Chemo-Enzymatic Modification of High-Lignin Content Fibers with Laccase

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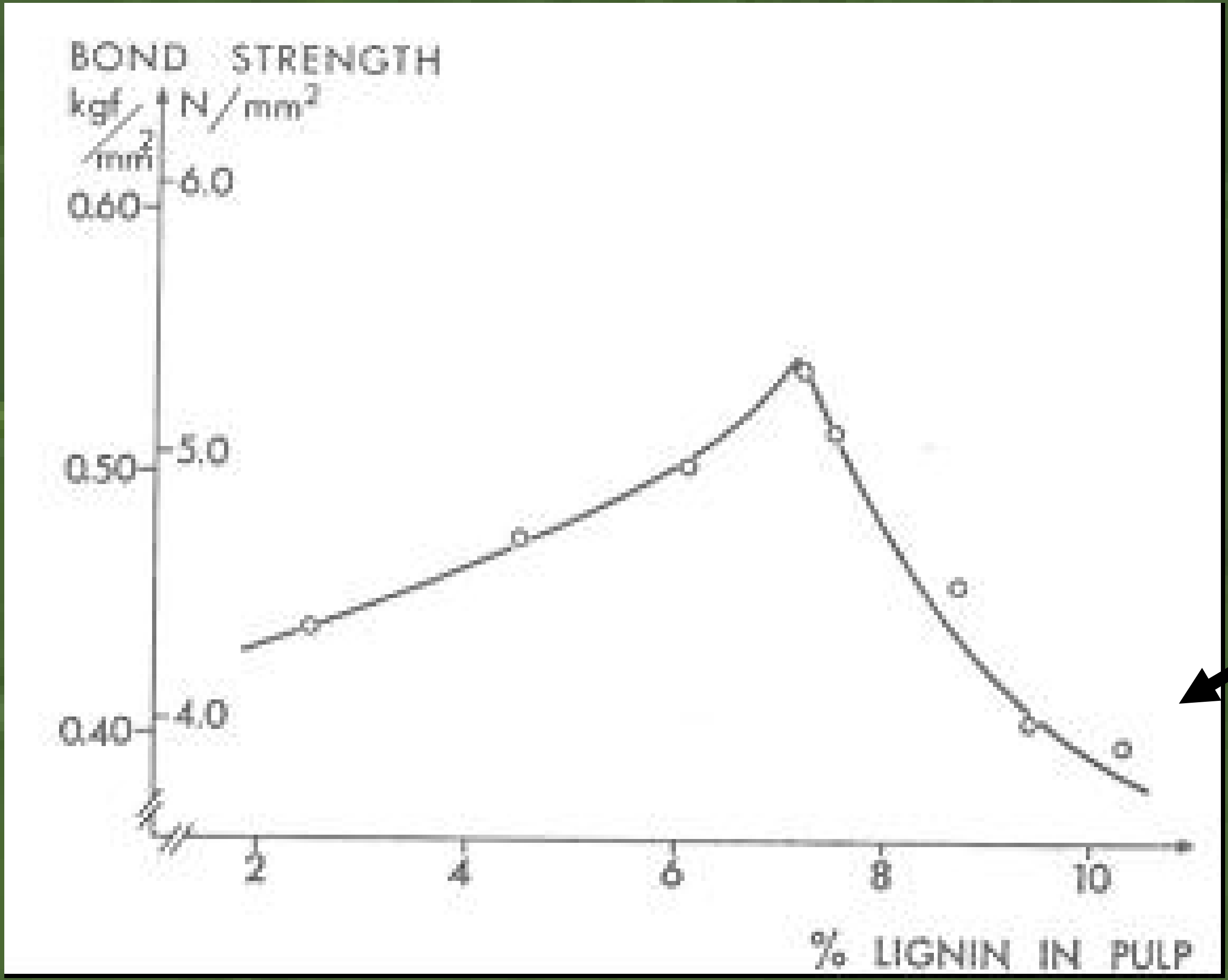
"If it Ain't Broke Don't Fix it?"

- Kraft pulping
  - Long, strong fibers
  - Energy and chemical recovery
  - 45-50% pulp yield for bleached grades

What happens when we pulp to higher lignin contents to preserve yield?

