**FINAL REPORT**

**Project title:** Use of Electronic Nose to Evaluate Aroma Quality of ‘Gala’, ‘Fuji’, ‘Delicious’ and ‘Granny Smith’ Apples Treated by 1-MCP and Stored in Controlled Atmosphere

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**Objectives:**
The goals of this 4-year project were extended gradually from developing alternative apple coatings to three additional goals: (1) Evaluate flavor of major apple varieties using GC and electronic nose during CA storage and simulated marketing for documentation of changes due to storage time, temperature, atmosphere, and 1-MCP pre-treatment (to determine flavor life as opposed to shelf life); (2) Develop fresh-cut apple technologies, including anti-browning, coating and pre-treatment before cutting; and (3) Continually develop and evaluate alternative apple coatings for major apple varieties.

**Significant findings:**
1. In previous year, we had worked on evaluation of flavor responding to storage time, atmosphere, and 1-MCP pretreatment in ‘Gala’ apple.  
   1). Controlled atmosphere significantly extended shelf life by maintaining high penetrometer reading (higher than 60 N after 3 months in CA storage, compared to < 60 N within 1 month in regular air [RA] storage). 1-MCP pre-treated ‘Gala’ apple showed firm texture during the entire 6-month storage period, although firmness was greater in CA than in RA.  
   2). Treatments that retarded softening also inhibited apple volatile (headspace GC) development compared to controls (RA). Canonical discriminant analysis of electronic nose data showed clear separation of controls + RA (non-pretreatment controls, and regular atmosphere storage), controls + CA, MCP + RA and MCP + CA.  
   This means that pre-treatment with MCP, storage in CA or both resulted in reduced volatile levels, but increased firmness compared to non-MCP treated fruit or RA storage.  
2. Significant findings for the last three years:  
   1). Developed and evaluated several alternative apple coatings.  
      - High gloss and low gas resistance coatings were developed from polyvinylacetate (PVA, a component of chewing gum).  
      - High gloss and high gas resistance coatings were developed from starch.  
      - Medium-low gloss and medium gas resistance coatings were developed from candelilla wax.  
      - Coatings were developed from corn protein (zein) with a wide range of gloss, whitening susceptibility, and gas resistance. This was accomplished by regulating zein and/or propylene glycol (PG) content. An optimal formulation had similar gloss duration, less whitening, and better gas-barrier properties than a commercial shellac product.  
      - Commercial (Shellac and carnauba wax) and experimental coatings (PVA, starch, candelilla and zein) were evaluated for shine, gas resistance, whitening, and effect on apple flavor and firmness (Bai et al., 2002a,b).  
   2). Developed fresh-cut apple technologies.  
      - 1-MCP, heat and ethanol vapor were used for pre-treatment of intact apple and the heat and ethanol pretreatments improved cut production stability (Bai et al., 2001).  
      - A dip solution for sanitation, anti-browning and flesh firming was developed as a post-cut apple treatment.
Several coatings were developed and tested on cut fruit using soybean oil emulsion, chitosan and carboxymethyl cellulose (CMC) (Bai et al., 2002f).

3. Apple flavor was evaluated for effect of 1-MCP pre-treatment, CA storage, and coatings.
- 1-MCP and CA/cold storage inhibited ‘Gala’ apple volatile production, and the effects could not be recovered by removing the fruit to regular air and ambient temperature.
- Proper post-storage coating treatments increased post-CA stored apple volatiles by altering fruit metabolism and trapping volatiles, thus building up their concentrations (Bai et al., 2002d).
- Canonical discriminant analysis of electronic nose showed a strong relationship with headspace GC data.

Methods:
1. Apple flavor evaluation: effect of 1-MCP pre-treatment, storage time, and atmosphere.
   Apples were pre-treated, stored, and shipped by Federal Express by Jim Mattheis from Wenatchee Washington.
   Freshly harvested, CA- or RA-stored ‘Gala’, ‘Delicious’, ‘Fuji’, ‘Braeburn’ and ‘Granny Smith’ were used. The flavor quality of fruit, +/- pre-treatment with 1-MCP were evaluated monthly during CA or RA storage and subsequent marketing by GC and electronic nose. Penetrometer reading, soluble solids content (measured by refractometer), and titratable acidity (using titrator with NaOH) were also measured.

