

Article

Proteomic and Metabolomic Profiling Reveals Peel- and Pulp-Specific Molecular Regulation During Fruit Development of *Selenicereus megalanthus*

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Abstract

Fruit ripening involves coordinated metabolic and molecular changes that shape quality traits, yet tissue-specific regulation in *Selenicereus megalanthus* remains unclear. Fruit development in *S. megalanthus* was investigated through an integrated analysis of pulp and peel across five developmental stages to elucidate tissue-specific metabolic and molecular regulatory dynamics. Proteomic profiling combined with targeted metabolomic analyses of sugars and phenolic compounds, multivariate statistics, and protein–protein interaction analysis was applied. A total of 411 proteins were identified in the pulp and 812 in the peel, of which 255 and 362 proteins, respectively, showed significant differential accumulation across development ($p < 0.05$), indicating higher regulatory plasticity in the peel. Multivariate analyses revealed clear stage-dependent reorganization of the proteome in both tissues. Functional annotation highlighted coordinated modulation of pathways related to cell wall remodeling, carbohydrate metabolism, antioxidant and detoxification systems, protein folding, and myo-inositol biosynthesis, directly associated with fruit texture and quality attributes. Metabolomic analyses revealed progressive sugar accumulation during ripening, with sucrose predominating at advanced stages, and pronounced tissue- and stage-dependent modulation of phenolic compounds, characterized by early enrichment in the pulp and sustained accumulation in the peel. Overall, these results demonstrate that yellow pitaya development involves tightly coordinated biochemical and regulatory mechanisms and identify the peel as a metabolically active tissue with relevance for postharvest management, fruit quality, and sustainable horticultural valorization.



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1. Introduction

Fruit ripening in non-climacteric species is characterized by progressive metabolic reprogramming rather than an abrupt ethylene-dependent transition, involving coordinated modulation of carbohydrate metabolism, antioxidant systems, and structural components of the cell wall. Among non-climacteric fruits, pitaya species have attracted increasing

attention due to their distinctive physiology and compositional dynamics during development. In particular, *Selenicereus megalanthus* exhibits marked stage-dependent changes in sugar accumulation and bioactive compounds, yet the molecular networks underlying tissue-specific regulation remain poorly understood [1,2].

S. megalanthus (K. Schum. ex Vaupel) Moran, commonly known as Colombian yellow pitaya, is a non-climacteric cactus fruit recognized for its sweet taste, white pulp, and exotic appearance [1]. Characterized by a spiny yellow peel and easily detachable bracts, it is primarily cultivated in Colombia, Israel, and Taiwan [2]. This species exhibits nocturnal anthesis, is not self-pollinating, and presents a longer phenological cycle compared to other pitaya species such as *Selenicereus undatus* [3,4].

Despite its high commercial value and growing consumer interest, the cultivation of *S. megalanthus* remains geographically limited due to specific edaphoclimatic requirements and postharvest challenges [2]. The fruit is primarily consumed fresh due to its sweet taste and distinctive appearance; however, it is also used in processed products such as juices, desserts, jams, and functional beverages. In addition to its sensory attributes, *S. megalanthus* is recognized for its nutritional value, containing soluble sugars, dietary fiber, vitamin C, phenolic compounds, and antioxidant metabolites that contribute to its functional potential and consumer appeal [1,2]. Fruit development involves a cascade of physiological, structural, and biochemical changes that define key quality parameters such as firmness, soluble solids content, and bioactive compound accumulation. Enzymes such as pectinases and cellulases play a central role in cell wall degradation, affecting texture and enhancing the release and bioavailability of antioxidant metabolites like flavonoids, particularly flavan-3-ols like catechin, as well as phenolic acids including gallic, chlorogenic, and caffeic acids, along with betacyanins and ascorbic acid [5,6].

Although some studies have addressed yellow pitaya's morphometric and postharvest aspects [1,2,6], the molecular mechanisms underlying its fruit development remain poorly understood. Integrated omics approaches, particularly proteomics and metabolomics, have proven essential for unraveling the biochemical complexity associated with fruit development in fleshy fruits [7,8]. Proteomics is a molecular technology that dynamically analyzes the accumulation and changes in the profile of proteins involved in a wide variety of biological processes in living organisms, organs, tissues, or cells [7]. Proteomics makes it possible to identify and quantify proteins that govern critical physiological processes, including stress responses, enzyme regulation, and structural remodeling of fruit tissues [8]. Metabolomics analyzes low molecular weight metabolites (≤ 1500 Da) qualitatively, quantitatively, and dynamically [9], making it possible to understand various biochemical changes that occur during maturation. The combination of these techniques provides a detailed view of how metabolic and enzymatic pathways shift over time, contributing to fruit quality and nutritional value.

This study aims to provide a molecular characterization of the pulp and peel of *S. megalanthus* fruit at five developmental stages through proteomics and metabolomics. Furthermore, as *S. megalanthus* remains less explored than other pitaya species, such as *S. undatus*, this study contributes new perspectives on the molecular and functional attributes of an underutilized cultivar with growing commercial relevance. The findings may also contribute to improving postharvest management strategies and supporting quality preservation in yellow pitaya production systems.

2. Materials and Methods

2.1. Experimental Design

The experiment followed a completely randomized design, with three biological replicates per fruit fraction (peel and pulp) at each developmental stage. Each replicate consisted of 10 fruits, totaling 150 fruits analyzed across the five stages of development.

2.2. Plant Material

Fruits of *Selenicereus megalanthus* ‘Colombian yellow’ were harvested from a commercial orchard in Ingaí city, Minas Gerais state, Brazil (21°24′24″ S, 44°56′30″ W), cultivated under open-field conditions [6]. Developmental stages were classified as S1—immature 1, S2—immature 2, S3—mature green, S4—early ripening, and S5—ripe, based on external traits and quality parameters (Figure 1). The classification was established based on peel color progression, bract detachment pattern, firmness, soluble solids content, and titratable acidity, together with visible structural changes in pulp consistency and seed development throughout maturation. After sanitization and selection, the peel (exocarp and mesocarp) and pulp (endocarp) were separated using a laboratory-grade knife and a non-reactive spatula, ensuring minimal mechanical damage and preventing sample contamination. The separated tissues were then homogenized and stored at −80 °C (Thermo Fisher Scientific, Waltham, MA, USA) for subsequent proteomic and metabolomic analyses.

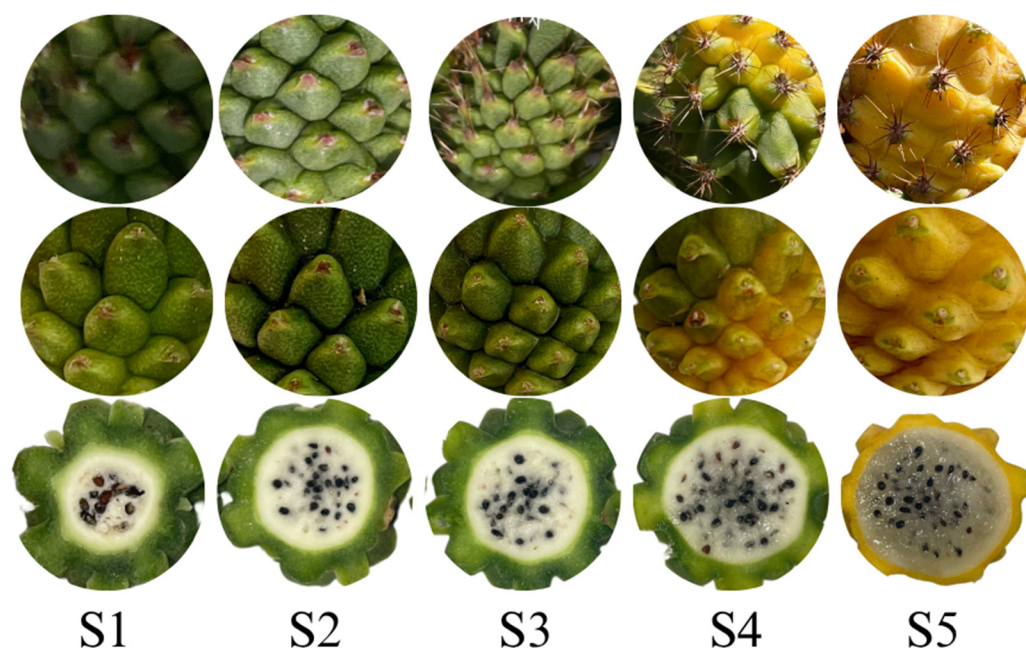


Figure 1. Developmental stages of *Selenicereus megalanthus* pitaya. For each stage (S1–S5), three visual representations are shown: (**top row**) whole fruits with intact bracts and areolar spines; (**middle row**) fruits after bract removal; (**bottom row**) longitudinally cut fruits showing internal pulp morphology. S1—Immature 1; S2—Immature 2; S3—Mature green; S4—Early ripening; S5—Ripe.

The orchard soil is classified as an Ultisol (Argissolo), typical of the region. Organic fertilization was performed using cattle manure and compost according to local agronomic practices. The vegetative period was characterized by a hot and relatively dry summer, followed by autumn with marked thermal amplitude and winter with low temperatures and occasional frost events. Cultivation was conducted under rainfed conditions, without supplemental irrigation. For pest management, a biological insecticide based on *Beauveria bassiana* (Boveril Evo®, Koppert, São Paulo, Brazil) was applied according to the manufacturer’s recommendations (five applications at 14-day intervals), mainly targeting thrips,

mites, and scale insects. Field monitoring was routinely performed, and occasional rotting cladodes were removed preventively; pruning sites were treated with Bordeaux paste as a protective measure.