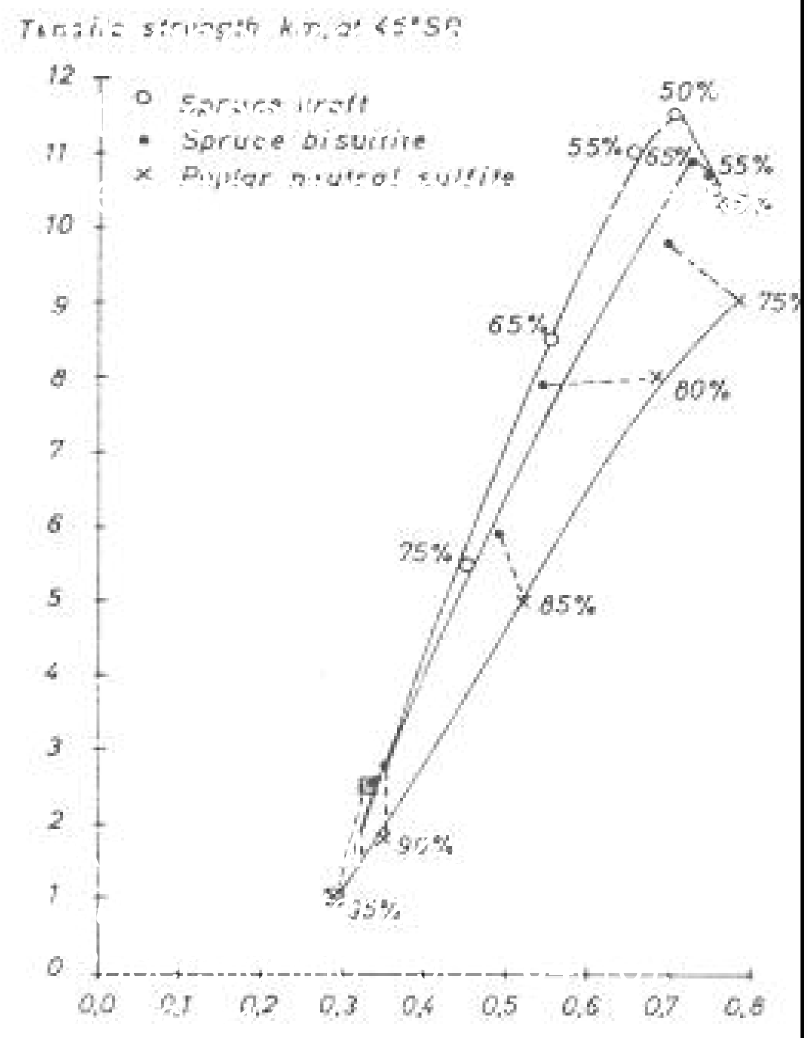
The Effects of Lignin on Fiber-to-Fiber Bonds

