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Investigation on Protein Pattern in Kiwifruit (*Actinidia deliciosa*)

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Abstract: Kiwifruit (*Actinidia deliciosa*) is a rich fruit for vitamins, proteins, sugars and minerals. Current study was focused on protein patterns of kiwifruit organs in Ramsar region (north of Iran). For this purpose current research was conducted to compare of electerophoretic protein patterns in leaves, fruits and seeds. Results of SDS-PAGE showed that the most number of protein bands were found in fruit, seed and leaf respectively. SDS-PAGE and two-dimensional (2D) electrophoresis analysis was carried out for above plant parts proteins. Based on results, actinidin, as main protease of kiwifruit, was expressed in seeds, fruits and leaves in different intensities. Results of SDS-PAGE and two-dimensional electrophoresis showed maximal and minimal differences for leaf and seed proteins respectively. Based on results, actinidin, as main protease of kiwifruit, with about 30 kDa mass and PI=3.5-4 was expressed more in fruits than leaves. An identical spot with mass about 29-45 kDa and PI=4 was found. 2D electrophoresis of proteins extracted from kiwi leaves showed 4 similar spots with mass bout 14-18 kDa and PI= 5-10.5 that are absent in fruits. Switch on and off and down or up regulation of some proteins may be diverse in different organs.

Key words: Kiwifruit · Proteome · Actinidin · Electrophoresis

INTRODUCTION

Iran is a country with the most significant increases (>20%) in the planted area during the previous decade [1]. The fruit of *Actinidia deliciosa* is highly rich in vitamin C (180 mg/100 g in Hayward cv), minerals, carbohydrates and proteins [2, 3]. Because of high antioxidant activity, most investigators have suggested use of kiwifruit [4-7].

Many reports have recommended that kiwifruit has laxative effects [3] and production this crop for biogas and biofuel [8]. Its promotion has improved in the world and Iran recently. Iran has a particular potential for increasing production area of kiwifruit and improving of its quality and quantitative yield. In addition using this potential, world production rank of Iran will be developed from seventh to better position [1].

Kiwifruit yield and quality is affected by environmental conditions [9, 10]. The Kiwellin, a protein from kiwifruit, was affected by environment conditions [11]. Proteome analysis was practical to research new proteins and for comparing proteins of plants that grew under different conditions [12, 13]. Kita *et al*. 2006 via two-dimensional polyacrylamide gel electrophoresis (2D- PAGE) recognized eleven proteins in the transgenic kiwifruit that their expression had been reduced and they demonstrated homologies to kiwifruit hypothetical protein, Osmotin I and photosynthesis associated protein [14].

Using an *in vitro* model, digestive activity of actinidin, the most abundant protein in kiwifruit, has been addressed [15]. SDS-PAGE is most widely used due to its validity and simplicity for describing gene expression of crop germplasm [16]. Therefore, this method has been used to study acclimatization in several plants [17-19].

Current research was conducted to compare electerophoretic protein patterns in leaves, fruits and seeds of kiwi.

MATERIALS AND METHODS

Kiwifruit (*Actinidia deliciosa*) (Hayward cultivar) has been well adapted, cultivated in Ramsar (Mazandaran Province, north of Iran with high relative humidity region) in large area and commercially exploited to date. Plant materials including fruits, seeds

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and leaves of eight-year-old kiwifruit in maturation period were supplied from Ramsar regions. All analyses were conducted at Medical Biology Research Center, Kermanshah University of Medical Sciences, Iran.

