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Proteomic analysis of apricot fruit during ripening

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ABSTRACT

Ripening of climacteric fruits involves a complex network of biochemical and metabolic changes that make them palatable and rich in nutritional and health-beneficial compounds. Since fruit maturation has a profound impact on human nutrition, it has been recently the object of increasing research activity by holistic approaches, especially on model species. Here we report on the original proteomic characterization of ripening in apricot, a widely cultivated species of temperate zones appreciated for its taste and aromas, whose cultivation is yet hampered by specific limitations. Fruits of *Prunus armeniaca* cv. Vesuviana were harvested at three ripening stages and proteins extracted and resolved by 1D and 2D electrophoresis. Whole lanes from 1D gels were subjected to shot-gun analysis that identified 245 gene products, showing preliminary qualitative differences between maturation stages. In parallel, differential analysis of 2D proteomic maps highlighted 106 spots as differentially represented among variably ripen fruits. Most of these were further identified by means of MALDI-TOF-PMF and nanoLC–ESI–LIT–MS/MS as enzymes involved in main biochemical processes influencing metabolic/structural changes occurring during maturation, i.e. organic acids, carbohydrates and energy metabolism, ethylene biosynthesis, cell wall restructuring and stress response, or as protein species linkable to peculiar fruit organoleptic characteristics. In addition to originally present preliminary information on the main biochemical changes that characterize apricot ripening, this study also provides indications for future marker-assisted selection breeding programs aimed to ameliorate fruit quality.

1. Introduction

Common apricot (*Prunus armeniaca* L.) is a member of the *Rosaceae* family; most of the apricot varieties cultivated for fruit production belong to this species, which originated in Central Asia [1] and was then disseminated in Middle East, Mediterranean basin and Northern Europe areas. Adaptation to different environments resulted in significant phenotypic diversification, so that at least four major ecogeographical groups are recognized [2]. Production of apricot for different uses, as fresh or processed fruits, is

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economically very relevant (3,210,194 MT in 2009, according to FAO data) in the Mediterranean countries and in Italy, where almost 60 and 15% of the world harvest is generated, respectively. Apricot cultivation is hampered by some specific problems, such as high sensitivity to diseases (brown rot and sharka) or environmental stresses (spring frost), insufficient fruit quality or ripeness, as well as restricted harvest period. Consequently, breeding programs have been undertaken in different countries to overcome these limitations. In this respect, local cultivars are a source of genetic diversity, exploitable to select new varieties with improved agronomical traits. Whereas cultivars of the European ecogeographical group have a restricted genetic base [3], the Italian germplasm retains a high level of genetic diversity [2].

Breeding of fruit trees is exceedingly time-consuming and the availability of biomarkers linked to agronomical favorable traits can greatly improve process efficiency. As far as apricot, up to now molecular markers have been identified by isozyme analysis [3], amplified fragment length polymorphism [4] or microsatellites [5] and they have been used to investigate genetic diversity. Extensive expressed sequence tags (ESTs) collections or microarrays have greatly extended genetic information on vegetables or fruit-producing species, including peach and apricot. In this context, Grimplet and coworkers generated a collection of 13,006 ESTs from *P. armeniaca* mesocarp at different stages of ripening [6], while a bioinformatic database of integrated genetic information for *Rosaceae* (http://www.rosaceae.org) is available since 2008, which contains data on peach, apple and strawberry genomes. However, functional genomic studies have demonstrated that information deriving from nucleotide data do not necessarily match the corresponding translated protein complement at a precise organism physiological moment (e.g. fruit development or ripening) [7]; in fact, different modifications may affect gene products, including post-transcriptional, co-translational and degradative ones, along with environmental factors. Integration of genomic with proteomic data is hence highly desirable, in order to better clarify underlying molecular mechanisms, as well as to identify reliable molecular markers for crop breeding or amelioration.

Fruit ripening is a complex physiological process with a remarkable impact on human nutrition; occurring biochemical changes eventually make fleshy fruits palatable and rich in nutritional and health-beneficial compounds. Biochemical processes include the degradation of chlorophyll and starch, the biosynthesis of pigments and volatile compounds, the accumulation of sugars and organic acids, as well as cell wall softening [8,9]. Detailed comprehension of genetic regulatory elements is central for a full understanding of fruit ripening; in this context, proteomics represents a powerful approach to characterize biochemical networks and to establish functional correlations between genotype and phenotype [10]. Thus, differential proteomic studies on immature and mature fruits have been accomplished on tomato [11,12], grape [13–20], orange [21,22], peach [23–28], strawberry [29], mango [30], papaya [31,32] and apple [33,34] pericarp; some investigations were conducted on selected tissues, such as exocarp or mesocarp [14–18,20–23,25,27,28,30,31]. These studies described a tissue-dependent proteome repertoire that present distinctive changes during fruit ripening. As far as apricot, information about its protein composition is very scant; this may be due to different reasons, including the lack of a species-specific database, the low protein content of this fruit and the high concentration of interfering substances (pigments, polysaccharides, polyphenols, etc.).

In this study, we report on the first proteomic characterization of the apricot fruit in relation to ripening. Fruits of *P. armeniaca* cv. Vesuviana, a regional variety cultivated in the South of Italy and renowned for its peculiar flavor and texture characteristics, were harvested at three maturation stages and proteins extracted. Differently from other species (such as grape, orange, etc.) whose skin and mesocarp tissues can be easily distincted/separated also at the unripe stage, experiments on apricot were performed on the whole fruit. A number of protein species differentially expressed as result of ripening state was identified by means of combined 2-DE and MS procedures. Their putative physiological role is here discussed in relation to the fruit maturation process.

2. Materials and methods

2.1. Fruit material and characterization of corresponding ripening stages

Five apricot trees (*P. armeniaca* cv. Vesuviana, Pellecchiella) were grown in a farmland in the surroundings of Naples, Italy, by using standard cultural practices. Based on surface color, fruit samples were harvested at three ripening stages: green (T0), yellow (T1), and deep-orange/red (T2) at 7, 9 and 11 weeks after anthesis, respectively (Table 1); they were selected for uniformity without any damage. For each ripening stage, about 25–30 fruits were harvested and then divided into three biological replicates. After collection, fruits were cleaned, removed of seeds, cut, frozen in liquid N_2 and stored at −80 °C, until used for protein extraction. In parallel, a similar number of fruit samples for maturity stage assessment were not frozen but immediately processed. Flesh firmness, total soluble solids content (SSC), titratable acidity (TA) and total antioxidant capacity (TAC) were measured. Firmness was determined by means of a penetrometer (Effegi, Milan, Italy) bearing a 8 mm probe, and is expressed as kg/0.5 cm^2. Apricot samples for SSC, TA and TAC determinations were homogenized, centrifuged at 15,000 ×*g* for 15 min, at 4 °C, and the corresponding supernatants were used. SSC was estimated by means of the Brix degree (°Brix) at 20 °C, as determined by an RFM330 Refractometer (Bellingham Stanley Ltd, UK). TA was determined by titrating a known volume of apricot supernatant with 0.1 N NaOH until reaching pH 8.1 with a HI9017 Microprocessor pHmeter (Hanna Instruments) and phenolphthalein as color indicator; it was expressed as content of malic acid per gram of fresh weight of fruits. TAC was determined according to [35] and expressed as micromoles of Trolox equivalents per gram of fresh weight of fruits. Measurements were performed in triplicate on samples from three independent extractions.

2.2. Protein extraction

Protein mining was performed by means of a TCA/acetone precipitation, followed by a phenol extraction [36]; a separate