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# Purification and characterization of actinidin from *Actinidia deliciosa* and its utilization in inactivation of α-amylase

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## Abstract

**Background:** Actinidin is an anionic thiol-proteinase predominant and unique to Chinese gooseberry or kiwifruit, whose strong digestibility enables proteins or enzymes vulnerable to digestion. The arrangement of active cysteine–thiol residues (Cys22-Cys65, Cys56-Cys98, and Cys156-Cys206) stabilizes the catalytic unit, thus allowing an effective Inhibition of α-amylase protein on exposure to the highest concentrations of actinidin under optimum conditions. When starch-rich foods are consumed with kiwifruit, starch digestion may be slowed by the inactivation of α-amylase (digestive enzyme), specifically reducing the blood sugar levels by hindering starch digestion that is helpful in diabetes mellitus. Thus, the study aimed at actinidin purification, optimization for maximal activity, and its demonstration as a potential to degrade α-amylase.

**Results:** Protease showed a molecular mass of 27 kDa on SDS-PAGE analysis. One factor at a time method was applied for process optimization, increasing the actinidin yield up to 176.03 U/mg. The enzyme was stable at a wide pH range; however, it was most active and stable at pH 7.5. The enzyme possessed half-life at 35 °C of 5.5 h, at 40 °C of 4.5 h, at 45 °C of 2.5 h, and at 50 °C of 1 h. Lineweaver–Burk plot showed Michaelis–Menten constant (*Km*: 3.14 mg/ml) and maximal velocity (*Vmax*: 1.428 mmol/ml/min) using casein. The actinidin activity was enhanced with $Ca^{2+}$ while it was inhibited by $Cd^{2+}$ and $Hg^{2+}$ ions. The α-amylase protein was successfully inactivated upon incubation with actinidin for 30 min; around ~ 85% of the α-amylase activity diminished. $IC_{50}$ for inhibition of α-amylase was 2.54 mg/ml for crude actinidin and 1.86 mg/ml for purified actinidin.

**Conclusions:** Purified Actinidin showed a 1.28-fold increase in proteolytic activity. The proteinase showed an active pH range of 3.5–8.5 under varied buffer conditions and thermostability up to 50 °C. The results revealed a significant potential utility of actinidin to retard amylase as it effectively degraded the amylolytic enzyme under in vitro conditions and could be beneficial for lowering glycemic response to ingested starch. However, further in vitro as well as in vivo studies need to be conducted under gastrointestinal conditions to establish the hypothesis.

**Keywords:** Kiwifruit, *Actinidia deliciosa,* actinidin, SDS-PAGE, MALDI–TOF MS, Characterization, α-amylase

## Background

Proteases have a key role in basic research because of their specificity to cleave peptide bonds used to elucidate protein sequence and structure. Commercially important proteases are obtained from a wide variety of living organisms (Baker et al. 1980). Kiwifruit belongs to the genus *Actinidia* and is a good source of actinidin protease. Actinidin is a plant-derived protease unique to *Actinidia sp.* It belongs to the cysteine or thiol protease

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group due to its functional and structural resemblance to papain (Baker 1980). Actinidin has a high potential for commercial use in meat tenderization, milk coagulation, and health-promoting diet supplements (Montoya et al. 2014). Different studies showed the antimicrobial effect of the actinidin enzyme against foodborne pathogens, pathogenic gram-positive and gram-negative bacterial strains (Lim 2012). Actinidin, being a protease, is capable of digesting many enzymes. Alpha-amylase is an essential enzyme present in saliva and pancreatic juice responsible for the degradation of large non-soluble starch into simple sugar moieties and influences the rate of carbohydrate digestion and its absorption (Peyrot des Gachons and Breslin 2016). Treatment of Type-II diabetes explicitly involves inhibition of starch processing proteins, such as α-amylase. Studies have reported alpha-amylase digestion via actinidin (Martin et al. 2017). The reduction of amylase protein due to the presence of actinidin is anticipated to lower the glycemic index from a starch-containing diet and help to lower the postprandial glucose level (Martin et al. 2017). While there have been few studies on the effects of actinidin on other digestive enzymes in vivo or in vitro, actinidin has recently been reported to digest human salivary amylase and its protease effect on salivary amylase function requires further clarification (Martin et al. 2017). Keeping into account the potential uses of actinidin, the present study focused on the purification and characterization of actinidin from *Actinidia deliciosa*. Different parameters were studied for enzyme characterization aimed at increasing its activity. Subsequently, characterized actinidin was used for the in vitro study of inhibition of α-amylase and also checked for its role in inactivating the digestive enzyme amylase via degradation under in vitro conditions.

