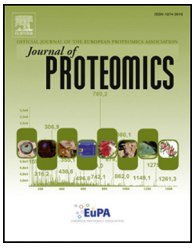
JOURNAL OF PROTEOMICS XX (2013) XXX–XXX

Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/jprot



A proteomic investigation of apple fruit during ripening and in response to ethylene treatment☆,☆☆

Qifa Zheng[a], Jun Song[a,*], Leslie Campbell-Palmer[a], Kristen Thompson[a], Li Li[b], Brad Walker[a], Yunsong Cui[c], Xihong Li[b]

[a]Agriculture and Agri-Food Canada, Atlantic Food and Horticulture Research Centre, 32 Main St., Kentville, NS., Canada B4N 1J5
[b]Key Laboratory of Food Nutrition and Safety (Ministry of Education), Tianjin University of Science and Technology, Tianjin 300457, PR China
[c]Acadia Statistical Consulting Centre, Acadia University, Wolfville, NS., Canada

ARTICLE INFO

Article history:
Received 18 October 2012
Accepted 11 February 2013

Keywords:
Proteins
Two-dimensional electrophoresis
LC/MS/MS
Fruit ripening and ethylene

ABSTRACT

A proteomic approach employing a two dimensional electrophoresis (2-DE) technique with SYPRO Ruby, a fluorescent stain with improved sensitivity and quantitative accuracy, was performed to separate the total proteins from apple fruit at different stages of ripening and senescence. After imaging and statistical analyses were performed on 2340 spots, a total of 316 spots, or approximately 13.5% of the total protein population, was found to be significantly changed in this study. Of the 316 proteins, 219 spots were only present at a specific ripening stage, while 97 spots were significantly different ($p<0.05$) throughout fruit ripening and in response to ethylene treatment. From 316 candidate spots, 221 proteins were further identified by liquid chromatography and mass spectrometry analysis with protein sequence and express sequence tag (EST) data searching. Analysis and identification of proteins revealed that apple fruit ripening is associated with increase of abundance of many proteins with functions such as ethylene production, antioxidation and redox, carbohydrate metabolism, oxidative stress, energy, and defense response. Ethylene treatment increased a group of unique proteins that were not present during normal fruit ripening and have not been previously reported. It also reduced some proteins involved in primary metabolism, including those of the last few steps of the glycolytic pathway. This study demonstrated the complexity and dynamic changes of protein profiles of apple fruit during ripening and in response to exogenous ethylene treatment. Identifying and tracking protein changes may allow us to better understand the mechanism of ripening in climacteric fruit.

Biological significance
Postharvest physiology and biochemistry has been conducted on apple fruit for many years. Ethylene plays an important role in ripening and senescence in many climacteric fruit. However, little information is available at the proteome level to investigate fruit ripening and effect of ethylene treatment. The significance of this paper is that it is the first study employing 2-DE and fluorescent dye in the investigation of the apple fruit ripening and influence of ethylene treatment. It reveals some significant biological changes in association with these events and demonstrates significant changed proteins under these conditions. Therefore, our study links the biological events with proteomic information and provides

☆ This article is part of a Special Issue entitled: Translational Plant Proteomics.
☆☆ Contribution no. 2393 of the Atlantic Food & Horticulture Research Centre, Agriculture & Agri-Food Canada.

* *Corresponding author*. Fax: +1 902 679 2311.
E-mail address: jun.song@agr.gc.ca (J. Song).

1874-3919/$ – see front matter
http://dx.doi.org/10.1016/j.jprot.2013.02.006

detailed peptide information on all identified proteins. Through the function analysis, those significantly changed proteins are also analyzed. These findings from this paper provide not only proteome information on fruit ripening, but also pave the ground for further quantitative studies using SMR to investigate certain proteins and pathways under the hypothesis involved in fruit ripening.

This article is part of a Special Issue entitled: Translational Plant Proteomics.