Proteome Analysis

Protein Extraction from Leaf, Fruit and Seed: 0.4 gram of leaf was powdered in liquid nitrogen. Then 2 ml of extraction buffer (include: 20 mM Tris, 1 mM EDTA, 20 mM CaCl2, 2% Mercaptoethanol and 2% Nonidet-P40) was added to sample. The sample was mixed with 8 ml of 10% cold trichloroacetic acid (TCA) in acetone and kept in an icy bath for 30 min. The sample was centrifuged at 15000 g for 10 min at 4°C, and the supernatant was discarded. The pellet was washed with cold acetone containing 20 mM DTT three times, centrifuged and vacuum-dried. The dried powder was solubilized in both lysis buffer of SDS-page containing: 8 mol/L urea, 2 mol/L Thiourea, Tris-Hcl 50 mM and lysis buffer of Two Dimensional gel electrophoresis containing: 8 mol/L urea, 2 mol/L Thiourea, 4% CHAPS, 2% ampholine, 80 mmol/L DTT, Tris-Hcl 40 mM. Mixture was incubated for 1 hour at 4°C and 2 ml lyses buffer (include 7 M Urea, 2 M thiourea and 1 mM PMSF) added. The mixture was incubated for 1 hour at 4°C and centrifuged for 20 minutes at 14000 g. Incubation and centrifuging were repeated as previously. Then 3 volumes of cold acetone containing 10% trichloroacetic acid was added mixture trichloroacetic acid 10% was added in cold acetone and after centrifuging, the sediment washed using cold acetone and solved in lyses buffer. For extraction protein from Kiwi fruit, the fruit was peeled; the Kiwi extracts were determined and centrifuged. Then 2 ml of fruit juice and 8 ml of 10% cold trichloroacetic acid (TCA) were mixed together and incubated for 1 hour at -20°C. After centrifuging the pellet was dried and dissolved in above lysis buffers. Seeds were separated from fruit and were grinded. 0.1 gram of seed powder and 1 ml of above lysis buffer were mixed; the sample was centrifuged at 15000 g for 10 min at 4 °C, and the supernatant was used for next step.

SDS-PAGE and 2D Electrophoresis: SDS-PAGE was conducted on 12.5% resolving gel and 5% stacking gel at an applied voltage of 150 volts under reducing and non-reducing conditions, according to the method of Lamemmli (1970) [20] with some modification. 15 µl of extracted protein and 5 µl of 5X sample buffer were mixed and the samples after heating for 5 minutes in boiling water, were applied on the gel. After electrophoresis coomassie blue R-250 staining was performed for 1 hour at room temperature. For two dimensional gel electrophoresis , the 18 cm IPG strips (pH 3-10) were rehydrated overnight with 350 mL of rehydration buffer (8 M urea , 0.5% CHAPS, 20 mM DTT, 0.5% v/v IPG buffers) in a reswelling tray (Amersham Biosciences) at room temperature, about 250 mg of protein were loaded; IEF was conducted with a Pharmacia Mutiphore II. The running condition was as follow: 500 V for 1 h in gradient, followed by 500 V for 3 h in step, and finally 3500 for 12 h. The focused strips were equilibrated for 15 min in 10 mL equilibration solution. The first equilibration was performed in a solution containing 6 M urea, 30% v/v glycerol, 2% w/v SDS, 1% w/v DTT, and 50 mM Tris-Hcl buffer, pH 8.8. The second equilibration was performed in a solution modified by the replacement of DTT by 2.5 w/v idoacetamide. Separation in the second dimension was performed by SDSPAGE. Gels were stained with silver nitrate [21].

Table 1: Timing of two dimensional PAGE isoelectric focusing

Step	Time (min)	Voltage (v) with 10 mA
1	60	500
2	180	500
3	720	3500

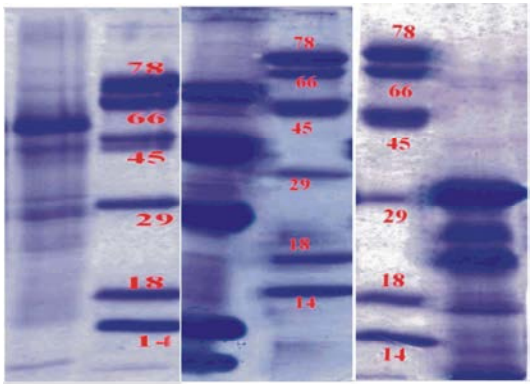
Protein size markers were proteins including standard proteins and ovotransferrin (78 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), actinidin (29 kDa), ß lactoglobulin (18 kDa) and lysozyme (14 kDa). This size marker was used previously by researchers [16, 22, 23].

RESULTS AND DISCUSSION

SDS-PAGE Results: Results of SDS-PAGE showed 11 protein bands for kiwi seeds (Figure 1.B) and 14 bands for fruit (Figure 1.C).

Results of SDS-PAGE for kiwi leaves (Figure 1.A) showed 13 protein bands that 4 of them located between 29 and 66 kDa more sharply and a protein with molecular mass about 30 kDa, commonly known as actinidin, is the most protein in Kiwifruit [24]. A 45 kDa protein was found and a protein with molecular mass larger than 29 kDa was found identical. Results of SDS-PAGE showed more number of protein bands in fruit, seed and leaf respectively.

