Measuring Sulfur Dioxide: A Perennial Issue

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Measuring SO_2: A Perennial Issue

- In the collaborative proficiency testing program managed by ASEV & CTS, the analysis of SO_2 often shows the largest variances of all the analytes tested
  - At least three different methods reported
  - Manual wet chemistry methods widely used
  - Reflects diversity of the industry
- SO_2 chemistry is complex
  - SO_2 is an intermediate on the path from fully reduce sulfur to fully oxidized sulfate
  - Highly reactive in solution chemistry—responds readily to other species in the matrix

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SO_2 in aqueous solutions

- When SO_2 gas is added to water, some of the SO_2 is hydrolyzed to form the acid H_2SO_3.
  - SO_2 (g) + H_2O (l) → H_2SO_3 (aq) (+ some SO_2 (aq))
- When $K_2S_2O_5$ (metabisulfite) is added to water, HSO_3^- (bisulfite) is formed
  - $K_2S_2O_5$ (s) + H_2O (l) → 2 HSO_3^- (aq) + 2 K^+ (aq)

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SO2 in wine or juice

- H_2SO_3 is a relatively strong acid, so at wine pH, most of the H_2SO_3 is ionized to form HSO_3^-
- Similarly, as the conjugate base of a strong acid, some of the HSO_3^- formed from meta will pick up a proton to become H_2SO_3
- At pH 3.0, about 7% of the SO_2 will be in the "molecular" form, the remainder will be primarily HSO_3^-
- At pH 4.0, less than 1% of the SO_2 will be in the "molecular" form

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SO_2 Adducts

- HSO_3^- will readily combine with a variety of compounds in wine or juice to form adducts
  - Acetaldehyde
  - Anthocyanins
  - Sugars
  - Pyruvate
  - Alpha-keto-glutarate
- The acetaldehyde adduct is stable at wine pH; a strong base such as NaOH is required to hydrolyze this adduct (the first step in most analyses of total SO_2)

Adduct Stability

- Anthocyanin adduct is not stable—it will hydrolyze readily with acid addition or to re-establish equilibrium if free SO_2 is consumed
- Sugars, pyruvate & alpha-keto-glutarate are moderately stable, but will hydrolyze with acid and to re-establish equilibrium.
- The stability of these adducts is critical to free SO_2 analysis, as most analytical approaches acidify the sample to drive the bisulfite to the H_2SO_3 (“molecular”) form

Free SO2 analysis schemes

- Most commonly used analyses start with an acidification step to convert the free SO2 to the molecular form
  - Direct titration or other reaction of the molecular form (Ripper)
  - Vacuum or air stripping (aeration/oxidation)
  - Diffusion across a gas permeable membrane (many flow injection systems)

Free SO_2 by Ripper

- Acidification of the sample
- Titration with Iodine
  - Starch endpoint
  - Potentiometric endpoints
- Can be done using an autotitrator to reduce the variability associated with manual titrations
- Subject to interference from the wine matrix

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Free SO_2 by Aeration Oxidation

- Sample is acidified to force HSO_3^- to molecular form
- gas stream or vacuum is used to strip molecular SO_2 from the sample to a peroxide trap
- SO_2 is oxidized by peroxide to sulfuric acid in the trap
- Sulfuric acid is titrated with NaOH to make SO_2 determination

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Release of bound SO_2

- Both Ripper and A/O start with an acidification step
- As many SO_2 adducts are acid labile, some bound SO_2 is released during these analyses
- “Free” SO_2 can better be characterized as “free” plus some dissociated bound SO_2
- The amount of dissociated bound SO_2 depends on the types of adducts present in the wines and the speed of the analysis

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SO_2 by Capillary Electrophoresis

- Capillary electrophoresis is an instrumental separation technique similar to GC or HPLC
- Separation conditions selected to avoid conditions which favor hydrolysis of SO_2 adducts
- Free SO_2 is separated from SO_2 adducts
- Results better reflect true free SO_2