## Methods

### Sample

*Actinidia deliciosa* cv. Abbot (fresh kiwifruit) was procured from Kiwifruit Orchard, YSP UHF, Solan (India), and samples were washed thoroughly with distilled water, placed on blotting paper air-dried, and stored at 4 °C until extraction for bioactive molecules was done.

### Chemicals

Casein, EDTA, TEMED, Bradford reagent, and α-amylase enzyme (*Aspergillus oryzae*) were purchased from Merck; starch, tyrosine, prestained protein ladder, DEAE Sepharose, and TCA were purchased from HiMedia Laboratories. Bromophenol blue was obtained from Genei and DTT from SERVA Electrophoresis GmbH, Heidelberg, Germany. All chemicals and reagents were of analytical grade.

### Assay for actinidin activity and protein estimation

Actinidin was assayed using casein (Kunitz 1947). The test mixture consisted of 1.8 ml 1% (w/v) casein and 0.2 ml actinidin. After a reaction time of 30 min at 37 °C stopped the reaction with 3 ml of cold 15% (w/v) TCA; control contained TCA prior to incubation and blank contained 0.2 ml distilled water in place of the enzyme. After incubation, assay mixtures were centrifuged at 4100 g for 15 min. The absorption of the supernatant was read at 280 nm with a UV–Vis spectrophotometer (Panomax PXUV-2601, New Delhi, India) to estimate the proteolytic activity of actinidin. One unit (U) of proteinase activity is the amount of proteinase enzyme, which liberates 1 µg of tyrosine per min per ml under standard assay conditions.

### Extraction of crude enzyme

The whole kiwifruit was processed in a food processor with an equal volume of ice-cold extraction buffer (0.1 M sodium phosphate buffer, 1.0 mM EDTA, and 1.0 mM DTT) containing 0.4 M sodium metabisulfite to avoid the enzyme-mediated browning reaction. The crude homogenate mixture obtained was subjected to centrifugation using a high-speed refrigerated research centrifuge (Eltek RC 4100 F) at 11,280.61 g for 10 min at 4 °C (Raynal et al. 2014). The supernatant was labeled as crude enzyme extract, checked for its activity, and stored at 4 °C.

### Purification of actinidin

#### *Ammonium sulfate precipitation*

The crude enzyme was subjected to 90% fractional salt precipitation with the $(NH_4)_2SO_4$ salt under steady temperature (4 °C) and gentle stirring. The saturated solution was mixed using a magnetic stirrer overnight. Subsequently, the mixture was centrifuged at 11300 g at 4 °C. The pellet obtained was resuspended into a minimal amount of extraction buffer (0.1 M sodium phosphate buffer, 1.0 mM DTT, 1.0 mM EDTA, pH 7.0) followed by dialysis against buffer containing 0.1 M sodium phosphate, 1.0 mM EDTA, and pH 7.0 buffer in a cellulose membrane. The protein concentration of dialysate was estimated by the Bradford method (Wingfield 2016).

#### *DEAE-anion exchange chromatography*

DEAE Sepharose column (10 × 1.5 cm) was pre-equilibrated with 1.0 M NaCl and 0.5 M NaOH, respectively. The dialysate (5 ml; 2.76 mg protein) was loaded on the column bed surface. The elution was performed with an increasing NaCl gradient (0.1 to 0.5 M) in sodium phosphate buffer (0.1 M, pH 8.0). The active protein

fraction(s) or elutriate were collected and analyzed for their activity and protein content. Fractions showing good activity were pooled and stored.

#### *SDS-PAGE analysis*

SDS-PAGE (12% (w/v)) analysis was performed, and 10 µl of the samples was loaded. A protein marker (HiMedia MBT092, Mumbai, India) was used for molecular mass analysis.

#### *MALDI–TOF MS/MS analysis*

The purity of actinidin was analyzed by MALDI–TOF MS. The molecular mass of the protease, the excised spots of the SDS-PAGE was trypsinized and given incubation (37 °C) overnight. The digests were dehydrated then treated with 0.1% TCA. This mixture (0.5 ml) was suspended in 0.5 ml of cyano-4-hydroxycinnamic acid and sprayed onto the MALDI–TOF mass spectrometry target plate. The peptides and spectra were analyzed and using Bruker Auto flex (Bruker Daltonics, Germany) and Bruker BioTools version 2.2.

### Effect of physicochemical factors on the activity of purified actinidin

#### *Effect of pH and temperature on actinidin*

To determine the effect of pH and buffer system for maximal actinidin activity, the specific activity of actinidin was measured at varying pH using five different buffer systems: sodium acetate buffer of pH 3.5–4.5, citrate buffer of pH 4.5–6, sodium and potassium phosphate buffer of pH 6–8, and Tris–HCl buffer (pH 7–8.5).

