DOI:10.1021/jf903835g

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

# Actinidin Enhances Protein Digestion in the Small Intestine As Assessed Using an in Vitro Digestion Model

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This paper describes an in vitro study that tests the proposition that actinidin from green kiwifruit influences the digestion of proteins in the small intestine. Different food proteins, from sources including soy, meat, milk, and cereals, were incubated in the presence or absence of green kiwifruit extract (containing actinidin) using a two-stage in vitro digestion system consisting of an incubation with pepsin at stomach pH (simulating gastric digestion) and then with added pancreatin at small intestinal pH, simulating upper tract digestion in humans. The digests from the small intestinal stage (following the gastric digestion phase) were subjected to gel electrophoresis (SDS-PAGE) to assess loss of intact protein and development of large peptides during the in vitro simulated digestion. Kiwifruit extract influenced the digestion patterns of all of the proteins to various extents. For some proteins, actinidin had little impact on digestion. However, for other proteins, 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 and pancreatin alone. In particular, enhanced digestion of whey protein isolate, zein, gluten, and gliadin was observed. In addition, reverse-phase HPLC (RP-HPLC) analysis showed that a 2.5 h incubation of sodium caseinate with kiwifruit extract alone resulted in approximately 45% loss of intact protein.

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

## INTRODUCTION

Green kiwifruit (*Actinidia deliciosa*) contains a highly active proteolytic enzyme, actinidin. 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. Actinidin has a broad optimal pH around 4 (*1*, *2*) and temperature optimum of 58−62 °C (*3*).

It has long been known that raw kiwifruit prevents the solidification of gelatin jellies, assumed to be due to the presence of actinidin. It has also been thought that actinidin aids the digestion of foods by hydrolyzing proteins more completely and more rapidly than mammalian digestive enzymes alone. Actinidin in concert with pepsin and pancreatin can be expected to give rise to a different array of peptides (potentially bioactive peptides) during both gastric and small intestinal digestion, compared to the action of pepsin and pancreatin alone. However, there is little published evidence for this.

Here we describe the findings of an in vitro study that investigates the effects of actinidin from green kiwifruit on simulated upper tract digestion (gastric plus small intestinal digestion). A two-stage in vitro digestion system based on the method of Savoie and Gauthier (*4*) was used to simulate the gastric and small intestinal phases of protein digestion. Digestion studies have been performed on a variety of food proteins including those derived from legumes, meat, milk, and cereals.

The findings of the gastric digestion stage are discussed in detail in a companion paper (*5*). Kiwifruit extract containing actinidin enhanced the digestion of a variety of food proteins under simulated gastric conditions and also resulted in a different peptide pattern compared to the action of pepsin alone. Here we analyze and report the effects of actinidin on simulated upper tract digestion (gastric plus small intestinal digestion).

## MATERIALS AND METHODS

**Materials.** Green "ready-to-eat" kiwifruit (*A. deliciosa* var. Hayward) were provided by ZESPRI International Ltd. and were preripened to a similar degree of firmness [firmness RTE (ready to eat) of 0.5−0.8 kgf, measured using a penetrometer (*6*)]. The fruit was stored at 4 °C until extraction was carried out. Eight different proteins were used in the study: sodium caseinate; whey protein isolate (WPI); gluten; gliadin; 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. SPI (SUPRO XT 34N IP) was obtained from The Solae Co., St. Louis, MO, and the wheat gluten (Amygluten 110) was obtained from Tate & Lyle, Amylum group, Aalst, Belgium. Collagen, gliadin, pepsin (porcine gastric mucosa; 800−2500 units/mg of protein),

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 Published on Web 03/16/2010 

and pancreatin (hog pancreas; 4 × USP) 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.** Extracts of green kiwifruit were prepared and analyzed for protein content, actinidin activity, and specific activity as described by Kaur et al. (*5*). Digestibility experiments were carried out in batches of five, simultaneously and using the same extract. Fresh extracts were prepared immediately prior to each batch run. All of the digestibility experiments that required the addition of kiwifruit extract were performed within 2 days of receiving kiwifruit to minimize the effect of any changes in actinidin activity in kiwifruit during storage.

**In Vitro Digestibility of Proteins.** For each protein source, digestion simulating gastric plus small intestinal conditions was carried out on the basis of the in vitro digestion method described by Savoie and Gauthier (*4*). The two-stage model system simulates gastric and small intestinal digestion, and the method for the gastric digestion phase of the model has been described in detail by Kaur et al. (*5*). In brief, protein equivalent to 70 mg of 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 the gastric digestion stage, the 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.