An 18 kDa protein and a protein with molecular mass larger than 18 kDa were found in for protein bands of fruit. The mass of these proteins was similar to beta-lactoglobulin [25]. Actinidin with mass about 30 kDa was the most abundant protein of fruit in kiwifruits.



(A) (B) (C)

Fig. 1: SDS-PAGE comparing of proteins extracted from leaves (A), seeds (B) and fruits (C) of kiwifruit.

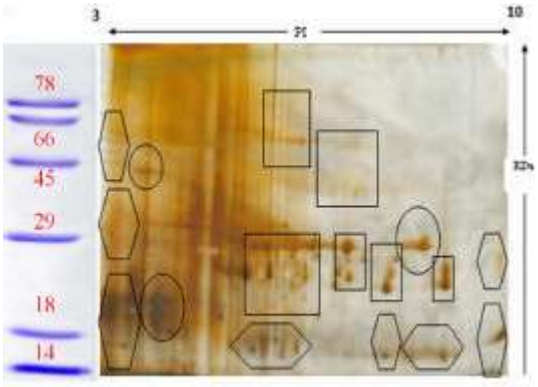


Fig. 2: Extracted proteins from kiwifruit leaves and separated by two-D Electrophoresis.

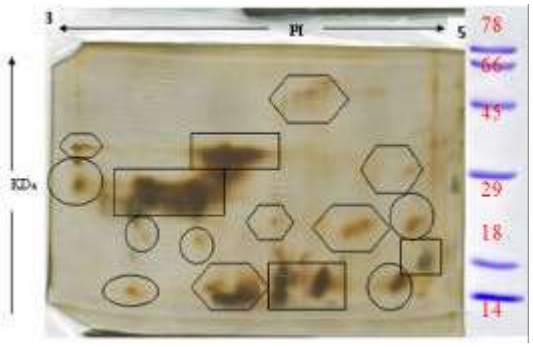


Fig. 3: Quantitative analysis of proteins which are expressed in kiwifruit fruits and separated by two-D Electrophoresis.

Podivinsky *et al*.1992 reported the similar results for actinidin content in kiwifruit [26]. They also declared insecticide effects of actinidin that protect the fruits. In total the highest variations of proteins was found in fruits and the lowest was for leaves. The differences among leaf and seed and fruit proteins were obvious, because leaves were exposing by environmental factors more than fruits and seeds; also protein types were affected by fruit maturity stage [27, 28]. The protein is affected by several conditions and factors in different organisms [29].

Two-Dimensional Electrophoresis Results: Like SDS-PAGE, the results of 2D electrophoresis showed variation and similarity for proteins of fruits and leaves of kiwifruits. 2D electrophoresis of proteins extracted from leaves of Kiwi (Figure 2) showed 4 similar spots with mass bout 14-18 kDa and PI= 5-10.5. 4 similar identical protein spots were found with molecular about 18-29 kDa and PI=5.5-9. All of these spots similarity had been shown by SDS-PAGE analyses. An spot was found with PI=9 in 18-29 kDa range. Actinidin expression (about 30 kDa and PI=3.5-4) was found more intensive in fruit than seeds and leaves. In total mainly the variation of proteins was due from Robisco subunits.

It seem to be they are subunits of Robisco enzyme; because Parry *et al*. 1987 reported 12 and 55 kDa molecular weight for these subunits [30]. Robisco is an important enzyme in Calvin cycle [31] in photosynthesis process. In addition low actinidin content was found for seed protein.

Two-dimensional electrophoresis of proteins extracted from kiwi fruits (Figure 3) showed 3 identical spots (14-18 kDa with PI=4.5). Actinidin with about 30 kDa mass and PI=3.5-4 was expressed more in fruits than seeds and leaves. An identical spot with mass about 29-45 kDa and PI=4 was fount.

CONCLUSION

SDS-PAGE results showed that the fruit has the most number of protein bands. The seed and leaf were found in following next orders respectively. Actinidin, as main protease of kiwifruit, was expressed in seeds, fruits and leaves in different intensities. Maximal and minimal differences were showed for leaf and seed proteins respectively. Actinidin, as main protease of kiwifruit, with about 30 kDa mass and PI=3.5-4 was expressed more in fruits than leaves. Switch on and off and down or up regulating pattern of some proteins are different in special plant parts.

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