2. Formulation of Coatings:
   Several experimental coatings were formulated. For starch, polyvinyl acetate (PVA), and candelilla wax coatings; emulsions or micro-emulsions were formulated with all components approved by FDA. The soluble solids content was about 20%. For zein coatings, 4-15% solutions were formulated with varying levels of PG (food grade plasticizer) that resulted in different levels in gloss, whitening, and gas permeability.

3. Evaluation of Coatings:
   Apples were commercially stored or freshly-harvested and shipped to Florida in refrigerated Publix Supermarket trucks.
   Both freshly harvested and stored fruits were coated with several experimental shiny or non-shiny coatings, stored under chilled and subsequent simulated marketing (ambient) conditions. The appearance of fruit was evaluated by a gloss reflectometer and sensory panel. Some fruit were submitted to a humidity chamber to evaluate whitening. Internal gases and volatiles were also measured and taste/texture evaluated by a sensory panel.

4. Fresh-cut apple:
   Five varieties of apples were harvested, pre-treated with 1-MCP, and shipped by Federal Express to Florida by Jim Mattheis from Wenatchee Washington.
   Intact apples were pre-treated with 1-MCP (in Wenatchee), heat or ethanol vapor in Winter Haven, FL, prior to cutting. Apple slices were then dipped into a sanitizing solution (chlorine or ethanol), a solution of anti-browning and firming agents +/- film-former (coating), drained, packaged in perforated plastic bags, and put in storage at 7°C.

Results and discussion:
1. Apple flavor evaluation: use of electronic nose for evaluation of aroma quality
   1a). Effect of 1-MCP pre-treatment, storage time, and atmosphere for ‘Gala’ apple.
   Controlled atmosphere significantly extended shelf life as determined by fruit firmness (maintenance of high penetrometer readings: > 60 N after 3 months in CA storage, compared to
1-MCP pre-treated ‘Gala’ apple showed firm texture for the entire 6-month storage period, although higher in CA than in RA. However, the treatments that retarded softening also inhibited apple volatile (headspace GC) development. Canonical discriminant analysis of electronic nose data showed clear separation among control + RA (non-pretreatment check, and regular atmosphere), control + CA, MCP + RA and MCP + CA. Samples have been prepared, analyzed for firmness and otherwise frozen for continued analysis from the other four varieties (‘Braeburn’, ‘Fuji’, ‘Red Delicious’, and ‘Granny Smith’), and will continue to be shipped to Winter Haven until the end of June. Therefore, the status of these samples cannot be reported at this time (see accompanying proposal).

1b). Coating resistance, apple varieties and flavor maintenance
Five experimental coatings with different resistance to gas exchange were used with freshly harvested and 20-week commercially pre-stored apples of ‘Delicious’, ‘Fuji’, ‘Braeburn’ and ‘Granny Smith’ varieties. The coated or non-coated apples were held at 20°C for up to 4 weeks, simulating commercial marketing conditions. The gas partial pressures inside the fruits for the various coatings ranged from 1 - 25 kPa CO₂ and 20 - 1 kPa O₂. Volatile composition, concentration and evaporation rates were measured during storage at 20°C. The coatings with intermediate gas resistance (carnauba-shellac mixture and candelilla wax) gave intermediate values of CO₂ and O₂ in the internal fruit atmosphere for ‘Delicious’, ‘Fuji’, and ‘Braeburn’ apple, and the highest concentrations of desirable volatiles, butyl acetate and 2-methylbutyl acetate, in the fruits. The coatings with highest gas resistance (shellac and shellac-protein) caused high internal CO₂, low O₂, resulting in anaerobic fermentation in ‘Braeburn’ and ‘Granny Smith’ apples. These coatings also resulted in excessive low molecular weight ethyl esters, trapped within the fruit. A small portion of the alcohols were evaporated from fruits compared to esters, this attributed to their high Henry’s law coefficients (Bai et al., 2002a,d).