To investigate the influence of temperature on purified actinidin, the assay was performed at 20 to 60° C in sodium phosphate buffer (0.1 M) pH 7.5 for 30 min.

#### *Effect of incubation time and substrate on actinidin*

To determine the effect of incubation time, actinidin activity was assayed at 10, 20, 30, 40, 50, 60, 70, 80, and 90 min in sodium phosphate buffer (0.1 M) of pH 7.5 at 37 °C.

The activity of purified actinidin was examined at various concentrations of casein (0.2 to 2.4 mg/ml) to assess its influence on the substrate concentration. The reaction was carried out in sodium phosphate (0.1 M) buffer of pH 7.5 for 50 min at 37 °C.

#### *Kinetics of purified actinidin*

The catalytic activity of actinidin was measured by studying the reaction velocities at different casein concentrations (0.2 to 1.0 mg/ml) under standard assay conditions. The reciprocal of velocities ($1/V$) vs. substrate concentration ($1/V$) was plotted to determine Michaelis–Menten constant ($K_{m)}$ and maximal velocity ($V_{max}$) values by Lineweaver–Burk plot. The enzyme kinetics were calculated using the Lineweaver–Burk equation: $1/V = (K_m/V_{max})\ (1/S) + 1/V_{max.}$

#### *Effect of temperature and different metal ions on actinidin stability*

The thermostability of the purified actinidin was tested by incubating enzyme at particular temperatures (30 °C, 35 °C, 40 °C, 45 °C, and 50 °C) for 6 h. After every 1-h interval, the activity of incubated actinidin was studied.

Influences of metal ions on actinidin activity were demonstrated. A few of the metal ions $Na^{2+}$, $K^{+}$, $Ca^{2+}$, $Mg^{2+}$, $Cd^{2+}$, $Fe^{2+}$, $Mn^{2+}$, $Hg^{2+}$, and $Ba^{2+}$ obtained from (2.0 mM each) NaCl, KCl, $CaCl_2$, $MgCl_2$, $CdCl_2$, $FeCl_2$, $MnCl_2$, $HgCl_2$, and $BaCl_{2,}$ were used respectively.

#### *α-Amylase inhibition assay*

A modified approach of Kazeem et al. (2013) and in vitro inhibition experiment were used to investigate the effect of actinidin on α-amylase. A total of 200 µL of actinidin (3 mg/ml) was taken in tubes along with the 200 µL of α-amylase solution (0.1–0.5 mg/ml) which was prepared in 0.20 M sodium phosphate buffer (pH 6.9). The solutions were preincubated at 25 °C, followed by the addition of 200 µL of 1% (w/v) starch solution. Another incubation was given to the reaction mixture at 37 °C for 10 min. 500 µL of DNS solution was added, and the mixture was water bathed at boiling temperature for 5 min and kept at rest for a few minutes. Finally, all the tubes were diluted with distilled water (5 ml) and at 540 nm absorption were recorded for each reaction mixture. The inhibitory activity of amylase was calculated as follows:

$$\text{Enzyme activity} = \frac{A_{540}(\text{test}) \times \text{Concentration of Standard } (\mu\text{moles}) \times \text{Dilution of enzyme}}{A_{540}(\text{standard}) \times \text{incubation time (10 min)}}.$$

One unit (U) of activity is defined as 1.0 mg of glucose equivalents released per min in the assay reaction (Kazeem et al. 2013).

### Statistical analysis

Experiments were performed in triplicate. The standard deviation (where applicable) was calculated using Microsoft Excel software, and the results were presented as

**Table 1** Summary of actinidin purification from *Actinidia deliciosa* Chev

| Purification step | Volume (ml) | Activity (U/ml) | Protein (mg/ml) | Total protein (mg) | Total enzyme activity(U) | Specific activity (U/mg) | Purification (fold) | Recovery (% yield) |
|---|---|---|---|---|---|---|---|---|
| CE | 452 | 59.67 | 1.05 | 475.48 | 26,970 | 56.82 | 1.00 | 100.00 |
| PP | 26 | 244.38 | 2.76 | 71.86 | 6353 | 88.54 | 1.55 | 23.55 |
| DEAE Sepharose | 15 | 403.52 | 2.93 | 43.95 | 6052 | 137.72 | 2.42 | 22.43 |

CE: Crude enzyme; PP: protein precipitate (dialysate)

Mean ± SD values. This helped to obtain the optimum/optimum range for each of the parameters analyzed.

