JOURNAL OF PROTEOMICS 94 (2013) 219–239

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jprot

Quantitative proteomic investigation employing stable isotope labeling by peptide dimethylation on proteins of strawberry fruit at different ripening stages☆,☆☆

Li Li[a], Jun Song[b,*], Wilhelmina Kalt[b], Charles Forney[b], Rong Tsao[c], Devanand Pinto[d], Kenneth Chisholm[d], Leslie Campbell[b], Sherry Fillmore[b], Xihong Li[a]

[a]Key Laboratory of Food Nutrition and Safety (Ministry of Education), Tianjin University of Science and Technology, Tianjin 300457, PR China
[b]Agriculture and Agri-Food Canada, Atlantic Food and Horticulture Research Centre, 32 Main St., Kentville, NS B4N 1J5, Canada
[c]Agriculture and Agri-Food Canada, Guelph Food Research Programs, Stone Road, Guelph, ON, Canada
[d]Institute for Marine Biosciences, National Research Council of Canada, Halifax, NS B3H 4H7, Canada

ARTICLE INFO

Article history:
Received 26 June 2013
Accepted 12 September 2013
Available online 25 September 2013

Keywords:
Proteins
Fruit
Volatiles
Anthocyanin
LC/MS/MS
Ripening

ABSTRACT

A quantitative proteomic investigation of strawberry fruit ripening employing stable isotope labeling by peptide dimethylation was conducted on 'Mira' and 'Honeoye' strawberry fruit. Postharvest physiological quality indices, including volatile production, total phenolics, total anthocyanins, antioxidant capacity, soluble solids and titratable acidity, were also characterized in white, pink and red fruit. More than 892 and 848 proteins were identified and quantified in the 'Mira' and 'Honeoye' fruit, respectively, using at least two peptides for each protein identification. Using the normalized ratio of protein abundance changes, proteins that changed two-fold or more were identified as proteins that are up- or down-regulated during fruit ripening. Among the quantified proteins, 111 proteins were common to both cultivars and represented five significant clusters based on quantitative changes. Among the up-regulated proteins were proteins involved in metabolic pathways including flavonoid/anthocyanin biosynthesis, volatile biosynthesis, antioxidant metabolism, stress responses and allergen formation. Proteins that decreased during fruit ripening were found to be responsible for methionine metabolism, antioxidant-redox, energy metabolism and protein synthesis. Our results show that strawberry ripening is a highly complex system involving multi-physiological processes made possible through changes in protein expression. This study provides new insights on the regulation of proteins during strawberry fruit ripening that lay the foundation for further targeted studies.

Biological significance
Research on the postharvest physiology and biochemistry of strawberry fruit as a model of non-climacteric fruit ripening has been conducted for many years. However, the mechanism(s) for the initiation and metabolic regulation of non-climacteric fruit ripening

☆ Identification and quantitation of proteins reveal a dynamic network of proteins in the regulation of strawberry fruit ripening and quality.
☆☆ Contribution No. 2420 of the Atlantic Food & Horticulture Research Centre, Agriculture & Agri-Food Canada.

* *Corresponding author*. Fax: +1 902 365 8455.
E-mail address: jun.song@agr.gc.ca (J. Song).

http://dx.doi.org/10.1016/j.jprot.2013.09.004

remains unknown. Little information on strawberry fruit ripening is available at the proteome level. This paper is the first report of a quantitative proteomic investigation of strawberry fruit ripening employing stable isotope labeling by peptide dimethylation. Postharvest physiological quality indices, including volatile production, total phenolics, total anthocyanins, antioxidant capacity, soluble solids and titratable acidity, were also characterized in ripening fruit. Significant biological changes associated with ripening were revealed and proteins that change significantly under these conditions were identified. Therefore, our study links the biological events of strawberry fruit ripening with proteomic information and provides insights into possible mechanisms of regulation. Proteins that changed during ripening were analyzed through function analysis, which provides new insights into metabolic changes occurring during ripening. Findings from this paper not only provide proteome information on fruit ripening, but also pave the way for further quantitative studies using SMR to investigate certain proteins and pathways involved in fruit ripening.