SO_2 in Model Wines with Binding Compounds

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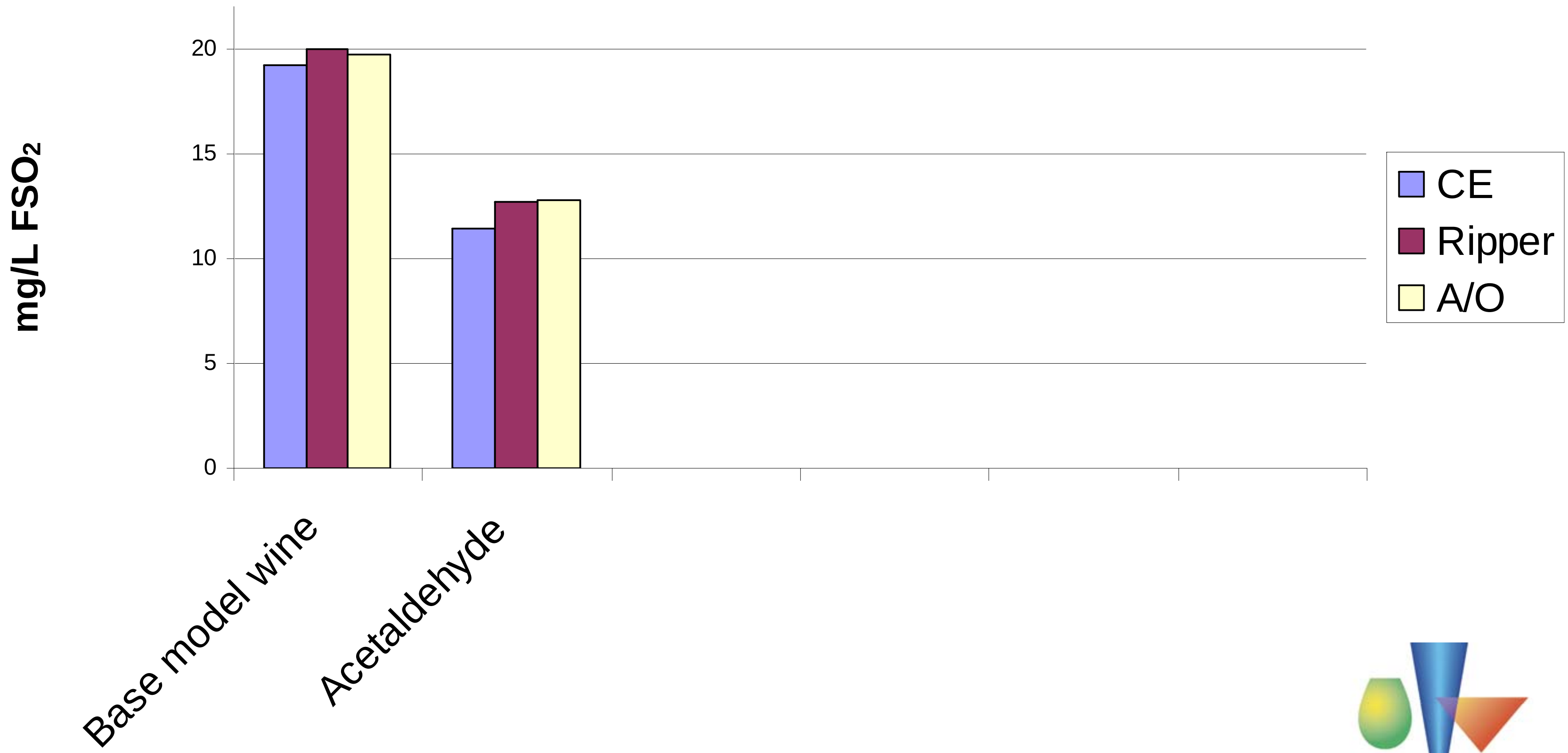
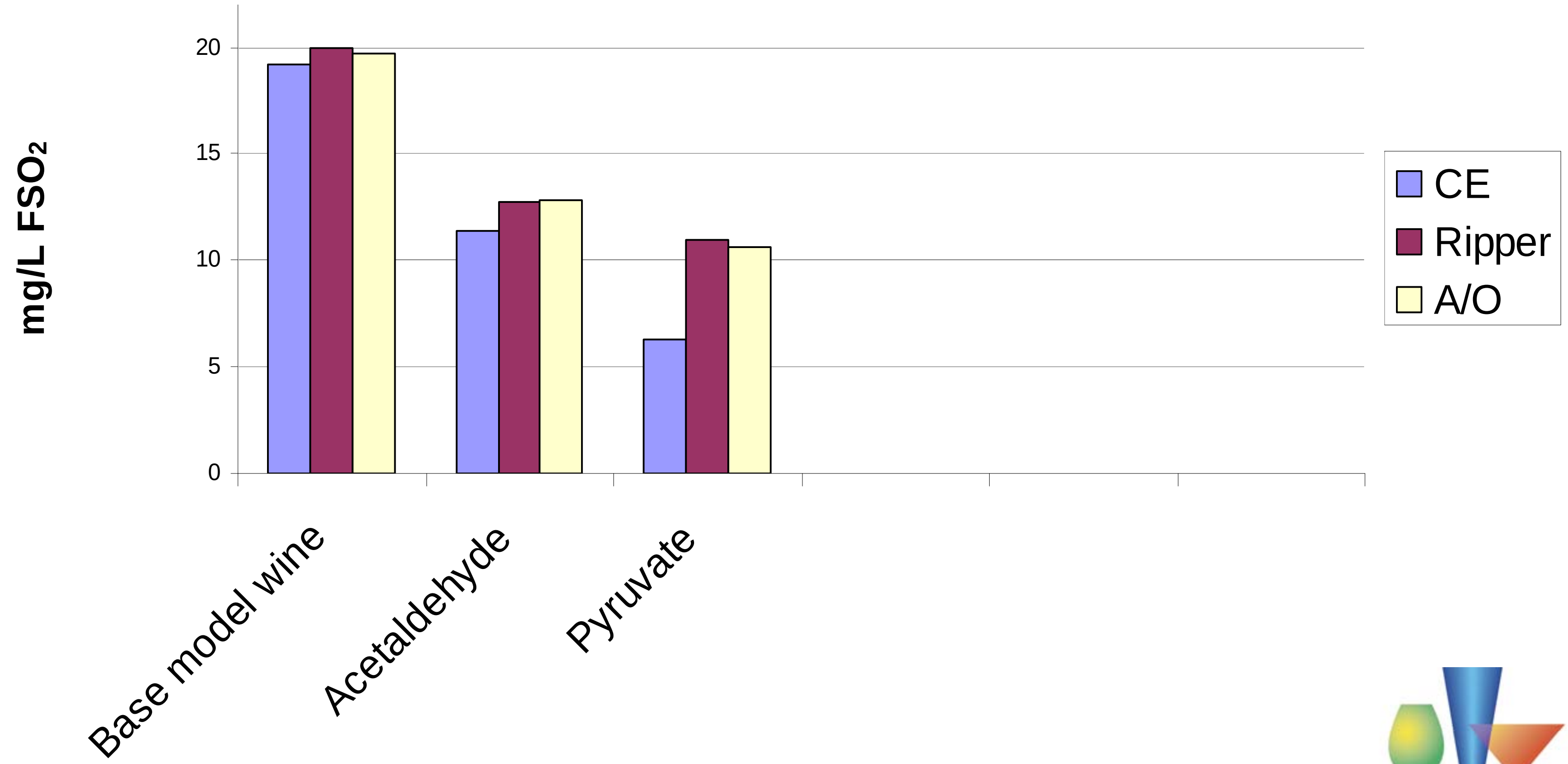
SO_2 in Model Wines with Binding Compounds