2.3. Shotgun Proteomics

Samples of *S. megalanthus* peel and pulp were lyophilized at $-30\text{ }^{\circ}\text{C}$ under a vacuum pressure of 0.998 mbar for 72 h in the absence of light. These controlled freeze-drying conditions were applied to ensure efficient sublimation of ice crystals while minimizing thermal degradation, enzymatic activity, and oxidative reactions, thereby preserving protein conformation and metabolite stability for subsequent proteomic and metabolomic analyses. After freeze-drying, the material was transported to the Vale Technological Institute (Belém city, Pará state, northern region of Brazil, $1^{\circ}27'18''\text{ S}$, $48^{\circ}30'9''\text{ W}$) for shotgun proteomic analysis, a high-throughput, untargeted technique, therefore eliminating the need to use standards. Protein extraction, quantification, and digestion were performed according to the procedure described by Nascimento et al. [10], while protein identification and data analysis were carried out as outlined by da Costa et al. [7]. The samples were analyzed on an NanoACQUITY UPLC ultraperformance liquid chromatography system (Waters, Milford, MA, USA). The chromatograph was coupled directly to an ESI-Q-ToF Synapt G2S mass spectrometer (Waters, Milford, MA, USA). Data analysis was performed using Progenesis QI software (version 4.2, Waters) for protein identification and quantification, using the Uniprot trembl database from UniProt (UniProtKB/swiss-prot, uniprot.org), following these criteria: false positive control was performed using a 1% (0.01) FDR. A minimum of two unique peptides per protein was adopted for validation of the identifications. The mass tolerance used in the searches was 20 ppm. For differential analysis, the data were normalized by intensity before statistical comparisons. Protein quantification was performed using the label-free quantification (LFQ) method. Identification was considered valid when the probability of accurate peptide identification exceeded 90% and for proteins 95%. Prediction of protein–protein interaction networks was based on a functional analysis done with STRING software version 12.0 (<http://string-db.org/>, accessed on 10 May 2025), utilizing homologous proteins from *Arabidopsis thaliana* as a reference species.

2.4. Quantification of Phenolic Compounds

To quantify the phenolic compounds, 2.5 g of fresh pulp and peel were extracted with 20 mL of HPLC-grade aqueous methanol, following a method adapted from Zitha et al. [5]. The analysis was performed using a Shimadzu HPLC system (Shimadzu Corporation, Kyoto, Japan) equipped with four high-pressure pumps (LC-20AT), an autosampler (SIL-20A), a degasser (DGU-20A5), a diode array detector (SPDM-20A), and a column oven (CTO-20AC). Phenolic separation was achieved on a Shim-pack ODS GVP-C18 column (4.6 mm $\times$ 250 mm, 5 μm) (Shimadzu Corporation, Kyoto, Japan) coupled with a corresponding guard column (4.6 mm $\times$ 10 mm, 5 μm). The mobile phases consisted of solvent A (2% acetic acid in deionized water) and solvent B (methanol:water:acetic acid, 70:28:2, v/v/v). The flow rate was set to 1.0 mL/min, with the column maintained at $35\text{ }^{\circ}\text{C}$ and an injection volume of 20 μL . Thirteen standards were used, namely catechin, resveratrol, vanillin, and gallic, chlorogenic, ferulic, caffeic, *o*-coumaric, *m*-coumaric, *p*-coumaric, syringic, rosmarinic and *trans*-cinnamic acids. The results were expressed as milligrams per 100 g of fresh weight ($\text{mg } 100\text{ g}^{-1}\text{ FW}$).

2.5. Determination of Soluble Solids, Fructose, Glucose, and Sucrose

Soluble solids content (SS, %) was measured in fresh, undiluted pitaya pulp using a digital refractometer (ATAGO PR-100, Tokyo, Japan), following the official method described by AOAC [11]. The determination of individual sugar contents, namely fructose,

glucose, and sucrose, was determined by high-performance liquid chromatography (HPLC), following a protocol adapted from Santos et al. [12]. Approximately 1 g of pitaya pulp was diluted in 5 mL of ultrapure water. After thorough homogenization, a 1 mL aliquot was collected and further diluted 1:2 with ultrapure water before chromatographic analysis. The analyses were performed using a Shimadzu® Prominence HPLC system (Shimadzu Corporation, Kyoto, Japan) equipped with a Shim-pack SCR 102HG analytical column and a matching pre-column. Detection was carried out using a refractive index detector (RID-20A) and a UV-VIS detector (SPD-20A), with the column oven maintained at 50 °C. The mobile phase consisted of 5 mM perchloric acid, delivered at a constant flow rate of 0.600 mL min⁻¹. Results were expressed as mg g⁻¹ fresh weight (FW).

2.6. Data Analysis and Statistical Treatment

Analyses were conducted in triplicate for each developmental stage. The metabolomic data, comprising phenolic compounds and sugars, were subjected to analysis of variance (ANOVA). When significant differences were identified by the F-test ($p < 0.05$), mean comparisons were performed using Tukey's test.

Principal component analysis (PCA) was performed separately for pulp and peel proteomic datasets using the protein abundance matrix, Z-score normalized across samples. PCA was applied to reduce data dimensionality and to visualize variance patterns and sample discrimination among developmental stages (S1–S5), with interpretation based on the first two principal components (PC1 and PC2).

Multiple Factor Analysis (MFA) was applied to integrate metabolomics data (phenolic compounds) and proteomics data (antioxidant enzymes) obtained across five fruit developmental stages (S1–S5), analyzed separately for pulp and peel tissues. Data matrices were structured considering developmental stages as observations and chemical and enzymatic variables as descriptors. Non-detected (ND) values were treated as zero prior to analysis.

Each data block was internally auto-scaled, ensuring equal weighting between metabolomics and proteomics datasets regardless of differences in absolute magnitude. MFA was performed using the FactoMineR package (version 2.8), implemented in R software (version 4.4.2, R Foundation for Statistical Computing, Vienna, Austria), considering both datasets as quantitative blocks. Interpretation was based on the first two principal components (PC1 and PC2), which explained the largest proportion of total variance. Variable contributions were assessed by the sum of contributions to PC1 and PC2 and visualized using a color gradient in the biplots.

Correlation analysis was performed to investigate the relationships between soluble sugars (glucose, fructose, sucrose and soluble solids) and enzymes involved in glycolysis, sucrose metabolism and anaplerotic carbon fixation during fruit development. Enzyme abundance data included multiple phosphoenolpyruvate carboxylase (PEPC) isoforms, as well as enolase (PGH1), phosphoglycerate kinase (PGK), glyceraldehyde-3-phosphate dehydrogenase (GAPDH/GAPC), triosephosphate isomerase and sucrose synthase. Pair-wise relationships were assessed using Pearson's correlation coefficient, retaining only statistically significant correlations ($p < 0.05$). Significant associations were visualized as a correlation network, in which nodes represent sugars or enzymes and edges represent positive or negative correlations, allowing the identification of coordinated patterns between sugar accumulation and carbohydrate-related enzymatic activity.

All statistical analyses were conducted using R software (version 4.4.0).

3. Results

3.1. Proteomics and Metabolomics

A total of 411 and 812 proteins were identified in the pulp and peel of *Selenicereus megalanthus* fruits, respectively (Tables S1 and S2). Among these, 255 proteins in the pulp and 362 in the peel showed differential accumulation with statistically significant variation across the five developmental stages ($p < 0.05$).

The accumulation patterns of these differentially abundant proteins throughout fruit development are presented as heatmaps in Figure 2A (pulp) and Figure 2B (peel). In both fractions, the heatmaps revealed the formation of several protein clusters across the developmental stages. In the peel heatmap (Figure 2B), a major cluster of proteins with higher abundance at stage S5 was observed in the upper portion of the figure.

