

Actinidin Enhances Gastric Protein Digestion As Assessed Using an in Vitro Gastric Digestion Model

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Consumption of kiwifruit has long been claimed anecdotally to assist in gastric digestion. This has generally been assumed to be due to the presence of the proteolytic enzyme actinidin; however, there is little published evidence supporting this assumption. This paper reports the findings of an in vitro study that examined the effect of kiwifruit proteases (actinidin) on the digestion of a range of common food proteins under simulated gastric conditions. An extract from green kiwifruit containing actinidin was prepared. Several protein sources derived from soy, meat, milk, and cereals were incubated in the presence or absence of the kiwifruit extract using an in vitro digestion system consisting of incubation with pepsin at pH 1.9, simulating gastric digestion in humans. The digests were subjected to gel electrophoresis (SDS-PAGE). For some protein sources, simulated digestion in the presence of kiwifruit extract resulted in a substantially greater loss of intact protein and different peptide patterns from those seen after digestion with pepsin alone. As an example, the addition of actinidin extract enhanced the digestion of α -, β -, and κ -caseins in sodium caseinate by 37, 33, and 48%, respectively. Under simulated gastric conditions, kiwifruit extract containing actinidin enhanced the digestion of some, but not all, food proteins over and above that found with pepsin alone.

KEYWORDS: Kiwifruit; *Actinidia deliciosa*; actinidin; in vitro protein digestion; SDS–polyacrylamide gel electrophoresis; actinidin

INTRODUCTION

Kiwifruit is a well-known fruit within the genus *Actinidia*. Some varieties of kiwifruit such as green kiwifruit (*Actinidia deliciosa* var. Hayward) contain a highly active proteolytic enzyme, actinidin, whereas others, such as ZESPRI® GOLD Kiwifruit (*Actinidia chinensis* var. Hort16A), have only trace levels of this enzyme (1).

Actinidin (EC 3.4.22.14) belongs to the family of cysteine proteases and contains a free sulfhydryl group essential for its activity. Some of the other thiol/sulfhydryl proteases include ficin, stem bromelain, fruit bromelain, chymopapain, and papain. The important features of actinidin include a wide pH range for catalytic activity and stability at high concentrations at moderate temperatures, but the enzyme is susceptible to oxidation, a feature in common with other plant thiol proteases.

It has long been known that raw kiwifruit prevents the solidification of gelatin jellies, and the first crude extract from kiwifruit containing proteolytic activity was reported by Arcus (2).

Anecdotal claims that the consumption of kiwifruit assists in gastric digestion have existed for many years. This effect has generally been assumed to be due to the presence of the proteolytic enzyme actinidin. There is little published evidence, however,

to support these assertions. Moreover, the action of actinidin in concert with pepsin may give rise to a different array of peptides during gastric digestion compared to the action of pepsin alone. Peptides released from food proteins during digestion may exhibit a diverse range of bioactivities (3).

Here, we describe an in vitro study that investigates the role of actinidin from kiwifruit in gastric digestion. In this study, we have hypothesized that an extract of kiwifruit (actinidin) as part of a meal may influence the digestion of protein, particularly in the stomach. An in vitro digestion system based on the method of Savoie and Gauthier (4) was used to simulate gastric protein digestion. Digestion studies were carried out on a range of food protein sources including those derived from legumes, meat, milk, and cereals.

MATERIALS AND METHODS

Materials. Green kiwifruit (*A. deliciosa* var. Hayward) used in the study were provided by ZESPRI International Ltd (New Zealand) and were preripened to a similar degree of firmness [firmness RTE (ready to eat) of 0.5–0.8 kgf, measured using a penetrometer (5)]. The fruit was stored at 4 °C until extraction was carried out. Nine different proteins were used in the study: sodium caseinate; whey protein isolate (WPI); gluten; gliadin; gelatin; soy protein isolate (SPI); collagen from bovine Achilles tendon (Type I); beef muscle protein; and zein. Sodium caseinate and WPI (Alanate 185 and WPI 895) were procured from Fonterra Co-operative Group Limited, Auckland, New Zealand. Gelatin was supplied by Gelita

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New Zealand Ltd., Christchurch. SPI (SUPRO XT 34N IP) was obtained from The Solae Co., St. Louis, MO, whereas the wheat gluten (Amygluten 110) was obtained from Tate & Lyle, Amylum group, Aalst, Belgium. Collagen, gliadin, and pepsin (porcine gastric mucosa; 800–2500 units/mg of protein) were purchased from Sigma-Aldrich Pty Ltd., St. Louis, MO. Beef muscle protein was extracted from beef mince by removing the fat using isopropyl alcohol. All of the protein sources were analyzed for nitrogen content using the Leco total combustion method (AOAC 968.06 using an elemental analyzer LECO FP528, St. Joseph, MI). All other chemicals and reagents used in the study were of analytical grade.