SO_2 in Model Wines with Binding Compounds



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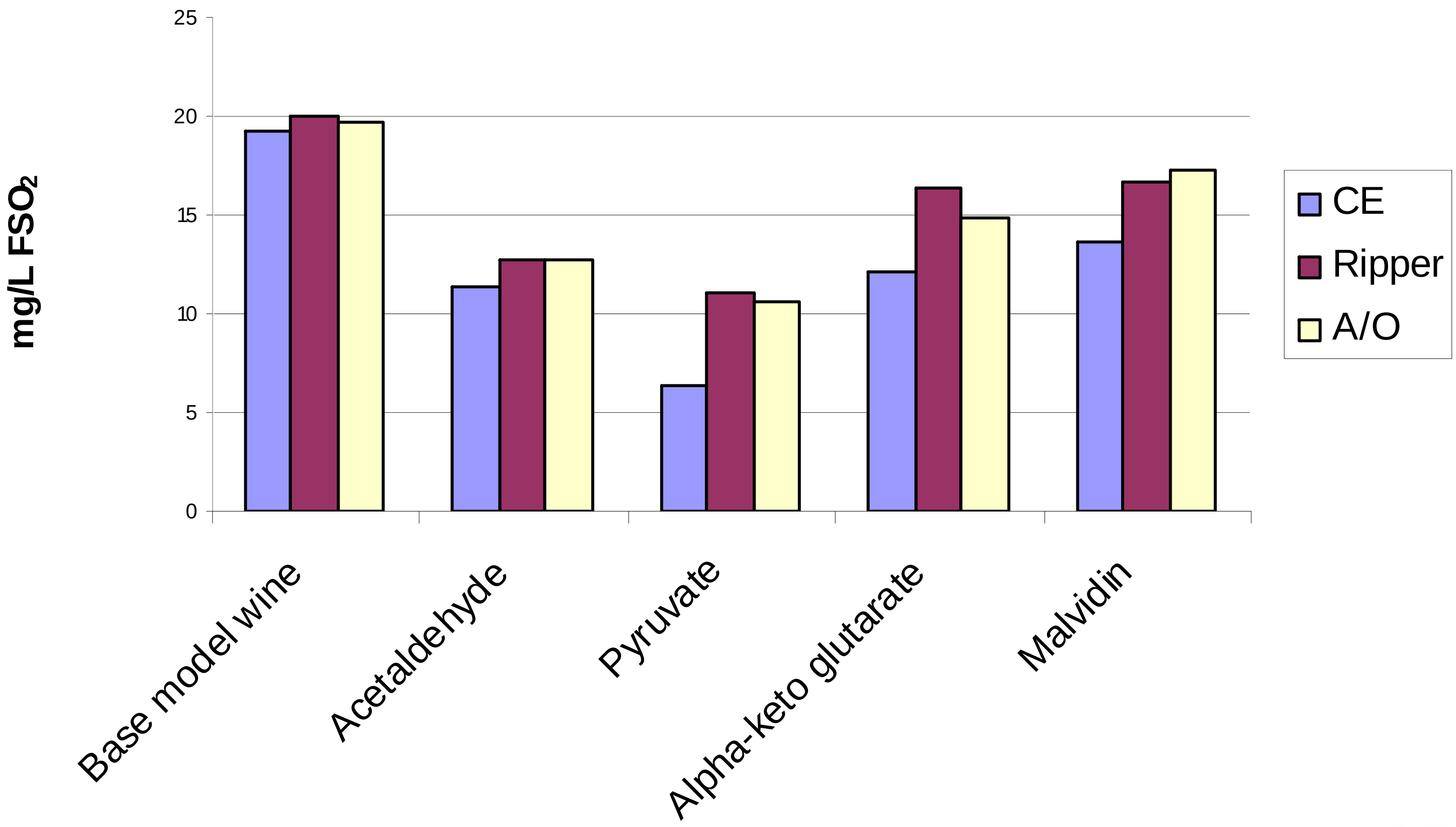

