

FINAL PROJECT REPORT

Project Title: Metabolomics: characterizing fruit with chemistry

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Other funding Sources

Agency Name: USDA-ARS
Amount requested or awarded:
Notes: ARS provides a permanent, full-time GS-9 Biological Sciences Technician and analytical instrumentation.

Total Project Funding: \$47,520

Budget History:

Item	2008		
Salaries	0		
Benefits	0		
Wages ¹	23,000		
Benefits	10,000		
Equipment	0		
Supplies ²	5000		
Travel	0		
Miscellaneous ³	9520		
Total	47,520		

¹ Time-slip employees

² Consumables, analytical standards

³ Instrument maintenance and repair

Objectives:

1. Characterize metabolites (phytochemicals) (500+ expected) that are detectable in apple fruit.
2. Generate an analytical library to rapidly identify apple metabolites (phytochemicals).
3. Apply the analytical library to characterize how storage duration, 1-MCP, and diphenylamine impact apple fruit metabolites (phytochemicals).

Significant Findings:Objectives 1 and 2.

1. 600+ apple peel phytochemicals were characterized including many that contribute to quality aspects including aroma, sweetness, sourness, nutritive value, color, and physiological disorders.
2. Chemical identifiers for peel phytochemicals characterized in the study were compiled in an analytical library to reference in future research.

Objective 3.

1. Changes in apple peel chemistry resulted from storage initiation, storage duration, and 1-MCP or DPA treatment.
2. 1-MCP treatment slowed or prevented changes in peel chemistry.
3. Peel from DPA treated apples contained more typical flavor phytochemicals, ethylene precursors, and novel putative antioxidants compared to untreated controls.
4. Differences in peel chemistry between controls and DPA-treated fruit were detectable 8 weeks and differences in some individual phytochemicals detectable 10 weeks prior to the onset of visible scald suggesting these phytochemicals may be useful for early scald diagnostic tests.
5. Changes in peel chemistry resulting from biochemical stress that leads to scald were far more extensive than previously expected.

Results and Discussion:*Biochemical characterization and library construction*

Chemical profiling of apple peel uncovered a very broad collection (600+) of known, newly discovered (in apples), and unknown metabolites. To evaluate a more complete set of phytochemicals, three different analysis procedures were employed (Fig. 1). Phytochemicals found and routinely measured included volatiles (apple aroma), sugars, acids, amino acids, pigments, vitamins, antioxidants, and compounds involved in energy storage, signal transduction, and building other phytochemicals. The results provided a more complete picture of changes in peel chemistry occurring during storage compared to previous work focusing on a few phytochemicals or groups of phytochemicals.

An objective of the current project was generation of apple phytochemical libraries using specialized software (AMDIS, NIST05, Chemstation) that “cleans up” analytical data for each metabolite and stores it in a format that can be used for future research. These libraries contain reference information from all of the phytochemicals characterized in this study. Progress was made laying some of the groundwork for compiling an on-line metabolic library using the data generated in this study for apples and other Rosaceous crops in Genomic Database for Rosaceae in cooperation with Dr. Dorrie Main. This information may aid biochemical and genetic comparisons to develop breeding strategies as well as fruit quality and storability research.

Broad or “comprehensive” phytochemical evaluation of fruit provides a means for understanding some of the changes occurring in response to production and postharvest practices that lead to desirable or undesirable characteristics in our fruit. Using these extensive analytical techniques leads to a better understanding of storage-related apple fruit biochemistry that is expected to be useful for developing new postharvest tools, or suggesting new areas of fruit chemistry that are related to edible quality or disorders. Developing comprehensive phytochemical evaluation

techniques and libraries, something that has not been previously accomplished in apple, pear, or cherry, is crucial to the work performed in this and later studies that generate new information related to apple storage and superficial scald.

DPA, 1-MCP, and storage duration alter apple peel chemistry

Comprehensive phytochemical evaluation techniques proved useful for distinguishing different treatments and effects of storage duration in ‘Granny Smith’ apples treated with 2000 ppm DPA, 1 ppm 1-MCP, or washed with water (untreated) and stored for up to 6 months in air at 33 °F. Peel was sampled at 0, 1, 2, and 4 weeks and 2, 3, 4, and 6 months. The apple peel chemistry of 1-MCP treated peel was immediately different from those of the control and DPA treated fruit (Fig. 2). 1-MCP treated peel was most similar to that of pre-storage fruit, although changes were still considerable. The peel chemistry of the control and DPA treated fruit were different around 4 weeks and increasingly so at longer storage durations.

