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# A proteometabolomic study of *Actinidia deliciosa* fruit development

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## ABSTRACT

Green-fleshed kiwifruit is worldwide appreciated for its flavor and macronutrients that are related to its physiological development. Fruit ripe stage for harvesting does not correspond to an optimal edible condition due to firmness/acidity pome characteristics; this is overcome with postharvest fruit processing. To describe the metabolic pathways/molecular effectors underlying *Actinidia deliciosa* cv. Hayward pome physiological development, kiwifruits were harvested at four growth stages (from fruit set to physiological maturity), and corresponding outer endocarp samples were analysed for metabolite and protein content. Combined NMR/LC-UV/ESI-IT-$MS^n$ procedures quantified 46 metabolites at these developmental stages; similarly, integrated 2D-DIGE/nLC-ESI-LIT-MS/MS analyses described corresponding proteomic changes. Quantitative protein dynamics showed that components related to disease/defense, protein destination/storage, metabolism, energy and cell structure functions were highly affected at specific moments of fruit development, suggesting a rationale to pomological and metabolite content characteristics at those times. Bioinformatic interaction prediction revealed a main network of differentially represented proteins, which may control metabolic changes in developing kiwifruit. Main pome allergens were also quantified, demonstrating their highest levels at the mature stage. By aligning kiwifruit development to a proteometabolomic representation, this investigation integrates previous metabolic observations and provides a reference framework for further physiological/nutritional studies, also allowing cross comparison among crop species.

*Biological significance:* Compared with some other fruits, green-fleshed kiwifruit is unique for its nutrient density, health benefits, and consumer appeal; in fact, it is exceptionally rich in vitamins, carotenoids, potassium, fibre and phytochemicals acting in synergy to achieve multiple health advantages. However, kiwifruit is allergenic, and although symptoms in most susceptible individuals are mild, severe reactions have also been described. In the course of their 6-month development, kiwifruit undergoes radical changes in its morphology and chemical composition; these modifications may highly affect fruit nutraceutical and allergenic properties. To gain a better understanding of the molecular processes regulating metabolite concentration during fruit development but also affecting general pomological characteristics, a time-course metabolomic and proteomic analysis of kiwifruit flesh tissues was undertaken. Combined information on modified levels of 46 metabolites and 241 proteins showed that molecular processes underlying central and secondary metabolism, energy, cell structure, protein destination/storage, disease/defense kiwifruit functions were highly affected during fruit development, providing a rationale to the corresponding changes in organic acids, sugars, amino acids, polyphenols, fatty acids, phospholipids and allergens content, but also to the corresponding modifications in pome firmness, pulp colour,

*Abbreviations:* AA, ascorbic acid; $C_2H_4$, ethylene; FA, formic acid; HMBC, $^1H$–$^{13}C$ heteronuclear multiple-bond correlation; HSQC, $^1H$–$^{13}C$ heteronuclear single quantum coherence; ROS, reactive oxygen species; TOCSY, $^1H$–$^1H$ total correlation spectroscopy

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protein and total solid content. By providing original information on fruit development, this multiomic study integrates previous metabolomic/transcriptomic observations in describing molecular mechanisms associated with nutritional and agronomic traits of great interest for kiwifruit molecular breeding.

## 1. Introduction

Hexaploid, green-fleshed kiwifruit (*Actinidia deliciosa* cv. Hayward) is worldwide appreciated for its high content of bioactive metabolites, which also allow fruit to tolerate protracted storage [1]. It is harvested at a physiological mature stage that does not correspond to an optimal edible condition, due to firmness/acidity pome characteristics. For fruit commercialization, a postharvest treatment is generally necessary, which consists of pome exposure to ethylene ($C_2H_4$) and/or low temperatures for further ripening [2,3]. Depending on treatment, kiwifruit displays a climacteric behaviour at temperatures close to 20 °C (during ripening in storage/conditioning rooms, even following cold storage) and a non-climacteric behaviour at temperatures < 10 °C (during cold storage) [4]. Thus, kiwifruit can be successfully stored at 0 °C for 6 months in the absence of $C_2H_4$ [5]. Kiwifruit metabolism during development is different from that of fruits from other plants since carbon is mostly accumulated as starch [6]. Starch storage arises only after the fruit has completed cell division, and continues until its complete development [7]. Starch degradation occurs after kiwifruit harvest, leading to polysaccharide conversion into soluble sugars at the edible stage [8].