1. Introduction

Apples (*Malus domestica* Borkh.) are one of the most popular fruit crops in the world, with a total production of ca. 66 million metric tonnes worldwide. In North America, the apple industry produced ca. 4.2 million tonnes of fruit in 2010. Apples have become part of our daily diet due to their nutritional value. The annual per capita consumption of apples was an estimated 7.2 kg [1]. Expanding the high-quality fruit market is key to meeting consumer demand and maintaining the apple industry. The quality of fresh fruit is measured using appearance, color, texture, flavor (taste and aroma) and nutritional value [2]. Apples are climacteric fruit that demonstrate a burst in respiration and ethylene production in association with fruit ripening after harvest, resulting in yellowing, decreased firmness, reduced acidity and formation of volatile compounds [3,4]. Developed technologies such as controlled atmosphere storage (CA) and ultra-low oxygen storage (ULO) maintain color and firmness, but are detrimental to flavor development [5]. Treatments that inhibit ripening by inhibiting ethylene biosynthesis (e.g. aminovinylglycine, AVG) [6] or action (e.g. 1-methylcyclopropene, 1-MCP) delay fruit ripening and senescence [7]. Following these treatments, fruit firmness can be maintained and volatile production partially recovered during storage, but they cannot reach the optimal levels seen in normal ripe fruit [8,9]. These results demonstrate that fruit ripening directly influences quality, and ethylene plays a very important role in the ripening process.

Molecular biological tools have been developed and used to study general plant metabolism [10]. These technologies have been applied to apple fruit to investigate and gain better understanding of ripening and senescence. Recently, molecular and genomic tools have been used to identify increasing numbers of genes involved in apple fruit ripening and senescence in relation to fruit firmness/texture [11], aroma volatile compounds [12–14], acidity [15], pigments [16], allergens [17] and other biological functions. In addition to the above identified genes, ethylene-related genes have been studied the most [14,18–21].

In comparison with other plant species, research on apple ripening has been hampered by the lack of appropriate genomic tools. Although the genome of apples has been sequenced [22], a recent public sequence databank that contains in excess of 325,020 ESTs for apple allows the identification of approximately 22,493 unigens (NCBI: http://www.ncbi.nlm.nih.gov/), and offers new opportunities to identify unknown genes and proteins.

The analysis of mRNA and gene expression may not predict the exact protein concentration and activity. Therefore, proteomics has become a key tool in system biology to provide quantitative and structural information about functional proteins and the dynamic state of the cell [23,24]. Proteomics is a systematic approach to study global changes in proteins and provides a linkage between the transcriptome and metabolome [25]. It reveals different protein expression patterns under different biological situations, such as development, stress, and postharvest treatments, and provides direct information on the proteins driving cell metabolism. Among proteomic techniques, two-dimensional electrophoresis (2-DE), a gel based technique, has been applied to resolve thousands of proteins simultaneously in order to facilitate peptide composition analysis, peptide sequencing and polypeptide identification using mass spectrometry (MS) [26]. This 2-DE-based proteomic technique has allowed the investigation of biochemical processes at the gene and protein levels; while the technique has mainly been applied to *Arabidopsis* and rice [23], it has also been used to identify proteins present in apple fruit samples with reasonable confidence [27,28]. Reports on strawberry, tomato and grapes have shown that the proteomic approach has potential to identify proteins involved in fruit ripening, the development of physiological disorders, and allergens [29–33]. These studies demonstrated that proteomics is a powerful tool that can be used to reveal the complicated protein population in specific biological samples, and the potential proteins involved in certain biological processes. Despite the current interest and application of the proteomic technique, very little data are available at the transcriptomic and proteomic levels for ripening and senescence in apple fruit.

In this study, a 2-DE based proteomic approach was applied to investigate changes in protein profiles at different stages of apple fruit ripening, and after treatment with exogenous ethylene. The objectives of this study were 1) to investigate the changes in protein profiles in association with fruit ripening stages and identify the most variable proteins during ripening and senescence; 2) to reveal the unique proteins that are affected by ethylene treatment; and 3) to establish the links between the physiological changes and the changes in protein profiles.

2. Materials and methods

2.1. Plant materials

Apples (*M. domestica* Borkh. cv. 'Golden Delicious') were harvested in 2005 on Oct. 04th from the Atlantic Food and Horticulture Research Centre, Agriculture and Agri-Food Canada, Kentville, Nova Scotia, Canada at the pre-climacteric stages, as determined by internal ethylene concentration and starch index. The internal ethylene concentration was $<0.1\ \mu L\ L^{-1}$ for immature fruit, and was determined on 14 apples. A total of 120 fruit were harvested equally from 10 trees.

Please cite this article as: Zheng Q, et al, A proteomic investigation of apple fruit during ripening and in response to ethylene treatment, J Prot (2013), http://dx.doi.org/10.1016/j.jprot.2013.02.006