## Results

### Extraction and purification of crude actinidin

A total of 452 ml of crude actinidin was obtained from 196 g of fresh kiwifruit with the enzymatic activity of 59.67 U/ml and 1.05 mg/ml of protein content (Table 1).

The crude actinidin was purified from other soluble proteins by dialyzing the supernatant after concentrating protein by 60% ammonium sulfate saturation. The dialysate contained many soluble proteins, which were further separated and purified through fractionation by DEAE-anion exchange chromatography. Thirty fractions (1.0 ml) were collected and assayed for both protein content and enzyme activity (Fig. 1a).

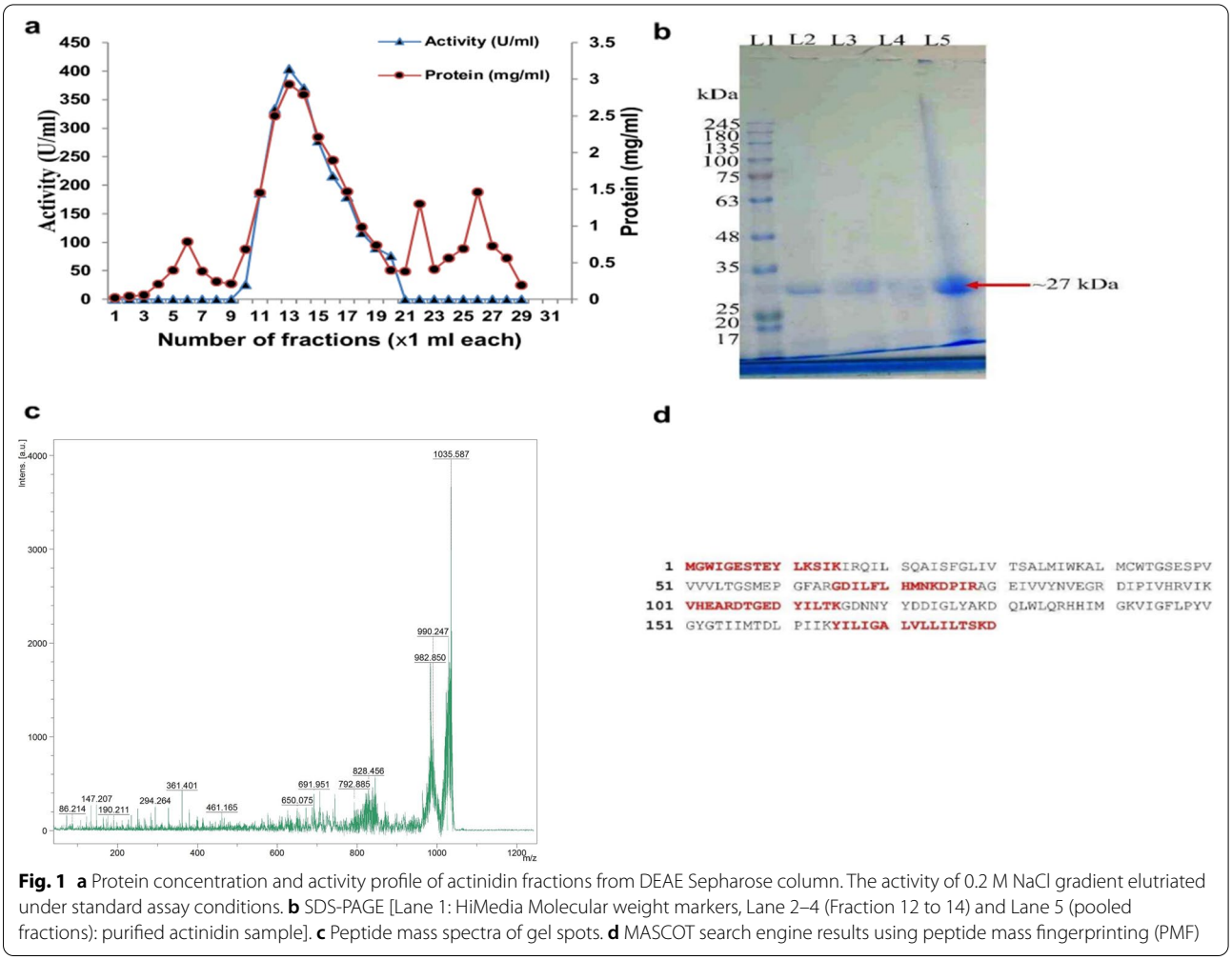


**Fig. 1** **a** Protein concentration and activity profile of actinidin fractions from DEAE Sepharose column. The activity of 0.2 M NaCl gradient elutriated under standard assay conditions. **b** SDS-PAGE [Lane 1: HiMedia Molecular weight markers, Lane 2–4 (Fraction 12 to 14) and Lane 5 (pooled fractions): purified actinidin sample]. **c** Peptide mass spectra of gel spots. **d** MASCOT search engine results using peptide mass fingerprinting (PMF)