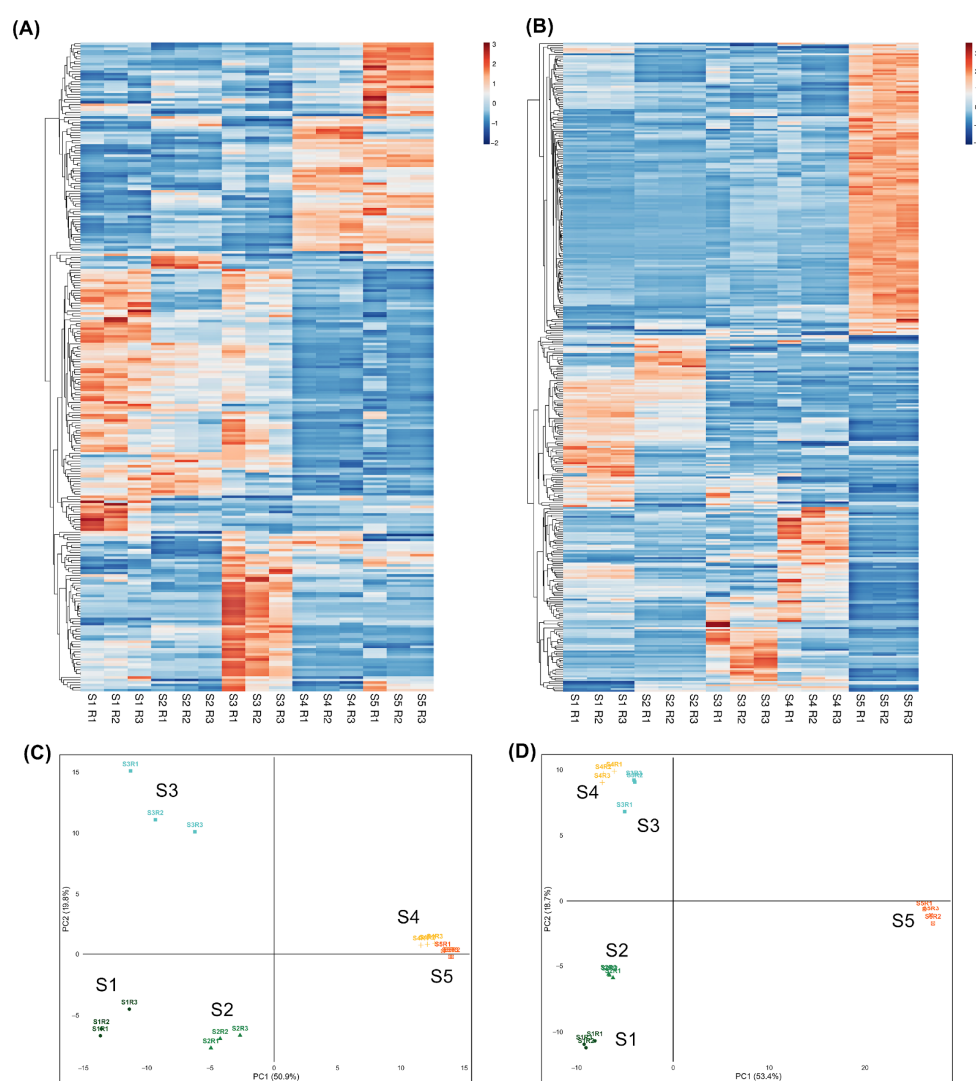


Figure 2. Heatmaps showing the relative abundance (Z-score) of statistically significant proteins ($p < 0.05$) identified in *Selenicereus megalanthus* across five developmental stages (S1 to S5), with three biological replicates per stage (R1 to R3). Protein abundance is color-coded from blue (low) to red (high), and hierarchical clustering was applied to rows to highlight stage-dependent expression patterns. (A) Heatmap of the 255 proteins from the pulp. (B) Heatmap of the 362 proteins from the peel. (C) PCA of the 255 proteins from the pulp. (D) PCA of the 362 proteins from the peel. In panels (C,D), colors represent biological replicates grouped according to developmental stages (S1–S5).

PCA based on the differentially accumulated proteins ($p < 0.05$) is shown in Figure 2C for the pulp and Figure 2D for the peel. In the pulp, PC1 and PC2 explained 50.9% and

24.1% of the total variance, respectively. Samples from S1 and S2 were positioned in the negative PC1 region, with S1 located in the lower-left quadrant and S2 in the lower-central region. In contrast, S4 and S5 were distributed in the positive PC1 region, with S4 occupying the upper-right quadrant and S5 the lower-right quadrant. The intermediate stage S3 was separated mainly along PC2, clustering in the upper-left quadrant.

In the peel, PC1 and PC2 accounted for 53.4% and 18.7% of the variance, respectively. S1 and S2 samples were positioned in the negative PC1 region, with S1 in the lower-left quadrant and S2 slightly higher along PC2. S5 samples were clearly separated in the positive PC1 region, whereas S3 and S4 were grouped in the upper-left quadrant, showing separation primarily along PC2.

Figure 3 presents the functional distribution of proteins identified in pitaya pulp and peel, highlighting the relative contribution of individual proteins within major functional processes. In both tissues analyzed (pulp and peel), the functional distribution of the identified proteins is presented in Figure 3, highlighting the relative contribution of individual proteins across the different functional processes. Proteins were grouped into nine functional categories, organized in the same order for both sets of panels, namely: photosynthesis/chloroplast (A and J), other enzymes/transport (B and K), others/uncharacterized (C and L), RNA/translation/processing (D and M), energy and central carbon metabolism (E and N), regulation/signaling (F and O), antioxidant defense/ROS (G and P), cell wall/carbohydrates (H and Q), and protein folding/stress response (I and R). This standardized organization enabled direct comparison between pulp and peel.



Figure 3. Functional distribution of proteins in pitaya pulp (A–I) and peel (J–R). Donut charts illustrate the relative contribution of individual proteins within each functional process, arranged in the same sequence across both sets of panels. Functional processes correspond to: (A,J) photosynthesis/chloroplast; (B,K) other enzymes/transport; (C,L) others/uncharacterized; (D,M) RNA/translation/processing; (E,N) energy/central carbon metabolism; (F,O) regulation/signaling; (G,P) antioxidant defense/ROS; (H,Q) cell wall/carbohydrates; and (I,R) protein folding/stress response. Slice size reflects the proportional abundance of proteins within each process, while color intensity indicates the share within the process (lighter to darker tones correspond to lower to higher contributions). Numeric identifiers within slices follow the format process.protein, in which the first number refers to the functional process and the second identifies individual proteins ranked by abundance within that process; only proteins with a relative contribution $\geq 5\%$ within each process are labeled in the charts.

In both tissues, proteins associated with photosynthesis and chloroplast-related functions (A and J) constituted one of the most representative functional categories, reflecting the predominance of components of the photosynthetic apparatus and pathways involved in energy conversion. In addition, proteins related to energy and central carbon metabolism (E and N), as well as RNA translation and processing (D and M), showed substantial contributions, indicating high metabolic and biosynthetic activity in both pulp and peel tissues. A relevant proportion of proteins was also classified as other enzymes/transport (B and K) and others/uncharacterized (C and L), underscoring the functional diversity and biochemical complexity of the pitaya proteome.

Functional categories related to antioxidant defense and reactive oxygen species (ROS) (G and P), cell wall and carbohydrate metabolism (H and Q), regulation and signaling (F and O), and protein folding and stress response (I and R) were consistently detected in both tissues. However, differences in their relative contributions among processes were observed. Within each category, only proteins with a relative contribution of at least 5% to the respective process were highlighted in the charts, thereby emphasizing the most representative components of the functional profile while ensuring clarity in data visualization.

3.1.1. Transcription Factors

Three transcription factors (TFs) were identified in the pulp proteome of *S. megalanthus* across all developmental stages: Growth-Regulating Factor (GRF), Myb-like domain-containing protein (MYB), and TATA-binding protein-associated factor BTAF1. Among these TFs, GRF and MYB showed statistically significant differences in accumulation across the five developmental stages ($p < 0.05$). Both proteins exhibited higher accumulation at stage S2 and lower accumulation at stage S5. GRF showed a pronounced variation, with a 37.92-fold change between stages S2 and S5. In the peel proteome, 29 TFs belonging to different families were identified across all developmental stages. Among these, three proteins were classified as auxin response factors (ARFs), and three auxin-responsive proteins belonging to the Aux/IAA gene family were also detected. Of the six auxin-related proteins identified in the peel proteome, only one Aux/IAA protein showed a statistically significant difference across the developmental stages ($p < 0.05$), with the highest accumulation at stage S5 and the lowest at stage S2, corresponding to a 1.55-fold change. The remaining ARF and Aux/IAA proteins showed relatively stable accumulation levels throughout fruit development.

3.1.2. Cell Wall Synthesis and Remodeling Enzymes Associated with Fruit Texture and Quality

In the fruit pulp, the direct involvement of enzymes associated with cell wall remodeling was limited. Only one isoform of β -galactosidase 14 (BGAL14) was identified in the pulp proteome, an enzyme involved in the removal of galactan side chains from cell wall polysaccharides, a process associated with tissue softening. In addition, a trichome birefringence-like (TBL) protein was detected, which is related to the chemical modification of cell wall polysaccharides, such as acetylation processes, influencing cell wall rigidity and architecture. These results indicate that, in the pulp, cell wall remodeling mechanisms occur in a punctual manner, whereas the proteomic profile is predominantly characterized by proteins associated with energy metabolism and stress responses.

In contrast, the fruit peel exhibited a broader and more diverse set of enzymes directly involved in cell wall synthesis, modification, and degradation, highlighting its central role in texture changes during fruit development and ripening. Three isoforms of cellulose synthase (CS) were identified in the peel proteome; however, only one isoform showed a statistically significant variation ($p < 0.05$) across the five developmental stages, with

maximum accumulation at stage S3 and minimum accumulation at stage S5, corresponding to an 82.30-fold reduction. This pattern indicates a progressive decline in the biosynthetic activity of structural polysaccharides at advanced stages of fruit development.

Enzymes involved in plant cell wall degradation were predominantly detected in the peel proteome, including pectin methylesterase (PME), two isoforms of pectinesterase (PE), and three isoforms of β -galactosidase (β -gal). Both PE isoforms showed higher accumulation at stages S4 and S5 ($p < 0.05$), indicating an intensification of pectin demethylation processes during the late stages of development. In addition, polygalacturonase inhibitor 2 (PGIP2) was identified, which is associated with the modulation of pectin degradation and the regulation of the balance between structural integrity and tissue softening.

Enzymes related to the modification of hemicelluloses and side chains of the cell wall were also identified in the peel, including two β -galactosidase isoforms, involved in the removal of galactans and arabinogalactans, and an α -L-arabinofuranosidase, responsible for the removal of terminal arabinose residues from arabinans, arabinoxylans, and pectins. These processes indicate the progressive disassembly of the polysaccharide matrix, favoring cell wall loosening.

In addition to degradation-related enzymes, proteins associated with cell wall synthesis and structural organization were detected in the peel, including a cellulose synthase-like protein D1, involved in the biosynthesis of structural polysaccharides; a 1,3- β -glucan synthase, associated with β -glucan synthesis and cell wall reorganization; and a glycosyltransferase containing a GT47 domain, related to the biosynthesis and modification of cell wall polysaccharides. Furthermore, an expansin-like B1 protein was identified, which is associated with cell wall loosening without direct hydrolytic activity, playing an important role in tissue softening and cell expansion.