2. Alternative coatings formulation and evaluation
2a). Comparison of several experimental coatings
Zein, starch, polyvinyl acetate (PVA), carnauba wax, and carnauba-polysaccharide (CPS) coatings were applied to ‘Delicious’ apples after removal from controlled atmosphere storage and compared with a commercial shellac coating. Coated apples were stored in air at 2 °C for two weeks and then removed to 21 °C for an additional two weeks to simulate marketing conditions. Gloss, internal O₂ and CO₂ partial pressures, weight loss, flesh firmness, and contents of sugars, acids and volatiles were measured on 0, 2 and 4 weeks after coating treatment. Starch- and carnauba-coated apples had high initial gloss, similar to that found for shellac-coated fruit. Gloss of all coated fruit decreased similarly during the 4 week evaluation period, although all of the coated fruit were glossier than uncoated controls. For uncoated apples, the differences of O₂ and CO₂ partial pressure between internal and ambient atmosphere were approximately 1 kPa at 2 °C, and increased by a further 2 kPa after transfer to 21 °C. Fruit coated with shellac and starch had more than 10 kPa CO₂, and less than 10 kPa O₂ at 21 °C. Zein-, PVA- and carnauba-coated apples showed a less modified internal atmosphere (6-7 kPa CO₂, 11-15 kPa O₂). Internal partial pressures of O₂ and CO₂ were inversely related for most coatings, except for the CPS coating, for which partial pressures of both CO₂ and O₂ were low. Carnauba-, PVA- and shellac-coated fruit lost less weight than uncoated fruit. Starch-, shellac-, and CPS-coated fruit were firmer than those from other coating treatments, and all coated fruit were firmer than uncoated control. Titratable acidity was higher in the fruit coated with CPS, starch, and shellac than in uncoated control. Ethyl alcohol and ethyl esters accumulated in
starch-, shellac-, and CPS-coated fruit kept at 2 °C, but, levels of these volatiles decreased after transfer of fruit to 21 °C (Bai et al., 2002a).

2b). Zein coating formulations
For zein coating, further research was applied by changing zein and PG (plastisizer) contents. Coating gloss was dependent on PG and zein concentrations. At least 4% (by weight) PG, was necessary for adequate gloss, however increasing levels of both compounds resulted in increased gloss. Whitening occurred on the coated fruit surface upon wetting due to high zein contents in the formulations. This occurred after transfer of coated-fruit from low storage temperatures to ambient marketing conditions, resulting in condensation of water vapor on the cold fruit surface. The whitening was reduced by decreasing zein content to less than 11%. Permeability of CO₂, O₂ and water vapor was strongly dependent on the zein content in the coating. Internal gases of coated ‘Gala’ apple were modified to 3 to 12 kPa CO₂ and 19 to 5 kPa O₂, respectively, by increasing zein content in the coatings. The gloss levels on ‘Gala’ apple surface varied due to zein and PG content in coating formulations from that of controls to levels observed for shellac-coated fruit. This was also dependent on the amount of zein and PG in the coating. An optimum formulation with 10% zein and 10% PG was developed, applied to ‘Gala’ apple, and was found to extend shelf life as well as maintain overall fruit quality comparable to a commercial shellac coating. The advantage of using an alcohol solvent is the reduction in drying time, but the shortcoming is increased coating cost and an increase in volatile organic compounds (VOC’s) which can become a regulatory issue. A commercial water soluble zein, sold by Freeman Industries, L.L.C (Tuckahoe, NY) does not have the gloss and other coating properties suitable for coating of apple fruits (Bai et al., 2002b).