For the small intestinal digestion phase, the method was as follows: after the 30 min incubation with pepsin at pH 1.9 (simulated gastric digestion), the remaining reaction mixture was adjusted to 24 mL with sodium phosphate buffer (pH 8.0, 0.1 M). Pancreatin (2.5 mL; enzyme/substrate ratio, 1:100 w/w in phosphate buffer) was added to simulate digestion in the small intestine. Pancreatin (according to USP specifications) is a substance containing enzymes, principally amylase, lipases and proteases, obtained from the pancreas of the hog, *Sus scrofa* Linné var. *domesticus* Gray (Fam. Suidae), or of the ox, *Bos taurus* Linné (Fam. Bovidae). 1× USP pancreatin contains, in each milligram, not less than 25 USP units of amylase activity, not less than 2.0 USP units of lipase activity, and not less than 25 USP units of protease activity (*7*). Mixtures were maintained at 37 °C and gently stirred (300 rpm) for a further 2 h, and the pH values of the controls and the treatment were maintained at 8.0 ± 0.1 throughout this step. Aliquots (5 mL) were taken after 90 and 150 min of digestion, and the pH of the aliquot immediately lowered to pH 2.0 before incubation in a boiling water bath for 5 min to quench enzyme activity. The aliquots were stored at −18 °C until further analysis.

There were two controls and one treatment for each protein source: (1) no enzymes added but incubated at pH 1.9 for 30 min and then at pH 8.0 for either 60 or 120 min (control−90, control−150); (2) a second control with pepsin (pH 1.9 for 30 min) and pancreatin solution (pH 8.0 for 60 or 120 min), but no kiwifruit extract (Act−90, Act−150); or (3) pepsin (pH 1.9 for 30 min) and pancreatin solution (pH 8.0 for 60 or 120 min) with kiwifruit extract (2.5 mL added at the beginning of the digestion; Act+90, Act+150). The digestion products were determined using SDS−polyacrylamide gel electrophoresis (SDS-PAGE).

The kiwifruit extract used for in vitro digestion had an average protein content, enzyme activity, and specific activity of 0.625 mg/mL, 26.4 U/mL, and 42.24 U/mg of protein, respectively (*5*).

**Tricine−Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis.** Tricine-SDS-PAGE was carried out using a Criterion cell (Bio-Rad Laboratories Pty Ltd., Auckland, New Zealand) to evaluate the protein digestion products after 90 min of incubation (30 min of simulated gastric digestion followed by 60 min of simulated small intestinal digestion) and 150 min (30 min of simulated gastric digestion followed by 120 min of simulated small intestinal digestion). The method used for SDS-PAGE analysis is described by Kaur et al. (*5*).

**Reverse-Phase (RP) HPLC Analysis of the Protein Digests.** RP-HPLC analysis of the sodium caseinate hydrolyzed with kiwifruit extract alone (2.5 mL added at the beginning of simulated digestion as described under In Vitro Digestibility of Proteins with no added pepsin or pancreatin) was carried out using a 1200 series Agilent HPLC equipped with a diode array detector (DAD) operating at 214 nm and an Agilent automatic injector (Agilent Technologies, Palo Alto, CA). A Jupiter 4 $\mu$ Proteo 90 Å C12 column (150 × 4.60 mm) supplied by Phenomenex NZ Ltd. (Auckland) was used, which was connected to a guard cartridge system and was operated at a flow rate of 1 mL/min at 35 °C. All samples were filtered through a 0.45 $\mu$m syringe filter before injection. Samples (20 $\mu$L) were injected and eluted for 10 min with solvent A [0.1%, trifluoroacetic acid (TFA) in Milli-Q water]. Peptides were then eluted using first a linear gradient of solvent B (60% acetonitrile in Milli-Q water and 0.1% TFA) ranging from 0 to 50% for 80 min followed by a second linear gradient from 50 to 75% for the next 10 min and then to 0% in the next 2 min. Data acquisition and chromatographic analyses were performed using EZ Chrom Elite software (version 3.3.1).

The degree of hydrolysis (DH) of the protein was determined using the method of Roufik et al. (*8*) and was based on the decrease in peak area of casein before and after hydrolysis. DH was calculated as

$$\text{DH (\%)} = 100 - (\text{peak area of casein after digestion} / \text{peak area of casein prior to hydrolysis}) \times 100$$

