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Research Article

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Proteome change of *Hardy kiwifruit* during softening

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ABSTRACT

The extracted proteins from the hardy kiwifruit, which stored at room temperature (25°C) for up to 7 days, were used for proteomic analysis in order to understand the changes of post-harvest hardy kiwifruit at proteomic level. After two dimensional gels electrophoresis (2-DE) was conducted, more than 1300 protein spots were detected. Among them, 39 differently expressed spots (P<0.05) were selected to be excised and analyzed using MALDI-TOF/MS, and finally 5 protein spots were confidently identified according to NCBI database. The results demonstrated that among the 39 protein spots expressed particularly induced in fruits softening.

Key words: Hardy Kiwifruit; proteome change; MALDI-TOF/MS

INTRODUCTION

Hardy kiwifruit(*Actinidia arguta* (Sieb. et Zucc.) Planch. ex Miq) is typical wild kiwi in the native Asia, which grows with a strong-growing vine with dense, dark green foliage. Located northeast of china, the fragrant white flowers appear in early June around Qianshan mountains [1]. The fruit, which ripens in late summer or fall, is about 1.88cm-6.88cm long, 10-20g weight, which is rich in vitamin C, starch and pectin [2]. It tastes much like the commercial kiwi fruit, to which it is closely related, but is somewhat sweeter and has smooth skin. The seeds are very small and not noticeable, so eating the fruits is somewhat like eating large seedless grapes [3]. They are very cold-hardy and will tolerate temperatures down to at least -20°c and probably much lower. However, the fruits often soften rapidly during the storage phase. So it is the bottleneck problem to how to keep the fruits fresh. Horticulture researchers have long been interested in biochemical characterization of fruit before, during and after storage [4,5] only few data on fruit development proteomics are available [6]. Similar to gene expression profiling, proteomics offers the opportunity to examine simultaneous changes and to classify temporal patterns of protein accumulation occurring in complex developmental processes [7].

In this study, we used 2-DE followed by MALDI-TOF/MS to investigate and analyze the biological function of the differently expressed proteins during the fruits softening in order to further explain the molecular mechanism of the biological process at proteomic level. This study will offer new insights to the physiological mechanism of hardy kiwifruit ripening and will provide theoretical evidence for better quality control of post-harvest hardy kiwifruit in practice.

EXPERIMENTAL SECTION

2.1 source of sample

The hardy kiwifruit were collected from the same tree in Qianshan mountain located in northeast china, then stored at 25±1 °C with 80-90% relative humidity for the durations of 1, 4, 7 days, respectively.

2.2 Protein preparation and 2-DE and staining

Protein extraction was performed using phenol protocol [8] with some modifications. 800 μg of proteins were applied to 24 cm pH 4-7 IPG strips. Isoelectric focusing was performed on a PROTEAN IEF system (Amersham) for a total of 62 kVh at 20 °C. Subsequently, the strips were sealed by 0.5% molten agarose in running buffer on 12% SDS-PAGE gels and run on the Ettan DALT twelve System at 1 W/gel for 1.5hr, then at 10W/gel for 7hr. The gels were stained with 0.02% Coomassie brilliant blue R250, 33%ethanol and 10% acetic acid [9]. Three technical replicates were performed for each treatment.

2.3 Image acquisition and data analysis

The CBB R250-stained 2-DE gels were imaged by a MAX imagescanner, and analyzed on the Image master 7.0. Images . The percentage volumes were used to designate the significant differentially expressed spots (ANOVA, P<**0.05).**

2.4 Protein identification by MALDI-TOF/MS

Samples were analyzed by a Bruker autoflex tof/tof II Proteomics Analyzer. Protein was used to calibrate the mass instrument using Bruker Peptide calibration standard. Parent mass peaks with a mass range of 500-4500 Da. A MS data search was performed over NCBInr database using the web of MASCOT MS/MS ions Searchy.

