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Research Article

Optimizing Extraction Conditions of Actinidin from Kiwifruit (*Actinidia deliciosa*)

Maysaa A. A. Al-Zubaidy
Biotechnology Branch, Department of Applied Sciences, University of Technology, IRAQ
*Author Email: maysaa2003@yahoo.com

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Abstract

Kiwifruit (*Actinidia deliciosa*) is one of many fruits that are rich of enzymes like Actinidin. Actinidin is considering a member of cysteine protease. In this study, different parameters and conditions were tested for optimal Actinidin extraction from kiwifruit. The tested parameters are optimum buffers, pH, Molarity, time, and amounts (gm) of kiwifruit to volume (ml) of buffer ratio. The best buffer for Actinidin extraction from kiwifruit was Sodium phosphate because it gave high activity, with casein as a substrate. The next experiments used sodium phosphate as an optimal buffer for Actinidin extraction and casein as a substrate, detected the optimal Actinidin extraction conditions were carried out at pH 7.0, 0.1 M of sodium phosphate, 2.5 min of extraction time, 1:0.5 (gm of kiwifruit fruit/ v of sodium phosphate buffer) extraction percentage, and 30 min of incubation time. Also this study showed that the maximum enzyme activity for Actinidin extracted from kiwifruit was at pH 7and at 30 min of incubation with casein as substrate.

Keywords: Actinidin, Kiwifruit, enzyme activity, Actinidin extraction.

الخلاصة

يعتبر في هذه الدرسة تم اختبار معايير مختلفة لمعرفة الظروف ألامثلى للأستخلاص أنزيم الأكتندن من فاكهة الكيوي. المعايير التي تم احتبارها في هذه الدراسة هي محاليل منظمة مختلفة , أرقام هيدروجينية , تراكيز مختلفة من المحلول ألمنظم ألامثل, فترات زمنية مختلفة لحضن الانزيم مع مادته الاساس (الكازئين) , و نسب أستخلاص مختلفة (وزن فاكهة ألاناناس ألى أحجام مختلفة من محلول المنظم ألامثل). بينت النتائج بأن أفضل بفر لاستخلاص انزيم الأكتندن من فاكهة الكيوي هو (فوسفات الصوديوم) لأنه أظهر أعلى فعالية مع مادته الاساس (الكازئين). كذلك بينت هذه الدراسة ان افضل رقم هيدروجيني لمحلول المنظم (فوسفات الصوديوم) للاستخلاص انزيم الأكتندن هو 7 وبتركيز 0.1مولر , فترة استخلاص 2.5 دقيقة , كانت افضل نسبة استخلاص انزيم الأكتندن هي1:0.5, وكانت فترة الحضن الامثلى لفعالية انزيم الأكتندن مع مادته الاساس (الكازئين) هي 30 دقيقة.

Introduction

The most common type of kiwifruit is the Green kiwifruit (*Actinidia deliciosa*). Like other plants, *Actinidia deliciosa* contains soluble and insoluble proteins. 60% of the soluble proteins is Actinidin [1], which is a cysteine protease (284mmol-*o*-nitrophenol/min per g DM) and it has sulfhydryl group which plays a main role for its activity [2] [3]. While other types of kiwifruit such as gold kiwifruit (*Actinidia chinensis)* have low levels of proteolytic enzyme [4]. Cysteine proteases present in many types of plants where they play essential roles in plant cellular functions which include removing abnormal or damaged proteins, controlling a lot of important enzymes and regulatory proteins, helping in the ripening of kiwifruit, and working as a mechanism of defense against pathogens (fungal and insect) [1] [5] [6]. In Kiwifruit, Actinidin accumulates at a high concentration in the fruit comparing with the other parts (roots, leaves, stem and crown) [4]. According to DNA sequence and proteins database, cysteine protease in kiwifruit is closely related to cysteine proteases that present in other plants, like papain in papaya, ficin in fig, and bromelain in pineapple [7] [8]. Actinidin has narrow industrial applications due to narrow range of substrate specify [9].