Taken together, these results demonstrate that while the pulp exhibits punctual signs of cell wall remodeling, the peel concentrates a classical and integrated set of enzymes involved in cell wall degradation, reorganization, and biosynthesis, reinforcing its determinant role in texture modulation and in maintaining the structural quality of the fruit throughout development and ripening.

3.1.3. Heat Shock Proteins

Several isoforms of heat shock proteins (HSPs) were detected in the proteomes of both pulp and peel of yellow pitaya. In the peel, 11 HSPs were identified, of which three showed statistically significant differences ($p < 0.05$) across the five developmental stages. In the pulp, approximately 14 HSPs were detected, and 11 of these exhibited statistically significant differences ($p < 0.05$), with predominant accumulation of six proteins at stage S1. In the entire proteome of *S. megalanthus*, only a single chaperonin was detected.

3.1.4. Enzymatic Antioxidant and Detoxification Systems and Phenolic Compounds

Several proteins involved in plant antioxidant and detoxification systems were detected in the proteomes of both pulp and peel of yellow pitaya. In the pulp, 15 proteins related to this system were identified, of which eight showed statistically significant differences ($p < 0.05$) across the five developmental stages. These included two isoforms of catalase (CAT, EC: 1.11.1.6), three isoforms of monodehydroascorbate reductase (MDHAR, EC: 1.6.5.4), and three isoforms of superoxide dismutase [Cu-Zn] (SOD, EC: 1.15.1.1). The highest accumulation of these proteins occurred mainly at stages S1 and S2.

In the peel, 22 antioxidant- and detoxification-related proteins were detected, with 11 exhibiting statistically significant differences ($p < 0.05$) across the five developmental stages. This group included seven isoforms of CAT and one isoform each of SOD, MDHAR, hydroperoxide dehydratase (HPXDH, EC: 4.2.1.92), and ascorbate peroxidase (APX, EC:

1.11.1.11). These proteins exhibited varied accumulation patterns across stages S3 and S5, with greater accumulation at stage S5.

Among the proteins related to antioxidant and detoxification processes that did not show statistically significant differences ($p > 0.05$) in both pulp and peel were glutathione reductase (GR, EC: 1.8.1.7), peroxidase (PX, EC: 1.11.1.7), isoforms of lactoylglutathione lyase (EC: 4.4.1.5), as well as additional isoforms of CAT, MDHAR, and SOD.

Phenolic compounds were detected in both pulp and peel of *Selenicereus megalanthus* throughout all developmental stages, with marked differences in composition and concentration depending on tissue and ripening stage (Table 1). Overall, the pulp exhibited significantly higher total phenolic levels at the early stages, whereas the peel showed a more heterogeneous and stage-dependent phenolic profile ($p < 0.05$). Significant differences among stages were confirmed by Tukey's test, as indicated by different letters in Table 1.

Table 1. Phenolic contents in *Selenicereus megalanthus* at different developmental stages.

Phenolic Acids Content	S1		S2		S3		S4		S5	
	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel
Gallic acid	5.4 ± 0.31 a *	0.07 ± 0.01 d	4.66 ± 0.56 b	ND	2.09 ± 0.06 c	0.07 ± 0.01 d	0.71 ± 0.09 d	0.17 ± 0.02 c	0.16 ± 0.02 e	0.36 ± 0.03 a
Chlorogenic acid	7.01 ± 0.27 a	0.60 ± 0.06 c	0.80 ± 0.02 e	0.08 ± 0.02 e	1.35 ± 0.12 c	0.84 ± 0.05 b	0.91 ± 0.06 d	9.07 ± 0.51 a	1.74 ± 0.10 b	0.53 ± 0.04 d
Catechin	182.44 ± 2.27 a	0.42 ± 0.01 d	17.13 ± 0.47 c	0.74 ± 0.04 b	40.22 ± 1.99 b	0.39 ± 0.03 d	14.57 ± 0.02 d	0.50 ± 0.03 c	11.96 ± 0.88 e	0.90 ± 0.06 a
Syringic acid	8.20 ± 0.24 a	1.14 ± 0.06 b	1.89 ± 0.14 c	1.01 ± 0.06 c	1.52 ± 0.07 d	1.21 ± 0.05 b	2.88 ± 0.24 a	1.17 ± 0.05 b	2.11 ± 0.11 c	1.34 ± 0.08 a
p-Coumaric acid	1.88 ± 0.11 a	1.04 ± 0.05 b	0.54 ± 0.03 b	1.01 ± 0.09 b	0.08 ± 0.00 c	1.44 ± 0.11 a	ND	0.63 ± 0.03 c	ND	1.16 ± 0.01 a
m-Coumaric acid	0.47 ± 0.02 a	0.34 ± 0.01 b	0.34 ± 0.03 b	ND	0.21 ± 0.01 c	0.23 ± 0.01 c	ND	0.45 ± 0.01 a	ND	0.48 ± 0.01 a
Caffeic acid	1.27 ± 0.08 b	0.39 ± 0.02 b	1.30 ± 0.08 a	0.54 ± 0.04 a	0.61 ± 0.03 e	0.25 ± 0.01 c	1.12 ± 0.07 c	0.42 ± 0.01 b	0.74 ± 0.06 d	0.41 ± 0.02 b
Vanillin	6.12 ± 0.41 a	0.91 ± 0.04 a	1.19 ± 0.13 c	ND	0.94 ± 0.04 d	0.94 ± 0.06 a	2.50 ± 0.14 b	0.60 ± 0.03 b	0.59 ± 0.06 e	0.90 ± 0.05 a
Resveratrol	4.67 ± 0.05 a	ND	4.61 ± 0.30 a	ND	ND	4.13 ± 0.03 b	ND	5.95 ± 0.25 a	ND	4.48 ± 0.16 b
Rosmarinic acid	1.89 ± 0.14 a	9.76 ± 0.17 a	1.39 ± 0.06 b	6.54 ± 0.48 b	1.33 ± 0.08 b	3.45 ± 0.07 c	1.38 ± 0.08 b	3.40 ± 0.23 c	ND	2.10 ± 0.03 d

* Means followed by the same letter in the row do not differ significantly according to Tukey's test at 5% probability ($p < 0.05$). ND: not detected.

In the pulp, catechin was the predominant phenolic compound at the initial stage (S1), reaching 182.44 mg 100 g⁻¹, which was significantly higher than the concentrations observed at all subsequent stages ($p < 0.05$). A pronounced and statistically significant decline in catechin content occurred during fruit development, with values decreasing to 11.96 mg 100 g⁻¹ at the mature stage (S5). Other phenolics in the pulp, including gallic acid, chlorogenic acid, syringic acid, vanillin, and caffeic acid, also showed significantly higher concentrations at S1 compared to later stages, followed by a general reduction or stabilization as ripening progressed ($p < 0.05$).

In the peel, hydroxycinnamic acids were the dominant phenolic class, with rosmarinic acid presenting the highest concentration at S1 (9.76 mg 100 g⁻¹), differing significantly from the other stages ($p < 0.05$). Chlorogenic acid showed a distinct accumulation pattern, reaching a significant maximum at S4 (9.07 mg 100 g⁻¹; $p < 0.05$). At the mature stage (S5), resveratrol became the predominant phenolic compound in the peel (4.48 mg 100 g⁻¹), with significantly higher levels than those detected at earlier developmental stages ($p < 0.05$), highlighting a tissue- and stage-specific modulation of phenolic composition during fruit development.

In the peel proteome, three isoforms of phenylalanine ammonia-lyase (PAL, EC: 4.3.1.24) and two isoforms of chalcone synthase (CHS, EC: 2.3.1.74) were detected across all developmental stages. Only one CHS isoform showed statistically significant variation ($p < 0.05$), with maximum and minimum accumulation at stages S3 and S2, respectively, corresponding to an 832.27-fold difference. In the pulp proteome, no enzymes directly associated with the phenolic compounds identified in Table 1 are detected.

In the pulp MFA, the first two components explained 77.9% of the total variance (PC1 = 54.8% and PC2 = 23.1%), indicating strong discriminative power. PC1 clearly

separated early (S1–S2) from late developmental stages (S4–S5), reflecting major metabolic shifts during pulp maturation (Figure 4).