Since genomic information are still not available on green-fleshed kiwifruit, research activity on this organism has been greatly facilitated after characterizing the draft genome of diploid yellow-fleshed *Actinidia chinensis* [9,10]. This allowed defining a genetic map of *A. deliciosa* chromosomes [11] and assaying ESTs underlying fruit flavor, health, colour and ripening [12,13]. Various proteomic investigations were also accomplished on green-fleshed kiwifruit through the identification of corresponding proteins based on yellow-fleshed fruit homologues. They consisted of differential studies where the treatment of pomes with specific chemical additives or temperatures during postharvest storage was evaluated; in some cases, fruits were analysed after their further ripening at room temperature. Thus, the effect of the treatment with exogenous $O_3$ [14,15], $C_2H_4$ [16], sodium nitroprusside [15], of chilling [16] or of combination of them [15,16] was assessed identifying differentially represented proteins in treated kiwifruits with respect to control. The effect of the treatment of kiwi plants with cytokinins having phytohormonal activity was similarly analysed on developing/harvested fruits eventually stored for different times under specific storage conditions [17]. These investigations reflected the attention of kiwifruit industry in evaluating potential agronomic procedures to increase pome size at harvest and technological approaches for maintaining fruit quality during its postharvest life. However, abovementioned proteomic studies were performed using silver staining-based 2-DE procedures, which suffer of well-known limitations due to a reduced accuracy in quantitative measurements; the latter were solved with the development of 2D-DIGE [18]. Various studies were also realized on kiwifruit metabolites by NMR and chromatographic approaches with the aim of evaluating corresponding concentrations during fruit development [19,20], after knockdown of enzymes involved in $C_2H_4$ biosynthesis [13] in the course of comparative assays on different plant genotypes/cultivars [6,20], or in plants treated with cytokinin phytohormones [17]. Similar investigations were also realized on specific parts of fresh kiwifruit [21,22] or on fruit juices obtained with specific food processing procedures [21].

All studies reported above emphasize the lack of proteomic information on metabolic pathways and molecular processes involved in kiwifruit development from early unripe to physiological ripe stage before fruit harvest. In this context, proteomics has largely been used to describe physiological changes occurring during development of many fruits, *i.e.* apple [23,24], orange [25–27], apricot [28], sweet cherry [29], strawberry [30–31], mango [32–33], date palm fruit [34], grape [35–44], pear [45–46], peach [47,48] and tomato [49,50]. The results of these studies have been summarized in dedicated reviews [51,52], where climacteric and non-climateric fruits were compared with the aim to tentatively highlight common variably represented metabolic pathways and protein effectors, and their relation with increased fruit respiration rate and $C_2H_4$ biosynthesis. In the present study, a proteo-metabolomic approach was used to fill this gap by comparing metabolite and protein levels in kiwifruit at four developmental stages. Results were subjected to bioinformatics analysis that suggested metabolic pathways and molecular processes/interactions important for fruit physiological development and nutritional characteristics.

**Table 1**
Stage parameters, and pomological and qualitative traits of kiwifruits sampled at developmental stages T0, T1, T2 and T3. Reported are data on: percentage of the fruit size with respect to the developed counterpart for commercial picking (% FS); BBCH scale; days post anthesis (DPA); fruit fresh weight (FW); fruit firmness; total soluble solid content (SSC); Chroma; Hue angle; total protein content (TP). ND, not determined. Reported values correspond to the mean ± SD. Measurements were performed as reported in Supplementary material.

| Developmental stage | % FS | BBCH scale | DPA | FW (g) | Firmness (N) | SSC (°Brix) | Chroma | Hue angle | TP (mg/g FW) |
|---|---|---|---|---|---|---|---|---|---|
|  T0 | 20 | 72 | 40 | 22.7 ± 1.2 | ND | 3.8 ± 0.3 | 29.5 ± 1.3 | 125.1 ± 5.4 | 0.40 ± 0.05 |
|  T1 | 50 | 75 | 72 | 50.9 ± 1.7 | ND | 4.8 ± 0.5 | 32.7 ± 1.8 | 121.3 ± 6.2 | 0.67 ± 0.09 |
|  T2 | 80 | 78 | 135 | 80.3 ± 2.0 | > 100 | 6.1 ± 0.2 | 41.6 ± 1.2 | 113.2 ± 3.7 | 0.63 ± 0.09 |
|  T3 | 100 | 82 | 175 | 104.9 ± 4.0 | 71.2 ± 2.1 | 7.4 ± 0.3 | 50.8 ± 1.1 | 109.2 ± 4.6 | 0.59 ± 0.08 |