However, many studies found that proteolytic enzymes (Actinidin) can play an important role in the digestive process since they have the ability to breakdown the proteins to their simple units (amino acid) by hydrolyzing their peptide bonds [10]. Other studies found that Actinidin can be used in many organic processes instead of many commercial enzymes that extract from microorganism, which are not appropriate for organic processes [11]. Many manufacturing process of cheese are using protease as coagulant enzyme [12]. Additionally, other studies suggested that actinidin helps in meat tenderizing [13] [14]. On the other hand, studies have showed that actinidin is responsible for allergic reactions in some European populations [15]. Here in this study, optimal conditions for actinidin extraction from kiwifruit had been determined. Different types of buffers, different buffer molarities of sodium phosphate, different sodium phosphate pH values, different time of extraction, and different amounts (gm) of kiwifruit to volume (ml) of buffer ratio had been used in this study.

Materials and Methodology

The fresh kiwifruit that have been used in this study were purchased from local Iraqi fruitier. All the chemicals and reagents that have been used in this study were supplied by Hi Media and sigma.

Determining the optimal buffer and pH for Actinidin extraction:

The Actinidin were extracted from a 25 gm of fresh kiwifruit that were blended in a blender with 50 ml of 0.1 M of four different buffers which include potassium phosphate (pH 7.0), sodium acetate at pH values ranking from 3 to 6, sodium phosphate (pH 7.0), and Tris-Hcl (pH 8 and 9) for 1 min. Also, the Actinidin were extracted from 25 gm of kiwifruit in 50 ml tap and distilled water for 1 min. At a high speed and at room temperature, the process of blending was carried out. After the blending process, the homogenate were filtered by using filter paper (whatman filter paper).By using the filtrate (crude enzyme), the protein concentration and Actinidin activity against casein were estimated.

Determining the optimal buffer molarity for Actinidin extraction:

A 25 gm of fresh kiwifruit were blended in a blender with 50 ml (0.05, 0.1, 0.2, 1, and 2 M) of sodium phosphate at pH 7 and at room temperature, then an optimum buffer molarity for Actinidin extraction were investigated. The resulted homogenate were filtered by using filter paper. The filtrate (crude enzyme) was used to determine the protein concentration and Actinidin activity against casein.

Determining the optimal time for Actinidin extraction:

An optimal time for Actinidin extraction were determined by blending 25 gm of fresh kiwifruit with 0.1 M of sodium phosphate at pH 7 and at various period of time (30 sec, 45 sec, 1 min, 1.5 min, 2 min, and 2.5 min). The resulted homogenate were filtered .The filtrate were used to determine the protein concentration and Actinidin activity against casein.

Determining the optimal extraction percentage:

Different amounts of kiwifruit to volumes (ml) of 0.1 M of sodium phosphate were applied to determine the optimal extraction percentage of Actinidin enzyme from fresh kiwifruit. The investigated percentage were 1:0.5, 1:1, 1:1.5, 1:2, and 1:3 (w:v). This experiment was done at pH 7 and at 2 min extraction time. Both of the protein concentration and the Actinidin activity was estimated.

Determining the optimal pH for Actinidin activity:

The Actinidin activity was estimated under various pH values from 4-9 respectively, with maintaining other parameter at their optimal level. The protein concentration and Actinidin activity were estimated.

Determining the optimal incubation time for Actinidin activity:

An optimal incubation time for Actinidin activity were estimated by incubating the

extracted Actinidin from fresh kiwifruit with casein for different period of time. The investigated periods of time were ranking from 10-50 min. with an interval of about 5 min. The protein concentration and Actinidin activity were determined.

Assay of proteolytic activity of Actinidin:

In this assay, the proteolytic activity of Actinidin against casein was investigated by Lower's method [16]. At 37 C, ° a mixture of 200 µl of extracted Actinidin and 1.8 ml of 1% (w/v) casein were incubated in water bath° for 30 min. After incubation, 3 ml of 15 % trichloroacetic acid (TCA) was added to stop the reaction. The mixture was centrifuged at 6000 rpm for 15 min. 3 ml of TCA was mixed with 1.8 ml of 1% casein to prepare a blank. Then 200 µl extracted enzyme was added. The blank was subjected to the same steps as the investigated samples by using distilled water instead of plant extract.3 ml of supernatant were applied to spectrophotometer cuvette. By measuring the absorbance at 280 nm, the proteolytic activity of Actinidin were estimated.