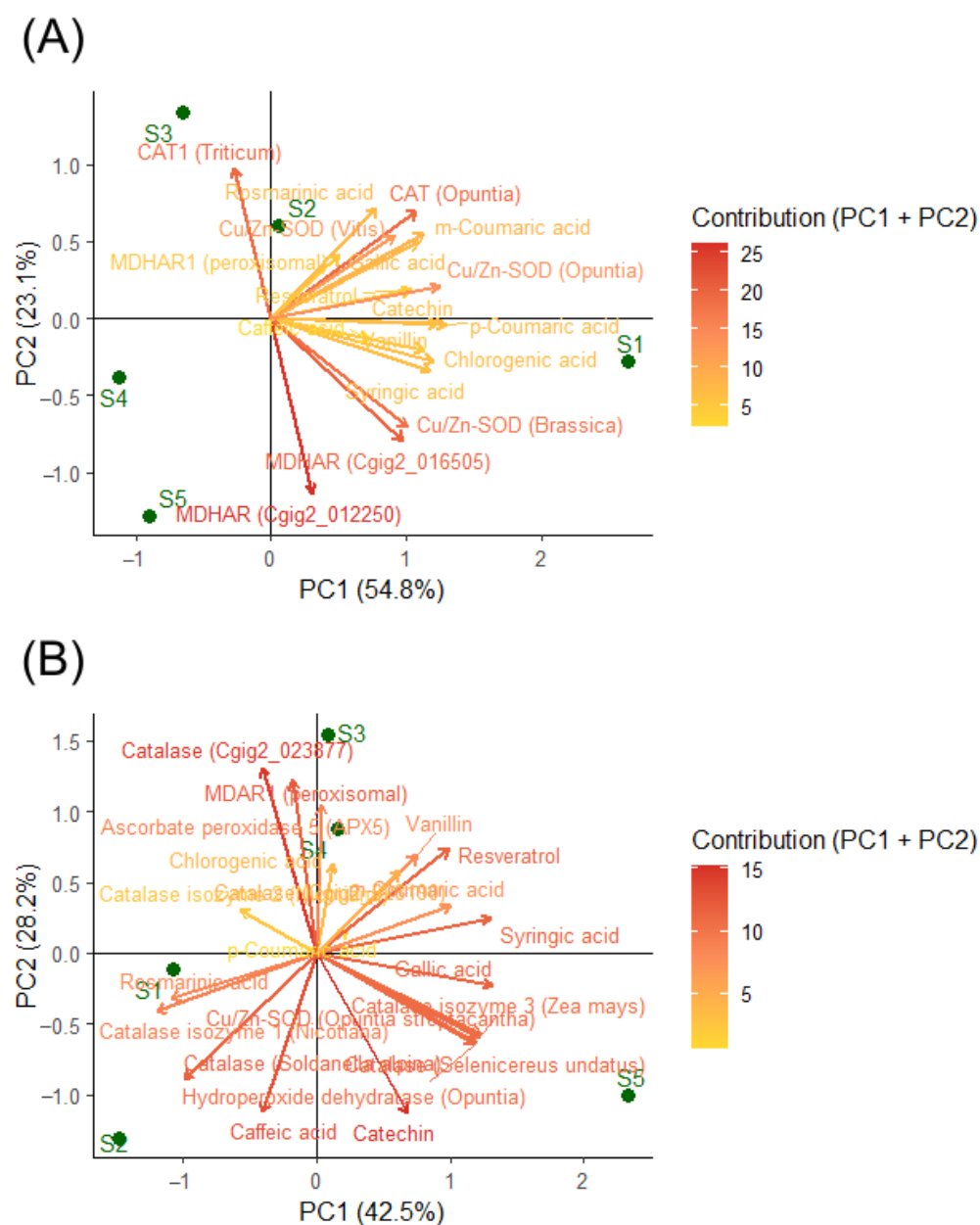


Figure 4. Multiple Factor Analysis integrating phenolic compounds and antioxidant enzyme data across fruit developmental stages. (A) MFA of the pulp. (B) MFA of the peel. Green points represent developmental stages (S1–S5), while vectors represent metabolomic and proteomic variables. Vector length and color intensity indicate the contribution of each variable to the first two principal components.

Stage S1, positioned on the positive side of PC1, was mainly associated with chlorogenic acid, p-coumaric acid, catechin, and Cu/Zn-SOD (Opuntia), indicating a higher relevance of phenolic compounds and specific antioxidant enzymes at early developmental stages. In contrast, S4 and S5, located on the negative side of both PC1 and PC2, were strongly associated with MDHAR (Cgig2_012250 and Cgig2_016505) and Cu/Zn-SOD (Brassica), suggesting a shift toward predominantly enzymatic antioxidant regulation during advanced stages of pulp development. Stage S3 occupied an intermediate position in the factorial space and was primarily associated with CAT1 (Triticum), characterizing a transitional metabolic phase. Overall, the MFA of the pulp indicates that developmental

progression is marked by a gradual reduction in the relative influence of phenolic compounds and an increasing contribution of antioxidant enzymes, consistent with metabolic reprogramming during fruit maturation.

In the peel MFA, the first two components explained 70.7% of the total variance (PC1 = 42.5% and PC2 = 28.2%), revealing a distinct organization compared to the pulp. Stage S3 was clearly separated in the upper quadrant of the factorial space and strongly associated with catalase (Cgig2_023877), peroxisomal MDAR, and APX5, indicating high antioxidant enzymatic activity in the peel at the intermediate developmental stage.

Late developmental stages (S4 and S5) clustered in the lower-right quadrant and were associated with catechin, caffeic acid, hydroperoxide dehydratase, and several catalase isoforms, suggesting a coordinated contribution of enzymatic and phenolic antioxidant mechanisms during advanced fruit development. In contrast, early stages (S1 and S2) were associated with rosmarinic acid, chlorogenic acid, and Cu/Zn-SOD, highlighting the role of the peel as a chemical defensive barrier during early fruit development. Overall, the peel MFA reveals a highly dynamic and integrated antioxidant system in which phenolic compounds and antioxidant enzymes act synergistically to protect the fruit throughout its developmental trajectory.

3.1.5. Myo-Inositol Biosynthesis Enzymes and Sugars

In the proteome of *S. megalanthus*, proteins involved in myo-inositol (MYO) biosynthesis were detected, including nine isoforms of inositol-3-phosphate synthase (MIPS, EC: 5.5.1.4) and two isoforms of inositol monophosphatase (IMP, EC: 3.1.3.25). Of these 11 enzymes, only one MIPS was identified in the peel, while all others were detected in the pulp. The highest accumulation of MIPS and IMP enzymes occurred between stages S2 and S4. Conversely, stage S5 was characterized by lower accumulation levels of these enzymes.

A significant increase ($p < 0.05$) in the contents of soluble solids (SS), glucose, fructose, and sucrose was observed across the developmental stages (S1–S5) in *S. megalanthus* fruits. Soluble solids increased from 6.35% at S1 to 20.41% at S5, indicating a progressive accumulation of soluble sugars. Sucrose was the main sugar accumulated at the mature stage (35.40 mg g^{-1}), followed by fructose (29.29 mg g^{-1}) and glucose (7.27 mg g^{-1}) (Table 2).

Table 2. Soluble solids and sugar in *Selenicereus megalanthus* pulp at different developmental stages.

Parameters	S1	S2	S3	S4	S5
SS (%)	$6.35 \pm 0.29 \text{ e}^*$	$10.31 \pm 0.12 \text{ d}$	$13.39 \pm 0.31 \text{ c}$	$16.40 \pm 0.14 \text{ b}$	$20.41 \pm 0.3 \text{ a}$
Glucose (mg g^{-1})	$1.21 \pm 0.07 \text{ d}$	$2.14 \pm 0.14 \text{ c}$	$3.31 \pm 0.49 \text{ b}$	$3.82 \pm 0.28 \text{ b}$	$7.27 \pm 0.11 \text{ a}$
Fructose (mg g^{-1})	$4.31 \pm 0.97 \text{ c}$	$3.75 \pm 0.44 \text{ c}$	$8.28 \pm 0.38 \text{ b}$	$21.27 \pm 0.73 \text{ a}$	$29.29 \pm 1.18 \text{ a}$
Sucrose (mg g^{-1})	$5.91 \pm 0.36 \text{ d}$	$7.55 \pm 0.05 \text{ d}$	$15.80 \pm 0.39 \text{ c}$	$24.39 \pm 0.29 \text{ b}$	$35.40 \pm 1.18 \text{ a}$

* Means followed by the same letter in the row do not differ significantly according to Tukey's test at 5% probability ($p < 0.05$).

Chromatographic analyses confirmed a continuous increase in soluble sugars throughout fruit development, particularly glucose and fructose in the pulp. Consistently, the proteomic profile of *Selenicereus megalanthus* pulp revealed an intense activation of central carbon metabolism, characterized by the accumulation of enzymes associated with glycolysis and carbon flux redirection. Fourteen isoforms of phosphoenolpyruvate carboxylase (PEPC, EC 4.1.1.31), together with key glycolytic enzymes, including enolase (ENO, EC 4.2.1.11), glyceraldehyde-3-phosphate dehydrogenase (GAPDH, EC 1.2.1.12), triosephosphate isomerase (TPI, EC 5.3.1.1), phosphoglycerate kinase (PGK, EC 2.7.2.3), and pyruvate kinase were significantly more abundant ($p < 0.05$), especially between stages S1 and S2. In addition, the detection of malate dehydrogenase and pyruvate dehydrogenase indicates an integrated glycolytic–respiratory metabolism, supporting energy supply and carbon

turnover that favor the accumulation of glucose and fructose rather than direct sucrose synthesis in the pulp.

As shown in Figure 5B, individual sugars (glucose, fructose, and sucrose) and soluble solids (SS) showed significant correlations ($p < 0.05$) with key carbohydrate-metabolism enzymes (PEPC, GAPDH, ENO, TPI, PGK, and SuSy), evidencing coordinated activity between enzymatic metabolism and sugar accumulation throughout fruit development. One isoform of sucrose synthase (SuSy, EC: 2.4.1.13) was identified in the pulp proteome. SuSy showed higher accumulation at stage S1 and lower accumulation at stage S4, corresponding to a 3.24-fold reduction.

