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EXTRACTION AND PURIFICATION OF ENZYME ACTINIDIN FROM KIWI FRUIT (*ACTINIDIA CHINENSIS*)

Rasha Adnan Kazem* and Adnan Wahhsb Habeeb

Department of Food Science, College of Agriculture, University of Kufa, Najaf, Iraq.

Abstract

The aim of this study is to extract, purify and estimate molecule weight of the actinidin enzyme from kiwi fruit. The extraction process is carried out using a buffer consists of 6% sodium chloride + 2% boric acid in which it results in having 39 units/mg quality activity for the actinidin. After that, the process of purification is started by concentrated the crude, that is resulted from the extraction process, by saline gradient using ammonium phosphate with a saturation ratio varying from (20-80)%. At 60% saturation, the highest enzymatic activity of 48.7 units/ml is achieved. Then, the dialysis was carried out using potassium phosphate and the specific activity resulted from this step is 128.3 units/mg protein. The purification process was completed using ion exchange chromatography column DEAE-Sephadex A-50 and a quality activity at this step is 192 units / mg protein. Then, gel filtration with a column Sephadex G-75 is used in which it results in having an enzymatic activity of 20.4 units /ml and a specific activity of 226.6 units/mg protein at 5.81 number of purification and 11.33% enzymatic yield. Using electrophoresis, the purification process reveals that there is one protein bundle which is one of the signs to the purity of the enzyme. Moreover, the molecule weight of actinidin enzyme which is 245000 Dalton has been determined.

Key words : extraction, purification, Enzyme actinidin, Kiwi fruit.

Introduction

Kiwi fruit *Actinidia chinensis* is a fruit which is very popular in the human dietary system due to its pleasant taste, high content of vitamin C and minerals (especially potassium, phosphorus and iron) and low calories. Nevertheless, Kiwi fruit has a good source of Foliate and its seeds contains large amount of Vitamin E. In addition, (Sharma and Vaidya, 2018) states that Kiwi juice contains many enzymes including proteins which are used in the food industry. There are many types of kiwi fruit and the main two types in the international trade market are (green kiwi fruit) and (golden kiwi-fruit). The most common type is the green one which consists from 83% water, 9% sugar, 3% fibers, 1% protein and a small amount of minerals (Drummond, 2013). Like other plant, kiwi fruit's protein has found in two types soluble and insoluble proteins. The largest percentage of the soluble protein,which is the most important enzyme, is the actinidin enzyme. This enzyme accumulates in the kiwi fruit compared to the other parts of the kiwi plant (root, leaves, stem and crown). The main advantage of this enzyme is that it plays an important role in dissolving large amount of proteins of food such as meat protein thus it makes it more tenderer (Zubaidy, 2017; Martin *et al*., 2017).

To extract this enzyme from kiwi fruit, the crude extract is used which can be obtained from the leaves or dry seeds and then treated with the extraction solution by using an appropriate technique which can penetrate different protein membranes. After that, the enzymes can be extracted using a buffer solution or freezing technique and rapid defrosting can be used to break down hard cell walls (Bugg, 2004; Aehle, 2007). Purification of enzyme occurs in a series of step separated each other which depends on physical and chemical properties of the enzyme to gradually separate it from the unwanted components and result in increasing the specific activity for the enzyme (Labrou *et al*., 2004).

Objective of the study

The aim of this study was to extraction and purification the enzyme actinidin from the kiwi fruit using modern technology and then calculate the molecule weight of the enzyme.

****Author for correspondence :*** E-mail: rashaa.alkaraawei@student.uokufa.edu.iq

Material and Methods

Materials

Kiwi fruit was purchased from the local markets of Najaf / Iraq.

Preparation of Samples.

The preparation of kiwi fruit that has free of pathological, insect and any other infection has been chosen. Then, it cleaned and washed to remove dirt and chemical pesticides. After that, the outer shell of the selected fruit has been peeled and remaining parts has been cut into small pieces with a thickness of 1.5 cm.