RESULTS AND DISCUSSION

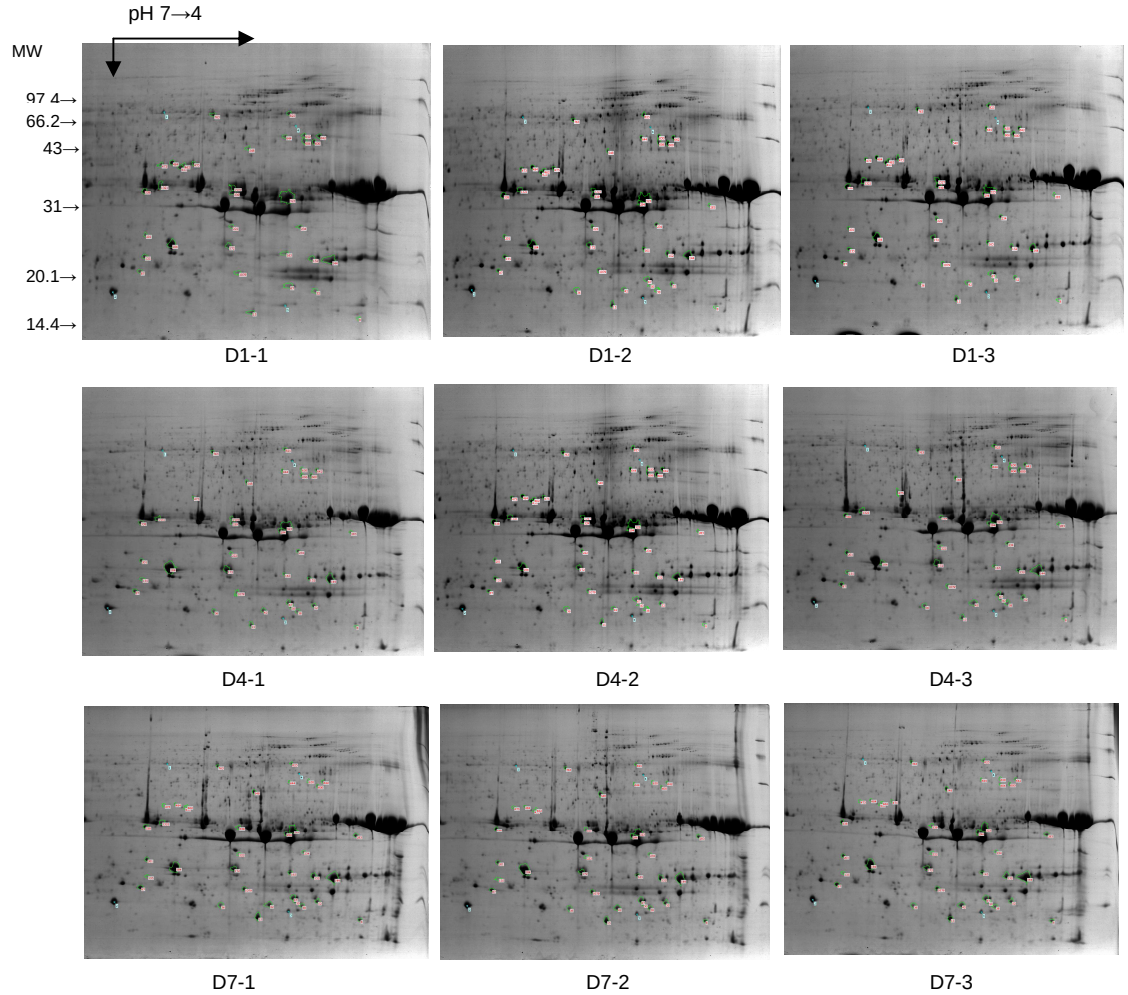

Fig. 1 Representative spot maps of Hardy kiwifruits. (D1:1d after harvest;D4:4d after harvest;D7:7d after harvest(Three technical replicates of each sample)