1-MCP, but not DPA, reduced ripening. Phytochemicals associated with the ethylene pathway and non-ethylene volatiles were strongly associated with longer storage durations and less so in the 1-MCP treated fruit. Conversely, less ripe fruit had more malic acid (responsible for sourness), certain amino acids, volatiles associated with “green” fruit and many other phytochemicals. Unlike levels of other amino acids, isoleucine, a building block of an important class of volatiles associated with ripe apples, increased with volatile production and ripening demonstrating the utility of our method for looking at many aspects of phytochemical production simultaneously instead of just the end products.

Comprehensive phytochemical profiling demonstrated its worth as a tool for providing a more complete picture of chemical changes occurring during apple storage than would be gained using more directed techniques. While control and DPA treated fruit may have not physically looked different in any way at 1-2 months, phytochemical differences were present well before visible symptoms of scald were observed. Following a broad array of phytochemicals rather than just volatiles or sugars and acids provides new areas to explore that may potentially lead to new tools for monitoring apple condition and storability much like chemical profiling tools commonly employed for monitoring human health.

Superficial scald and the apple peel chemistry

Clear differences in peel chemistry between control and DPA treated fruit were observed following 1 month of storage and the patterns continued to diverge until 6 months (Fig. 2). Scald incidence (rated 1-4; 1 = 0%, 2 = less than 25%, 3 = less than 50%, and 4 = greater than 50%) was also rated at all sampling periods (Fig. 3). Slight scald symptoms appeared at 3 months storage, approximately 2 months following detection of differences in apple peel chemistry. In a parallel experiment, ‘Granny Smith’ apples were treated with 2000 ppm DPA at harvest or following 1, 2, or 4 weeks or 2 months cold storage to determine when DPA treatment became ineffective. Delayed treatments after 1 or 2 weeks were partially effective, while treatment after 4 weeks, the point where peel chemistry began to differ, was not (Fig. 2). This suggests that a significant amount of the biochemical events that lead to scald development occurred prior to 4 weeks storage. In effect, 2-4 weeks was the “point of no return” for scald development.

Statistical data mining techniques identified phytochemicals most associated with the different phases of scald inception and development. Major groups of phytochemicals were associated with untreated (scalded peel or peel that would develop scald) or DPA treated (unscalded) fruit. The presence of many phytochemicals that increased in peel from untreated fruit appeared to result from biochemical stress that changes chemical structure and, potentially, function. Likewise, compounds associated with DPA treated fruit decreased in untreated peel during storage. While typical volatiles increased in DPA treated apples, different volatile phytochemicals, that may affect flavor, were produced in scalded tissue. Other compounds with antioxidant activity were more

prevalent in DPA treated fruit as storage duration increased indicating their depletion in the control fruit may be related to oxidative biochemical stress.

Individual phytochemicals that changed prior to actual symptom development were uncovered. These groups included more than 51 phytochemicals whose fluctuations could potentially be used to diagnose peel that will develop scald up to 10 weeks prior to appearance of the symptoms. Differences between individual scald-associated phytochemicals in control and DPA treated peel increased or decreased and other phytochemicals continued to appear up to 6 months storage.

In a parallel experiment, the wax layer, cuticle layer, and live epidermal and hypodermal cells were analyzed separately to localize phytochemicals within the entire peel structure. Many phytochemicals associated with scald-induction are in live epidermal and hypodermal cells, where changes would more likely lead directly to tissue death and browning. Given the nature of these phytochemicals, associations with crucial tissue functions such as structural integrity and cellular communications are possible.

Conclusions and future directions

Comprehensive profiling of 600+ apple peel phytochemicals proved a valuable tool for discovering key changes in apples affecting quality and storability. By understanding these changes, it may be possible to develop tools that predict quality changes before they occur and reduce losses during storage or marketing.

Further research examining factors influencing scald development (harvest maturity, cultivars, storage environments) are needed to validate which phytochemicals are effective markers. Similarly, identification of changes in apple peel chemistry that cause scald will identify phytochemicals and genes that could also be used as benchmarks for breeding new cultivars that are not susceptible to scald.

