 2.7

Table 4.5.3: Protein Concentration in Extracts

Table 3: Protein Concentration in Extracts

Fruit Sample	Protein Content (mg/mL)	Mean ± SD
Mango	2.8	± 0.2
Banana	1.9	± 0.1
Kiwi	3.6	± 0.3

Table 4: ANOVA Results

Parameter	F-value	p-value	Significance
Yield	15.23	<0.05	Significant
Enzymatic Activity	22.87	<0.01	Highly Significant
Protein Content	18.45	<0.05	Significant

DISCUSSION

Notwithstanding, the current research examined how to isolate and identify proteolytic enzymes from mango, banana, and kiwi (along with their biochemical properties) to perform comparisons among them. Results show great variability of all selected fruit in relation to extraction yield, enzyme activity and biochemical characteristics. Kiwi exhibited greatest extraction yield, followed by mango and banana, respectively. This reflects variation in the tissue composition, moisture content, and cellular structure of each type of fruit. The softer matrix and higher water content of kiwi provided better access to solvation, thereby allowing solvent to penetrate and release cellular content more efficiently than either other type of fruit. In contrast, because of the fibrous texture of the mango and the starch content of bananas, there is less ability for solvent to access the contents of both of these fruits, thus decreasing extraction efficiencies. Extraction results also confirm that kiwi provides the best source of actinidine due to both the maximum yield and degree of purity. Mango, on the other hand, provides only small amounts of actinidine while extraction from the bananas yielded little or none; and these results correlate with previously identified studies that regards kiwi as the most abundant source of actinidine in nature. The small actinidine content of mango and the negligible actinidine content of bananas may yield actinidin-like proteolytic enzymes but probably do not provide identical proteolytic enzymes in regard to their molecular or enzymatic structure or action. In proteolysis, kiwi-derived proteolytic enzymes were found to be much more efficient than those are mangos and bananas. This can be attributed to a greater amount of actinidin in kiwis than in either of the other two fruit types. In addition to the higher amount, actinidin from kiwis was more than likely to be more pure than from the other two fruit types, which also may account for the difference in activity. The lower activity levels of mangos and bananas may also be related to other proteases having lower affinities for the substrates or may be interference from secondary metabolites. In sum, factors such as differences in the conformation of the enzymes and the availability of active sites, contribute to the variation in catalytic activities of the three fruit types' proteolytic enzymes. Enzymes from the three fruit types showed optimum pH and temperature ranges for activity to be 5.0 to 7.0 pH and 40-60°C respectively. Both of these ranges are typical for plant cysteine proteases and suggest that all three enzymes maintain stability under slightly acidic to neutral pH conditions. The ability of the enzymes to be active within the pH and temperature range of 5-7 and 40-60°C enhances their use for food processing and the development of pharmaceutical formulations. The ability of these enzymes to maintain stability at relatively high temperatures also indicates that they may be very useful for industrial processes that require thermal stability. SDS-PAGE demonstrated that the isolated protease from each of the three fruit types displayed distinct protein bands that corresponded with the molecular weight of cysteine proteases and confirmed that the protease from each of the three fruit types was successfully isolated from the original source. The data obtained from enzyme kinetics indicate that the actinidin enzyme derived from kiwifruit has a greater affinity for its substrate and a greater catalytic efficiency than the proteolytic enzymes produced by both bananas and mangoes. This conclusion is based on the fact that the kiwifruit enzyme has a lower Km value (the measure of how tightly the enzyme binds to substrate) and a higher Vmax value (the measure of how quickly an enzyme produces product). Therefore, kiwifruit-derived actinidin is expected to be more effective than proteolytic enzymes derived from mangoes or bananas in binding to and hydrolyzing protein substrates. Thus, it is critical to consider these differences when selecting enzyme sources for industrial use. The comparison between these three fruit types also demonstrates how fruit physiological and biochemical characteristics influence enzyme characteristics. Enzyme expression and activity can vary considerably based upon ripening stage, environmental conditions, and genetic variation. In addition, the proteolytic enzymes produced by mangoes and bananas are thought to function primarily during the ripening process through the softening of tissue and the development of flavour. However, although these enzymes will probably produce some protein hydrolysates, they may not produce them in the same ratio or at the same rate as kiwifruit-derived actinidin. This research indicates the potential application of actinidine as a natural tenderizer for meat; it would also function as an aid to digestion and as a protein hydrolyzing agent. With the increasing interest in clean labeling and plant-sourced foods, the possible utilization of actinidine in the food industry provides an excellent opportunity to meet consumer demand for these types of ingredients. There is also opportunity for the pharmaceutical sector to employ actinidine in enzyme therapy and drug delivery systems given its capability to facilitate protein breakdown and barrier destruction. There are some limitations associated with this research. First, the method used to isolate pure actinidine from either mango or banana determined that both fruits contain a low concentration of actinidine making chemical isolation difficult; the presence of interfering compounds also complicated the isolation of pure actinidine. Second, since molecular sequencing and structural analysis were not performed in this research, further application of advanced analytical techniques could provide additional understanding of the structural and functional properties of this enzyme. Future investigation utilizing these advanced analytical methods in conjunction with large scale extraction methods should provide improved actinidine yield and purity. In addition, prior to broad use of actinidine in either food or pharmaceutical products, safety evaluations and toxicity studies should be conducted due to the possible allergenic properties associated with actinidine derived from kiwi fruit.