Preparation of Kiwifruit Extract. The fruit was peeled and pureed. A small quantity (180 mL/kg of fruit pulp) of 0.05 M ice-cold phosphate buffer (pH 6.0) containing 0.4 M sodium metabisulfite was immediately mixed with the pulp to avoid enzyme oxidation. The pulp was then passed through a muslin cloth, and the filtrate obtained was centrifuged at 13089g for 30 min at 0 °C. The supernatant was immediately stored at 4 °C for protein and enzyme activity analysis.

Determination of Enzymatic Activity and Protein Content. The enzyme activity of the extract was measured immediately following preparation using the method of Boland (6). The actinidin substrate, *N*- α -CBZ-lys-*p*-nitrophenol hydrochloride (*Z*-lys-pNp) (Sigma, Sigma-Aldrich Pty Ltd.) was dissolved in deionized distilled water at a concentration of 1.2 g/L. An aliquot of substrate (100 μ L) was added to 2.85 mL of 0.05 M phosphate buffer, pH 6, directly in a cuvette. After spontaneous hydrolysis of *Z*-lys-pNp had been ascertained (10–20 s), 25 μ L of kiwifruit extract (enzyme solution) was added and the change in absorbance was measured for 300 s at 348 nm using a spectrometer. Enzyme activity (micromoles of product formed per minute per milliliter or units per milliliter) of the extract was calculated using $\Delta\epsilon = 5400$ (7). Results were also expressed as specific activity quoted as units per milligram of protein (8). Protein content of the extract was measured using the dye binding method (9).

To study the effect of actinidin extraction buffer on the digestion of the proteins, a control sample of zein [which is easily oxidized (10)] was digested in the presence of actinidin extraction buffer along with pepsin and pancreatin, and it was found that the buffer had no observable effect on the digestion of zein.

In Vitro Digestibility of Proteins. For each protein source, the extent of gastric digestion was determined using an in vitro model based on the method of Savoie and Gauthier (4). In brief, 70 mg of protein source nitrogen was suspended in 17 mL of 0.1 M HCl in a jacketed glass reactor and stirred for 5 min. The reactor jacket was connected to a circulatory water bath to maintain its temperature at 37 ± 1 °C. The pH was adjusted to 1.9 and the solution made up to 19 mL with distilled water. Pepsin solution (2.5 mL; enzyme/substrate ratio, 1:100 w/w in 0.1 M HCl) was then added to start the hydrolysis. After 30 min, pepsin was inactivated by changing the pH to 8.0 using 1 M NaOH. During digestion, pH was maintained at 1.9 ± 0.1 for controls and treatments by automatic titration with 0.5 M NaOH, using a pH controller attached to a peristaltic pump. Aliquots were taken after 0 min (control) and 30 min of digestion, and the pH was immediately adjusted to 8.0. The aliquots were incubated in a boiling water bath for 5 min to quench any enzyme activity. These digestion products were then stored at -18 °C until further analysis.

There were two controls and a treatment: (1) no enzymes added (control); (2) a second control with pepsin solution, but no kiwifruit extract (Act-); (3) pepsin solution with kiwifruit extract (2.5 mL) included at the beginning of the digestion (Act+). The digestion products were determined using SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

Digestibility experiments were carried out in batches of five, using the same extract. Fresh extracts were prepared immediately prior to each batch. All of the digestibility experiments that required the addition of kiwifruit extracts were performed within 2 days to minimize any changes in the actinidin activity in kiwifruit during storage.

Tricine-Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis. Tricine-SDS-PAGE was carried out using a Criterion cell system (Bio-Rad Laboratories Pty Ltd., Auckland, New Zealand) to evaluate the protein digestion products. Protein digests for each incubation time were diluted to 0.2% using Tricine sample buffer (Bio-Rad) containing β -mercaptoethanol and heated at 100 °C for 4 min. Tricine-SDS-PAGE was performed on 10–20% gradient Tricine gels (Bio-Rad) at a constant voltage of 125 V, followed by staining for 30 min in Coomassie

Brilliant Blue R (CBB, 0.3% in 20% isopropanol and 10% acetic acid) and then destaining for 1 h in isopropanol/acetic acid solution (10% each). Quantification of intact proteins and polypeptides was carried out by gel scanning densitometry (Molecular Imager Gel Doc XR, Bio-Rad Laboratories Pty Ltd.) followed by analysis, using the associated software (Quantity One 1-D Analysis, Bio-Rad Laboratories Pty Ltd.).