2c). Determination of appropriate coatings for different apple varieties
The gas permeabilities of shellac and several experimental coating formulations, including candelilla wax and shellac-carnauba were measured. These coatings, selected to have a very wide range of gas permeabilities, were applied to freshly harvested and 5-month commercially stored ‘Delicious’, ‘Fuji’, ‘Braeburn’ and ‘Granny Smith’ apples. The coated apples were monitored during storage of 2 or 4 weeks at 20 C for changes in internal gases level, respiration rate and other quality parameters (surface gloss, weight loss, flesh firmness, Brix, titratable acidity and ethanol content). The shellac coating resulted in maximum fruit gloss, lowest internal O₂, highest CO₂, and least loss of flesh firmness for all of the varieties. The ‘Granny Smith’ fruit coated with shellac had very low internal O₂ (< 2 kPa) with both freshly harvested and 5 month-stored apples. The freshly-harvested ‘Braeburn’ fruit had high internal CO₂ (25 kPa). This excessive modification of internal gas induced an abrupt rise of the respiratory quotient, prodigious accumulation of ethanol in both ‘Braeburn’ and ‘Granny Smith’, and flesh browning at the blossom end of ‘Braeburn’. In addition the shellac coating gave a substantial accumulation of ethanol in freshly harvested and 5 month-stored ‘Fuji’. Candelilla wax and carnauba-shellac coatings maintained more optimal internal O₂ and CO₂ levels, and resulted in better quality for ‘Fuji’, ‘Braeburn’ and ‘Granny Smith’ apples, although even these coatings may present too much of a gas barrier for ‘Granny Smith’. In general, the gas permeabilities of the coatings were useful as an indicator of differences in coating barrier properties, but did not account for differences in pore blockage. The polyethylene coating formed the lowest barrier for water vapor loss and the least resistance to CO₂ and O₂ exchange, as indicated by the relatively high values of internal O₂ and low internal CO₂, with no retardation of softening. Thus, coatings with very high permeability do not provide a sufficient barrier to gas exchange and water loss, except possibly for CA-stored ‘Granny Smith’. For ‘Fuji’ and ‘Braeburn’ fruit, the optimum coatings seem to be those with intermediate permeability, like the candelilla wax or carnauba-shellac. These avoided excessive build-up of ethanol, but helped somewhat to preserve firmness
and protect against weight loss. The ‘Delicious’ apples, especially those from CA storage, seemed to do well with shellac coating, which gave the most change in internal atmosphere without causing excessive ethanol production, and maintained firmness compared to other coating treatments. In general, the performance of coatings on apples was related to their O₂ and CO₂ permeabilities, with higher permeability resulting in lower internal CO₂ and higher internal O₂. However, this trend was reversed for two of the coatings, presumably because of differences in the ability of these coatings (carnauba-shellac and candelilla) to block pores in the fruit peel (Bai et al., 2002c).

3. Fresh-cut apple quality

3a). Pre-treatments of whole fruit affect cut-fruit shelf life

1-MCP pre-treatment. No ethylene production was detected in 1-MCP-pretreated slices for 7 days after slicing, but ethylene levels increased sharply by 14 days, in conjunction with severe deterioration and microbial growth. Respiration rate was inhibited by 1-MCP pretreatment during two weeks storage. However, dark mold pitting was exhibited by 11 days on both of pretreated and control slices, but was more severe on 1-MCP-pretreated slices. This corresponded to an increase in Chromameter a* and decrease in L* values by 14 days. Visual quality was scored using a scale of 9 = excellent, 7 = good, 5 = fair, 3 = poor, 1 = terrible. A score of 5 was considered the threshold of marketability. Both control and 1-MCP-pretreated slices lost shelf-life by 14 days, with the 1-MCP slices rated lower in visual quality than controls. Important aroma compounds, measured by headspace GC, in 1-MCP pretreated slices showed higher levels than control initially, but this was reversed one week after slicing. Control fruit generally lost volatiles from initial levels 7 days after slicing, probably due to off-gassing once the peel barrier was removed. The coating applied in this experiment turned out not to be a good barrier to gases, and may have been permeable to the aroma volatiles as well.

Heat pre-treatment. Ethylene production from heat-pretreated apple slices was very low: 0, 0.1 and 0.6 ul kg⁻¹ h⁻¹ at 0, 7 and 14 days, respectively, compared to 3.1, 3.7 and 5.2 ul kg⁻¹ h⁻¹ for control slices. Heat pretreatment of whole fruit also decreased respiration rate, generally delayed loss of flesh firmness (sig. 0 days), and increased L* values and visual shelf-life to 14 days of subsequent apple slices (sig. 14 days). Heat pre-treatment resulted in higher initial a* values than controls, but this difference could not determined by eye. However, levels of important aroma compounds were lower in heat-pretreated slices than controls after 7 days.