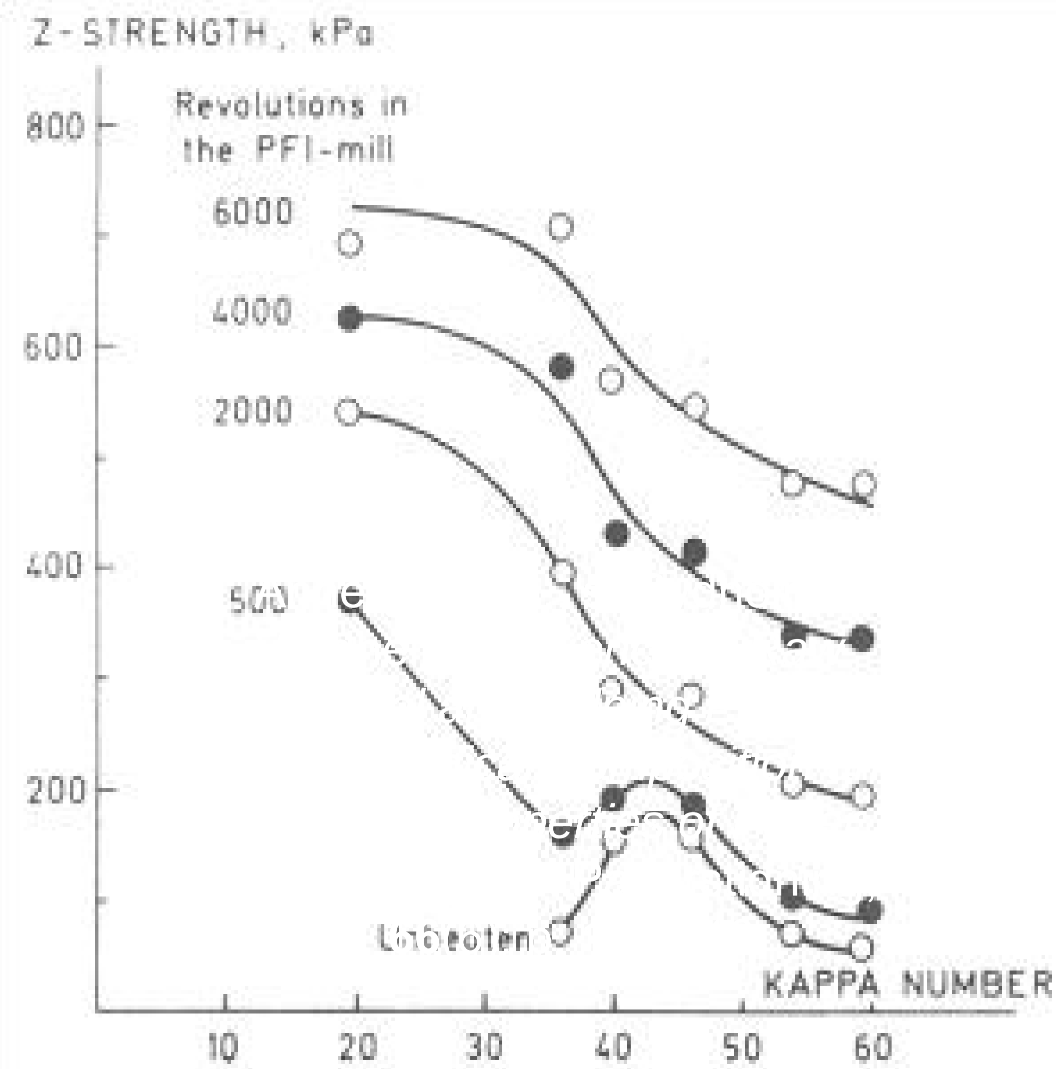


High-Kappa Kraft

Mohlin, U.-B. and N. Hartler, *Cellulose Fiber Bonding.* Svensk Papperstiding, 1975. **78**(8): p. 295-299.

The Effect of Lignin Content on Paper Strength Properties





High-Yield Kraft Fibers

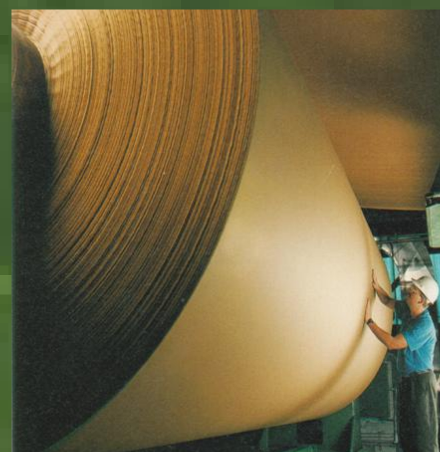
Stop kraft cook earlier =High-Kappa Kraft Pulp

- Poor bonding characteristics limit applications
  - High lignin content
    - Surface lignin content prevents bonding
    - Stiff fibers – conformability
    - Restriction of fiber swelling

Can the properties of high-yield kraft pulps be improved to broaden their range of applications?

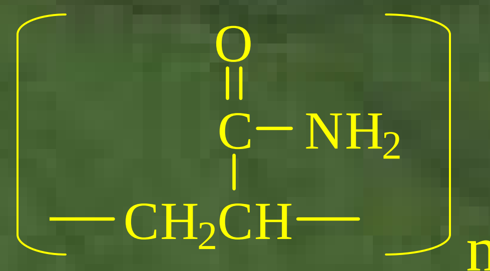
What is currently being done to modify fibers to affect pulp quality?

Tree choice



Chipping

Pulping



Papermaking chemicals

Next Generation?