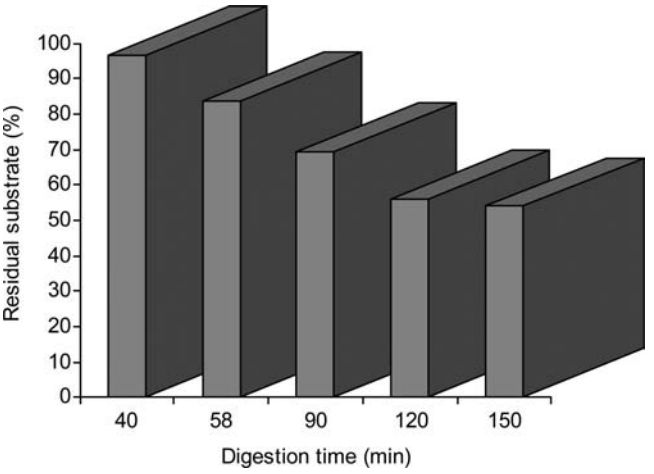


**Figure 1.** Extent of sodium caseinate digestion in the presence of kiwifruit extract alone (no pepsin and pancreatin) determined using RP-HPLC analysis. Reaction conditions were as follows: 30 min at pH 2.0 and 37 °C followed by 120 min at pH 8.0 and 37 °C.

## RESULTS

**Sodium Caseinate.** Sodium caseinate was chosen as a model protein to explore the efficacy of protein digestion with kiwifruit extract alone without the presence of pepsin and pancreatin (**Figure 1**). On the basis of RP-HPLC analysis, incubation of sodium caseinate with the kiwifruit extract resulted in increased protein digestion over time with approximately 45% protein digestion occurring after 150 min.

Intact casein protein and its products after digestion with pepsin and pancreatin either with or without added kiwifruit extract were analyzed using SDS-PAGE (gel not shown). The undigested sodium caseinate contained three major bands of 34, 30, and 27 kDa, which were identified as α-, $\beta$-, and $\kappa$-caseins. The proteins and peptides that remained undigested or partially digested during the simulated gastric phase were further digested during the small intestinal stage. After in vitro digestion with pepsin and pancreatin for 90 min, either in the absence or in the presence of kiwifruit extract, sodium caseinate was virtually completely digested with almost no peptides > 3.5 kDa being observed. The exceptions were two minor bands corresponding to molecular weights between 3.5 and 6.5 kDa.