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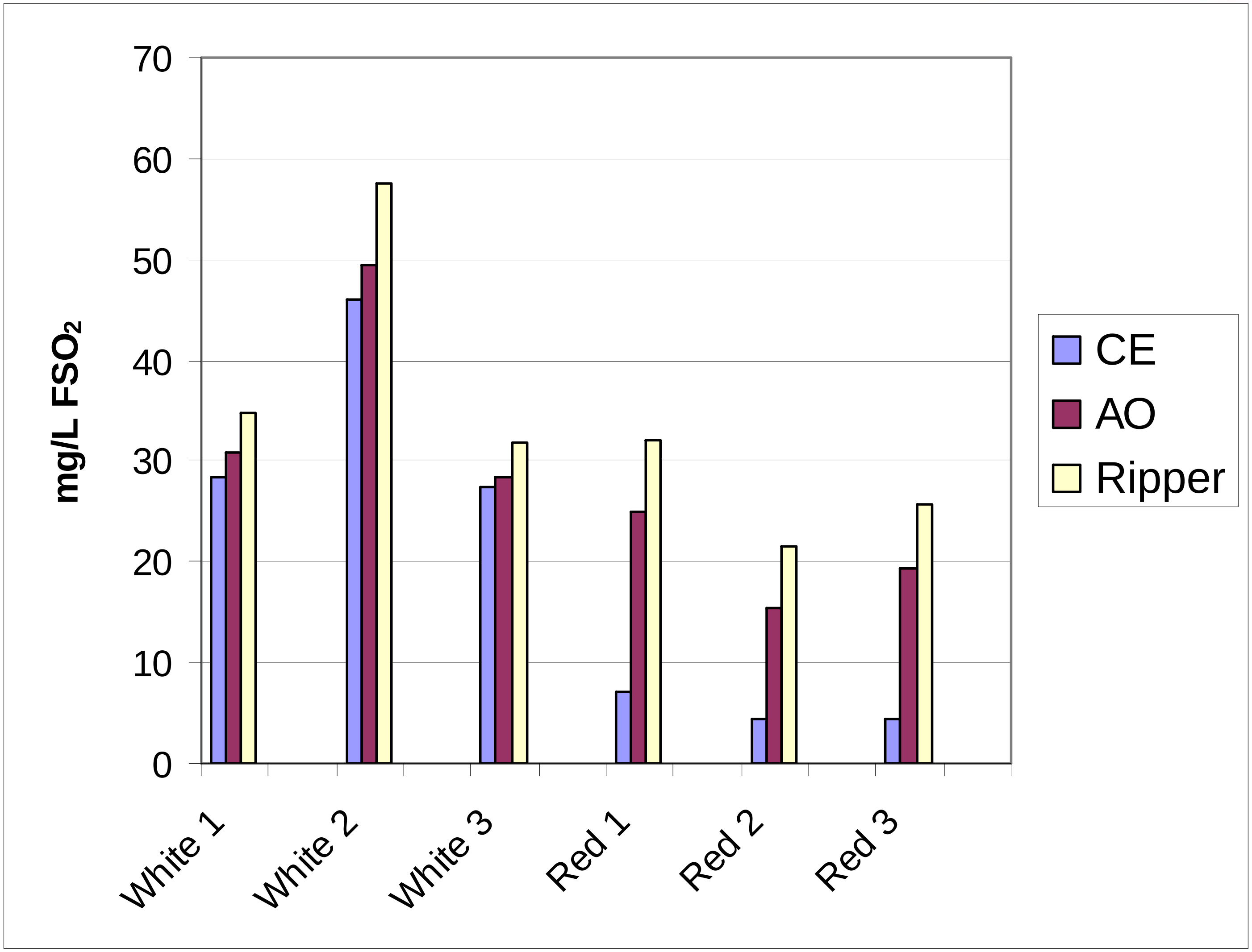
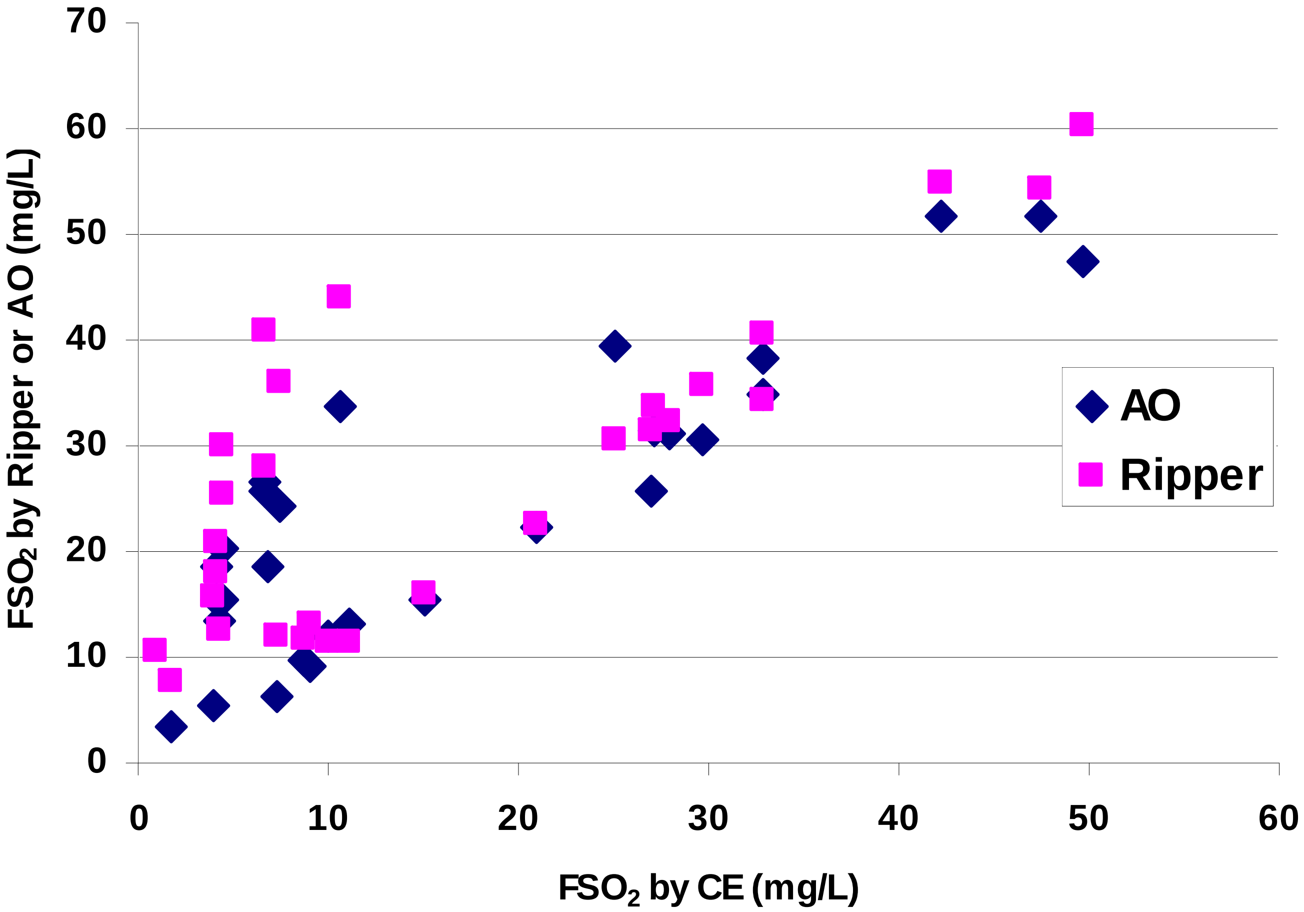
Free SO_2 by CE, Ripper & A/O