Assay for Protein concentration:

The protein concentration within samples were estimated and determined by Bradford method [17].

Results and Discussion

Buffers and pH for Actinidin extraction:

Many factors effect on enzymes extraction, Buffer is one of the most essential factors that might effect on enzymes extraction. In this study, the different extraction buffers were used as shown in Figure 1. Sodium phosphate buffer was used at pH 7.0 for Actinidin extraction which gave the highest specific activity for Actinidin (133.25U/mg) .The lowest specific activity for Actinidin (40 U/mg) was got when sodium acetate buffer at pH 3.0 was used for Actinidin extraction. Depending on the obtained data in Figure 1, sodium phosphate (pH 7.0) was evaluated to be the optimal buffer for Actinidin extraction. Sodium phosphate buffer (pH 7.0) has been used for more examination.

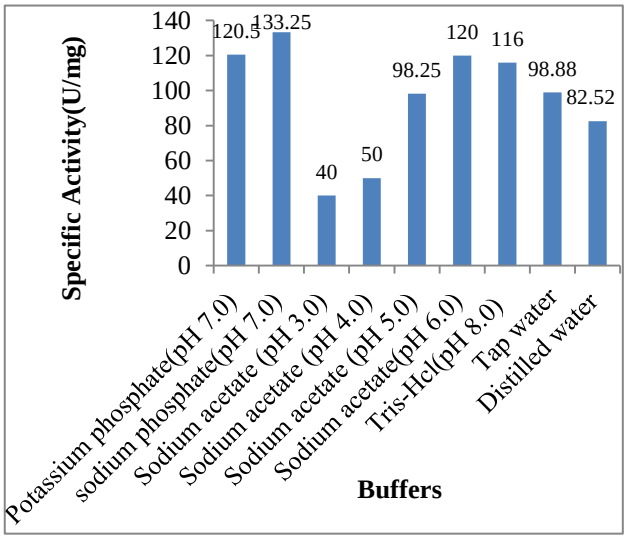

Figure 1: Effect of buffer and pH on Actinidin extraction from kiwifruit.

This result is similar to that obtained from [18] [19] which detected that sodium phosphate was an optimum buffer for proteolytic extraction from pineappl. [20] [21] showed that Actinidin has a wide pH activity range. Also this result is in accordance with the result obtained from [22] [23] [24], which demonstrated that the best pH for proteolytic activity was at pH 7.

Optimal buffer molarity for Actinidin extraction:

Molarity is another factor that effects on enzymes extraction. The specific activity of extracted Actinidin was the highest (133 U/mg) when the molarity of sodium phosphate at pH 7 was 0.1 M while the lowest specific activity for Actinidin extraction was (66.2 U/mg) when the molarity was 2 M (see figure 2). According to the obtained data in figure2, 0.1 M of sodium phosphate at pH 7.0 is the optimal molarity.

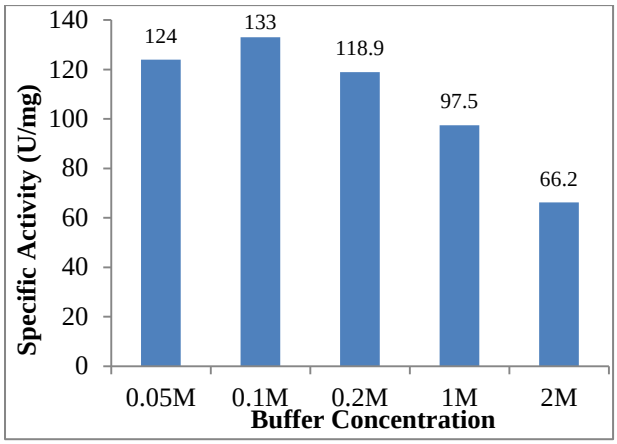

Figure 2 Effect of buffer concentration on Actinidin extraction from kiwifruit.