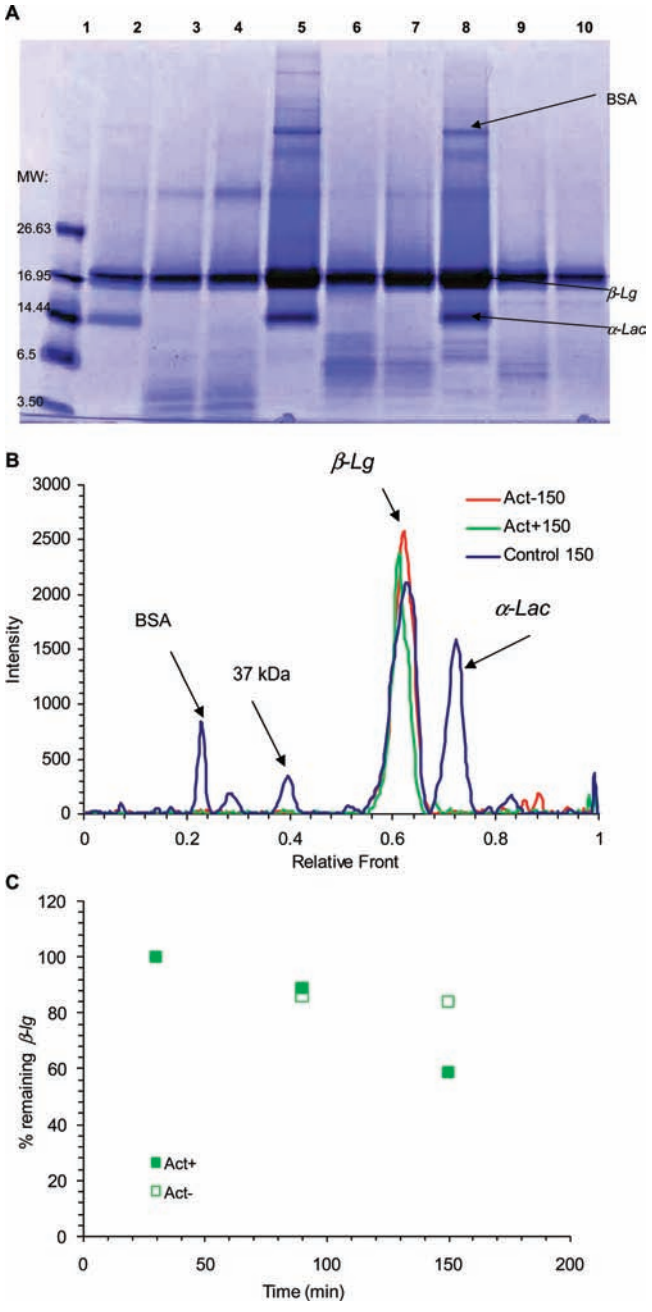


**Figure 2.** Tricine-SDS-PAGE electrophoretogram (**a**) and densitogram of the tricine-SDS-PAGE gel (**b**) of whey protein isolate (WPI): lane 1, molecular weight marker (kDa); lane 2, WPI incubated at pH 1.9 for 30 min without any added enzymes (control-30); lane 3, WPI hydrolyzed with pepsin (pH 1.9) for 30 min (Act−30); lane 4, WPI hydrolyzed with pepsin and actinidin (pH 1.9) for 30 min (Act+30); lane 5, WPI incubated at pH 1.9 for 30 min and then at pH 8.0 for 60 min without any enzymes (control); lane 6, WPI hydrolyzed for 30 min at pH 1.9 with pepsin and then for 60 min at pH 8.0 with pancreatin (Act−90); lane 7, WPI hydrolyzed for 30 min at pH 1.9 with pepsin and then for 60 min at pH 8.0 with pancreatin in the presence of kiwifruit extract (Act+90); lane 8, WPI incubated at pH 1.9 for 30 min and then at pH 8.0 for 120 min without any enzymes (control); lane 9, WPI hydrolyzed for 30 min at pH 1.9 with pepsin and then for 120 min at pH 8.0 with pancreatin (Act−150); lane 10, WPI hydrolyzed for 30 min at pH 1.9 with pepsin and then for 120 min at pH 8.0 with pancreatin in the presence of kiwifruit extract (Act+150). (**c**) Effect of actinidin extract (solid symbols) on the hydrolysis of $\beta$-Lg in WPI. Percent remaining $\beta$-Lg was calculated by the decrease in the intensity of densitogram bands compared to the control protein.

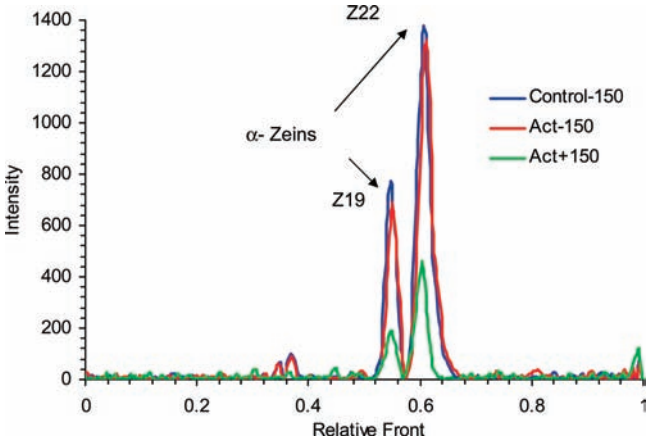


**Figure 3.** Densitogram of the tricine-SDS-PAGE gel of zein.

No bands were observed in gels of the hydrolysates after digestion for 150 min.

**Whey Protein Isolate.** The SDS-PAGE gel and densitogram for WPI after digestion with pepsin/pancreatin for 90 and 150 min either with or without kiwifruit extract are shown in **Figure 2a**,**b**. BSA and α-Lac were previously shown to be completely digested after 30 min of simulated gastric digestion (*5*). In contrast, $\beta$-Lg was poorly digested after 90 min of incubation (30 min of simulated gastric and 60 min of simulated small intestinal conditions) and was only slightly more digested after 150 min of incubation (30 min of simulated gastric and 120 min of simulated small intestinal conditions). After 150 min of digestion with pepsin and pancreatin, 84% of the original $\beta$-Lg remained undigested compared with 59% remaining $\beta$-Lg when kiwifruit extract was present along with pepsin and pancreatin (**Figure 2c**). A number of peptides between 10 and 4 kDa were generated after 90 min of incubation in the absence of kiwifruit extract. When the kiwifruit extract was present, only some of these peptides (8 and 4 kDa) were observed. After 150 min of incubation, in the absence of kiwifruit extract, some of the peptides generated during 90 min of incubation remained undigested. However, in the presence of kiwifruit extract all of the generated peptides were completely digested after 150 min of incubation. The latter finding suggests that, whereas actinidin may not enhance the digestion of the intact WPI protein over and above that of pepsin and pancreatin, it may catalyze the further hydrolysis of peptides generated during in vitro digestion.

The different electrophoretic patterns observed for WPI controls after simulated gastric and small intestinal digestion could be due to changes in solubility of some protein components (for example, breakdown of insoluble aggregates) under alkaline conditions (pH 8).