FSO_2 by Ripper & A/O vs FSO2 by CE



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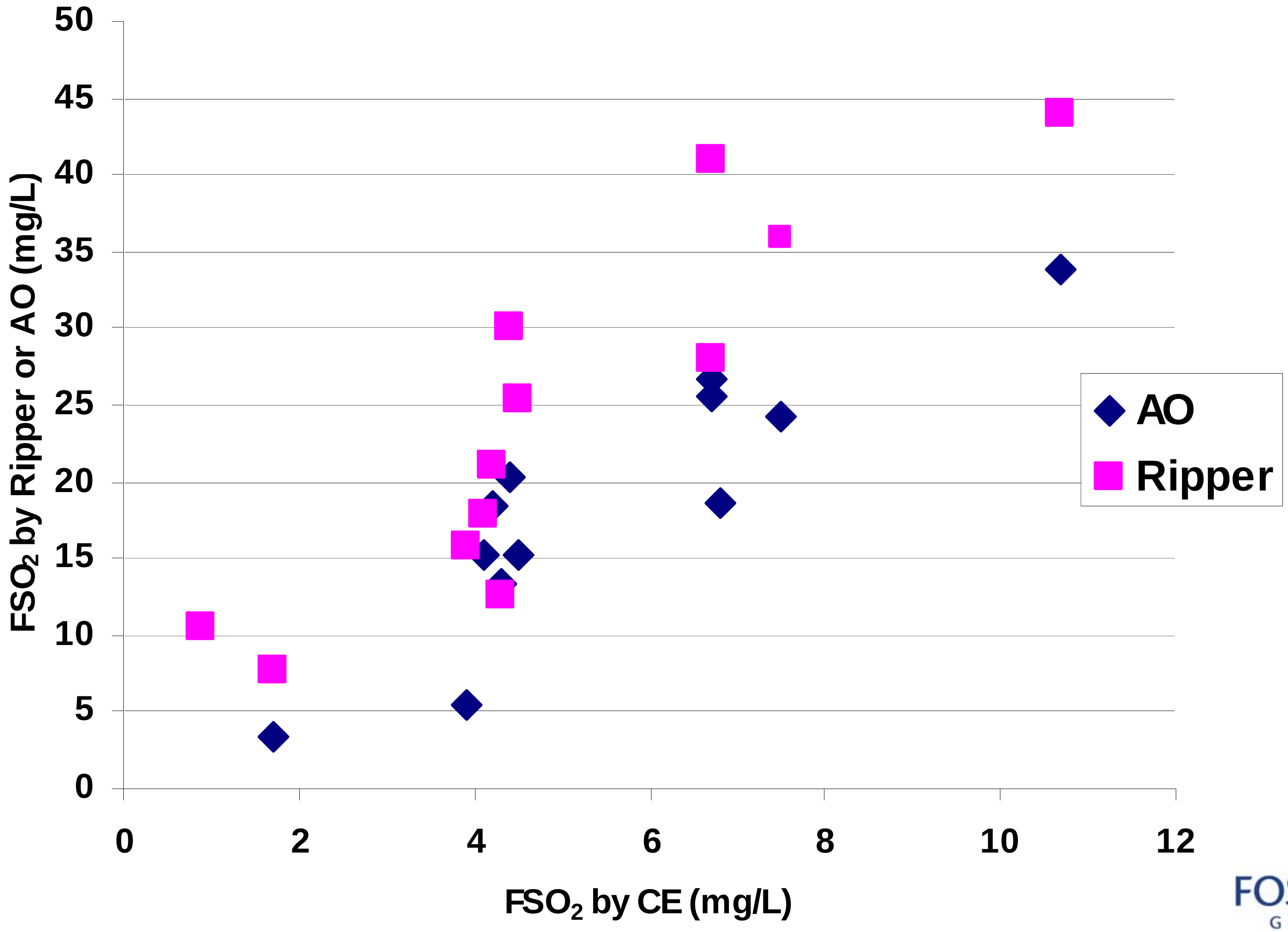
FSO_2 by Ripper and AO vs FSO_2 by CE



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Flow Injection Analysis

- Automated systems for analysis
  - Free & Total SO2
  - Volatile acidity
- Continuously streaming system into which samples are introduced
  - May contain different modules depending on analysis
  - Reagent addition, mixing lines, incubation lines, gas permeable membranes, etc.
- Usually based on a colorimetric assay, but other approaches are possible

Flow Injection Analysis for SO_2

- Again usually based on reacting SO2 with a reagent to form a colored product
  - Pararosaniline is common but there are others
- The first step is often to acidify the sample, forcing the SO2 to the molecular form, which is then separated from the wine matrix via a gas permeable membrane
- The color-forming reagent is then added, followed by a mixing/incubating step. The colored product is then measured by the instrument's spectrophotometer

The Good and the Bad of FIA

- The Good
  - High throughput
  - Good reproducibility
  - Relatively easy to operate