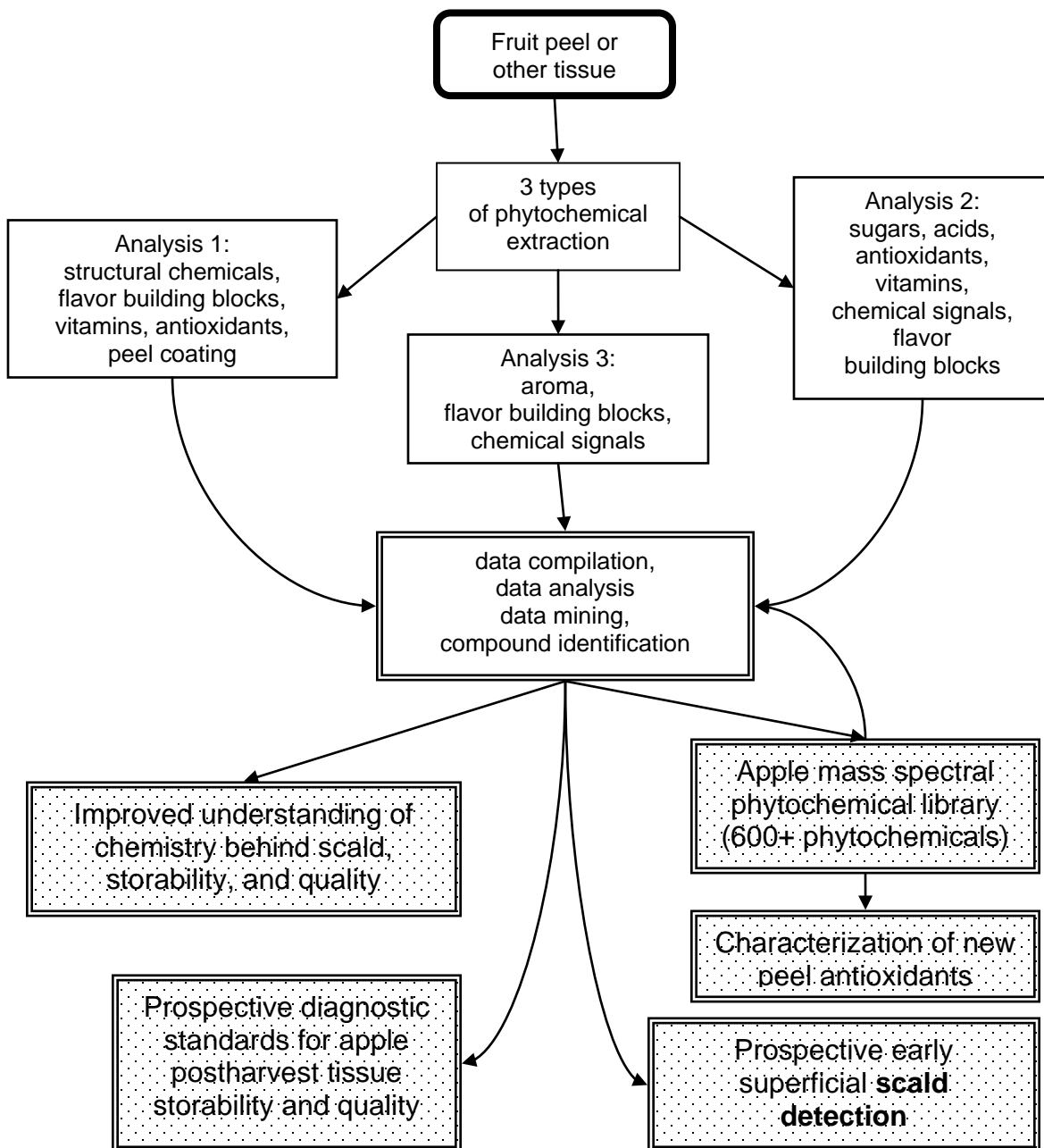


Fig. 1. Peel sample processing, sample analysis, data compilation/analysis, and experimental outcomes flow chart.

