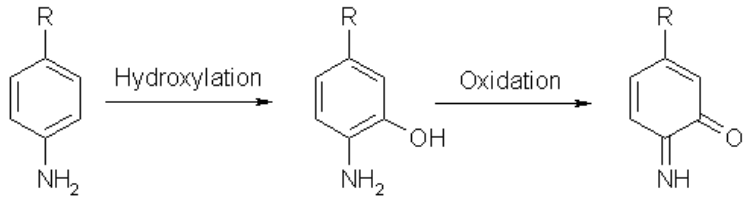
# CPPs in Polyphenol Oxidase Enzyme Catalysis

## Background

Polyphenol oxidase (PPO), a major contributor to the undesirable browning effects in fruits and vegetables (Martinez and Whitaker, 1995), catalyzes the reaction shown below.

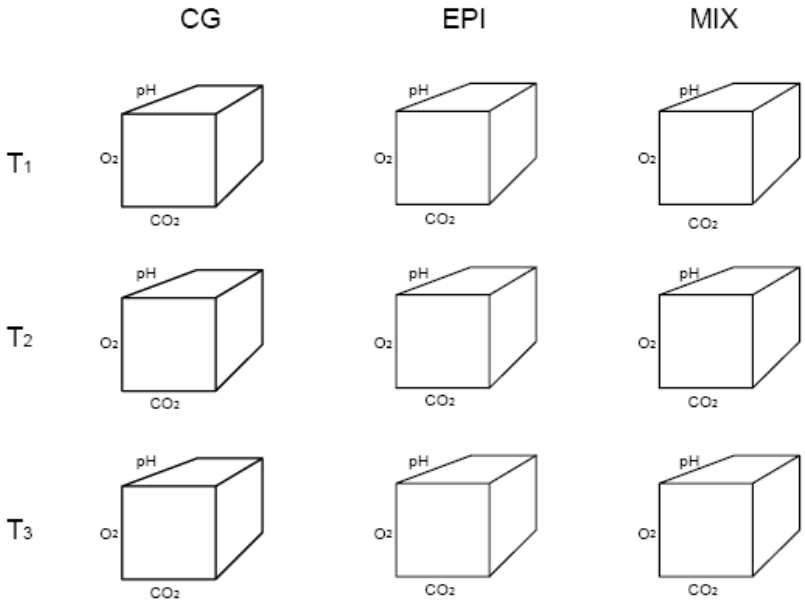



## Objective: Characterize Enzyme Performance Issues

Enzymatic browning in fruits and vegetables is directly proportional to PPO activity measured as the consumption of molecular oxygen. To minimize enzymatic browning during processing, fruits and vegetables must be maintained under conditions that suppress PPO activity. The objective in this illustrative study is to identify the critical process parameters and characterize the enzyme activity in terms of the critical process parameters.

## Performance Data: Summary

The experimental data used in the present case study was obtained from the literature (Bro, 1996; Heimdal et al., 1997) involving PPO obtained from fresh iceberg lettuce. PPO activity was measured by a polarographic PPO assay as nanomoles of O2 consumed per second. Studies were conducted under the following conditions: (a) five O2 levels (0, 5, 10, 20, 80%), three CO2 levels (0, 10, 20%), three substrate types (CG, EPI, Mix), three pH values (3.0, 4.5, 6.0), and three temperatures (5, 20, 30 degC).



A summary of the data is shown below:

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      pH        Subs          Temp            CO2             O2          Activity
 Min.   :3.0   CG :135   Min.   : 5.00   Min.   : 0   Min.   : 0   Min.   :  0.00
 1st Qu.:3.0   EPI:135   1st Qu.: 5.00   1st Qu.: 0   1st Qu.: 5   1st Qu.:  3.00
 Median :4.5   MIX:135   Median :20.00   Median :10   Median :10   Median : 19.00
 Mean   :4.5             Mean   :18.33   Mean   :10   Mean   :23   Mean   : 42.66
 3rd Qu.:6.0             3rd Qu.:30.00   3rd Qu.:20   3rd Qu.:20   3rd Qu.: 61.00
 Max.   :6.0             Max.   :30.00   Max.   :20   Max.   :80   Max.   :272.00
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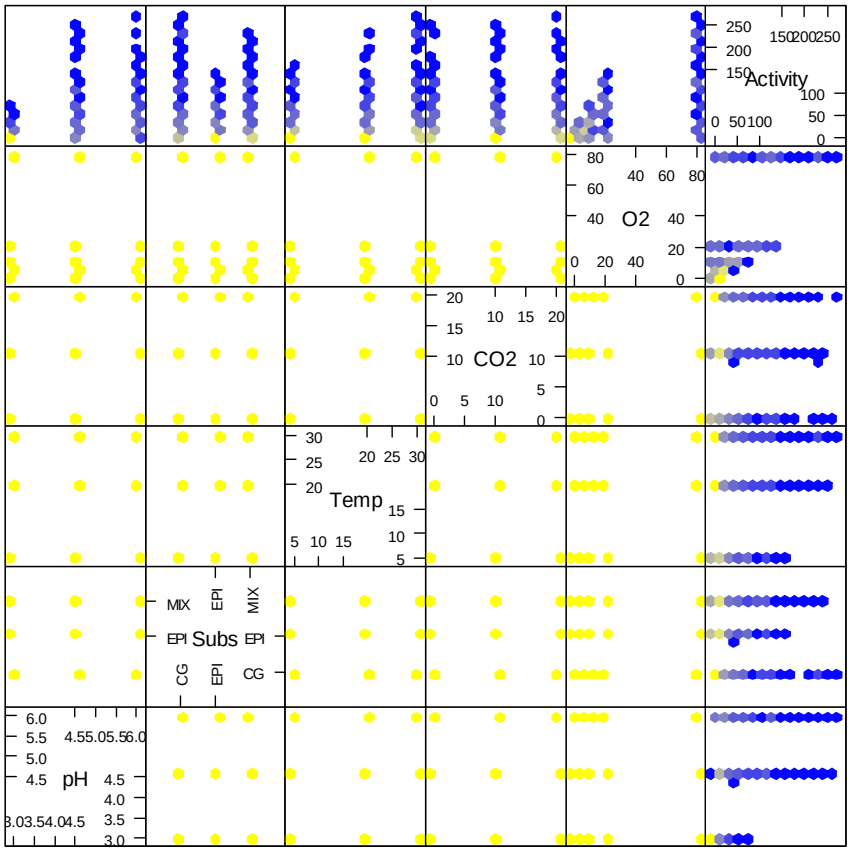
## Performance Data: Experimental Space Coverage

All conditions were varied independently (factorial design of 405 conditions) and each PPO activity measurement was run in duplicate (810 activity measurements). A two-dimensional (hexagonal binning) scatterplot displays the coverage of the experimental space.




Scatter Plot Matrix

The scatterplot is comprised of a 15 x 15 matrix of hexagon bins containing a count of the number of runs that fall within the boundaries of the bin. Count values are displayed as a color gradient running from yellow (low count) to blue (high count).

## Critical Process Parameters: Identification and Prioritization

A Random Forest analysis is run on the data set using substrate, pH, Temp, CO2 and O2 as predictors (potential control points) and PPO Activity as the response (quality metric). The results, summarized in the table below, reveal that the Random Forest model explains

95.5% of the variance in the data and can predict the PPO activity with a Root Mean Square Error (RMSE) of only 11.9 nmol of O2/sec. The high % indicates a high degree of confidence in the analysis

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Call:
 randomForest(x = dims, y = Activity, mtry = 4)
               Type of random forest: regression
                     Number of trees: 500
No. of variables tried at each split: 4