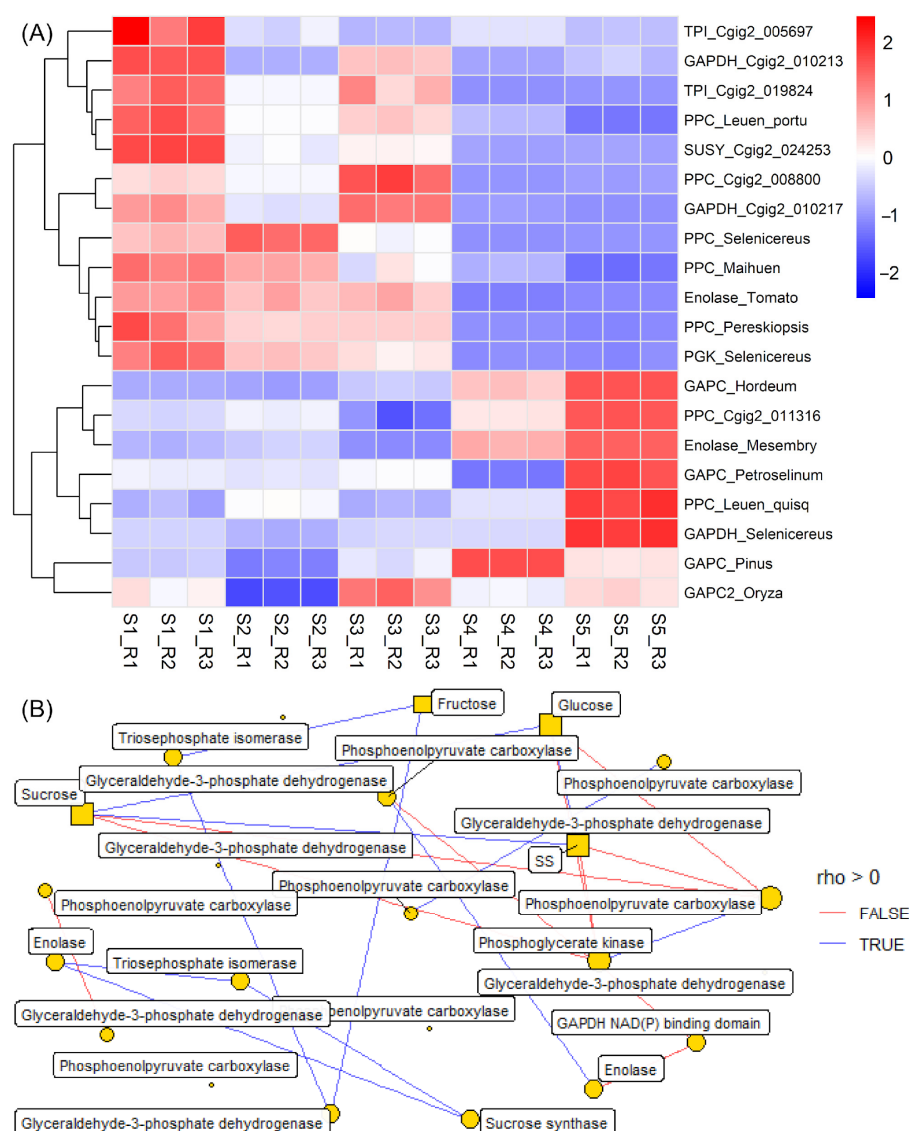


Figure 5. Integration of sugar metabolism enzymes and carbohydrate accumulation during *Selenicereus megalanthus* fruit development (pulp). (A) Heatmap and hierarchical clustering of enzymes involved in carbohydrate metabolism across the five developmental stages (S1–S5), showing stage-dependent changes in protein accumulation. Values are displayed as z-scores. (B) Correlation network between individual sugars (glucose, fructose, sucrose), soluble solids (SS), and key carbohydrate-metabolism enzymes. Blue edges indicate significant correlations ($p < 0.05$), while red edges represent non-significant associations. The network highlights coordinated enzymatic regulation associated with sugar accumulation during fruit development.

3.2. Protein–Protein Interaction

Figure 6 presents the protein–protein interaction (PPI) network obtained from the list of genes encoding all the proteins discussed above, graphically illustrating the physical or functional interactions among these proteins within the cell. Different line colors indicate distinct types of recognized or potential interactions. Not all submitted genes showed interactions recognized by the database.

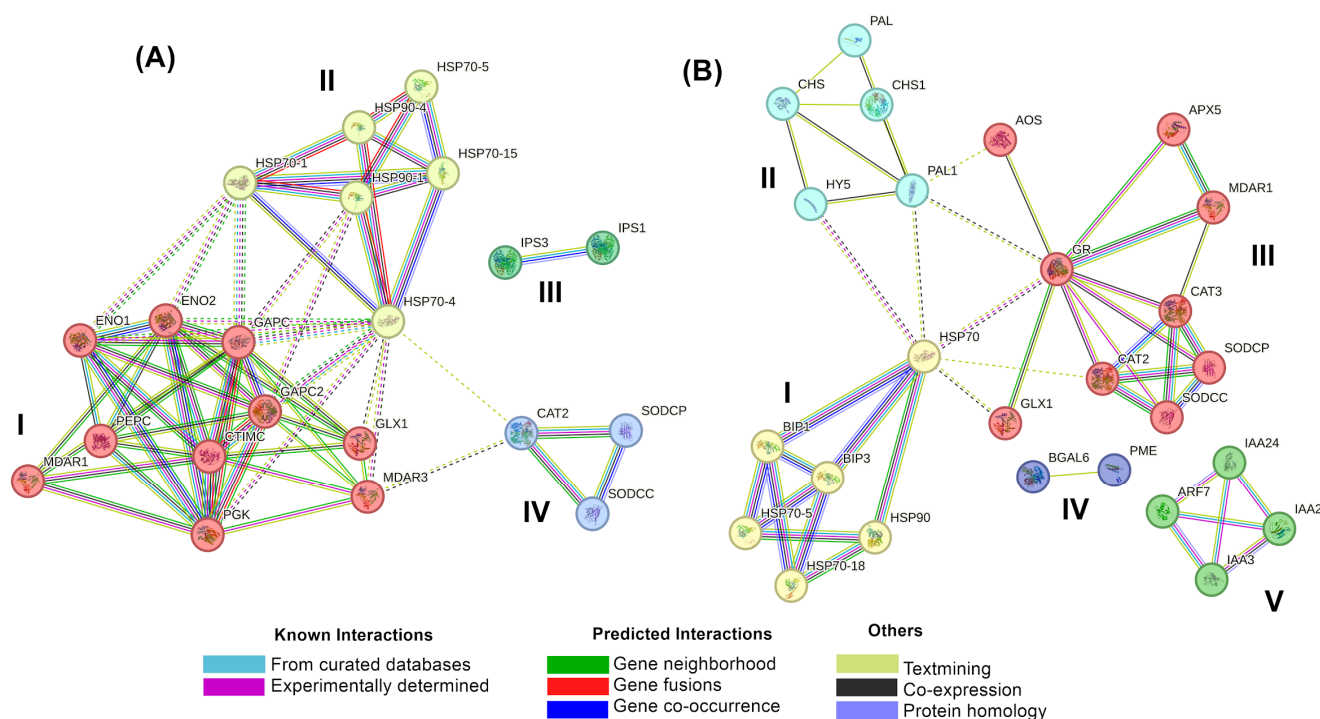


Figure 6. Protein–protein interactions (PPI) generated by the list of genes encoding all proteins discussed, when compared with equivalent proteins in the *Arabidopsis thaliana* database. The different line colors indicate distinct types of interactions recognized or prospected. (A) PPI discussed in the pulp with four clusters identified: I-Glycolytic process; II-Chaperone-mediated protein folding; III-Inositol biosynthesis; IV-Cellular response to ozone. (B) PPI indicating the interactions of the proteins discussed in the peel, with five clusters identified: I-Protein refolding; II-Regulation of flavonoid biosynthesis; III-Detoxification of Reactive Oxygen Species; IV-Mucilage metabolism involved in seed coat development and mucilage extrusion from the seed coat V-AUX/IAA family and Auxin binding.

Four clusters were identified for the pulp (Figure 6A) and five clusters for the peel (Figure 6B) of yellow pitaya. In the pulp, clusters I and II showed the highest number of interactions and were associated with the glycolytic pathway and protein folding, respectively. In the peel PPI network, interactions were observed among clusters I (protein folding), III (ROS detoxification), and V (Aux/IAA family and auxin binding). Cluster V showed strong interactions between the transcription factor ARF7 and the three members of the Aux/IAA family.

4. Discussion

The identification of a large number of proteins in both pulp and peel highlights the metabolic complexity underlying *S. megalanthus* fruit development. The higher number of proteins and differentially accumulated proteins detected in the peel compared to the pulp indicates greater proteomic dynamism in this tissue, likely reflecting its protective, structural, and regulatory roles during fruit maturation. Clustering patterns observed in the heatmaps reveal coordinated changes in protein abundance across developmental

stages, consistent with stage-specific metabolic reprogramming. Notably, the accumulation of a distinct set of proteins at stage S5 in the peel suggests intensified biological activity during the late developmental phase, potentially associated with ripening-related processes. These trends are further supported by PCA, which clearly discriminated developmental stages based on proteomic profiles, confirming that *S. megalanthus* fruit development is accompanied by marked and systematic shifts in protein accumulation. Collectively, these proteomic changes underpin the activation and modulation of multiple biological processes throughout fruit growth and maturation, which are explored in detail in the following sections.

The combined representation in Figure 3 provides an integrated overview of the functional allocation of proteins in pitaya pulp and peel, allowing direct comparison between tissues. Overall, the same functional categories are represented in both tissues, while differences are observed in the relative contribution of proteins within each process, suggesting tissue-specific metabolic emphasis. Based on this global overview, individual functional processes are discussed in detail in the following sections.

Within this functional context, regulatory mechanisms associated with fruit development were further explored through the analysis of transcription factor-related proteins. Fruit development and ripening are regulated by genetically controlled networks involving multiple phytohormone signaling pathways, in which transcription factors act as central regulators of gene expression [13,14]. The identification of a diversity of TF families in the peel suggests tissue-specific regulatory strategies during *S. megalanthus* fruit development [8]. The pronounced decrease in GRF accumulation from early to late developmental stages supports its established role in promoting cell proliferation and organ growth during initial phases of fruit development. GRFs are typically highly expressed in actively growing tissues, with their abundance declining as organs approach physiological maturity [15]. Similar regulatory patterns have been reported in tomato and apple, where GRF genes positively influence fruit size, weight, and early cell production [16,17]. In *S. megalanthus*, the higher GRF accumulation observed at stage S2 likely reflects its involvement in pulp expansion, while the reduced abundance at stage S5 coincides with the cessation of significant biomass accumulation during full ripening.