RESULTS

The extract prepared from kiwifruit had an average enzyme activity and specific activity of 26.4 U/mL and 42.24 U/mg of protein, respectively.

Sodium Caseinate. The major caseinate constituents are α_{s1} -, α_{s2} -, β -, and κ -casein, in the proportion of 3:0.8:3:1 by weight (11, 12). The undigested sodium caseinate contained three major bands on SDS-PAGE (Figure 1a) of 34, 30, and 27 kDa, which could be α -, β -, and κ -caseins (CN). Densitometry of the gel provided quantitation of the bands (Figure 1b). After digestion with pepsin alone (Act-), 66, 56, and 63% of α -, β -, and κ -caseins remained undigested, respectively. Several smaller peptides appeared with the main peptides being approximately 22, 16, 15, 10, 6, 4, and 3 kDa in size. In contrast, after digestion with both pepsin and actinidin (Act+), the 16, 15, 6, and 3 kDa peptides were present but the other peptides were almost completely digested. Furthermore, α -, β -, and κ -caseins were 36, 33, and 48% more digested (30, 23, and 15% remaining α -, β -, and κ -caseins, respectively) in the presence of pepsin and actinidin compared to incubation with pepsin alone (Figure 1).

Whey Protein Isolate (WPI). WPI is composed of mainly β -lactoglobulin (β -Lg) and α -lactalbumin (α -Lac) proteins (electrophoretogram not shown). β -Lg exists naturally in milk as a dimer of two monomeric subunits associated via noncovalent bonds (13), so the protein would be expected to exist as a monomer under gastric conditions. However, a small band was observed at 37 kDa, consistent with a dimer of β -Lg. α -Lac was completely digested after 30 min of incubation with pepsin, and the addition of kiwifruit extract thus could not enhance the digestion of this protein over and above that of pepsin. β -Lg appeared to be resistant to digestion in the presence of pepsin and actinidin as there was no reduction in the amount of β -Lg after incubation with pepsin and kiwifruit extract compared to that prior to incubation. Intensity of the band at 37 kDa was observed to be greater in the presence of both pepsin and actinidin than in the presence of pepsin alone. The most likely explanation for this is that there were some insoluble aggregates present in the WPI, which solubilized in the presence of actinidin. Many low molecular weight peptides (< 6.5 kDa) were observed in the hydrolysates after 30 min of digestion both in the presence and in the absence of kiwifruit extract.

Zein. The digestion products present after simulated gastric digestion conducted using an in vitro model were analyzed using Tricine-SDS-PAGE (results not shown). No bands were observed on the gel for either the unincubated zein or the zein incubated with pepsin either with or without kiwifruit extract. The lack of any bands could be related to poor solubility of zein at pH 2.0.

Soy Protein Isolate (SPI). The SDS-PAGE pattern of the intact SPI showed that the SPI was composed of lipoxigenase (94 kDa), β -amylase (60 kDa), α' -, α -, and β -subunits (80, 76, and 49 kDa) of β -conglycinin (7S globulin), acidic subunits (40, 35, and 31 kDa) of glycinin (11S globulin), and basic subunits (25, 21, and 18 kDa) of 11S globulin (14–16). The 31 and 18 kDa bands were more clearly observed in the digested protein samples. The densitogram of the gel is shown in Figure 2.

Digestion with pepsin for 30 min resulted in the disappearance of many bands corresponding to intact soy proteins along with the appearance of many bands below 30 kDa. The digested