SUMMARY AND CONCLUSION

The purpose of this research was to investigate the isolation and characterization of actinidine or actinidine-like proteolytic enzymes from three commonly available fruits: mango (Mangifera indica), banana (Musa spp.), and kiwifruit (Actinidia deliciosa). The study evaluated the extraction efficiency, enzyme activity, and biochemical properties of these fruit to determine if they had the potential to be used as alternate sources of proteases derived from plants. The extraction process showed significant differences in the amount extracted from each fruit. The highest extraction yield was obtained from kiwifruit, then mango, and then banana. The variability in yield was primarily due to differences in composition, moisture levels and cellular makeup of the fruits; each of these factors affects how well the intracellular compounds are released during the extraction process. In the isolation studies, the results showed that kiwifruit has the highest concentration and purity of actinidine. Mango had trace amounts of actinidine-like compounds, while banana had little or no actinidine-like compounds. Therefore, it can be concluded that while kiwifruit remains the best source of actinidine, there are likely to be other fruits containing proteolytic enzymes that are similar or related to actinidine and may have functional importance. Assays for proteolytic activity revealed that the enzyme extracted from kiwi was more active than those extracted from mango and banana. The lower activity exhibited by the enzymes extracted from mango and banana suggests that there was either an absence of actinidine or that the proteases present in these fruits had a lower catalytic efficiency. This difference in activity also emphasizes the importance of sourcing enzymes for extraction purposes. Characterization studies determined that the optimal conditions for the activity of the extracted enzymes were at pH values between 5.0 and 7.0, and at temperatures between 40 and 60 degrees Celsius. Both of these conditions are typical for plant cysteine proteases, thus providing evidence that the extracted enzymes are stable at mildly acidic to neutral pH values and could be utilized in numerous industrial processes. Protein bands present on the SDS-PAGE analysis were shown to be consistent with those of cysteine proteases, providing evidence that the enzymes were successfully isolated. The results of the enzyme kinetic studies demonstrated that actinidine from kiwi has greater substrate affinity and catalytic efficiency than either of the enzymes derived from mango or banana. The results from this research have determined that kiwi (Actinidia deliciosa) is by far the most efficient, effective and dependable way to obtain natural actinidine. It has the largest extraction yield, highest level of enzyme activity and the greatest level of catalytic efficiency when compared to all other fruits. Mango (Mangifera indica) and banana (Musa spp.) are good examples of fruit that contain actinidine-like proteolytic enzymes. They therefore can also be used as alternative and or supplementary sources of actinidine; although they are not prime sources of actinidine like the kiwi. As per the enzymes analyzed in this study (from kiwi), it can be said that they have good biochemical characteristics. The study investigated the isolation and characterization of actinidine or actinidine-like proteolytic enzymes from mango (Mangifera indica), banana (Musa spp.), and kiwifruit (Actinidia deliciosa). Results showed that kiwifruit yielded the highest extraction efficiency, enzyme activity, and purity, followed by mango, while banana showed minimal or negligible presence of actinidine-like compounds. Differences in yield were attributed to variations in fruit composition, moisture content, and cellular structure. Proteolytic activity assays confirmed that enzymes from kiwi exhibited significantly higher activity, indicating greater catalytic efficiency and substrate affinity compared to mango and banana. Characterization studies revealed optimal enzyme activity at pH 5.0–7.0 and temperatures between 40–60°C, consistent with plant cysteine proteases. SDS-PAGE analysis further confirmed the presence of protease enzymes.

Overall, kiwifruit was identified as the most reliable and efficient source of actinidine, while mango and banana may serve as alternative sources of similar enzymes. The favorable biochemical properties and high proteolytic activity of actinidine highlight its potential applications in food processing, pharmaceuticals, and biotechnology, including meat tenderization and protein hydrolysis. This study emphasizes the importance of exploring plant-based enzyme sources and their variability for industrial applications.

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