Reparation of crude plant extract

The crude extract of kiwi fruit has been prepared as described in (Aworth and Nakai, 1986). The preparation method begins by mixing 50 g of kiwi fruit pulp after cutting into 1.5 cm with 175 ml of extraction solution consisting of 6% sodium chloride + 2% boric acid at ratio (1:4) (weight:volume). Thereafter, the cooled centrifuge is thoroughly mixed at a speed of 6000 rpm and filtered. Finally, crude was collected and part of it was used to subsequently measure enzymatic activity.

• Measurement of enzymatic activity: The enzymatic activity was determined by the method described in (Aworth and Nakai, 1986) where it prepared by adding 0.1 mL of crude that has been extracted and then filtered. The odor was quietly separated and the absorbance was measured along a 280 nm wavelength.

• Measuring Protein Concentration: To measure the protein concentration, 1 mL of crude was added as it described in the method (Lowry *et al*., 1951). Then, the absorption was determined along with a 600 nm wavelength and according to the protein concentration of the standard curve.

Enzyme Purification

• Concentration of the Enzyme using Ammonium phosphate and dialysis: Using the method described in (Aworth and Nakai, 1986), the deposition of enzymes is obtained by adding a certain weight of ammonium phosphate to the enzymatic extract with a saturation ration gradually ranging from (20-80)% respectively. To determine the optimal concentration of extraction then dialysis is used. After dialysis, thus it will be ready for use in the next step.

• Ion Exchange Chromatography: The method described in (Biosciences, 1999) is used to prepare solutions. It starts by passing the enzymatic solution from the previous step to the DEAE Sephadex A-50 ion exchange column. Than the washing step was then performed and the absorption of the separate parts resulting from this step was tracked at a wavelength of 280 nm. The rinsing was then carried out on the proteins surrounding the negative ion exchanger. Analytical activity of the parts was estimated, their activity and protein concentration were estimated.

Table 1: Percentages of the materials used in the preparation of polyacrylamide gel.

T	Used materials		
1	Storage gel 40%	3.75 mL	6 mL
2	Solution gel stacking buffer 0.5 molari	5.25 mL	-
3	Solution gel separation buffer 1.5 molari	-	5.25 mL
4	Solution SDS 10%	500 mL	500 mL
5	TEMED	15 mL	15 mL
6	Distilled water	5.4 mL	3.1mL
7	Solution 1.5% Ammonium persulphate Postpone adding it to the pre-casting process	150 mL	150 mL

• Gel Filtration Chromatography using Sephadex G-75: The gel is prepared according to the instructions of the Swedish pharmacia company. The enzymatic solution resulting from ion exchange on the gel surface has been passed quietly and gradually on the sides of the column in order to make sure that the enzymatic solution has been distributed homogeneously on the surface of the gel. Then, elution was done by sodium acetate buffer and separated parts from the column were then collected in tubes. The absorption was estimated for that separated parts at 280 nm wavelength. Also, the enzyme activity was measured to the separated peaks after plotting the relationship between the number of discrete parts with respect to absorption at 280 nm. Finally, the active parts were collected, measured in size and estimated their effectiveness and protein concentration.

Confirm Purity and determine Molecular Weight

The method of (Laemmli, 1970) has been used in electrophoresis to determine the purity of the enzyme and estimate the molecular weight in the presence of SDS (sodium dodecyl sulfate).

The ingredients described in table 1 were used and mixed to prepare the separation gel at a concentration of 12.5%. then, it was added directly in glass plates of the electrophoresis device and left to get harden and then pouring the 10% concentration. After electrophoresis and removal of the bromophenol blue from the gel, the distance that both of the bromophenol blue and standard proteins migrated has been estimate this has been used to estimate the relative movement for the purified enzyme and the standard proteins according to the following equation.

$$\text{Relative movement (Rm)} = \frac{\text{Distance travelled by protein packets (cm)}}{\text{Distance travelled by dye (cm)}}$$

Table 2: Steps to Clean Actinidin from Kiwi Fruit.