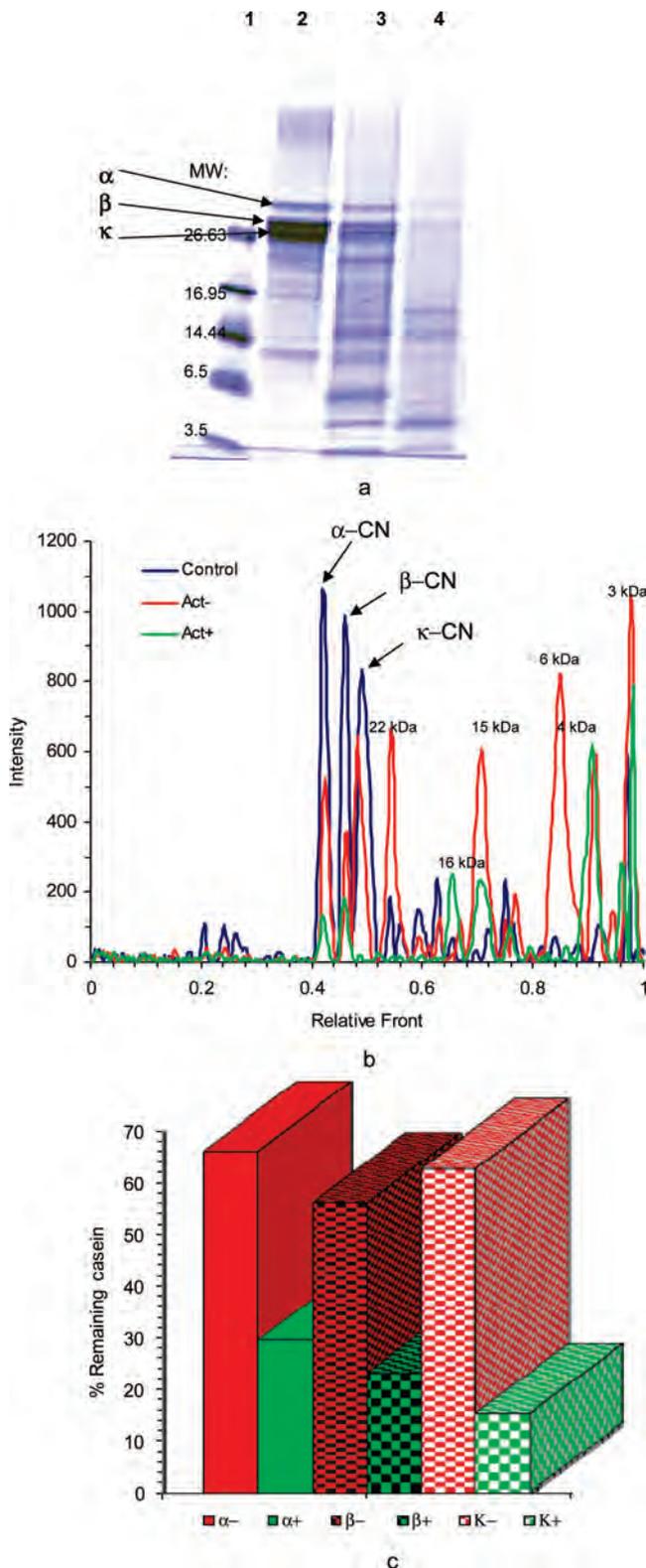


Figure 1. Tricine-SDS-PAGE electrophoretogram (a) and densitogram of the Tricine-SDS-PAGE gel (b) of sodium caseinate after 30 min of digestion with pepsin either with or without kiwifruit extract: lane 1, molecular weight marker (kDa); lane 2, sodium caseinate incubated at pH 1.9 for 30 min without any added enzymes (control); lane 3, sodium caseinate hydrolyzed with pepsin (pH 1.9) for 30 min (Act⁻); lane 4, sodium caseinate hydrolyzed with pepsin and actinidin (pH 1.9) for 30 min (Act⁺). (c) Effect of pepsin and actinidin extract (green color) on the hydrolysis of α -, β -, and κ -caseins (percent remaining casein was calculated by the decrease in the intensity of densitogram bands compared to the control protein).

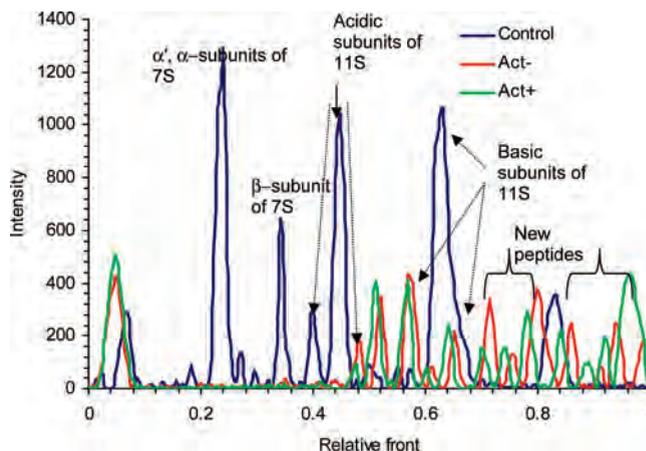


Figure 2. Densitogram of the Tricine-SDS-PAGE gel of soy protein isolate after 30 min of digestion with pepsin either with or without kiwifruit extract.