**Zein.** Zein, one of the typical prolamins, is a major storage protein of corn (*Zea mays*) and is more hydrophobic than any other prolamins (*9*). This tends to render it insoluble in aqueous solutions but soluble in alcohol (*10*). Zeins are classified according to their solubility, molecular weight, and immunological response as α-, $\beta$-, $\gamma$-, and $\delta$-zein (*11*−*14*). α-Zeins are the most abundant (75−85% of the total) and are divided into two types, Z19 and Z22, with approximate weights of 22 and 25 kDa, respectively (*15*).

The digestion products of zein after in vitro digestion with pepsin and pancreatin either with or without kiwifruit extract were analyzed using tricine-SDS-PAGE (gel not shown), and the densitogram is shown in **Figure 3**. The water-soluble proteins present in the zein used in this study consisted of α-zeins with molecular weights of 24 and 20 kDa. Similar results have been reported by Malumba et al. (*16*).

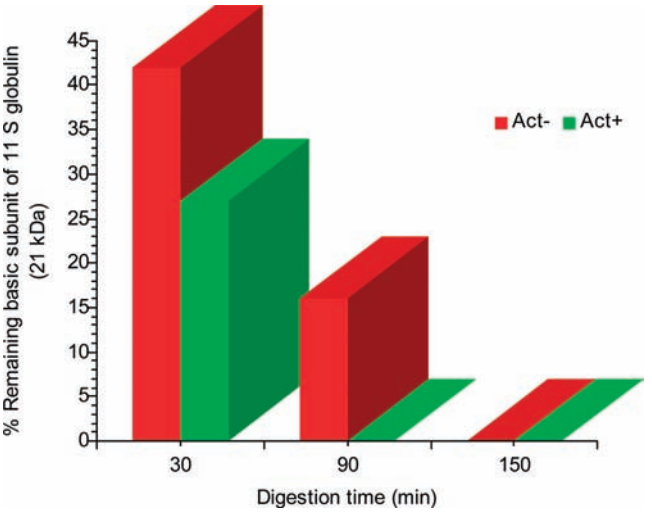


**Figure 4.** Effect of actinidin extract on the hydrolysis of the basic subunit of 11S globulin (21 kDa).

The insolubility of zein in the solutions/buffers used for the digestibility study made it difficult to study digestion over shorter digestion times. Thus, the results for 150 min of digestion only are presented here. Most of the Z19 and Z22 α-zeins remained undigested (91 and 93% residual proteins, respectively) after 150 min of digestion (30 min at pH 1.9 with pepsin and 120 min at pH 8.0 with pancreatin) in the absence of kiwifruit extract. However, the addition of actinidin led to significant digestion of both components as observed from the gel and densitogram, with 61 and 62% residual Z19 and Z22 α-zeins, respectively.

**Soy Protein Isolate.** The proteins present in SPI are discussed by Kaur et al. (*5*).

The 11S basic polypeptides (18, 21, and 25 kDa), which were quite resistant toward pepsin hydrolysis (the gastric stage), were digested to a considerable extent during simulated small intestinal digestion (data not shown). Upon further digestion of the peptic digests with pancreatin, the intensity of all of the new bands (that appeared during gastric digestion) decreased over time in both samples digested with and without kiwifruit extract. However, the decrease in intensity of the bands in the protein sample without kiwifruit extract was observed to be greater than its counterpart sample with kiwifruit extract. The SDS-PAGE results suggested that kiwifruit extract had some effect on the in vitro digestion of some subunits such as 11S basic polypeptide (21 kDa) but had almost no positive effect on the digestion of other subunits (**Figure 4**).

**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 5a**). The main components were myosin, actin, troponin, and tropomyosin (*5*).

When further digestion of the peptic hydrolysates was carried out using pancreatin, which simulated gastric plus intestinal digestion, most of the bands corresponding to the identified major proteins disappeared, suggesting their complete or nearly complete digestion. In addition, some new bands appeared corresponding to peptides with molecular weights of 45, 38, and 30 kDa after 150 min of digestion, which may have been derived from the digestion of large proteins such as nebulin or titin. Overall, kiwifruit extract did not appear to greatly enhance protein digestion after simulated "gastric plus intestinal digestion". However, the material represented by the band at 26 kDa appeared to be more digested in the presence of kiwifruit extract (**Figure 5b**).