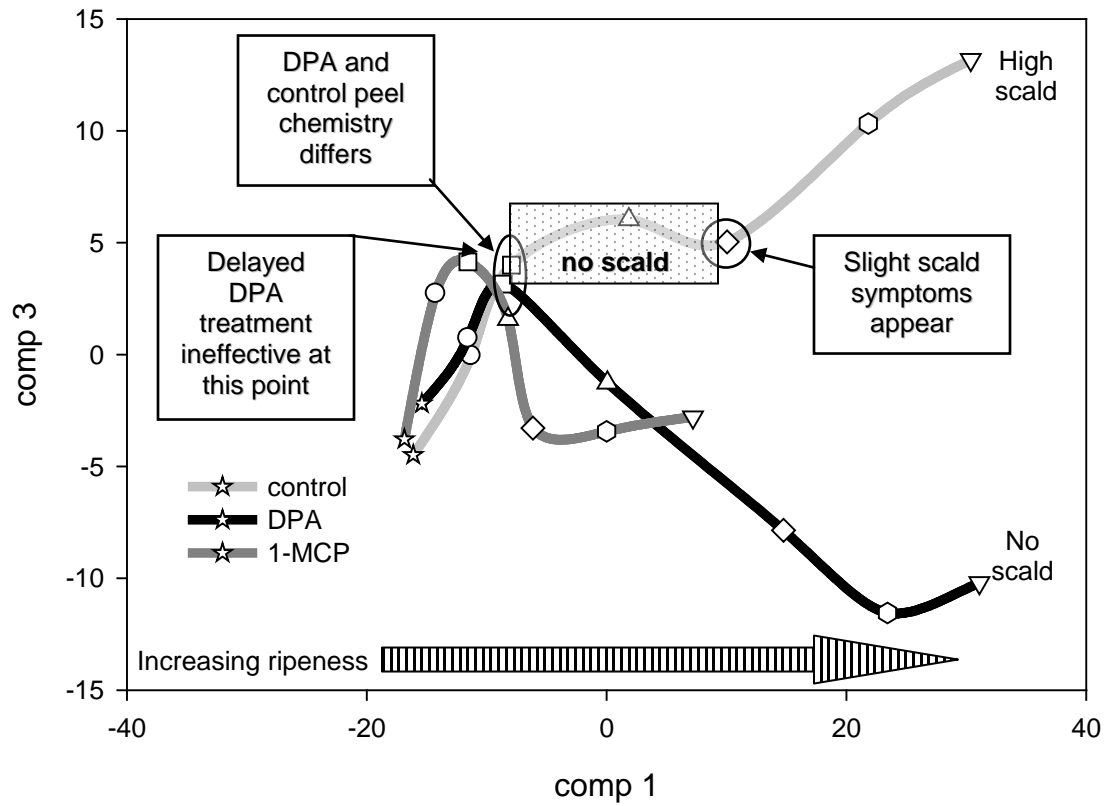


Fig. 2. Graph (PCA scores plot) reflecting total peel chemistry changes with respect to relative ripeness and scald development. After harvest apples were treated with 1 ppm 1-MCP, 2000 ppm DPA, or untreated. 600+ phytochemicals were evaluated in peel sampled from each treatment at 1 week (**star**), 2 weeks (**circle**), 4 weeks (**square**), 2 months (**triangle**), 3 months (**diamond**), 4 months (**hexagon**), and 6 months (**inverted diamond**) of 33°F air storage.