proteins included lipoxygenase, β -amylase, 7S globulins, and two of the 11S acidic polypeptides (35 and 40 kDa). The 11S basic polypeptides (18, 21, and 25 kDa) appeared to be still present. The electrophoretic pattern of SPI digested with pepsin and actinidin was similar to that of SPI digested with pepsin alone; however, the intensity of some of the bands differed. The band at 31 kDa (11S acidic polypeptide) appeared to be more completely hydrolyzed in the presence of kiwifruit extract after 30 min (**Figure 2**); moreover, other bands below 10 kDa were observed to have greater intensity in the sample digested in the presence of actinidin.

Beef Muscle Protein. The SDS-PAGE results showed that the beef muscle contained many proteins ranging in molecular weight from approximately 15 to 220 kDa (**Figure 3**). The main components were myosin, actin, troponin, and tropomyosin. The bands observed in the gel corresponded, respectively, to β -actinin (130 kDa); myosin-heavy chain (MHC, 220 kDa); α -actinin (95 kDa); actin (43 kDa); tropomyosin- β chain (39 kDa); troponin T (35 kDa); tropomyosin- α -chain (33 kDa); myosin-light chain (MLC1, 23 kDa); troponin I (21 kDa); troponin C (18 kDa); and myosin-light chain (MLC2, 16 kDa) (17). High molecular weight components such as nebulin and titin (600–800 and 2500 kDa, respectively) could not be resolved on the present SDS-PAGE.

The SDS-PAGE pattern of the protein digested for 30 min in the presence of pepsin and kiwifruit extract was quite different from the one of protein digested with pepsin alone. All of the bands above 25 kDa were observed either to decrease in intensity or to disappear completely, which suggests that actinidin enhanced the digestion of beef muscle protein under conditions present in the stomach (**Figure 3b**). As an example, the percent remaining Troponin T was 77 and 62%, respectively, when digested with pepsin alone and pepsin + actinidin, which shows 15% higher digestibility of Troponin T when kiwifruit extract was present. Several high molecular weight bands also appeared after digestion with pepsin, which were not present after digestion with pepsin and actinidin. These may be hydrolyzed peptides from the very large proteins such as nebulin and titin. In addition, a thick and diffuse band (< 10 kDa) appeared in both the pepsin and pepsin + actinidin digests.

Collagen. Gel electrophoresis of the collagen incubated without proteolytic enzymes showed the presence of high molecular weight multimers that did not enter the gel (gel not shown) along with some faint bands corresponding to molecular weights of 110, 93, 68, and 23 kDa. The bands were clearly visible in the protein hydrolyzed by pepsin for 30 min (results not shown). One band at