3.1 2-DE proteome gel analyses for hardy kiwifruit during harvest softening

The results revealed a consistent protein pattern in their expression levels on the gels and more than 1300 protein spots could be reproducibly exhibited. Among the 39 differentially expressed protein spots(Fig.1), 15 proteins (spot 8, 36, 38, 42, 50, 52, 67, 87, 144, 184, 201, 283, 294, 548 and 1079) are expressed with up-regulation, while 20 proteins (spot 135, 165, 222, 234, 338, 339, 453, 462, 473, 475, 484, 600, 606, 642, 644, 650, 763, 825, 1321 and 1527) are expressed with down-regulation in the 2-DE spectrum of proteome in fruit softening process. Protein spot

15 and 152 are expressed with down-regulation at first and up-regulation afterwards, while protein spot 172 and 323 are expressed with up-regulation at first and down-regulation afterwards during fruit softening process. As it is seen, for most of the identified proteins, experimental Mr and pI were in reasonable agreement with the theoretical values of the matched proteins. However, differences between the experimental and theoretical values of Mr and pI for some identified proteins were also noticeable (Table 1). The phenomena could be due to numerous factors, such as the lack of genomic information for the organisms, expression in different organ-isms, different gene expression products, or post-translational modifications of the same gene expression [10,11].

Tab.1 Identification of differentia proteins extracted from ripe softening fruits of Hardy kiwifruit with MALDI-TOF/MS and database searching

Spot	Protein change	Accession NO.	Homologous protein	Species	Theoretical MW(D)/PI	Experimental MW(kD)/PI	Expect	score
36	↑	gi|413933080	Hypothetical protein ZEAMMB73_547815	*Zea mays*	114450/8.11	13.25/5.56	0.013	**90***
283	↑	gi|441482380	Thaumatin-like protein	*Actinidia chinensis*	25677/4.55	28.875/4.4	0.56	**63**
606	↓	gi|146215976	Actinidin Act1b	*Actinidia arguta*	42365/4.91	42/4.83	0.048	**74***
644	↓	gi|146215976	Actinidin Act1b	*Actinidia arguta*	42365/4.91	43/4.99	0.0041	**84***
650	↓	gi|146215976	Actinidin Act1b	*Actinidia arguta*	42365/4.91	44/4.83	0.011	**80***
825	↓	gi|225428086	PREDICTED: V-type proton atpase subunit B 1	*Vitis vinifera*	54367/5.04	57/4.97	6.90E-08	**132***

a) Protein description is in NCBI database(Swissprot).
b) Accession number was recorded as a reference for the identification in NCBI database(Swissprot).
c) Coverage of protein sequence by the peptides was used for spot identification.
*d) * is standing for significant of protein scores greater than 73.*
e) Increased (↑) or decreased (↓) compared with D1fruit during fruits softening.

3.2 Protein spots 2-DE gel analyses for hardy kiwifruit during harvest softening

The 39 spots from 2-DE gels were subjected to mass spectrometry analysis. Finally, 5 protein spots, including spot 36, 606, 644, 650 and 825, were confidently identified according to NCBI database (or SwissProt) database which protein scores greater than 73 (or 58) are significant (Table 1).

3.3 Hypothetical protein ZEAMMB73_547815 (spot 36) was up-regulated expressed during the fruits softening (1d to 7d), which involved in organelle transport. Three protein spots (spot 606, 644 and 650) were identified to the same protein of *Actinidin Act1b* (gi|146215976 in NCBI database). The protein modification or degradation maybe causes this experiment result. *Actinidin Act1b* of all above three proteins kept ≥2-fold down-regulated during the fruits storing from 1d to 7d at 25°C. *V-type proton ATPase subunit B1* (spot 825) associated with ATP hydrolysis was also more than 2-fold down-regulated expressed on the 4d and 7d compared to the 1d during storage at 25°C.