          Mean of squared residuals: 145.9641
                    % Var explained: 95.35
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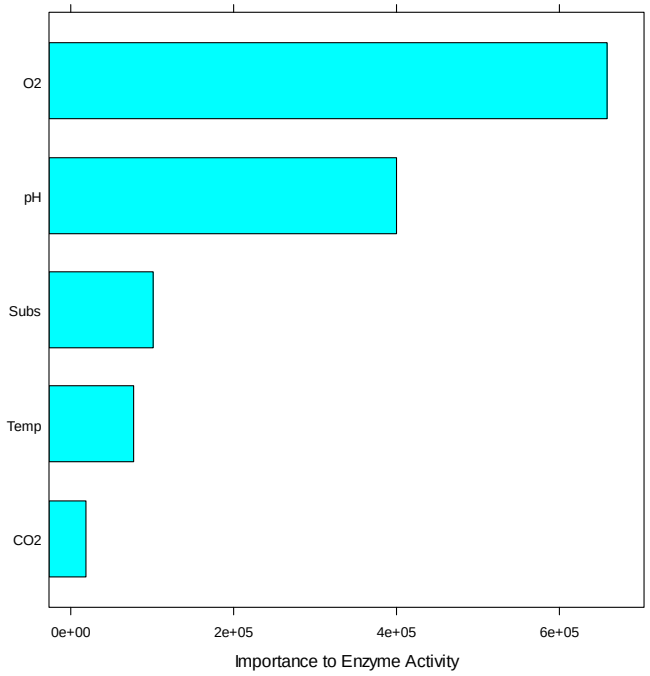
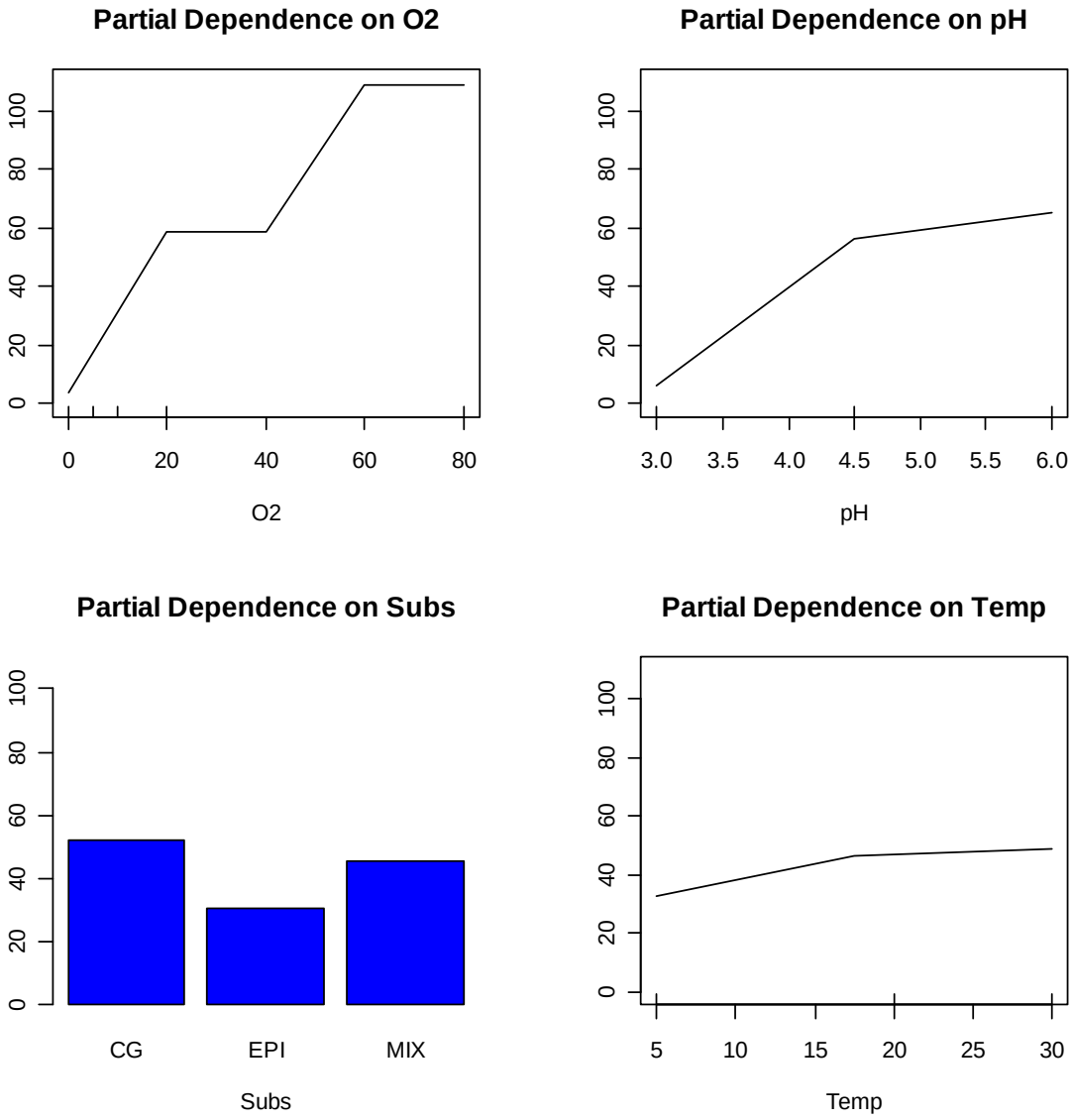
A first powerful feature of Random Forest regression is that it provides a prioritized list of variables with regards to how critical they are to performance prediction—that is, to what extent are they critical process variables.




Random Forests ascertains the importance of a predictor variable (potential control point) by permuting ("shuffling") predictor values and measuring the effect on the prediction error. Predictor variables that exhibit strong effects are deemed important, which provides a direct means of establishing whether a predictor variable is a CCP. As illustrated below, oxygen concentration and pH are important CCPs.