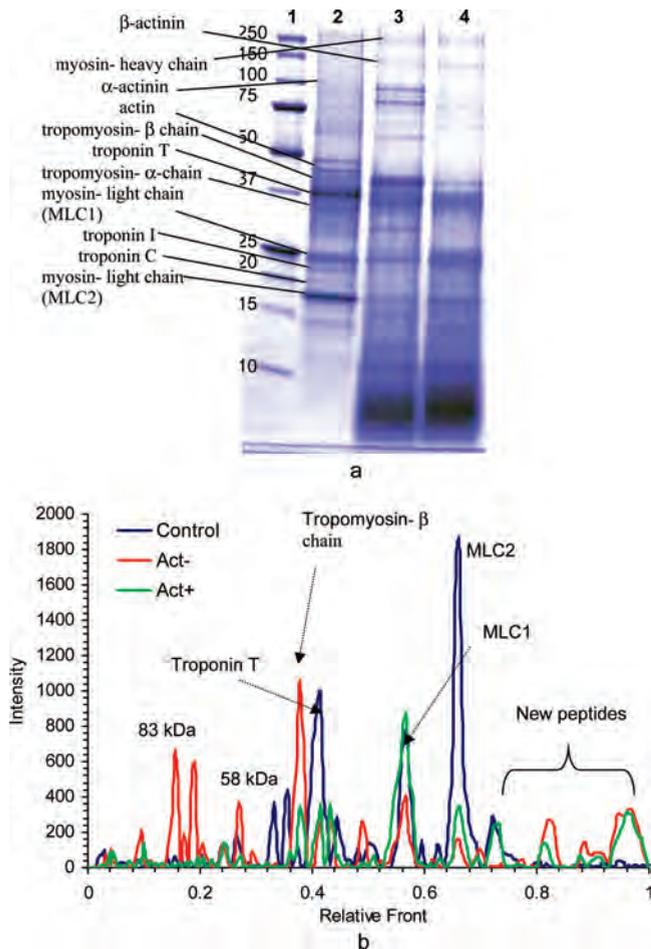


Figure 3. Tricine-SDS-PAGE electrophoretogram (a) and densitogram of the Tricine-SDS-PAGE gel (b) of beef muscle protein after 30 min of digestion with pepsin either with or without kiwifruit extract: lane 1, molecular weight marker (kDa); lane 2, beef muscle protein incubated at pH 1.9 for 30 min without any added enzymes (control); lane 3, beef muscle protein hydrolyzed with pepsin (pH 1.9) for 30 min (Act⁻); lane 4, beef muscle protein hydrolyzed with pepsin and actinidin (pH 1.9) for 30 min (Act⁺).

231 kDa was observed in addition to the high molecular weight aggregates (γ -components and other), which could be the dimer (β -component). Two bands appeared around 127 and 112 kDa, which could be the characteristic $\alpha 1$ (I) and $\alpha 2$ (I) type I collagen bands. Other new peptides of 50 and 29 kDa also appeared in the pepsin-digested protein. However, in the sample digested with pepsin in the presence of actinidin, the bands at 62 and 58 kDa were much darker than those observed in the pepsin-digested sample.

Gelatin. Gelatin is a denatured form of collagen and is commercially prepared from skins and skeletons of cattle and pigs by alkaline or acidic extraction (18, 19).

For gelatin incubated without either pepsin or kiwifruit extract (control), protein bands were observed with molecular weights equivalent to that of the γ -component (high molecular weight multimers), $\alpha 1$ - and $\alpha 2$ -chains of collagen (gel not shown). There were also a number of bands corresponding to proteins smaller than the α -chains of collagen. The numerous bands observed for gelatin with molecular weight less than the α -chains could be the result of the severe protein hydrolysis during extraction of gelatin. During the conversion of collagen to gelatin, the inter- and intramolecular bonds linking collagen chains as well as some peptide bonds are broken, generating many low molecular weight components (18, 20).

The electrophoretic pattern of the protein digested with pepsin (both in the presence and in the absence of kiwifruit extract) was similar to the control sample, except for the appearance of a new peptide corresponding to 30 kDa.

Gluten. Wheat proteins are divided into monomeric and polymeric proteins depending on whether they have single- or multiple-chain polypeptides (21). The monomeric proteins comprise gliadins and the albumins/globulins. Gliadins are usually divided into α -, β -, γ -, and ω -gliadins, with molecular weights ranging from 30 to 80 kDa. ω -Gliadins are clearly separated from the other gliadins, with the highest molecular weight (21). Glutenins are the polymeric proteins, which are composed of low molecular weight (LMW) and high molecular weight (HMW) glutenin subunits. The molecular weights of HMW (A subunits) subunits fall roughly in the size range 80–120 kDa, whereas the LMW subunits are further composed of the B (MW 40–55 kDa), C (MW 30–40 kDa; corresponding to α - and γ -gliadin type LMW subunits), and D subunits (corresponding to ω -gliadin type subunits) (22).

Five bands corresponding to HMW glutenins were clearly observed along with some bands corresponding to LMW B-glutenins and gliadins (electrophoretogram not shown). However, LMW C-glutenins could not be clearly resolved by the present SDS-PAGE, which could be due to the overlap of molecular weight of LMW C-glutenins and the gliadin fractions. It has been reported that LMW subunits are not easily resolved by one-step SDS-PAGE (21).

Incubation in the presence of pepsin digested all of the glutenin and gliadin proteins and subunits > 75 kDa. However, no difference was observed when kiwifruit extract was included in the incubation mixture.

Gliadin. Gliadins are the alcohol-soluble fraction of gluteins, belonging to the proline- and glutamine-rich prolamins family. They are usually divided into α -, β -, γ -, and ω -gliadins, on the basis of their mobility on one-dimensional Acid-PAGE (21).