- The Bad
  - Requires high throughput—not a good tool for running samples intermittently
  - Maintenance must be done routinely
  - As an acidification step is commonly the first step in the analysis, results may be subject to the issue of release of some bound SO_2 during the analysis

Clinical Analyzers

- Widely used in the wine industry for enzymatic analyses
  - Acetic acid
  - Malic acid
  - Residual Sugars
  - Ammonia

- Can also be used to run some colorimetric assays—the most widely used is the NOPA method for amino nitrogen
- There are colorimetric methods for SO_2 which have been or could be adapted for use on clinical analyzers

The Good and Bad of Clinical Analyzers

- The Good
  - Medium to high throughput, but can accommodate single samples
  - Operating costs are reasonable
  - Relatively easy to operate
  - Flexible—can be used to run a wide range of assays
- The Bad
  - Initial purchase price can be high
  - Maintenance must be done routinely
  - Once again, look at the chemistry—some methods will be subject to the issue of dissociation of bound forms

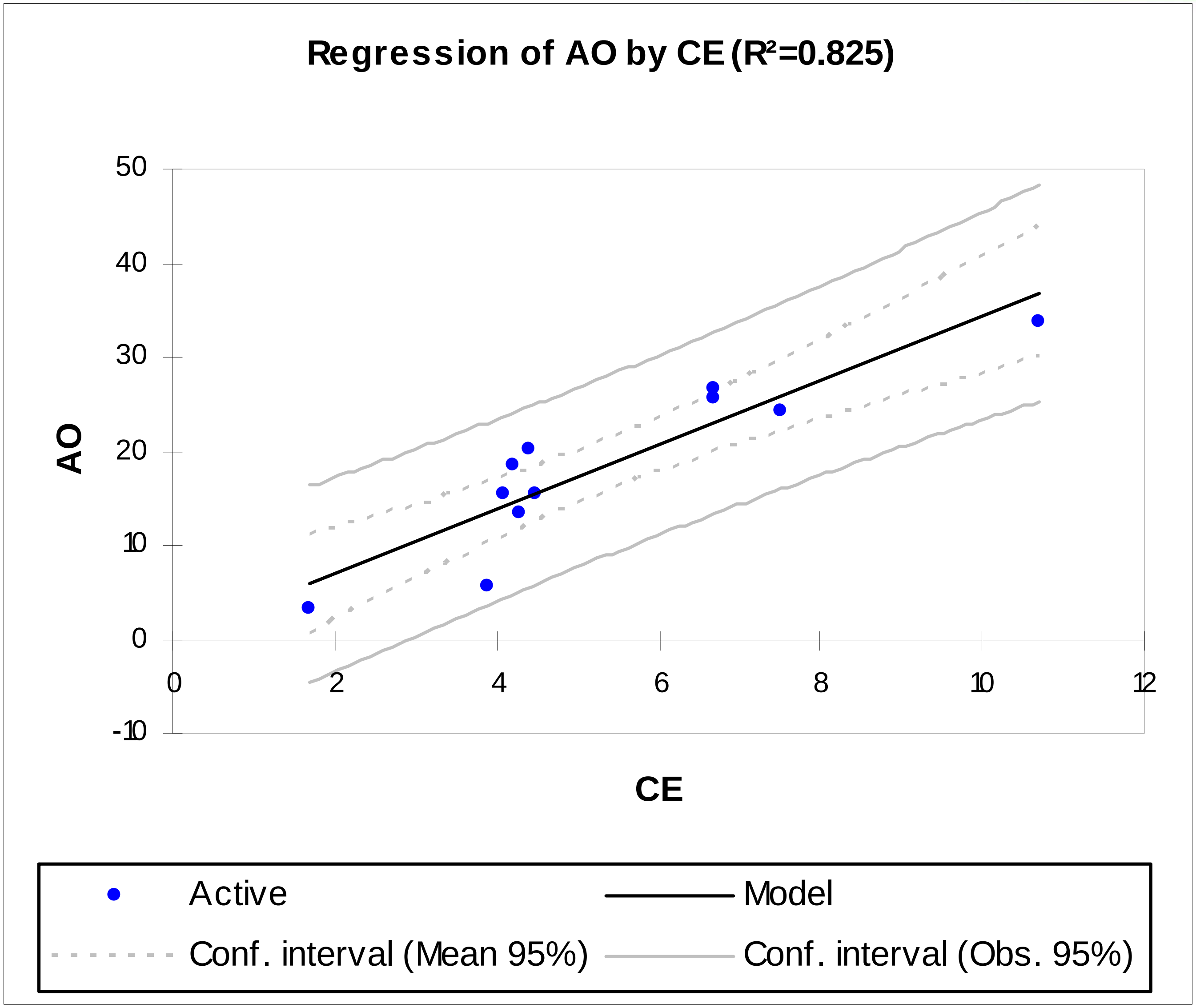
Measuring Sulfur Dioxide: A Perennial Issue

- The analyses widely used by the wine industry for FSO_2 can be characterized as measuring "free" SO_2 plus some fraction of "bound" SO_2