- Enzymes from wood-degrading fungi can be used for altering pulp properties

Cellulase

- **Enzymes modify fibers rendering them more flexible and aid drainage on paper machines**

Hemicellulase

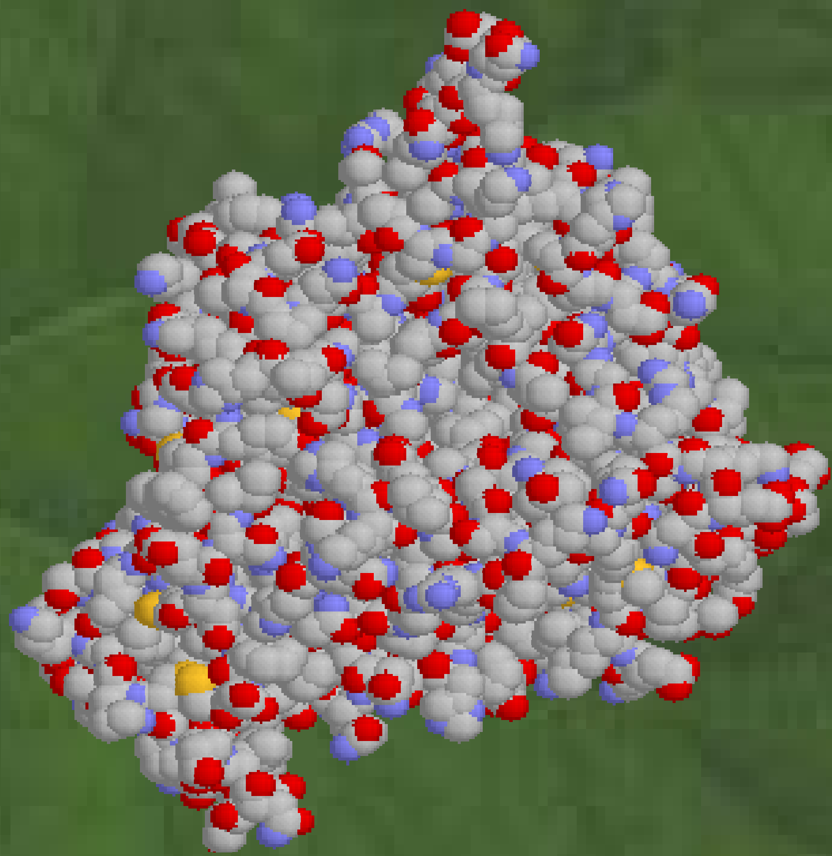
- **Enzymes modify fibers to aid in bleaching**

Ligninases

- **Manganese peroxidase, Lignin peroxidase, and LACCASE**
- **Employed primarily for delignification studies**
- **Laccase different than MnP and LiP**