The given result is similar to that obtained from a study conducted by Ketnawa *et al.* in 2012, [25] they showed that optimal proteolytic (Actinidin) extraction from kiwifruit obtained at 0.1 M of phosphate Buffer.

Extraction time:

Extraction time is another factor that might effect on enzymes extraction. In this study, various times for Actinidin extraction were set as shown in Figure 3. The specific activity of extracted Actinidin was enhanced 151 U/mg when the time was after 2 minutes while it decreased 114 U/mg when the time was in 30 sec., the reason of these results is attributed to the low releasing of Actinidin from kiwifruit. Whereas 0.45 sec., 1, 1.5, 2.5 and 3 minutes gave low specific activity 126, 132, 137, 145 and 136 U/mg respectively, and the reason is the increasing temperature for blender which effects on total activity for Actinidin enzyme. Decreasing in activity at high temperature degrees might change the structure of the enzyme that leads to block the active sites, with denaturation of enzyme.

This result is similar to that obtained from a study conducted by Abdulrahman *et. al*. [26] they have found that the best extraction time for proteolytic was 1.30 minWhile Glider and Hargrove [27] have found that best time for extraction from pineapple was 2 min.

Extraction ratio:

Different extraction ratios were used to determine the best ratio for Actinidin extraction. The specific activity was 147 U/mg when the ratio was 1:0.5 (w:v) as shown in (Figure 4), while the specific activity was 135 U/mg when the ratio was 1:1 (w:v). According to the obtained data in (Figure 4), 1:0.5 (w:v) was the best ratio for Actinidin extraction. Other ratios were 1:1.5, 1:2 and 1:3, (w:v) that gave specific activity 121, 112 and 90 U/mg respectively, because the protein concentration increased.

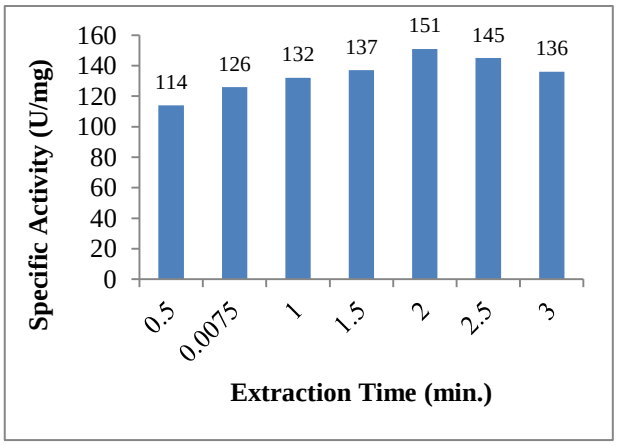

Figure 3: Effect of extraction time on Actinidin extraction from kiwifruit.

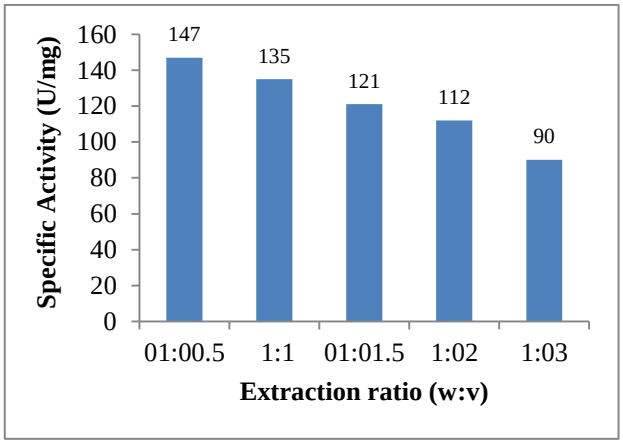

Figure 4: Effect of extraction ratio on Actinidin extraction from kiwifruit.

The best extraction ratio for proteolytic (Actinidin enzyme) from kiwifruit was 1:2 (w:v) that have found by Abdulrahman *et.al.* [26]. While Ketnawa *et.al.* [28] demonstrated that 1:1 (w:v) was better extraction ratio for Actinidin extraction from kiwifruit.