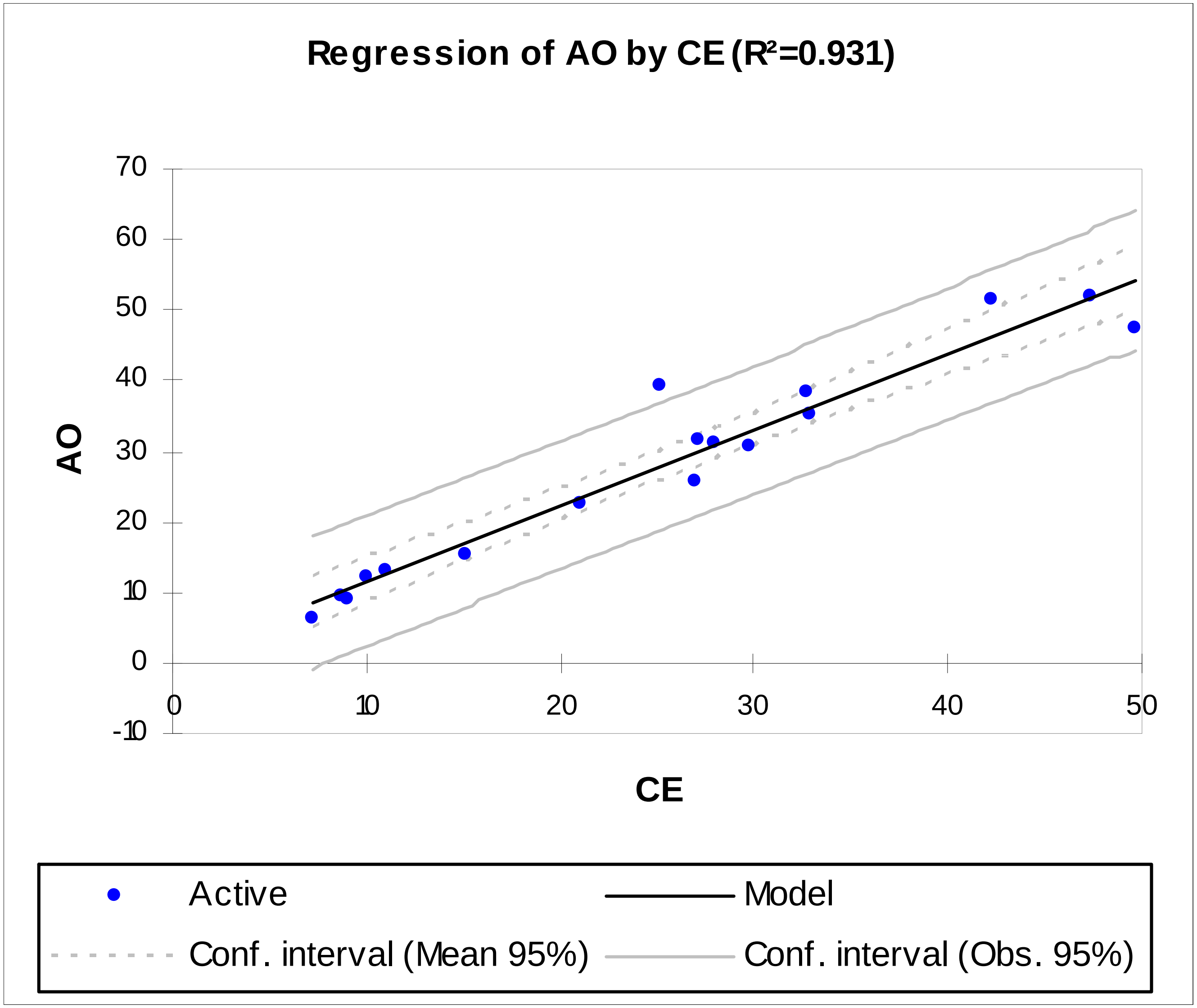
- Do the less stable adducts contribute to the stability of the wine, i.e., do they function as a pool of SO_2 which is released as the "true" free SO_2 is consumed?

- Questions?

Regression of AO FSO2 against CE FSO2 for red wines



Regression of AO FSO2 against CE FSO2 for white wines



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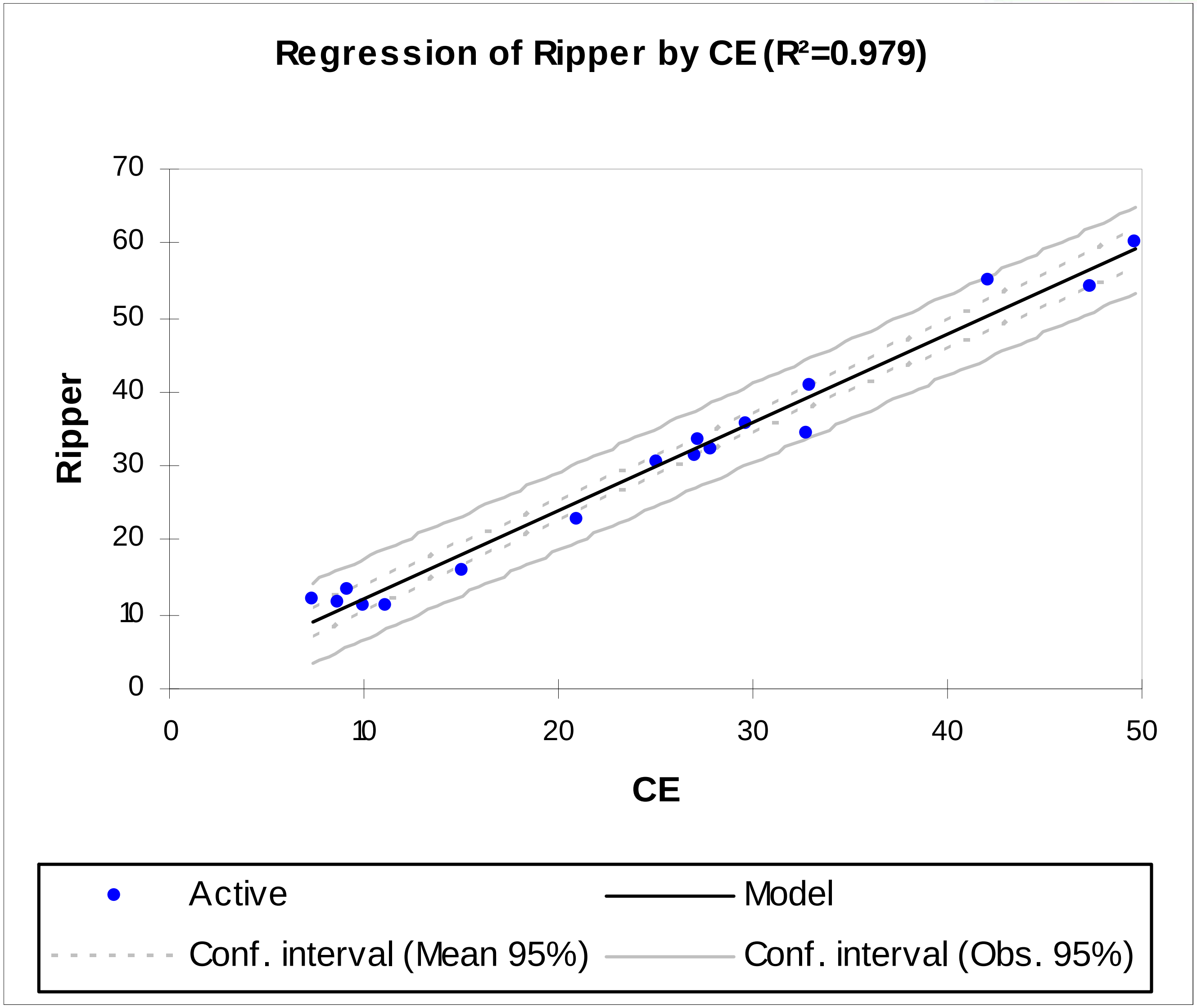
Regression of Ripper FSO2 against CE FSO2 for white wines