Elution profile showed a prominent peak (enzyme activity) at 12–14 fraction(s). The actinidin was purified up to 2.42-fold. The overall yield and activity of actinidin are summarized (Table 1). The purified enzyme sample was stored at -20 °C for further studies. In this analysis, the crystalline enzyme displayed an activity of 137 U/mg.

#### The molecular mass of actinidin

SDS-PAGE was used to examine the fractions (Lane 2, 3, and 4) with varied enzyme activity immediately after separation. Figure 1b shows that several fractions had a single major band with a comparable molecular weight (27 kDa). Pooled fractions (lane 5) had a greater intensity, suggesting that the actinidin had been purified.

#### Actinidin/protease detection by MALDI–TOF MS

To identify the protease, the peptide fragments (Fig. 1c) from MALDI–TOF MS were applied to the MASCOT search engine using peptide mass fingerprinting (PMF). The corresponding spots were identified match protease. Figure 1d shows an amino acid sequence alignment of peptides derived from pure actinidin following trypsin digestion, showing the matching peptides highlighted in red. The best match had a mascot score of 49 with a mass of 20,283 Da, which corresponds to *Cucurbita pepo* subsp. *pepo*. 33.0% of sequence coverage for peptidase was identified.

#### Effect of physicochemical factors on the activity of purified actinidin

One factorial at a time approach was followed for the characterization of purified actinidin for different parameters. The optimum for each parameter was also found/confirmed using the statistical tools (Table 2).

#### Effect of temperature and pH on enzyme activity

Actinidin showed maximal activity of 138.2 U/mg at 37 °C (Fig. 2a).

However, an optimum range for actinidin activity was obtained between 35 and 40 °C temperature (Table 1). The activities of actinidin were analyzed at varying ranges of pH (3.5 to 8.5) (0.1 M) buffering capacity (Fig. 2b). The purified actinidin exhibited strong activity for a pH range between 6.0 and 8.0. The maximum specific activity of 147.6 U/mg with NaPB (sodium phosphate) was observed at pH 7.5 (Table 2). Variations in substrate pH showed the highest specific activity in a neutral pH environment. Actinidin retained 76% and 88% of maximal activity at pH conditions of 6.0 and 8.0.

**Table 2** Optimum process parameters for the characterization of purified actinidin

| Parameter | pH | Time (min) | Temperature (°C) | Substrate (mg/ml) |
|---|---|---|---|---|
| Optimum range | 7.5 | 50–60** | 35–40** | 1.2–1.4** |

*: Similarity, $p \leq 0.05$. **: Similarity, $p \leq 0.01$

#### Effect of substrate and time on enzyme activity

Maximum activity of 166.3 U/mg was observed at 1.2 mg/ml casein concentration. The substrate concentration of 1.2–1.4 mg/ml was optimum to obtain maximum enzyme activity (Table 2, Fig. 2c). Further raising the casein concentration to 2.2 mg/ml resulted in a 76% reduction in activity. According to the observed data (Fig. 2d), the purified actinidin showed increasing activity up to 50 min of incubation time, where it reached the maximum activity of (176 U/mg). The optimum range for enzyme activity was recorded at a time period of 50–60 min after incubation with casein (Table 2).

#### Kinetics of purified actinidin

The $V_{max}$ and $K_m$ of actinidin were determined using specified concentration of casein (0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml). Kinetic values of the actinidin were plotted using the Lineweaver–Burk reciprocal plot (Fig. 2g). $K_m$ value of 3.142 mg/ml and a $V_{max}$ value of 1.428 mmol/ml/min were estimated. The *Kcat* of actinidin was found to be 13.16 per minute with a catalytic efficiency of 0.069 /mg/sec. The kinetic parameters of actinidin showed decreased $K_m$ and increased *K*cat for the enzyme and can be related to the enzyme's increased activity.

#### Thermostability of actinidin

The thermostability of purified actinidin was studied after 6 h incubation at specific temperatures (30 to 50 °C). The enzyme displayed a half-life of 6 h at 30 °C, 5.5 h at 35 °C, 4.5 h at 40 °C, 2.5 h at 45 °C, and 1 h at 50 °C. The thermostability profile (Fig. 2e) of actinidin strongly suggests that the enzyme was most stable at 35 °C. Thus, an increase in temperature up to 50 °C drastically reduced the proteolytic activity of actinidin.