1. Introduction

Strawberry (*Fragaria* × *ananassa*) fruit is one of the most popular fruit crops consumed worldwide and is recognized for its nutritional and flavor quality. Molecular biological tools have also been developed to study general plant metabolism and have been applied to investigate strawberry fruit ripening and senescence. Strawberry genes that were differentially expressed during fruit ripening were identified [1–3]. Later, DNA microarray technology was applied and identified that the alcohol acyl transferase (SAAT) gene plays a crucial role in volatile production of strawberry fruit [4]. Recently, genes related to ethylene reception [5], firmness [6], allergens [7] and anthocyanin biosynthesis [8–10] during strawberry fruit ripening and senescence have been reported. Investigations of metabolic events in the receptacle and achene tissues of strawberry fruit during fruit development and ripening revealed that both metabolic synchrony and speciation exist in strawberry fruit [11]. Despite the intensive biochemical and physiological studies using genomic and molecular approaches, very few data are available at the proteomic level to aid in our understanding of fruit ripening and senescence.

Research on fruit ripening has made great progress [12,13], but it has been hampered by the lack of appropriate genomic and other molecular biological tools. Using short-read technology without a physical map or reference genome, the genome of the woodland strawberry (*Fragaria vesca*) was sequenced and identified 33,264 protein coding genes [14]. In addition, a recent public sequence databank that contains in excess of 10,855 ESTs for strawberry (*Fragaria ananassa*), allows the identification of approximately 1004 proteins and offers new opportunities to identify unknown genes and proteins (NCBI: http://www.ncbi.nlm.nih.gov/).

Due to protein modifications and alterations that reflect functional states in the cell, the analysis of mRNA and gene expression may not correspond with functional proteins and truly reflect the dynamic state of the cell [15]. Proteomic techniques have emerged as a new platform to investigate biological systems. Among the proteomic techniques, 2-dimensional electrophoresis (2-DE) has been widely applied in plant proteomic research. 2-DE based proteomic technology has shown the potential to identify proteins present in strawberry fruit samples with reasonable confidence [16–18]. Reports on strawberry, tomato, pear and grape have shown that the proteomic approach has potential to identify proteins involved in fruit ripening, the development of physiological disorders, and allergens [18–24]. These studies have demonstrated that proteomic technology is a powerful tool to reveal the complicated protein population in specific samples associated with physiological status, which can reveal the proteins involved in specific biological processes. To date, the application of proteomics has mainly used 2D gel based approaches, with only a few proteomic studies on the ripening and senescence of strawberry fruit. These studies investigated allergens in strawberry fruit [7], and the accumulated proteins in 'Queen Elisa' elite genotype during fruit ripening, but did not relate them to physiological changes [18]. More proteomic investigations are required to better understand the regulation of fruit ripening and to link the proteome changes with quality traits.

Quantitative proteomics is a rapidly developing field that has shown the potential to expand our understanding of biology and disease. A few quantitative proteomic strategies that employ specific labeling techniques have been developed and applied to plant proteomic studies [25–28]. Recently, a new labeling strategy that involves the methylation of peptide amino groups via reductive amination with isotopically coded formaldehydes was reported [29]. This labeling technique was previously shown to be quantitative using standard peptides [30]. In addition, a labeling scheme that allowed the simultaneous analysis of three samples, while retaining a mass difference of 4 Da with the inclusion of formaldehyde labeled with ^{13}C was developed [31]. This stable isotope dimethyl labeling technique was described to be a straightforward and cost-effective labeling strategy to detect differences in abundance ratios over two orders of magnitude for both global proteomic research and protein modifications [32].

In this study, we have conducted quantitative proteomic analysis employing stable isotope dimethyl labeling to quantitatively investigate protein profiles of strawberry fruit tissues harvested at different stages of ripeness. The objectives of this study were to determine the protein profile changes associated with different fruit ripeness and to establish links between protein profile changes and ripening physiology to provide a more in-depth understanding of strawberry fruit ripening at the proteomic level. The biological significance of protein changes is