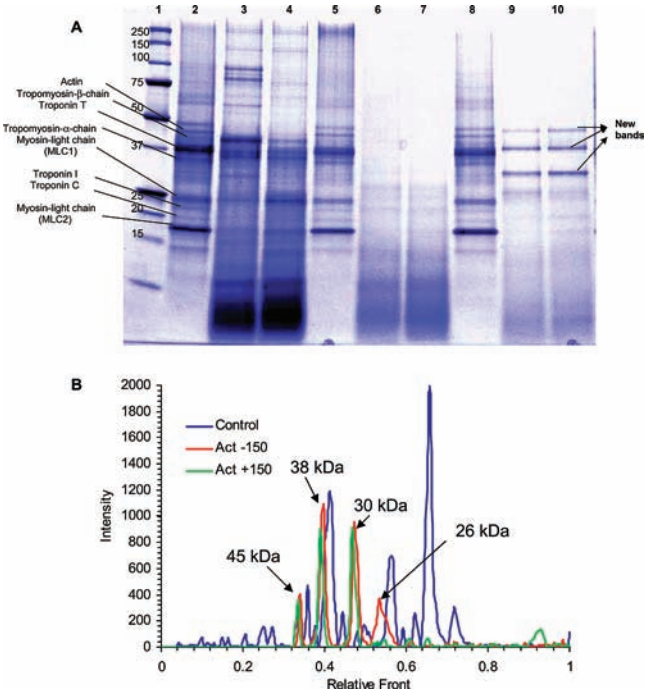


**Figure 5.** Tricine-SDS-PAGE electrophoretogram (**a**) and densitogram of the tricine-SDS-PAGE gel (**b**) of beef muscle protein: lane 1, molecular weight marker (kDa); lane 2, beef muscle protein incubated at pH 1.9 for 30 min without any added enzymes (control−30); lane 3, beef muscle protein hydrolyzed with pepsin (pH 1.9) for 30 min (Act−30); lane 4, beef muscle protein hydrolyzed with pepsin and actinidin (pH 1.9) for 30 min (Act+30); lane 5, beef muscle protein incubated at pH 1.9 for 30 min and then at pH 8.0 for 60 min without any enzymes (control); lane 6, beef muscle protein hydrolyzed for 30 min at pH 1.9 with pepsin and then for 60 min at pH 8.0 with pancreatin (Act−90); lane 7, beef muscle protein hydrolyzed for 30 min at pH 1.9 with pepsin and then for 60 min at pH 8.0 with pancreatin in the presence of kiwifruit extract (Act+90); lane 8, beef muscle protein incubated at pH 1.9 for 30 min and then at pH 8.0 for 120 min without any enzymes (control); lane 9, beef muscle protein hydrolyzed for 30 min at pH 1.9 with pepsin and then for 120 min at pH 8.0 with pancreatin (Act−150); lane 10, beef muscle protein hydrolyzed for 30 min at pH 1.9 with pepsin and then for 120 min at pH 8.0 with pancreatin in the presence of kiwifruit extract (Act+150).

**Collagen.** Under the in vitro conditions that simulate both gastric and intestinal digestion, the intensity of bands corresponding to the β-component and α-chains decreased, suggesting digestion of these proteins had taken place (gel not shown). Furthermore, the high molecular weight multimer bands gradually decreased in intensity, denoting hydrolysis. All of the new peptides formed during simulated gastric digestion were completely digested during simulated intestinal digestion.

**Gluten.** SDS-PAGE of the wheat gluten showed five bands corresponding to high molecular weight (HMW) glutenins along with some bands corresponding to low molecular weight (LMW) B-glutenins and gliadins (gel not shown). However, LMW C-glutenins could not be clearly resolved by the present SDS-PAGE, which is consistent with the findings of MacRitchie and Lafiandra (*17*), who reported that LMW subunits are not easily resolved using one-step SDS-PAGE.

Upon digestion under simulated intestinal conditions, the intensity of the bands formed during gastric digestion decreased further. The bands corresponding to 20−30 kDa disappeared, which could be due to the solubilization and then digestion of the globulins in the salt solution during intestinal conditions. Gluten incubated for 150 min in the presence of kiwifruit extract was

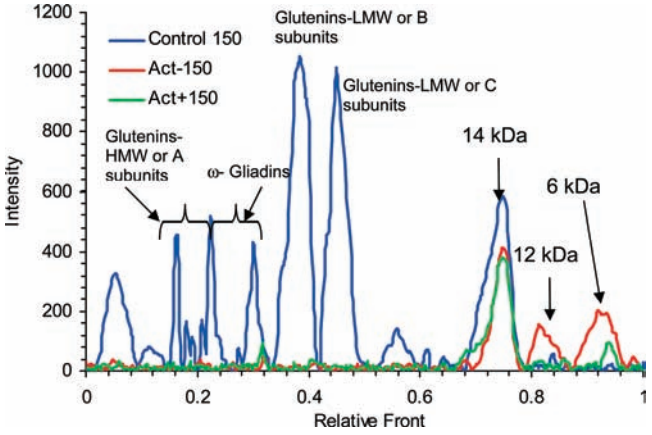


**Figure 6.** Densitogram of the tricine-SDS-PAGE gel of gluten.

digested to a greater degree than its non-kiwifruit extract counterpart, as observed from the densitogram (**Figure 6**).