Effect of substrate pH on Actinidin activity:

Different values of substrate pH were examined to determine the best substrate pH for Actinidin extraction (see figure 5). At pH 7.0, Maximum Actinidin specific activity was148.2 U/mg as shown in (Figure 5). Other pH's of casein were 4,5,6,8 and 9 with specific activity 49, 75, 96,134.5 and 128.2 U/mg respectively.

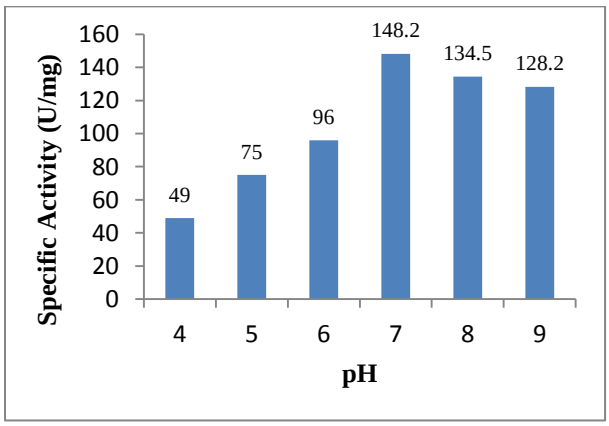

Figure 5: Effect of substrate pH on Actinidin activity.

Both Ferreira *et.al*. [29] and Abdulrahman *et. al*. [26] found pH 7.0 was best pH for proteolytic (Actinidin) activity. The pH of enzyme environment can have several effects of the activity of the enzyme in several ways. First, environmental pH effects on enzyme stability, the enzyme might denaturized at excess acidity or alkalinity. Second, the association of the substrate with the enzyme may influence by the reaction mixture pH ma. Third, each enzyme has a specific optimum pH which considers the maximum enzyme activity, but the enzyme is stable within certain limits less and more the optimum [30].

Effect of incubation period on Actinidin activity:

According to the obtained data in (Figure 6), (148 U/mg) was the Maximum Actinidin specific activity at 30 min. after incubation with casein as substrate. While other specific activity, above and below than 30 min incubation time, were decreased.

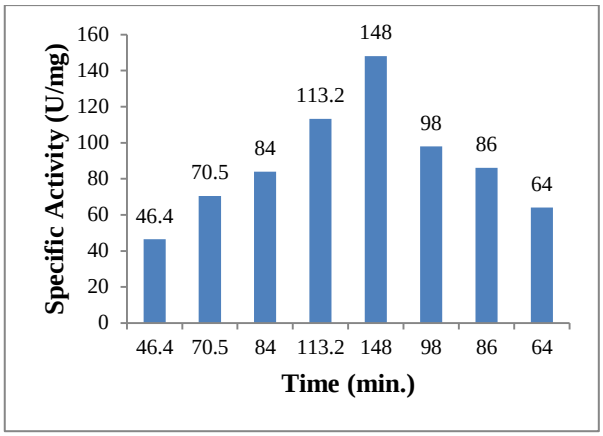

Figure 6: Effect of incubation period on Actinidin activity.

The best incubation period for proteolytic (Actinidin) activity after incubated with casein was 50 min that found by Abdulrahman *et. al*. (26). While 30 min. incubation time was best time for proteolytic (Actinidin) activity after incubated with gelatin as substrate that found by Priya *et.al* [31].

Conclusions

Many studies have demonstrated that Actinidin plays an essential role in many applications like industrial, domestic and commercial. This research aims to determine the optimal conditions for extraction and proteolytic activity. In this study, Actinidin have been extracted from kiwifruit which obtained from local Iraqi store. Extraction process has used different parameters and conditions in purpose to determine the optimal conditions for Actinidin extraction and enzyme activity. According to the obtained data from this study, Optimal Actinidin extraction was obtained with sodium phosphate buffer at pH 7 and 0.1 M at 1:0.5 extraction ratio for 2 min mixing time. Also this study found that optimal Actinidin specific activity was at pH 7 and for 30 min incubation time.

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