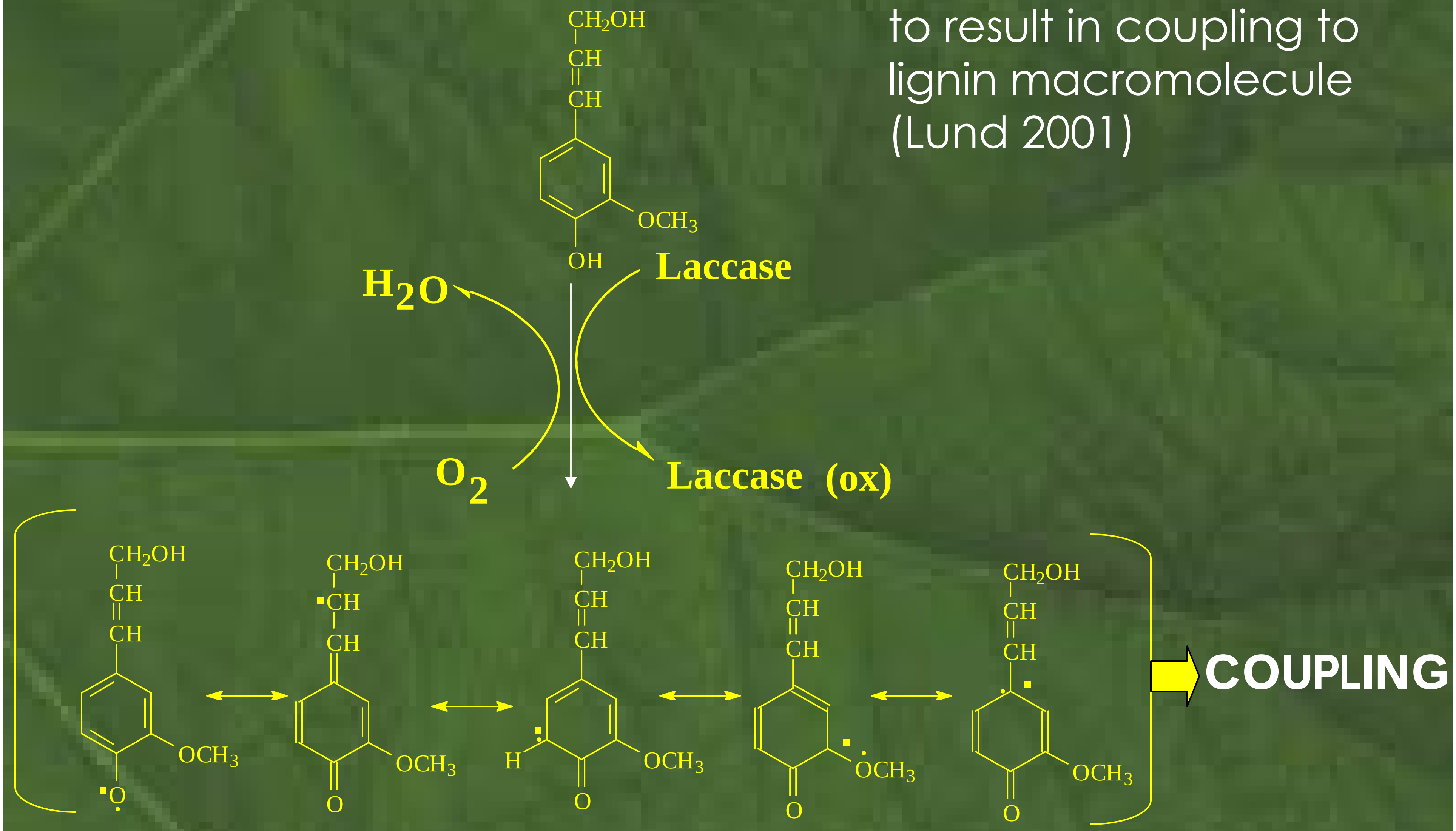
Laccase

- Japanese lacquer tree (*Rhus vernicifera*)
- Basidiomycete fungi and plants
- Oxidoreductase enzyme
- Biodelignification studies with kraft pulps
- Polymerizes monolignols in solution to form polymers with lignin-like properties



Laccase

- Oxidation of monomeric phenols has been shown to result in coupling to lignin macromolecule (Lund 2001)