## Critical Process Parameters: Extent of Criticality

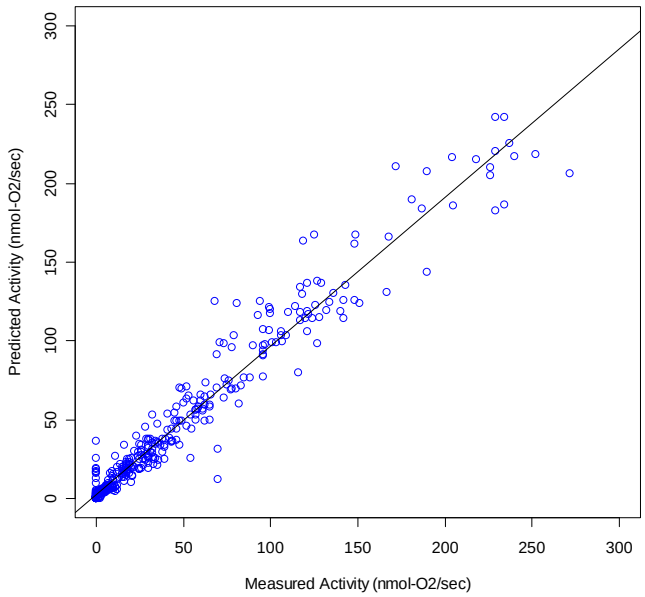
A second powerful feature of Random Forest regression is that it provides a graphical depiction of the marginal effect of a given process parameter (the derivative; slope) on the assay performance measure—that is, the amplification factor—while averaging out the effect of all the other parameters. Highly critical process parameters exhibit steep slopes in partial dependence plots.




As illustrated above, PPO activity is increased with increased values of O2, pH and temperature. By contrast, PPO activity is decreased with increased values of CO2 and varies with selection of the substrate

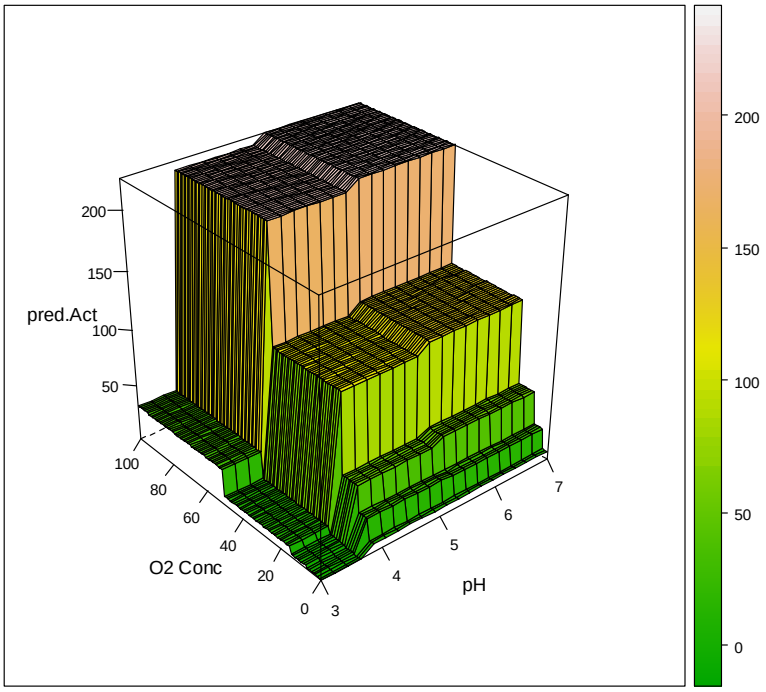
## Assay Performance: Ability to Forecast

Random Forests provide excellent predictive capabilities. Shown below is a comparison of the measured and predicted PPO Activity values for the data set and the least squares line for the correlation. Perfect prediction would place all the points on top of a 45 degree angle line.




## Assay Performance: Visualization

Once we have identified the critical process parameters, we can visualize their impact on enzyme performance by predicting the performance relative to the two most significant critical process parameters and the performance metric (PPO Activity), and displaying the results as a wireframe model.



## References

Andersson, C.A. (2000). “Exploratory Multi-Variate Data Analysis with Applications to Food Technology.” Ph.D. Thesis, Royal Veterinary and Agricultural University, Denmark.

Bro, R. and Heimdal, H. (1996). “Enzymatic Browning of Vegetables. Calibration and Analysis of Variance by Multi-Way Methods.” Chemometics and Intelligent Lab Systems. Vol. 34, p. 85.

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Heimdal, H.; Bro, R.; Larsen, L.M. and Poll, L. (1997). “Prediction of Polyphenol Oxidase activity in model solutions containing various combinations of chlorogenic acid, (-)-epicatechin, O2, CO2, temperature, and pH by multiway analysis.” J. Agric. Food Chem., Vol 45, 2399.

Martinez, M.V. and Whitaker, J.R. (1995). “The Biochemistry and Control of Enzymatic Browning.” Trendsin Food Science & Technology. Vol. 6, p. 195