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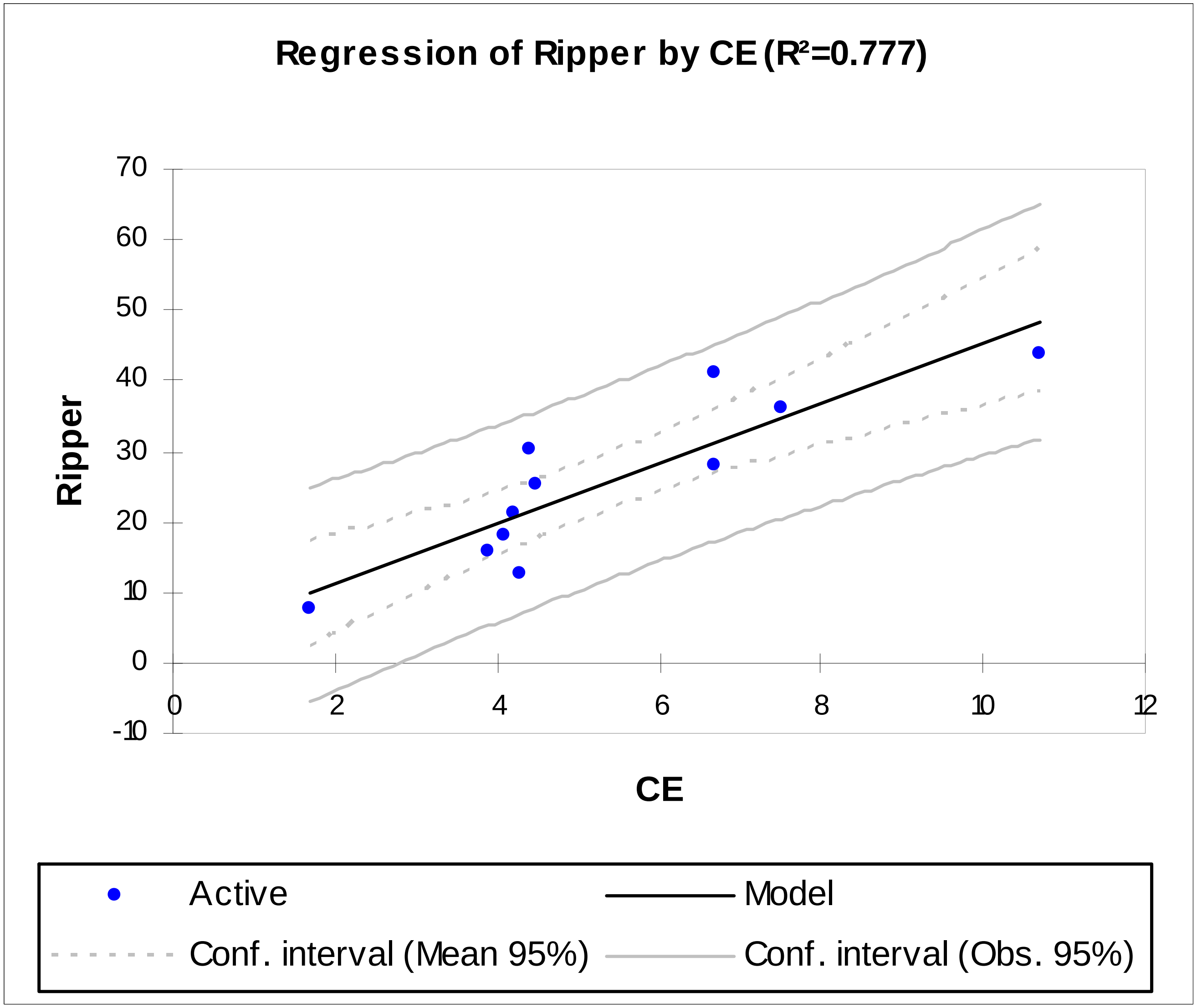

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