The molecular weights of gliadins vary from 30 to 80 kDa, with ω -gliadins clearly separated from the others, with highest molecular weight (21) (Figure 4). However, the latter are not clearly resolved in the present SDS-PAGE gel. During in vitro digestion under simulated gastric conditions (pepsin digestion for 30 min), all of the major protein fractions present in the undigested gliadin (82, 60, 49, 43, 38, 36, and 32 kDa) were either partially or completely digested. The peptide profile of gliadin digested with pepsin was similar to that after digestion with pepsin and kiwifruit extract, except that the peptide concentration was less after digestion with pepsin alone. On the basis of the densitogram (not shown), it would appear that digestion was greater with pepsin alone compared to digestion with pepsin and actinidin. The peptide pattern also differed between these samples, with actinidin-digested protein showing some additional peptide bands at 11 and 10 kDa (not seen clearly in the gel presented here). Surprisingly, the intensities of the bands corresponding to 30, 25, and 23 kDa were observed to be higher for actinidin-digested protein than for their pepsin-only counterparts.

DISCUSSION

The kiwifruit used in the present study had an average protein content of 0.89 mg/g of fruit. Similar protein levels for kiwifruit have been reported previously (8). The enzyme activity of the extract determined in this study was comparable with those reported by Lewis and Luh (23). However, actinidin activity in kiwifruit is known to vary markedly depending on fruit growth stage, postharvest storage, and the part of the fruit being tested. The average actinidin yield reported by Lewis and Luh (23) was 0.5 g of protein/kg of fruit with an average specific activity of

42 U/mg, with $K_m = 91 \mu\text{M}$ and $k_{\text{cat}} = 101 \text{ s}^{-1}$ using *N*- α -CBZ-lys-*p*-nitrophenol at pH 6.0 at 25 °C. Boland and Hardman (7) determined the specificity of actinidin (prepared as the sulfenyl thiosulfate derivative and activated with dithiothreitol) using Z-lys-pNp to be similar to those of papain and ficin. The relative amount of kiwifruit to food protein used in the study was approximately 1 kiwifruit (wt 100 g) to 1.3 g of protein N (or approximately 1 kiwifruit to 8.13 g of food protein).

In this study, actinidin (as part of a kiwifruit extract) appeared to markedly enhance the digestion of several food protein sources under simulated gastric conditions when determined using an in vitro model. This effect was most notable for sodium caseinate, beef muscle protein, and some SPI digestion products (Table 1). In contrast, actinidin appeared to have little effect on the gastric digestion (in vitro model) of WPI, collagen, gelatin, gluten, and gliadin. Sodium caseinate appeared to be extensively hydrolyzed under simulated gastric conditions, which is in accordance with the findings of Guo et al. (13), who reported that caseins are rapidly and extensively hydrolyzed by pepsin at pH 2. However, in the presence of kiwifruit extract, digestion was markedly greater than with pepsin alone. Actinidin did not have any

detectable effect on WPI digestion under simulated gastric digestion conditions. Pepsin completely digested α -Lac, irrespective of the presence of actinidin. β -Lg was observed to be stable in the acidic environment of the simulated gastric digestion. It has been previously reported by other authors that this protein resists peptic hydrolysis and reaches the upper portion of the small intestine mostly intact (24, 25).

Due to the poor solubility of zein at pH 2.0, SDS-PAGE results for the digestion of zein under simulated gastric conditions could not be determined.

Actinidin has been studied for some commercial applications, such as meat tenderization, gelatin, whey, and soy protein hydrolysis (23, 26–29). In this study for beef muscle protein, all of the bands corresponding to high molecular weight proteins, including MHC, β -actinin, α -actinin, and actin, were hydrolyzed to a much greater extent in the presence of actinidin and pepsin than with pepsin alone. The band at 58 kDa, identified as desmin, which is clearly observed in non-actinidin-digested sample, also disappeared completely in the actinidin-digested protein. The results are consistent with those reported by Lewis and Luh (23), who reported that actinidin caused significant tenderization of broiled steak by hydrolyzing the myofibrillar proteins. In that study it was reported that 43% of the myosin and most of the other proteins present in the undigested beef muscle protein were digested by the action of actinidin over and above that of pepsin alone.

Actinidin did not have much effect on collagen digestion but may have influenced gelatin digestion to some extent under simulated gastric conditions. Collagen (type I), a major constituent of intramuscular connective tissue, has its molecules assembled in fibrils and immobilized by cross-links that are preferentially formed between the triple-helix and globular domains. It has been reported that kiwifruit juice can degrade the globular domains of the denatured collagen (i.e., gelatin), but it cannot cleave the triple-helical domain of collagen, which gives collagen the rigid supermolecular structure, in the pH range of 3–7 (26). The reported optimal pH for actinidin activity is about 4 when using food proteins such as gelatin (1) or myofibrillar proteins (27) as substrates. However, in the present study, the pH used to simulate stomach conditions was 1.9, and this lower pH may have reduced any effect of actinidin on gelatin digestion. Wada et al. (28) suggest that kiwifruit protease could degrade the insoluble collagen of cattle Achilles tendon into collagen subunit chains (β - and α -chain at pH 3.3 and 6.0 but not at pH 2.7–2.9). Collagen and gelatin are notoriously resistant to hydrolysis by proteases because of their high levels of proline and hydroxyproline. These amino acids prevent the flexibility of the protein backbone that facilitates binding to the active site of the enzyme.