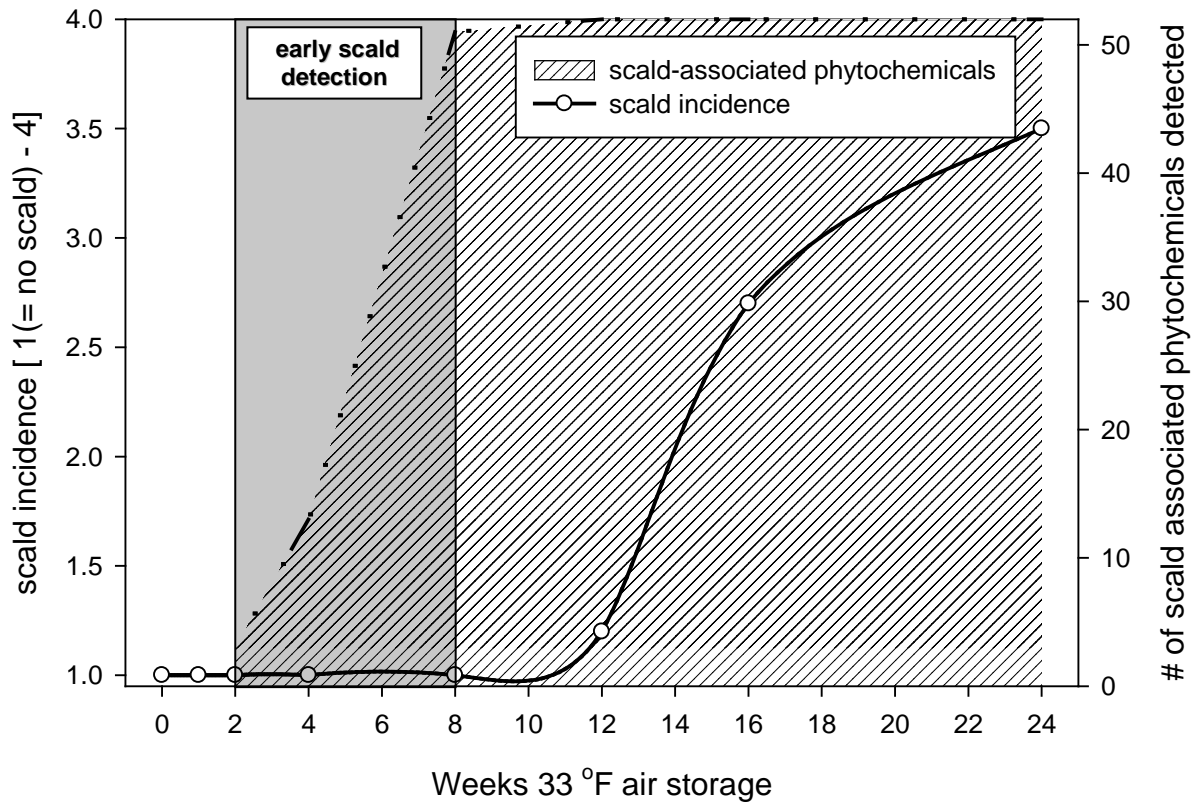


Fig. 3. Number of prospective early scald diagnostic markers compared to actual scald incidence. Differences in some prospective scald markers were found as early as 2 weeks after harvest increasing to 51 at 8 weeks of storage. Scald symptoms were slight at 12 weeks storage.

Metabolomics: Characterizing Fruit with Chemistry (*Rudell*)

Executive Summary

Project outcomes:

1. Prospective diagnostic markers for early superficial scald detection.
2. An apple fruit mass spectral phytochemical library containing descriptions of 600+ individual phytochemicals.
3. Partial characterization or identification of novel putative antioxidants and various flavor and quality related phytochemicals.
4. Improved understanding of the peel chemistry behind superficial scald, storability, and quality to direct and expedite future research.

Significant Findings:

1. 600+ apple peel phytochemicals were characterized including many that contribute to quality including aroma, sweetness, sourness, nutritive value, color, and physiological disorders
2. Chemical identifiers for peel phytochemicals characterized in the study were compiled in an analytical library to reference in future research.
3. Changes in apple peel chemistry resulted from storage initiation, storage duration, and 1-MCP or DPA treatment.
4. 1-MCP treatment slowed or prevented changes in peel chemistry.
5. Peel from DPA treated apples contained more typical flavor phytochemicals, ethylene precursors, and novel putative antioxidants compared to untreated controls.
6. Differences in peel chemistry between controls and DPA-treated fruit were detectable 8 weeks and differences in some individual phytochemicals detectable 10 weeks prior to the onset of visible scald suggesting these phytochemicals may be useful for early scald diagnostic tests.
7. Changes in peel chemistry resulting from biochemical stress that leads to scald were far more extensive than previously expected.

Future directions:

1. Continue to find peel phytochemicals that link scald to cultivar, harvest maturity, CA storage, and other factors.
2. Continue to identify phytochemicals that are important to fruit maturation, ripening, and scald.
3. Identify phytochemical/gene associations that trigger or control scald for use as harvest/storage management and breeding selection tools.
4. Evaluate prospective early superficial scald diagnostic markers for use as storage management tools.
5. Use apple peel chemistry to increase the understanding of scald and related disorders to find pre-harvest predictive tests.