Protein changes during hardy kiwifruit harvest softening are associated with the interaction of various aspects. There is little information regarding the proteome in the biological processes. As a consequence, the present work conducted a comprehensive proteomics analysis during fruits softening and senescence as a means of beginning to understand the molecular mechanisms.

3.4 Hypothetical protein ZEAMMB73_547815 (gi|413933080) was confidently identified by MALDI-TOF/MS data of spot 36. This catalytic (head) domain has ATPase activity and belongs to the larger group of P-loop NTPases [12]. Myosins are actin-dependent molecular motors that play important roles in muscle contraction, cell motility, and organelle transport. The head domain is a molecular motor, which utilizes ATP hydrolysis to generate directed movement toward the plus end along actin filaments [13-15].

3.5 Actinidin Act1b

was confidently identified by MALDI-TOF/MS data of three spot 606, 644 and 650. The score of spot 650 is greater than spot 606, and both of two spots were down-regulated expressed during the fruits softening which are similar in PI. Peptidase C1A subfamily (MEROPS database nomenclature) composed of cysteine peptidases (CPs) similar to papain, including the mammalian CPs. Most members of the papain subfamily are endopeptidases. Some exceptions to this rule can be explained by specific details of the catalytic domains like the occluding loop in cathepsin B which confers an additional carboxydipeptidyl activity and the mini-chain of cathepsin H resulting in an N-terminal exopeptidase activity. They are responsible for protein degradation in the lysosome. Papain-like CPs are synthesized as inactive proenzymes with N-terminal propeptide regions, which are removed upon activation. In addition to its inhibitory role, the propeptide is required for proper folding of the newly synthesized enzyme and its stabilization in

denaturing pH conditions. Residues within the propeptide region also play a role in the transport of the proenzyme to lysosomes or acidified vesicles. Also included in this subfamily are proteins classified as non-peptidase homologs, which lack peptidase activity or have missing active site residues [16,17].

3.6 PREDICTED: V-type proton ATPase subunit B1 was confidently identified by MALDI-TOF/MS data of spot 825, which was down-regulated expressed during the fruits softening. V/A-type ATP synthase (non-catalytic) subunit B. These ATPases couple ATP hydrolysis to the buildup of a H+ gradient, but V-type ATPases do not catalyze the reverse reaction. The Vacuolar (V-type) ATPase is found in the membranes of vacuoles, the golgi apparatus and in other coated vesicles in eukaryotes. Archaea have a protein which is similar in sequence to V-ATPases, but functions like an F-ATPase (called A-ATPase). A similar protein is also found in a few bacteria. This subfamily consists of the non-catalytic beta subunit [18].

Protein spot 283 expressed with up-regulation during fruit softening was identified as *thaumatin-like protein*(gi|441482380)of *Actinidia chinensis* which was belong to *Actinidia* Lindl though its score was less than 73. Several members of the thaumatin protein family display significant in vitro inhibition of hyphal growth and sporulation by various fungi. Thaumatins are pathogenesis-related (PR) proteins, which are induced by various agents ranging from ethylene to pathogens, and are structurally diverse and ubiquitous in plants [19]. The proteins are involved in systematically acquired resistance and stress response in plants, although their precise role is unknown [20]. The thaumatin-like proteins isolated from kiwi fruit or apple appear to have their allergenic properties minimally reduced by gastroduodenal digestive processes, but not by heating [21,22]

CONCLUSION

The conclusions of the present study were as follows: 2-DE was used followed by MALDI-TOF/MS to investigate and analyze the biological function of the differently expressed proteins during the hardy kiwifruit softening. More than 1300 protein spots were detected. Among them, 39 differently expressed spots (kept ≥2-fold) were selected to be excised and analyzed using MALDI-TOF/MS, and finally 5 protein spots were confidently identified according to NCBI database. The results demonstrated that among the 39 protein spots expressed particularly during fruits softening. All of them are involved in the regulation of hardy kiwifruits ripening and senescence. The study will enable future detailed investigation of gene expression and function linked with kiwifruits ripening.

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