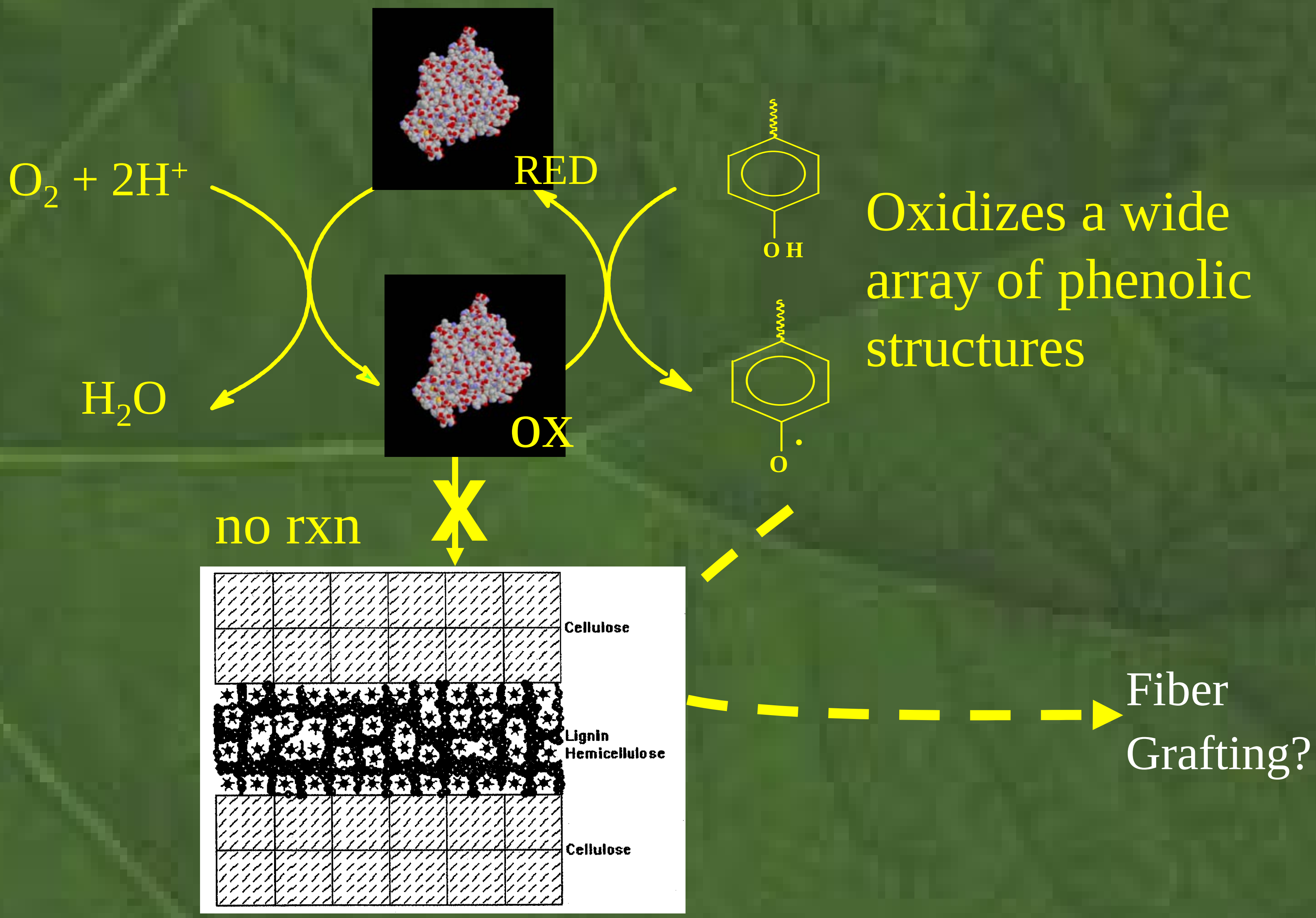


Rationale

- There has been limited work in pulp and paper research exploiting laccase's polymerizing ability to modify pulps.
- The high surface lignin content of high-kappa kraft pulps* makes them prime candidates for reaction with laccase.

*Laine, 1994

Hypothesis



RED
OX
$O_2 + 2H^+$
H_2O
OH
O
no rxn
Oxidizes a wide array of phenolic structures
Cellulose
Lignin
Hemicellulose
Cellulose
Fiber Grafting?