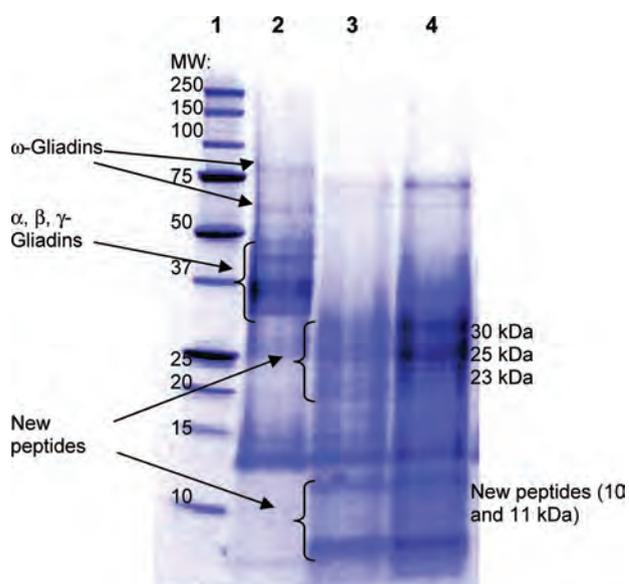


Figure 4. Tricine-SDS-PAGE electrophoretogram of gliadin after 30 min of digestion with pepsin either with or without kiwifruit extract: lane 1, molecular weight marker (kDa); lane 2, gliadin incubated at pH 1.9 for 30 min without any added enzymes (control); lane 3, gliadin hydrolyzed with pepsin (pH 1.9) for 30 min (Act⁻); lane 4, gliadin hydrolyzed with pepsin and actinidin (pH 1.9) for 30 min (Act⁺).

Table 1. Summary of the Effect of Kiwifruit Extract (Actinidin) on the in Vitro Digestion of Different Proteins under Simulated Gastric Conditions (30 min)

| protein | effect of actinidin (+ or -) | comments |
|---------------------|------------------------------|---|
| sodium caseinate | + | the % remaining α -, β -, and κ -caseins was 30, 23, and 15% when digested with pepsin + actinidin compared to 66, 56, and 63% when digested with pepsin alone; this shows enhancement of α -, β -, and κ -casein digestion by 37, 33, and 48% in the presence of actinidin |
| WPI | - | no positive effect on digestion |
| zein | - | data unavailable due to low solubility of the protein |
| SPI | + | small effects on the hydrolysis of some bands (at 31 and 21 kDa) in the presence of actinidin |
| beef muscle protein | + | significant enhancement of digestion of all the proteins above 25 kDa; as an example, the remaining Troponin T was 77 and 62% when digested with pepsin alone and pepsin + actinidin, respectively; this shows 15% higher digestibility of Troponin T in the presence of actinidin |
| collagen | - | no effect on digestion |
| gelatin | - | no effect on digestion |
| gluten | - | no effect on digestion |
| gliadin | - | no positive effect on digestion; actinidin appeared to decrease the digestion by pepsin |

Very few studies have been conducted to investigate the effects of kiwifruit protease on protein sources other than meat. Recently, the functional properties of enzymatically hydrolyzed soy proteins derived using actinidin have been reported (29). The soy flour hydrolysates obtained had a higher solubility than the unhydrolyzed soy flour across the full pH range (pH 1–11), which was attributed to the increased charge and hydrophilicity of the resulting peptide molecules, as well as to a reduction in the size of the peptides (30).

In conclusion, the present *in vitro* study provides clear evidence that actinidin can provide enhanced digestion of several food proteins under gastric digestion conditions when examined using an *in vitro* assay simulating gastric digestion. In particular, enhanced digestion of sodium caseinate, beef muscle protein, and, to some extent, SPI was observed in the simulated gastric system. This supports the proposition that actinidin can enhance the digestion of food proteins and that digestion with actinidin may give rise to different types of peptides. The work will now be extended to observe the effects of actinidin on the digestion of proteins *in vivo*.

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