#### Effect of metal ions on actinidin activity

The modulating effect of selected metal ions on actinidin was studied. The results showed that the exposure of actinidin to $Ca^{2+}$ ions enhanced the enzyme activity of 173.5 U/mg (Fig. 2f). This could be due to the stabilization of the tertiary structure of actinidin, preventing the

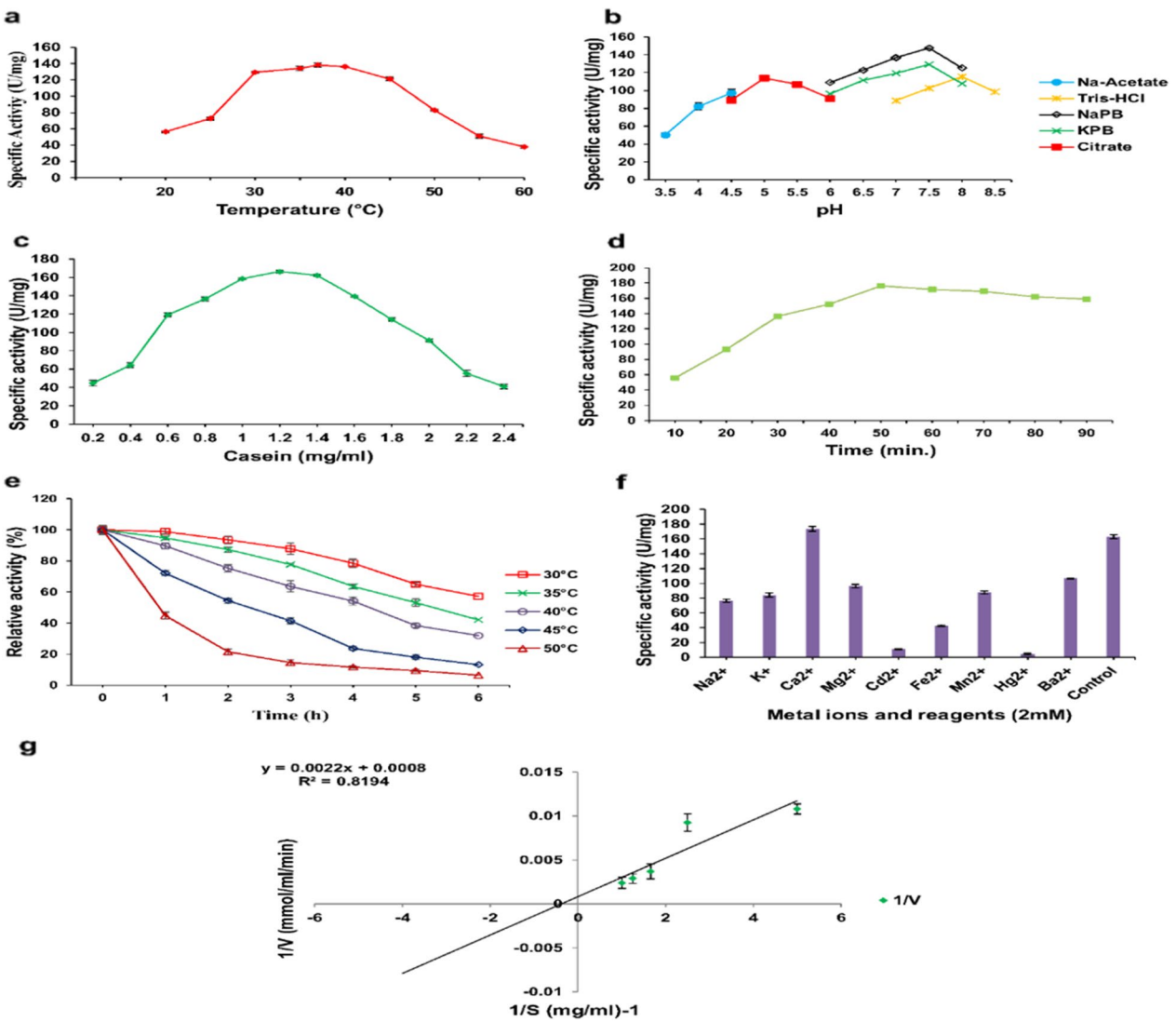


**Fig. 2** **a** Effect of temperature on purified actinidin activity. **b** Effect of pH (3.5–8.5) on actinidin activity. **c** Effect of the substrate (casein) on the actinidin activity. **d** Effect of incubation time on the actinidin activity. **e** Effect of temperature on actinidin stability. **f** Influence of metal ions (2 mM) on actinidin activity. **g** Lineweaver–Burk plot of purified actinidin using casein as a substrate

