Short Communication

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Quantitative Comparison of the Proteolytic Enzyme Actinidin in Fresh Kiwifruit and the Commercial Kiwifruit Extract KWD+®

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ABSTRACT

The aim of the present study was to analyse and to compare the proteolytic enzyme actinidin content of fresh kiwifruit (*Actinidina deliciosa*) and the commercial kiwi extract KWD+®. The total protein extract was quantified by Bicinchoninic Acid Assay subjected to electrophoretic separation on an SDS-PAGE gel by "Coomassie brilliant blue" stain, the identification of Actinidin-A by immunoassay and the quantification with the corresponding purified standard. The actinidin concentration of KWD+® was about 5 fold higher than fresh kiwifruit (from 4.1 to 0.8mg of actinidin per grams of the sample). The standardization to actinidin in KWD+® allows to stablish a concrete dosage to improve the digestion of some proteins difficult to digest such as gluten, soy protein or beef muscle.

Keywords: *Actinidina Deliciosa*; Kiwifruit Extract; Actinidin; Proteolytic Enzyme; Gluten; Digestion

Introduction

Kiwifruit (*Actinidia deliciosa*) is an exceptional source of nutrients such as vitamin C, and other bioactive components related to its healthy effects such as metabolic health, digestion, antioxidant activity and immune function [1].One of the best-known properties of kiwi consumption is the improvement of protein digestion due to the presence of actinidin (EC 3.4.22.14), a proteolytic enzyme [2]. There are several *In vitro* and *In vivo* studies that show the proteolytic effect of actinidin from kiwifruit in different food proteins such as gluten, soy protein or beef muscle [3-6]. The aim of this research work was to study the actinidin content of a new kiwifruit extract, and to compare it with the whole kiwifruit.

Materials and Methods

Materials

The commercial kiwifruit extracts (KWD+®) provided by Pharmactive Biotech Products together with the fresh kiwifruit in powder form (which consists of a fresh kiwifruit freeze-dried), were stored in darkness at room temperature previous to analyses.

Quantification of Actinidin and Total Protein Content

The fresh kiwifruit powder(KP) and KWD+® samples were previously prepared according to Martin (2016) to extract the protein components. Briefly, 500 mg of sample was subjected to a non-reductive lysis of the lipids by incubation for 16h at 4 °C in the presence of 2 mL of buffer. The resultant solution was centrifuged, and the soluble phase (supernatant that contains actinidin) was stored. The total protein extract was quantified by BCA (Bicinchoninic Acid Assay; Thermo Fisher Scientific, Massachusetts, USA), which was subjected to electrophoretic separation on an SDS-PAGE gel (12%). The total protein profile was performed by "Coomassie brilliant blue" stain (BioRad, California, USA) and the identification of Actinidin-A by immunoassay using a polyclonal antibody anti-actinidin A (anti-Cp2, Agrisera antibodies, Vännäs, Sweden), and visualised with an anti-rabbit peroxidase as secondary antibody. The quantitative analysis of Actinidin was performed with the purified standard Actinidin (KiwiEnzyme Ltd, New Zealand), the concentrations employed were between 1 to 200 µg/µL. In addition, a pattern of BSA concentrations was performed,

with a range between 0.5 at 40 µg/µL. The quantification of Actinidin and total protein content was performed by digital images on protein gels and blots using the processing software Fiji (https://fiji.sc/).

Results and Discussion

Actinidin and Total Protein Quantification

Coomassie brilliant blue staining of the KWD+® and KP samples have a major band between 30-35 KDa, which was identified as Actinidin A using immunoblot techniques (antibodies reactive against Actinidin A), with more than 70% of its total protein content as can be seen in Figure 1A Apart from actinidin A, other three bands of lower molecular weight were observed, which contribute 18-28 % to the total protein content of each sample (data not shown). Nevertheless, KWD+® sample showed about 5-fold higher levels of Actinidin A compared to the fresh kiwifruit (KP) samples (Figure 1B) P≤ 0.01. Kiwifruit has a low protein content, being the actinidin more than 50 % of the total protein content, which agrees with the results obtained by other authors [7-11]; in the case of the new KWD+®, the actinidin content (in percentage) represent more than 80% (82±9 %) of the total protein, and KP was slightly lower (74±5 % Figure 1A). However, the actinidin content in KWD+® (4.1±0.9 mg actinidin/g sample) was significantly higher compared to KP (0.8±0.3 mg Actinidin/ g of sample; (Figure 1B)).

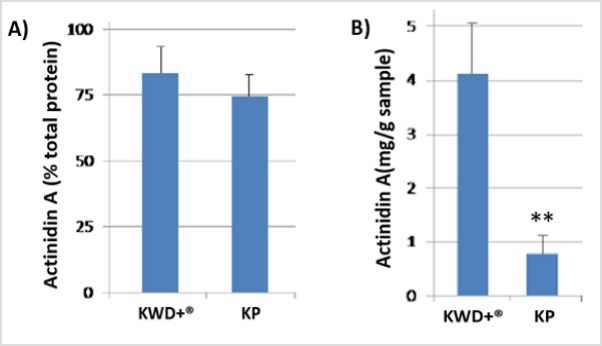

Figure 1:

Actinidin A concentration based on A) total protein content (%) and B) Actinidin A content (mg/g sample) in KWD® and KP samples

Conclusion

The extraction processing technology may increase the actinidin concentration at least 5-fold in the KWD+® extract compared to the fresh kiwifruit, and therefore, the development of a new kiwifruit extract standardized to actinidin may improve the digestion of specific proteins difficult to digest using an adequate dosage.

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