The identification of ARFs and Aux/IAA proteins in the peel proteome indicates the presence of an active auxin-responsive regulatory system throughout fruit development. ARFs are TFs that directly bind to auxin-responsive elements in the promoters of target genes, functioning either as transcriptional activators or repressors depending on their structural domains [18]. Aux/IAA proteins act as short-lived repressors of auxin signaling, interacting with ARFs through conserved domains and modulating their transcriptional activity [19]. Auxin-mediated transcriptional regulation depends on the dynamic balance between ARF activation and Aux/IAA-mediated repression. Elevated auxin levels promote the degradation of Aux/IAA proteins, releasing ARFs and enabling downstream gene expression, whereas low auxin availability maintains ARF repression [20]. In the peel of *S. megalanthus*, the relatively stable accumulation of most ARFs and Aux/IAA proteins across developmental stages, together with the modest variation observed for a single Aux/IAA protein, suggests that auxin signaling operates as a modulatory mechanism rather than a dominant driver of stage-specific developmental transitions.

Overall, these results indicate that transcriptional regulation in *S. megalanthus* fruits is influenced by the action of GRF, playing a prominent role in initial pulp growth, while auxin-related regulatory systems contribute to the coordinated development and ripening of the peel.

CS plays a central role in cell wall biosynthesis, and the marked reduction observed from S3 to S5 indicates a strong decline in cellulose deposition during the late stages of fruit

development. The substantial 82.30-fold decrease in CS accumulation is consistent with the transition from cell wall construction to disassembly processes during ripening. Such a shift reflects the reprogramming of cell wall metabolism from structural reinforcement to controlled loosening, a hallmark of fruit softening.

PE enzymes are considered key regulatory points in the assembly and disassembly of cell wall networks, as they catalyze pectin demethylation, rendering the cell wall more susceptible to degradation by PG [6,21]. The increased accumulation of PE isoforms at stages S4 and S5 suggests an intensification of pectic matrix remodeling during the late stages of development. This process promotes changes in cell adhesion and wall porosity, which are directly associated with texture softening and quality loss during ripening.

In addition to PE activity, the identification of multiple β -galactosidase isoforms in the peel indicates the progressive removal of galactan and arabinogalactan side chains from pectins and hemicelluloses, contributing to the dismantling of the polysaccharide network. The presence of α -L-arabinofuranosidase further supports the active remodeling of hemicellulosic and pectic domains, reinforcing the role of the peel as the primary tissue governing texture changes. The detection of expansin-like B1 suggests that non-hydrolytic mechanisms also contribute to cell wall loosening by disrupting hydrogen bonding between cellulose microfibrils and matrix polysaccharides, facilitating wall extensibility.

Conversely, enzymes associated with cell wall synthesis and structural reorganization, such as cellulose synthase-like protein D1, 1,3- β -glucan synthase, and GT47 glycosyl-transferases, indicate that cell wall remodeling in the peel is not exclusively degradative. Instead, it reflects a dynamic balance between degradation and reorganization, allowing controlled softening while maintaining structural integrity, which is essential for fruit quality during ripening.

In the pulp, the limited detection of cell wall-related enzymes, restricted mainly to a single β -galactosidase isoform and a trichome birefringence-like (TBL) protein, suggests that cell wall remodeling plays a secondary role in texture changes within this tissue. The predominance of proteins related to energy metabolism and stress responses indicates that pulp softening may be more closely associated with turgor changes and metabolic adjustments rather than extensive polysaccharide disassembly.

In a previous study on *Selenicereus megalanthus*, peel firmness was shown to gradually decrease from the first to the fourth developmental stage, followed by a pronounced reduction between stages S4 and S5, with firmness values declining from 78.09 to 54.4 N [6]. The concurrent decline in CS accumulation, the increased presence of PE and β -galactosidase enzymes, and the observed 23.69 N reduction in firmness between stages S4 and S5 collectively support the interpretation that pitaya softening is initiated at stage S4 and fully established at stage S5. Together, these molecular and physicochemical changes highlight the central role of peel-driven cell wall remodeling in determining fruit texture and overall quality during development and ripening.

HSPs were initially associated with protective mechanisms in plants in response to excessive heat; however, their role has since been expanded to include protection against a wide range of environmental stresses. HSPs are more frequently synthesized and activated when cells are exposed to unfavorable conditions and play a fundamental role in maintaining protein homeostasis. In addition to stress protection, HSPs assist in the correct folding of newly synthesized proteins, prevent protein misfolding, and participate in protein transport and degradation processes [22]. HSPs also contribute to the growth and development of plant organs, including fruits, even under non-stress conditions, as their presence has been reported in the absence of external stress stimuli [23,24]. In this context, the detection of multiple HSP isoforms in both pulp and peel suggests that these proteins play a constitutive role during yellow pitaya fruit development.

HSPs and antioxidant enzymes are considered primary regulators of stress responses in plant cells. Under adverse environmental conditions, coordinated transcriptional control involving transcription factors and protein kinases enhances the production of HSPs and antioxidant enzymes as a defensive strategy [25]. However, considering the previously discussed behavior of antioxidant enzymes and the lack of clear indicators of stress during yellow pitaya development, the accumulation of HSPs—particularly at the initial developmental stage—likely reflects a cellular effort to ensure the correct folding of newly synthesized proteins. Failures in protein folding and the consequent loss of macromolecular function would severely compromise downstream biological processes essential for fruit development. The detection of only a single chaperonin in the entire *S. megalanthus* proteome further reinforces the importance of HSPs in safeguarding protein folding and stability throughout fruit development.

Various biological processes in plant cells continuously generate reactive oxygen species (ROS) through electron transport chains located in different organelles [26]. Although ROS act as essential signaling molecules at controlled concentrations, excessive accumulation can result in cellular damage [27]. To prevent oxidative stress, plant metabolism relies on a coordinated antioxidant system composed of enzymatic and non-enzymatic components. Due to the limited and transient nature of non-enzymatic antioxidants, enzymatic antioxidants play a predominant role in maintaining cellular redox balance, as they function continuously throughout development [28]. This enzymatic system contributes to fruit protection against oxidative damage, enhances tissue resistance, preserves nutritional quality, and supports extended postharvest shelf life [8]. The relatively balanced accumulation of antioxidant- and detoxification-related proteins across the developmental stages suggests the absence of a pronounced oxidative stress condition during fruit development. Instead, the observed protein profiles indicate a regulatory adjustment of ROS levels aimed at maintaining cellular homeostasis and ensuring the proper functioning of the diverse biological processes occurring throughout yellow pitaya development.

Regarding phenolic metabolism, the presence of PAL and CHS isoforms throughout development confirms the continuous operation of the phenylpropanoid and flavonoid biosynthetic pathways in yellow pitaya. PAL is the key enzyme initiating phenylpropanoid biosynthesis, whereas CHS is the first rate-limiting enzyme in flavonoid synthesis [7,29]. The stable accumulation of PAL isoforms across stages indicates controlled phenolic production, while the significant variation observed for one CHS isoform reflects stage-dependent modulation of flavonoid biosynthesis. The diversity and temporal variation in phenolic compounds observed in pulp and peel suggest the coexistence of synthesis, interconversion, and degradation processes throughout fruit development, making it difficult to associate individual metabolites with single enzymatic patterns.

The phenolic profile of *S. megalanthus* exhibits clear tissue- and development-dependent modulation, indicating that secondary metabolism is dynamically reorganized throughout fruit growth and ripening (Table 1). In the pulp, early developmental stages were characterized by elevated levels of flavan-3-ols and simple phenolics, particularly catechin and gallic acid, followed by a pronounced decline at later stages. This pattern suggests that early accumulation of these compounds plays a protective role during phases of intense metabolic activity, cell division, and tissue expansion, whereas their reduction during ripening reflects a shift in metabolic priorities [30]. As development progressed, the phenolic composition became more diversified, with compounds such as syringic, caffeic, and chlorogenic acids persisting at lower but relatively stable levels, indicating a transition from high-concentration antioxidant pools to a more balanced phenolic profile in mature pulp.

When compared with results previously reported for red-skinned pitaya with white flesh (*Selenicereus undatus*), similar temporal trends in pulp phenolic dynamics are evident, particularly the early predominance and subsequent decline in catechin- and gallic acid-derived phenolics during fruit development [4]. However, the present study reveals a distinct tissue-specific metabolic strategy in *S. megalanthus*, with the peel maintaining higher and more sustained levels of hydroxycinnamic and complex phenolic acids, notably rosmarinic and chlorogenic acids, across developmental stages. This sustained phenolic investment in the peel underscores its role as a primary defensive barrier, supporting prolonged antioxidant capacity and protection against oxidative and environmental stress during maturation. Collectively, these findings indicate that while phenolic downregulation during ripening may represent a conserved feature of pitaya pulp development, the peel adopts a differentiated metabolic strategy, emphasizing phenolic-based defense throughout fruit ontogeny.

The MFA further supports the existence of coordinated and tissue-dependent antioxidant strategies during *S. megalanthus* fruit development, as illustrated in Figure 4. The integrated structure observed for pulp and peel indicates that phenolic compounds and antioxidant enzymes are functionally interconnected and jointly modulated along development, rather than responding independently. In the pulp (Figure 4A), the multivariate associations suggest a progressive rebalancing of redox control, in which enzymatic antioxidant activity predominates at earlier stages and is gradually complemented by phenolic compounds as ripening advances. In contrast, the peel (Figure 4B) displays a tighter coupling between ROS-scavenging enzymes and phenolic acids, consistent with its protective function and continuous exposure to oxidative challenges. This stronger integration in the peel reinforces its role as a primary defensive barrier, relying on both enzymatic and non-enzymatic antioxidant components to maintain redox homeostasis throughout maturation. Overall, the MFA highlights that fruit development is accompanied by coordinated adjustments within antioxidant networks, emphasizing the importance of considering enzymatic systems and secondary metabolites as part of an integrated biochemical response rather than isolated traits.