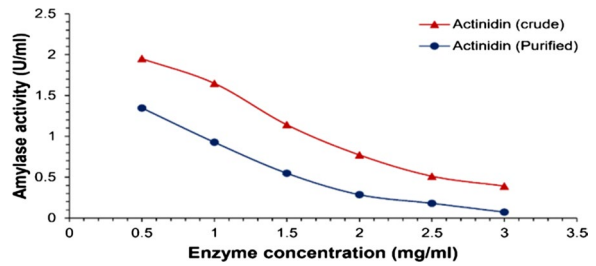


**Fig. 3** α-amylase activity after 10 min of exposure to actinidin: control (water + α-amylase) showed 2.43 U, filled square actinidin (crude), and filled circle actinidin (purified)

inactivation of protease. The minimum concentrations of $Hg^{2+}$ and $Cd^{2+}$ induced substantial inhibition of actinidin, whereas $Na^{2+}$, $K^{+}$, $Mg^{2+}$, $Fe^{2+}$, $Mn^{2+}$, and $Ba^{2+}$ caused moderate inhibition of enzyme activity.

### α-Amylase inhibitory assay

α-Amylase inhibition potential of the actinidin was determined. Upon treatment of α-amylase by actinidin at varied concentrations (0.5 mg/ml to 3 mg/ml) revealed 95% decreased amylolytic activity (1.95 U to 0.391 U, Fig. 3).

## Discussion

In the present investigation, actinidin (EC.3.4.22.14) was extracted from *Actinidia deliciosa* Chev. Abbot was purified and characterized. For the concentration of actinidin in the homogenate, successive methodologies were carried out. The earliest study on actinidin involved the centrifugation of ripe kiwifruit homogenate pulp followed by dialysis (Rahmawati et al. 2020). Firstly, salting out using ammonium sulfate, then DEAE-anion exchange chromatography was used, which was found to be most effective in the purification of many enzymes. McDowall (1970) prepared a crystalline anionic protease with the thawing of frozen mature kiwi, accompanied by centrifugation and anion exchange chromatography of the clarified fruit juice. The crystalline enzyme displayed an activity of 388 mU/mg. In addition, his demonstration that the active crystallized compound was actinidin is reinforced by our experiments focused on the observations using high-throughput analytical techniques. Purification experiments also found that the resulting active enzyme had a broad pH optimum, thermostability, and efficient digestibility of the substrate. Earlier, scientific evidence has highlighted that molecular mass for each actinidin form was between 27,000 ± 300 Da, which was based on the electrophoretic mobility of the molecule. The difference in size was due to the presence of different isoforms of actinidin. Sugiyama et al. (1996) discovered six acidic isoforms with various pI values with indistinguishable *N*-terminal sequences. The difference in molecular weights of actinidin isoforms could be due to various factors such as the number of kiwifruit cultivars, limitations of methods, or lack of studies of isoforms during the electrophoretic method (Chao 2016). The prior study determined 23,883.1 Da mass through MALDI–TOF MS (Chao 2016). For a deeper analysis of kiwifruit proteases, mass spectrometry of 2-D zymographic spots was done, and an *N*-terminal peptide of 1035 Da mass was obtained (Larocca et al. 2010). In another study, isoforms of actinidin with 31.2 to 23.9 kDa molecular mass were identified using MALDI–TOF MS (Baker 1980).