Purification steps	Volume (ml)	Activity (U / ml)	Protein (mg / ml)	Specific activity (U / mg)	Total activity (U)	Fold	Yield %
Crude enzyme	150	15.6	0.4	39	2340	1	100
Ammonium phosphate precipitation (20-80)% and dialysis	10	77	0.6	128.3	770	3.289	32.90
Ion exchange	15	38.4	0.2	192	576	4.92	24.61
Gel filtration	13	20.4	0.09	226.6	265.2	5.81	11.33

The enzyme is determined by plotting the relationship between the logarithm of molecular weights of standard proteins versus their relative motion (Relative Mobility)

Results and discussion

Extraction of Enzyme

Actinidin enzyme was extracted using an extraction solution consisting from sodium chloride and boric acid, giving 15.6 units / mL enzymatic activity, 39 units / mg specific activity and 0.4 mg/ml protein content as shown in table 2. This result is approximately considered to be more comparable to (Kaur *et al.*, 2010) method of extraction where it gave enzymatic activity of 26.4 units / ml and the specific activity of 42.24 units / mg protein using phosphate buffer (pH 6.0) as an extraction solution. Furthermore, the result is also considered comparable to (Sharma and Vaidya, 2018) method of extraction where it gave enzymatic activity of 0.22, specific activity of 0.52 units/mg protein and the amount of protein content of 0.4 mg/mL using phosphate buffer as an extraction solution.

Purification of the Enzyme

• Concentration of the Enzyme using Ammonium phosphate and Dialysis: Fig. 1 illustrates the step-by-step diagram to the enzymatic activity of the actinidin with respect to the ammonium sulphate at a saturation ratio ranged between (20-80)%. The enzymatic activity is found to be 13.4 units / mL, 29.3 units /mL, 48.7 units / mL and 23.2 units / mL at saturation ratio of ammonium

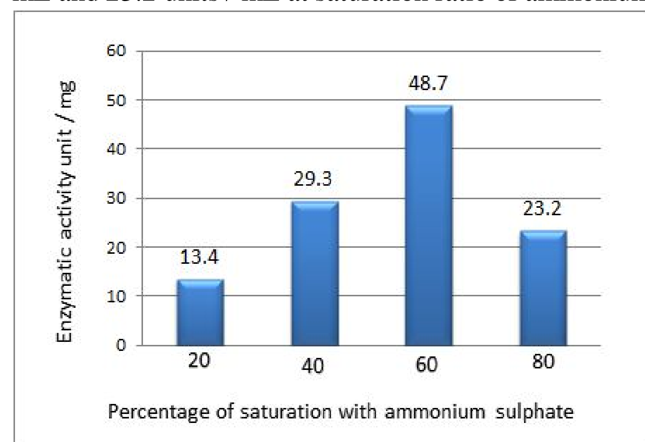

Fig. 1: Enzymatic activity of actinidin extracted from kiwifruit using different saturation ratio of ammonium sulphate.

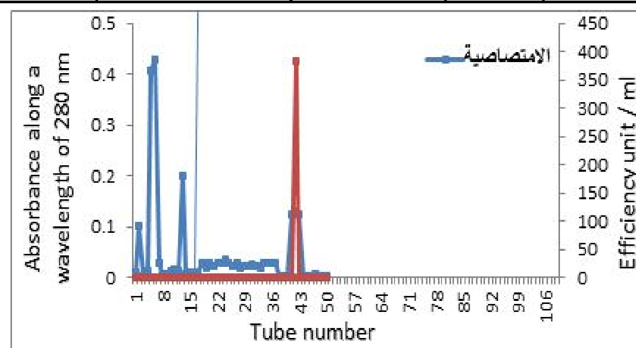

Fig. 2: Ion exchange chromatography to purify the enzyme actinidin extracted from kiwi fruit To find enzymatic activity using DEAE-Sephadex A-50 column.