**Gliadin.** The molecular weights of gliadins range from 30 to 80 kDa (*13*). Upon digestion using in vitro conditions that simulate small intestinal digestion, the peptides formed during the simulated gastric phase of digestion were further digested as observed from the decrease in intensity of those bands, particularly when actinidin was present (**Figure 7**). After 90 min of digestion, all of the bands present in the gliadin control lane corresponding to protein >30 kDa in size had completely disappeared, suggesting complete digestion of these proteins. However, many peptides with molecular weights of <30 kDa could still be observed, which were further, but not completely, digested after 150 min of digestion. A positive effect of kiwifruit extract on digestion was observed after gastric plus intestinal digestion. It should be noted that the intensity of the bands for the undigested gliadin "control" (incubated but no enzyme added) was much greater after incubation under simulated gastric conditions compared to simulated gastric and intestinal conditions. This may be due to the lower solubility of intact gliadin proteins in the reaction mixture at pH 8.0 compared to pH 2. However, as the protein was further digested, peptides, which were more soluble, were released, which can be seen in the SDS-PAGE gel. Overall, actinidin appeared to enhance the digestion of gliadin under simulated gastric plus intestinal conditions.

## DISCUSSION

There is a lack of information in the literature regarding the effects of kiwifruit protease on protein digestion or the possible generation or breakdown of peptides (potentially bioactive) by actinidin. Using an in vitro assay that simulates both the gastric and small intestinal phases of digestion, we found that kiwifruit (actinidin) influences the in vitro digestion of several food proteins, particularly under simulated gastric conditions, and thus may have an impact on overall protein digestion.

Actinidin markedly enhanced the digestion of several food protein sources under simulated gastric conditions alone (*5*). This effect was quite significant for sodium caseinate, beef muscle protein, and SPI, whereas the other proteins, such as WPI, collagen, gluten, and gliadin, appeared to be least affected by actinidin (**Table 1**). This provides evidence that kiwifruit as part of a meal may influence the digestion of a variety of food proteins under gastric conditions and may help ameliorate feelings of overfullness in the stomach.

Incubation of the gastric digestion products with pancreatin under simulated small intestinal conditions resulted in further digestion of the undigested proteins and the peptides formed

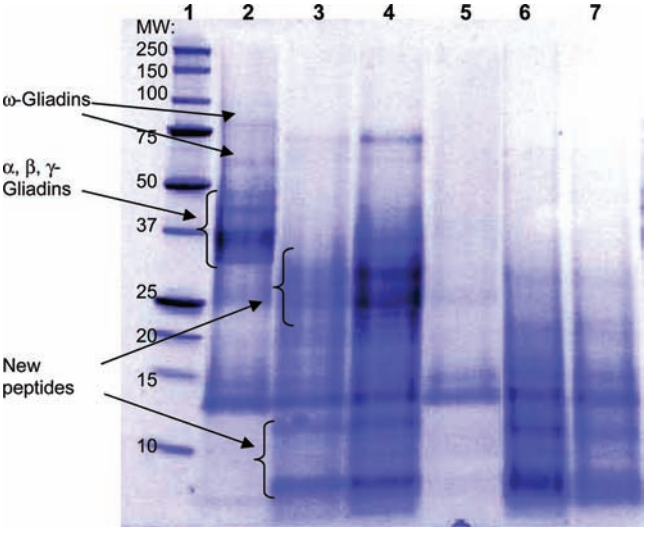


**Figure 7.** Tricine-SDS-PAGE electrophoretogram of gliadin: lane 1, molecular weight marker (kDa); lane 2, gliadin incubated at pH 1.9 for 30 min without any added enzymes (control−30); lane 3, gliadin hydrolyzed with pepsin (pH 1.9) for 30 min (Act−30); lane 4, gliadin hydrolyzed with pepsin and actinidin (pH 1.9) for 30 min (Act+30); lane 5, gliadin incubated at pH 1.9 for 30 min without any enzymes (control−150); lane 6, gliadin hydrolyzed for 30 min at pH 1.9 with pepsin and then for 120 min at pH 8.0 with pancreatin (Act−150); lane 7, gliadin hydrolyzed for 30 min at pH 1.9 with pepsin and then for 120 min at pH 8.0 with pancreatin in the presence of kiwifruit extract (Act+150).

during gastric digestion. At the end of the in vitro digestion (both simulated gastric and intestinal digestion), actinidin enhanced or changed digestion over and above that of pepsin and pancreatin alone for WPI, zein, beef muscle protein, collagen, gluten, and gliadin (**Table 1**). The digestion of SPI was least affected by the addition of actinidin. Sodium caseinate was completely digested at the end of the digestion period, both in the presence and in the absence of actinidin. Because of the more open and flexible structure of caseins, they are more susceptible to proteolysis.