Proteolytic activities of actinidin showed maximum degradation at 37 °C on various globular and fibrous proteins (Chalabi et al. 2014). However, a study showed maximal activity at 45 °C incubation temperature. This could be due to different pH environments and buffer conditions. The enzyme stability is critical of its pH. It plays a major role in the solubility of substrates, influences enzyme structure and association with its substrate, and causes denaturation at the extreme alkaline or acidic environment for most of the enzymes (Butts et al. 2016). Previous investigations also supported the present study where optimal proteolytic activity for actinidin was obtained at pH 7 (0.1 M) of phosphate buffer (Al-Zubaidy 2017). Incubation time plays an important role in determining the capabilities of any enzyme. In prior studies, different substrates such as gelatine, hemoglobin, and casein have been used to examine the proteolytic effects actinidin enzyme (Li et al. 2021). The $K_m$ values of protease enzymes depend upon their affinity with the substrate, depending upon their amino acid sequences (Homaei and Etemadipour 2015). The activity of free and immobilized actinidin was studied, and $K_m$, $K_{cat}$, were observed to be 12.5 M and 29.2 $s^{-1}$ for free, whereas 15.92 M and 20.74 $s^{-1}$ for immobilized enzyme. This suggests actinidin has good specificity for casein (Homaei and Etemadipour 2015). Zhang et al. (2017) reported an 80% loss of residual activity of actinidin at 50 °C after 30 min. Homaei and Etemadipour (2015) determined half-life of the actinidin enzyme to be 4 min at 80 °C. Sugiyama et al. (1996) reported a moderate increase in the residual activity of actinidin upon the addition of $Ca^{2+}$ (2 mM) ions. Ions usually bind at the active sites on proteases' charged residues, affecting their cleavage rate by acting as activators or inhibitors. For example, thermolysin, a peptidase, catalyzes hydrolysis on the binding of zinc metal ions, and removal of zinc ions results in the inactivated enzyme (Cheng et al. 2021). The increased activity of the actinidin on by calcium ions ($Ca^{2+}$) is attributed to their role in preserving the structure/conformity of actinidin which is significant for its catalytic activity (Svendsen 2016). The proteolytic ability of sulfhydryl actinidin enhances upon the addition of a reducing agent. Hence, cysteine as a reducing agent caused the increased catalytic activity of actinidin by dissociating the disulfide bridges present in the actinidin structure. When disulfide bridges get dissociated, a free disulfhydryl group is obtained with increased catalysis (Kaur et al. 2015).

The amylase activity on quickly edible starch renders smaller saccharides such as disaccharides and trisaccharides, which are then additionally hydrolyzed to glucose. Amylase being highly susceptible to digestion with actinidin may inhibit starch digestion resulting in the hindrance of glucose production. However, slow digestible or resistant starch persists as leftovers into the gastrointestinal tract to a large extent. The resultant malto-oligosaccharide levels usually represent the extent of granular starch decomposition. Thus, actinidin was able to bind with ES complex as well as a free enzyme. This suggested that the protease enzyme involves cleavage of protein residue permitting chemically changed nature of the protein, thereby preventing the breaking down of polysaccharides into oligosaccharides into disaccharides into glucose. Actinidin being able to work at a wide range of pH can effectively degrade human salivary amylase when pepsin cannot exhibit maximum (Al-Zubaidy 2017).

Besides, kiwifruit bearing a strong buffering capacity could help the kiwi protease in expressing its activity against amylase and other proteins (Martin et al. 2017). This could be helpful in delaying the absorption of starch on consuming kiwi. A previous study showed digestion of human salivary amylase actinidin, causing 83% loss in amylase function (Martin et al. 2017). And also, actinidin might be used to investigate the degree of proteolytic cleavage of amylases from various sources.

## Conclusions

Proteases from plant-derived sources are becoming incredibly popular in the food industry, biological research, and their potential therapeutic applications. In this study, a successful attempt was made to isolate, purify, and characterize one such plant protease (actinidin). Characterization of actinidin resulted in obtaining a 1.28-fold increase in actinidin activity. The protease remained catalytically active within a pH range of 3.5–8.5 and effectively inhibited $\alpha$-amylase. Inhibiting carbohydrate digesting enzymes such as $\alpha$-amylase acts as a key concept toward diabetes treatment. Though the present study showed the involvement of actinidin enzyme in $\alpha$-amylase inhibition, further in vitro as well as in vivo studies need to be conducted.

### Abbreviations

SDS-PAGE: Sodium dodecyl sulfate–polyacrylamide gel electrophoresis; MALDI–TOF MS: Matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry; Da: Dalton; EDTA: Ethylenediaminetetraacetic acid; DTT: Dithiothreitol; ES: Enzyme substrate; DEAE: Diethylaminoethlyl; $IC_{50}$: Concentration of enzyme which results in 50% inhibition; kDa: Kilodaltons; $V_{max}$: Maximal velocity; Km: Michaelis constant; TCA: Trichloroacetic acid; $K_{cat}$: Catalytic efficiency of enzyme; OD: Optical density; DNS: 3,5-Dinitrosalicylic acid; UV–Vis: Ultraviolet–visible.

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### Authors' contributions

VKD and VC conceived the original idea, carried out the field experiments, collected data for the study, and wrote the first draft of the manuscript. SSK, DS, and HP read the first draft of the manuscript for necessary corrections. DS and HP finalize the final draft after correction. All authors read and approved the final manuscript for submission.

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### Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

## Declarations

### Ethics approval and consent to participate

The studies involved in this article did not include animals or human participants as research objects.

### Consent for publication

Not applicable.

### Competing interests

No competing interests exist.

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