**AGRICULTURAL RESEARCH FOUNDATION**

**FINAL REPORT**

**FUNDING CYCLE 2014 – 2016**

**TITLE:** Inhibition of Discoloration of Minimally Processed Apples and Potatoes Thru the Application of Plant Byproduct Extracts

**RESEARCH LEADER:** Michael H. Penner (Food Science & Technology, OSU, Corvallis)

**COOPERATORS:** This project had no formal co-investigators/cooperators.

**SUMMARY:** Initial work focused on the development of assay protocols for monitoring the activity of potato and apple polyphenol oxidases (PPO) in the presence of potential inhibitors. Such assays are essential for screening plant extracts and other potentially inhibitory compounds for their ability to limit fruit/vegetable browning. The results from this work included the development of a pH-jump method for the determination of PPO activity in potato and apple juices (see “Procedures” below). This assay allows us to obtain qualitative preliminary data as to the relative inhibitory nature of a variety of test solutions. The assay was used to assess the pH stability of potato PPO and the general inhibitory nature of sodium chloride. Acids, as related to pH, and salts are both being considered as ingredients for PPO inhibition. The incremental nature of the inhibitory/stability effects seen in these initial studies illustrated the need for more quantitative approaches. Thus, we began a systematic study of practical approaches for obtaining partially-purified PPO preparations appropriate for quantitative kinetic analyses. This work led to the development of a protocol for the preparation of PPO acetone powders. These PPO preparations have thus far proved appropriate for assessing inhibitory compounds. For example, the PPO acetone powder-based assays demonstrated that a small but significant time-dependent inhibition of potato PPO occurs as a result of the presence of an alkali extract of wheat straw. This type of data is useful in leading our future experiments aimed at enriching the inhibitory compounds. Similar experiments have quantified the effect of incorporating ultrasound treatments for PPO inactivation. This same assay system was also used to show that potato PPO differs in properties from the mushroom enzyme, in particular with respect to the phenomenon of substrate inhibition. This latter finding is significant because the mushroom enzyme is the one for which the most information is available and, thus, its relationship with the potato and apple enzymes is relevant for guiding future work. This project is ongoing. The primary objective of current and future work in this area remains the same as that for the ARF-funded research, to develop food-grade inhibitory systems that will retard discoloration of fresh-cut fruits and vegetables and thus decrease food waste.

**OBJECTIVES:** The principle objective of the work has been to develop an economically viable process for controlling browning of fresh-cut and minimally processed apple and potato products. Sub-objectives have included the characterization of the primary enzyme responsible for discoloration (*i.e.*, polyphenoloxidase) and the effect of food-relevant parameters on this enzyme’s activity.

**PROCEDURES:**

Assay #1 🡪 “pH jump technique” - The assay is based on preparing vegetable/fruit juices, via homogenization in appropriate buffers (200 mM sodium phosphate) at pHs for which the PPO enzyme is stable but inactive (similar to a resting state). Activity-modifying compounds of interest can then be added to the fruit/vegetable juices prior to initiating the reaction by instantaneously “jumping” the pH to one at which the enzyme is active. In this assay approach the soluble components of the original fruit/vegetable, e.g. potato or apple, are present during the assay.

Assay #2 🡪 “PPO acetone powders” - The key to this assay is the preparation of the enzyme-containing acetone powder. Several studies were done to determine appropriate pH, ionic strength, and solvent conditions. Optimum conditions are defined with respect to total enzyme recovery and maximum specific activity. The outcome of these studies is a method in which the PPO acetone powder is prepared by making the PPO containing fruit/vegetable juice 50% in acetone (V/V), at pH 5 and 50 mM sodium phosphate. This preparation is then used at mg per mL in phosphate buffer, with added activity-modifying compounds, to collect initial velocity enzyme kinetic data. In this assay approach the soluble components of the original fruit/vegetable are not present during the assay.

**SIGNIFICANT ACCOMPLISHMENTS:** To date, the research outcomes of greatest relevance are the two assay approaches developed for the screening of PPO inhibition. These methods will be described an upcoming publication and, thus, be available for application by others interested in this field. The rationale for assuming the methods per se are of greatest significance are based on the PI’s observation that major advances in scientific disciplines often occur as a result of advances in methodology.

**BENEFITS & IMPACT:** This research resulted in heretofore unavailable methods for the screening of compounds for their impact on fruit and vegetable discoloration. This advance is expected to allow higher throughput screening of compounds than previously possible. This, in turn will accelerate the rate at which economically-relevant food-grade inhibitors can be developed.

 **ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM:** None.

**FUTURE FUNDING POSSIBILITIES:**  Plausible future funding sources include the USDA NIFA program, yet-to-be-identified private organizations emphasizing sustainable agriculture and specialized commodity groups.