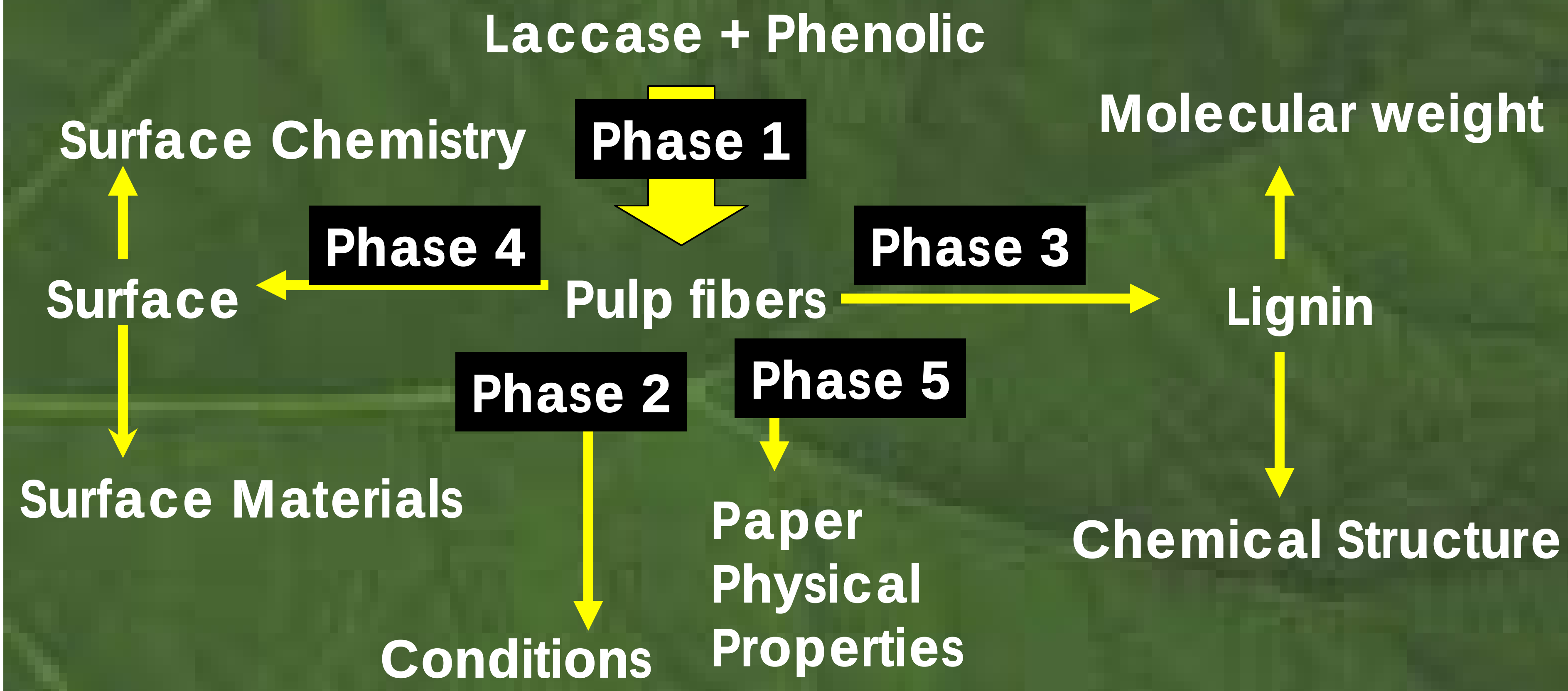
Possibilities…

- Polymerization of phenols with themselves (self-condensation)
- Polymerization of phenols with fiber
- Polymerization of fiber lignin

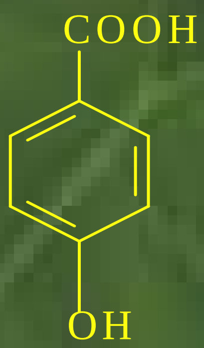
Objectives

- Devise and evaluate the feasibility of a system utilizing laccase to co-polymerize (graft) water soluble compounds with high kappa kraft pulp
- Determine if lignin was the main target for modification of the laccase-facilitated grafting system
- Determine conditions where the laccase-facilitated grafting system was the most effective for modifying fibers
- Evaluate the effects of the laccase-facilitated grafting treatment on paper strength properties and surface chemical properties

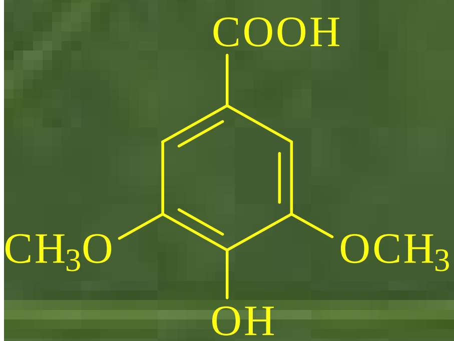
Overview


Laccase + Phenolic
Phase 1
Pulp fibers
Phase 4
Surface
Surface Chemistry
Surface Materials
Phase 2
Conditions
Phase 5
Paper Physical Properties
Phase 3
Lignin
Molecular weight
Chemical Structure