The progressive increase in soluble solids and individual sugars reflects the intense metabolic activity associated with fruit ripening. The continuous accumulation of sugars throughout *S. megalanthus* development is consistent with the physiological process of reserve degradation, such as starch, which favors the accumulation of free sugars at advanced developmental stages. Similar trends have been reported for yellow pitaya fruits, where starch conversion into soluble sugars becomes more pronounced during ripening [2]. Compared with other pitaya cultivars, the sugar profile of *S. megalanthus* is characterized by higher accumulation of simple sugars and disaccharides, particularly sucrose. Chromatographic data reported for *S. undatus* and *S. costaricensis* indicate lower sugar concentrations at comparable developmental stages [31]. Although higher absolute sugar values have been reported for *S. megalanthus* in studies harvesting fruits at later stages, differences among studies may be attributed to harvest timing, genetic background, edaphoclimatic conditions, and methodological factors [2]. Nevertheless, the results obtained here confirm sucrose predominance and the natural accumulation of sugars during maturation of *S. megalanthus* cultivated in Minas Gerais, Brazil, highlighting its potential for fresh consumption.

Although ethylene and abscisic acid (ABA) are recognized as central regulators of fruit ripening in fleshy fruits [13], their roles differ substantially between climacteric and non-climacteric species. In non-climacteric fruits, ripening progression is not driven by a pronounced ethylene burst but rather by progressive metabolic reorganization. Previous studies on pitaya species have described developmental changes characterized by gradual

shifts in sugar accumulation, firmness, and biochemical composition rather than climacteric ethylene-dependent behavior [4,6]. In this context, the coordinated modulation of carbohydrate-metabolism enzymes, antioxidant systems, and cell wall-remodeling proteins observed here suggests that hormonal signaling may operate upstream of the metabolic adjustments identified, even though hormone levels were not directly quantified in the present study.

The detection of key gluconeogenic and glycolytic enzymes, together with enzymes from the Krebs cycle, indicates a dynamic metabolic flux in which glucose synthesis, degradation, and storage occur simultaneously to meet the increasing energetic and biosynthetic demands of fruit development. The higher accumulation of gluconeogenic enzymes at the initial stages, combined with the early accumulation of SuSy, suggests that sugar metabolism is particularly active during early development, supporting both growth and reserve formation.

MIPS is an enzyme that irreversibly isomerizes D-glucose-6-phosphate into 1D-myoinositol 3-phosphate, which is subsequently converted into MYO through the action of IMP [32]. Although seven isoforms of inositol exist in nature, MYO is the most abundant and predominant form. This cyclitol plays a vital role in plant physiology and biochemistry, acting as a precursor for various structural and signaling molecules involved in the regulation of numerous metabolic processes. MYO is associated with carbohydrate metabolism, auxin biosynthesis, uronic acid and pentose biosynthesis, mineral nutrition, membrane biogenesis, and cell wall formation [32]. It is also essential for the structure of several lipid signaling agents that regulate complex cellular signaling pathways, such as stress responses, ascorbic acid biosynthesis, and auxin perception [33]. Rebecca, Boyce, and Somasundram [34] isolated and identified MYO crystals in the pulp of red pitaya (*Hylocereus polyrhizus*). Although MYO was not directly monitored in the present study, the presence of MIPS and IMP enzymes suggests the biosynthesis of MYO in pitaya pulp as a secondary metabolite involved in multiple physiological processes. Xie et al. [2] identified inositol contents in the pulp of yellow pitaya with white pulp, ranging from approximately 6 to 4 mg g⁻¹ in intermediate and mature fruits, respectively. The higher accumulation of MIPS and IMP enzymes observed between stages S2 and S4 likely reflects periods of intense biosynthetic activity, corresponding to phases of marked physical and chemical development of the fruit. In contrast, stage S5 is associated with fruit ripening, during which biosynthetic reactions typically decline.

The clustering pattern observed in the pulp PPI network indicates a strong tendency for glycolytic- and protein-folding-related proteins to interact with each other. This suggests coordinated production and functional integration of these proteins to ensure the efficiency of the biological processes they regulate or participate in, particularly those related to energy metabolism and protein homeostasis. In the peel, the strong interactions among clusters associated with protein folding, ROS detoxification, and auxin signaling highlight the close functional relationship among these biological processes. The interaction between ARF7 and members of the Aux/IAA family supports the coordinated regulation of auxin responses in yellow pitaya. Together, these interactions indicate that the simultaneous presence of these proteins is likely required to mediate auxin-responsive processes and maintain cellular homeostasis during fruit development.

Taken together, the integrative proteomic and metabolomic framework applied in this study provides a foundation for future investigations aimed at linking molecular regulation with physiological performance and postharvest behavior of *S. megalanthus* fruits. The coordinated modulation of antioxidant systems, phenolic metabolism, carbohydrate pathways, and regulatory proteins along development raises testable hypotheses regarding how specific developmental stages may differentially influence fruit resilience, shelf life,

and nutritional quality after harvest. From an applied perspective, the identification of stage-dependent metabolic signatures offers opportunities to refine harvest timing and postharvest handling strategies, particularly for minimizing softening, oxidative degradation, and quality losses. Future studies combining targeted metabolomics, hormone profiling, and postharvest stress assays may further clarify how these biochemical networks respond to storage conditions and abiotic challenges. More broadly, this systems-level approach contributes to advancing knowledge in fruit physiology, fruticulture, and horticulture by demonstrating how integrated omics analyses can support both fundamental understanding of fruit development and the design of strategies aimed at improving quality and sustainability in pitaya production chains.

Although this study provides comprehensive insights into the proteomic and metabolomic dynamics of *S. megalanthus* during development, some methodological limitations should be acknowledged. Hormonal profiling was not performed, preventing direct assessment of ABA- and ethylene-mediated regulation. Additionally, proteomic detection is inherently influenced by protein abundance and extraction efficiency, which may limit the identification of low-abundance regulatory proteins. Future studies integrating transcriptomics, targeted hormone quantification, and functional validation assays would further refine the molecular framework proposed here.

From a practical perspective, the identification of stage-dependent metabolic signatures and peel-specific regulatory mechanisms provides valuable information for optimizing harvest timing, improving postharvest management strategies, and supporting the development of value-added products based on pulp and peel fractions.

5. Conclusions

This study provides an integrated molecular perspective on *Selenicereus megalanthus* fruit development by combining proteomic and metabolomic approaches across distinct maturation stages. The results demonstrate that fruit growth and ripening are accompanied by coordinated and tissue-specific biochemical adjustments involving antioxidant systems, carbohydrate and myo-inositol metabolism, transcriptional regulation, and cell wall remodeling. Clear differences between pulp and peel highlight contrasting metabolic strategies, with the peel exhibiting greater proteomic dynamism and sustained investment in defense- and structure-related pathways throughout development. These findings reinforce the view of the peel as a metabolically active tissue rather than a passive byproduct. Although this study provides a comprehensive integrative analysis of proteomic and metabolomic dynamics, it remains exploratory in nature, as functional validation experiments and direct quantification of hormonal regulators were beyond its scope. Overall, this work advances the understanding of yellow pitaya developmental biology and provides a robust biochemical framework to support future studies in postharvest physiology, horticultural quality, and the sustainable valorization of pitaya tissues within integrated production systems.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae12030303/s1>, Compounds (HPLC). Table S1: Differentially accumulated proteins in the pulp. Table S2: Differentially accumulated proteins in the peel.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

AOAC	Association of Official Analytical Chemists
APX	Ascorbate peroxidase
ARF	Auxin response factor
Aux/IAA	Auxin/indole-3-acetic acid proteins
BTAf1	TATA-binding protein-associated factor 1
BGAL 14	β -Galactosidase 14
CAT	Catalase
CHS	Chalcone synthase
CS	Cellulose synthase
Da	Dalton
EC	Enzyme Commission number
ENO	Enolase
FW	Fresh weight
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GC-MS	Gas chromatography–mass spectrometry
GR	Glutathione reductase
GRF	Growth-regulating factor
GT47	Glycosyltransferase family 47
HPLC	High-performance liquid chromatography
HPXDH	Hydroperoxide dehydratase
HSP	Heat shock protein
IMP	Inositol monophosphatase
LC	Liquid chromatography
MDHAR	Monodehydroascorbate reductase
MFA	Multiple factor analysis
MIPS	Inositol-3-phosphate synthase
MYB	Myb-like transcription factor
MYO	Myo-inositol
ND	Not detected
PCA	Principal component analysis
PE	Pectinesterase
PEPC	Phosphoenolpyruvate carboxylase
PGK	Phosphoglycerate kinase
PGIP2	Polygalacturonase inhibitor 2

PME	Pectin methylesterase
PPI	Protein–protein interaction
PX	Peroxidase
RID	Refractive index detector
ROS	Reactive oxygen species
SS	Soluble solids
SOD	Superoxide dismutase
STRING	Search Tool for the Retrieval of Interacting Genes/Proteins
SuSy	Sucrose synthase
TBL	Trichome birefringence-like protein
TF	Transcription factor
TPI	Triosephosphate isomerase
UniProt	Universal Protein Resource
UV-Vis	Ultraviolet–visible (detector/spectroscopy)

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