sulphate of 20%, 40%, 60% and 80% respectively. It is very clear that the enzymatic activity has been improved by increasing the saturation ratio of ammonium sulphate to reach its maximum value of 48.7 units /mL at 60% saturation ratio. Then, the enzymatic activity decreased drastically to 23.2 at 80% saturation ratio. After that, the precipitate was collected from centrifuged and dialysis was done using special bags after activating them with sodium phosphate buffer, previously used, at 4°C for 24 hours taking into consideration replacing phosphate buffer every 6 hours. At this point, the enzymatic activity was estimated to be 77 units/ml, the specific activity is 128.3 units/mg and the protein concentration is 0.6 mg/mL with number of purification od 3.28 and 32.90% enzymatic yield. This results came closely to that in some other previous studies. In (Piero *et al.*, 2011), ammonium sulphate of 70% saturation ration was used to extract and purify actinidin enzyme from kiwi fruit and obtain

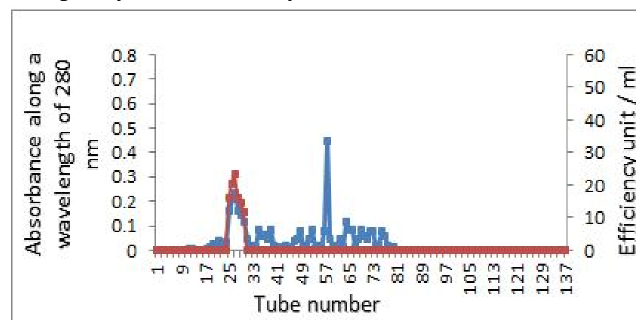

Fig. 3: Chromatography of gelatin filtration to purify the enzyme actinidin extracted from kiwi fruit Using Sephadex G-75.

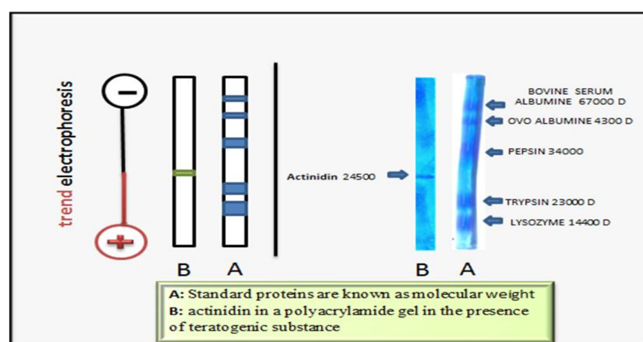

Fig. 4: Electrophoresis of actinidin and standard proteins in acryl amide ge In the presence of SDS to estimate molecular weight.

129.6 unit/mL specific activity with enzyme yield of 91% and the number of purification of 4. While in (Chalabi *et al.*, 2014), ammonium sulphate of 60% saturation was used to obtain specific activity of 85.6 units / mg protein and the enzyme yield reached 95%. The reason for that difference is due to the use of different concentrations of ammonium sulphate to modify the charges on the surface of the enzyme and cause distortion to the water layer surrounding the protein and thus, It lowers the solubility and deposition of protein (Englard and Seifter, 1990).