Ethanol pre- and post-treatments. In addition to ethanol vapor pre-treatment of intact fruit, a post-slicing ethanol dip treatment was conducted for comparison to vapor pre-treatment. Slices were dipped in 70% ethanol for 30 sec. prior to application of coating. Both of ethanol vapor pre-treatment of intact apple and ethanol dipping of slices generally decreased ethylene production and respiration rate. Ethanol-dipped slices lost marketability by 8 days, caused by ethanol-induced translucency. Control slices lost shelf life because of dark mold pitting by 10 days. However, ethanol vapor pretreated slices maintained visual quality, higher L* and lower a* values throughout the experiment. For aroma compounds, dipping slices in 70% ethanol immediately increased ethyl acetate and propanoate concentrations which remained elevated up to 14 days. Ethanol vapor pretreatment stimulated the production of most volatiles, especially ethyl esters, but inhibited butyl acetate production.

For fresh-cut ‘Gala’ apple, pre-treatment of intact fruit with heat is very effective for prolonging visual shelf-life of slices at the expense of aroma quality, while ethanol vapor is beneficial to both visual and aroma shelf life. Sensory studies need to be conducted, however, to confirm the effects of these pretreatments on cut slice flavor (Bai et al., 2002c).

3b) Post-processing dip maintains quality and extends the shelf life of fresh-cut apple

An aqueous solution with hypochlorite as a sanitizer; sodium erythorbate (isoascorbate), N-acetylcysteine and 4-hexylresorcinol as reducing and anti-browning agents; and Ca propionate as
A firming agent was developed for post-processing dip of fresh-cut ‘Gala’ apple. The additional effect of edible coating materials to the aqueous solution of additives was also investigated. The edible coating film-forming agents were soybean oil emulsion, chitosan and carboxymethyl cellulose (CMC), which were expected to form a protective layer on the cut surface of the apple wedges, decreasing water loss and other deteriorating factors due to cutting. Apple slices were dipped in aqueous solutions of sanitizer, with or without anti-browning and firming agents (additives), and with or without film-formers. Treated slices were then allowed to drain for 1 hour at 5.5 °C before placement in perforated polyethylene bags (20 X 18 cm, thickness 30 µm, with ten 1.5 mm holes) and stored at 5.5 °C for up to 14 days. Slices dipped in water (control, containing hypochlorite only) lost marketable quality within a day, because of severe browning accompanied by a sharp decrease of hue angle (h°ab), and lightness (L*), and an increase in a* and b* values. Slices dipped in the aqueous solution plus additives maintained cut surface color, inhibited ethylene production, maintained firmness, and maintained the major aroma of apple. However, these slices exhibited green mold after 8 days of storage. Addition of soybean oil emulsion reduced water loss, whereas chitosan and CMC did not, although water loss was not a problem for polyethylene packaged products. The wedges dipped in coatings had volatile levels similar to controls for desirable aroma compounds, but had less off-flavored volatiles (ethanol, acetaldehyde and ethylacetate) found in control slices. These results suggest that a dip with a sanitizer, firming agent, and reducing/anti-browning agents is beneficial of fresh-cut apple quality. Addition of film-formers did not retain important aroma compounds as well as the aqueous additive treatment, while chitosan did not reduce decay as has been reported for whole fruits (Bai et al., 2002f).

Budget
Total 3 years: $106,000

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1Whatever was not used in equipment or travel was used in supplies
2Travel $ were used for travel from Florida to Washington state to report results each year

Publications:
Bai, J., Baldwin, E.A. and Hagenmaier, R.H. 2002a. Alternatives to shellac coatings provide comparable gloss, internal gas modification, and quality for ‘Delicious’ apple fruit. Accepted for publication in HortScience 37:559-563.