Among the whey proteins, $\beta$-Lg was more resistant to digestion than α-Lac, and this difference is related to the different structural conformations of the two proteins and their relative stabilities under acidic conditions (*18*). The pepsin-resistant $\beta$-Lg (WPI) digestion in the simulated intestinal conditions was considerably enhanced in the presence of actinidin. It has been reported that predigestion by pepsin makes the $\beta$-Lg structure more susceptible to proteolysis by other proteases (*8*, *19*−*21*). Gluten was observed to be more digestible than gliadin. Furthermore, the effect of actinidin on the digestion of gluten was also more pronounced. Collagen and zein were among the least digestible proteins.

The peptides present after digestion with gut proteases and kiwifruit (actinidin) differed in many cases from those present after digestion with gut proteases alone. It is possible that some of the peptides generated in the presence of actinidin may have biological activity and also possible health benefits. Equally, the known bioactive peptides formed from food proteins during normal digestion may not be formed. These peptides will be examined more closely using mass spectrometry in a forthcoming companion study. The current work is limited by the need to assume that the in vitro results are valid. The work will now be extended to observe the effects of actinidin on the digestion of proteins in vivo.

The present in vitro study provides clear evidence that actinidin can provide enhanced digestion of a variety of food proteins under small intestinal digestion conditions. In particular, en-

**Table 1.** Summary of the Effect of Kiwifruit Extract (Actinidin) on the in Vitro Digestion of Different Proteins under Simulated Gastric and Small Intestinal Conditions (Gastric Phase, 30 min; Small Intestinal Phase, 2 h)

| | effect of actinidin (+ or −) | | comments | | |
|---|---|---|---|---|---|
| protein | gastric digestion | small intestinal digestion | gastric digestion | small intestinal digestion | overall effect of actinidin |
| 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 | the protein was completely digested after 150 min of digestion both in the presence and in the absence of actinidin | considerable enhancement of protein digestion (α-, β-, and κ-caseins) during gastric phase |
| WPI | − | + | no positive effect on digestion | after digestion with pepsin and pancreatin, 84% of original β-Lg remained undigested after 150 min of digestion compared to 59% residual protein when kiwifruit extract was present along with pepsin and pancreatin | no effect on gastric protein digestion but enhancement of small-intestinal digestion of β-Lg |
| zein | | + | data unavailable due to low solubility of the protein | after 150 min of digestion with pepsin and pancreatin, 91 and 93% of the original Z19 and Z22 α-zeins remained undigested, respectively; however, in the presence of kiwifruit extract along with pepsin and pancreatin, only 61 and 62% of these proteins remained undigested | improved digestion during small intestinal phase |
| SPI | + | − | small effects on the hydrolysis of some bands (at 31 and 21 kDa) in the presence of actinidin | no positive effect of actinidin on overall protein digestion was observed; however, the 11S basic polypeptide (21 kDa) appeared to be more digested in the presence of actinidin | slight effects on SPI digestion during both gastric and small intestinal phases |
| beef muscle protein | + | − | significant enhancement of digestion of all 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 | little enhancement of digestion in the presence of actinidin | improved gastric digestion considerably but little effect on small intestinal digestion |
| collagen | − | + | no effect on digestion | slight enhancement of digestion by actinidin | slight positive effect observed during small intestinal digestion only |
| gluten | − | + | no effect on digestion | considerable effect of actinidin on digestion | improvement of small intestinal digestion; no effect observed during gastric digestion |
| gliadin | − | + | no positive effect on digestion; actinidin appeared to decrease the digestion by pepsin | significant enhancement of digestion | improvement of small intestinal digestion only |

hanced digestion of WPI, zein, gluten, and gliadin was observed in the simulated upper tract digestive system. This provides further evidence that actinidin can enhance the digestion of food proteins and lends support to a role for dietary kiwifruit as a digestive aid. The present work is subject to the limitation of all in vitro studies; it is an attempt to replicate and understand a natural process. Work is now underway to extend this work to animal studies, which will be reported in due course.

## ACKNOWLEDGMENT

We thank Zhao Zhuo for assistance with the SDS-PAGE work.

## NOTE ADDED AFTER ASAP PUBLICATION

The version of the manuscript posted online on March 16, 2010, had errors in several figures. The figures shown in the version posted online on March 26, 2010, are correct.

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**Received for review November 1, 2009. Revised manuscript received January 24, 2010. Accepted February 25, 2010. We acknowledge funding from ZESPRI (New Zealand) for this work. Support for the development of the in vitro digestion model system from the Foundation for Research, Science and Technology (Contract MAUX0703), Wellington, New Zealand, is also acknowledged.**