• Ion Exchange Chromatography: The ion exchange chromatography process was performed as a second step in purifying the enzyme actinidin resulting from previous step. As it can be seen in fig 2, three different protein peaks with free of enzymatic activity has shown in the washing phase which indicated that these three protein peaks include Proteins of positive charge (Cation) and their collapse in the washing stage because of the charge repulsion between them and the exchanger material. Then, The recovery of the enzyme was carried out by linear gradient method using sodium chloride solution (0.05-0.2) molar at a flow rate of 30 ml /h resulting in appearing one protein peak. The enzymatic activity of this peak was measured at a wavelength of 280 nm and it was observed that it has an analytical activity in it. After that, parts of this peak which included tubes from (41-46) has collected to measure their size, activity and protein concentration. It was found that the enzymatic activity is 38.4 units / mL, specific activity is 192 units / mg protein with 4.92 number of purification and 24.61% enzymatic yield as it can be seen in table 2. These results are comparable to those from some previous purification studies. (Lewis and Luh, 1988) used affinity EDTA column to purify actinidin enzyme from kiwi fruit and obtain specific activity of 56.70 units/mg with enzymatic yield of 26%, while. (Alirezaei *et al.*, 2011) obtained a specific activity of 8.3 units / mg protein when using ion exchange column DEAE-Sephadex A-25.

• Gel filtration chromatography: Fig. 3 have represented the result of filtration chromatography step in which it showed the presence of two protein peaks while measuring the absorbance along with wavelength of 280 nm. When measuring the activity of the enzyme, it was found that the peak that contains the enzymatic activity was limited to tubes (31-25) out of 134 tubes. Then, all tubes have collected to estimate the enzyme size and activity. The result showed that the enzymatic activity is 20.4 units/mL, specific activity 226.6 units/mg protein with 5.81 number of purification and 11.33% enzymatic yield as it can be seen in table 2. This result is comparable to (Richards, 2014) results obtained from two peaks results that found when the actinidin enzyme of kiwi fruit was purified in a Gel filtration chromatography step using Superdex-200 column. Both of those peaks had an enzymatic activity of 39.5 units / mL with an enzymatic yield of 31%.

• Confirm the purity and set the molecular weight: Fig. 4, shows the results of the electrophoresis of the enzyme actinidin purified from kiwi fruit to confirm the purity of the enzyme and to ensure that it is free from any other proteins or enzymes as well as determine molecular weight. The results of shows the emergence of one protein bundle in the gel, which is one of the signs of the purity of the enzyme. This also indicates that the steps and conditions used in extracting and purifying the enzyme were as efficient as obtaining a single protein bundle of actinidin to ensure its purity.

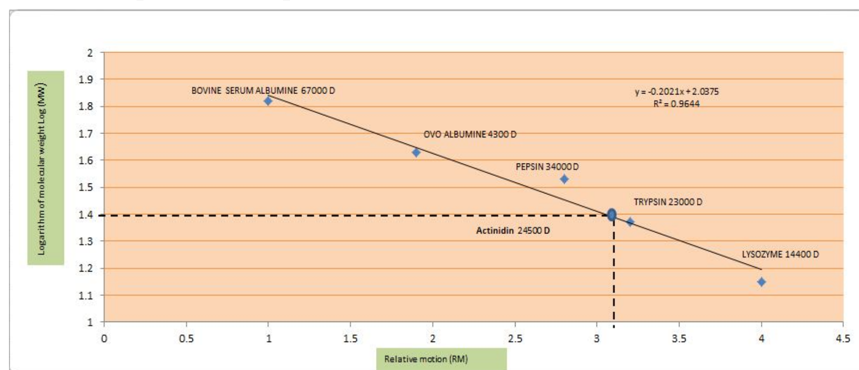

Fig. 5: Curve estimation of actinidin molecular weight by electrophoresis method In polyacrylamide gel and in the presence of SDS.

Fig. 5, shows the relationship between the molecular weight logarithm and the relative motion of proteins used to estimate the molecular weight of pure enzyme by electrophoresis. After measuring the relative motion, the molecule weight can be estimated which is found to be 24500 Dalton. This results is consistent in comparing with

other previous studies. (Lucas *et al.,* 2007) use electrophoresis and found that the molecule weight of actinidin is 25000 Dalton. While (Grozdanovic *et al.,* 2012) use electrophoresis to estimate the molecule weight of actinidin of 23883 Dalton.

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