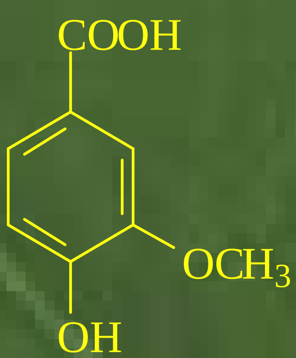
Phase 1: Initial Experiment



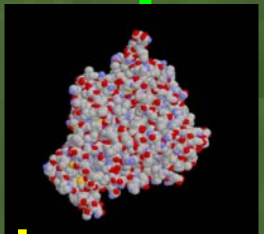
4-Hydroxybenzoic acid



Syringic acid



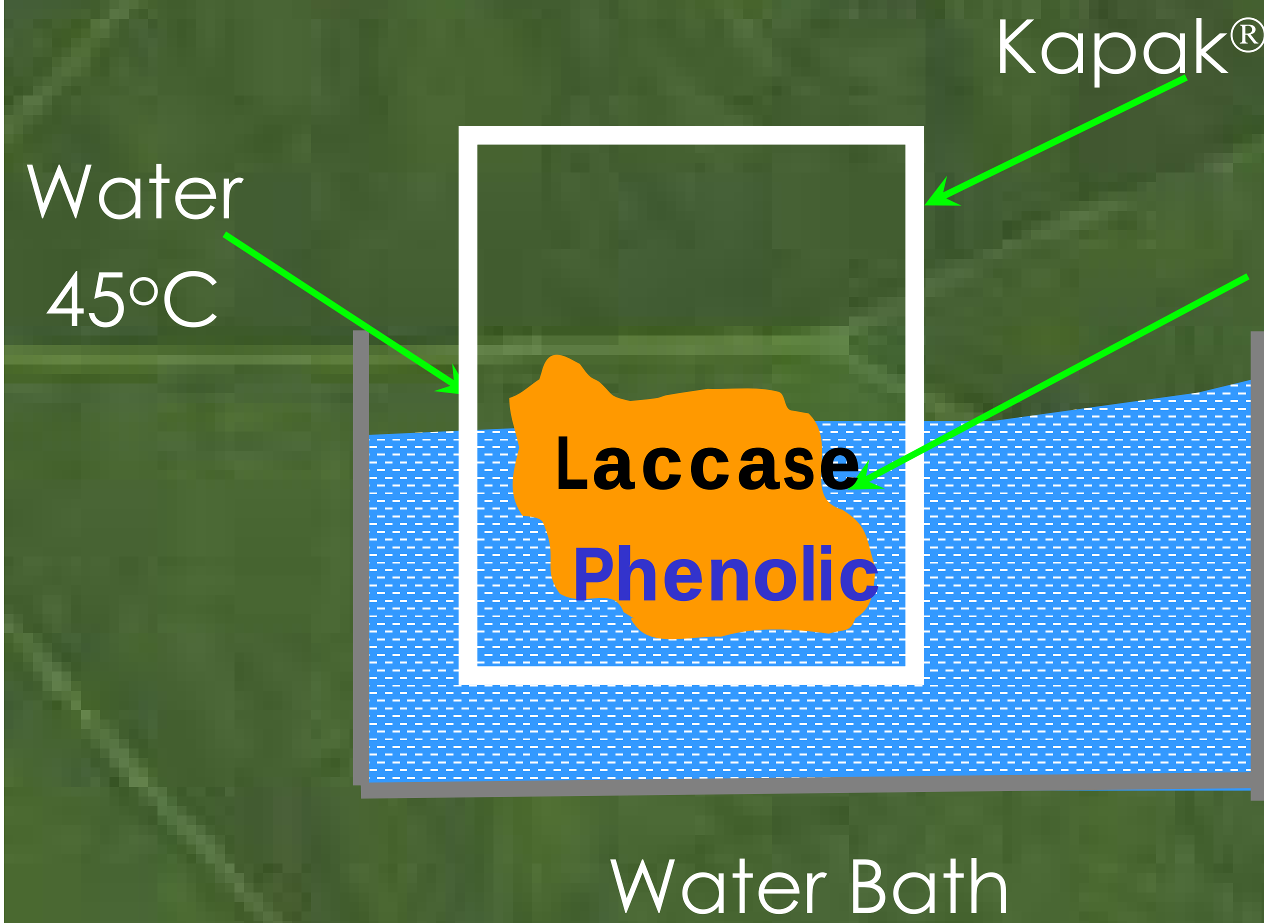
Vanillic acid



Laccase

- Kappa Number
- Carboxylic Acid Groups
- X-ray Photoelectron Spectroscopy

Experimental



Pulp

- **Kappa 90**
- **20% csc**
- **pH 4.5**

1. **Phenolic**
2. **Laccase**

Pulp before and after treatment

Control and Brownstock
TB:20.2

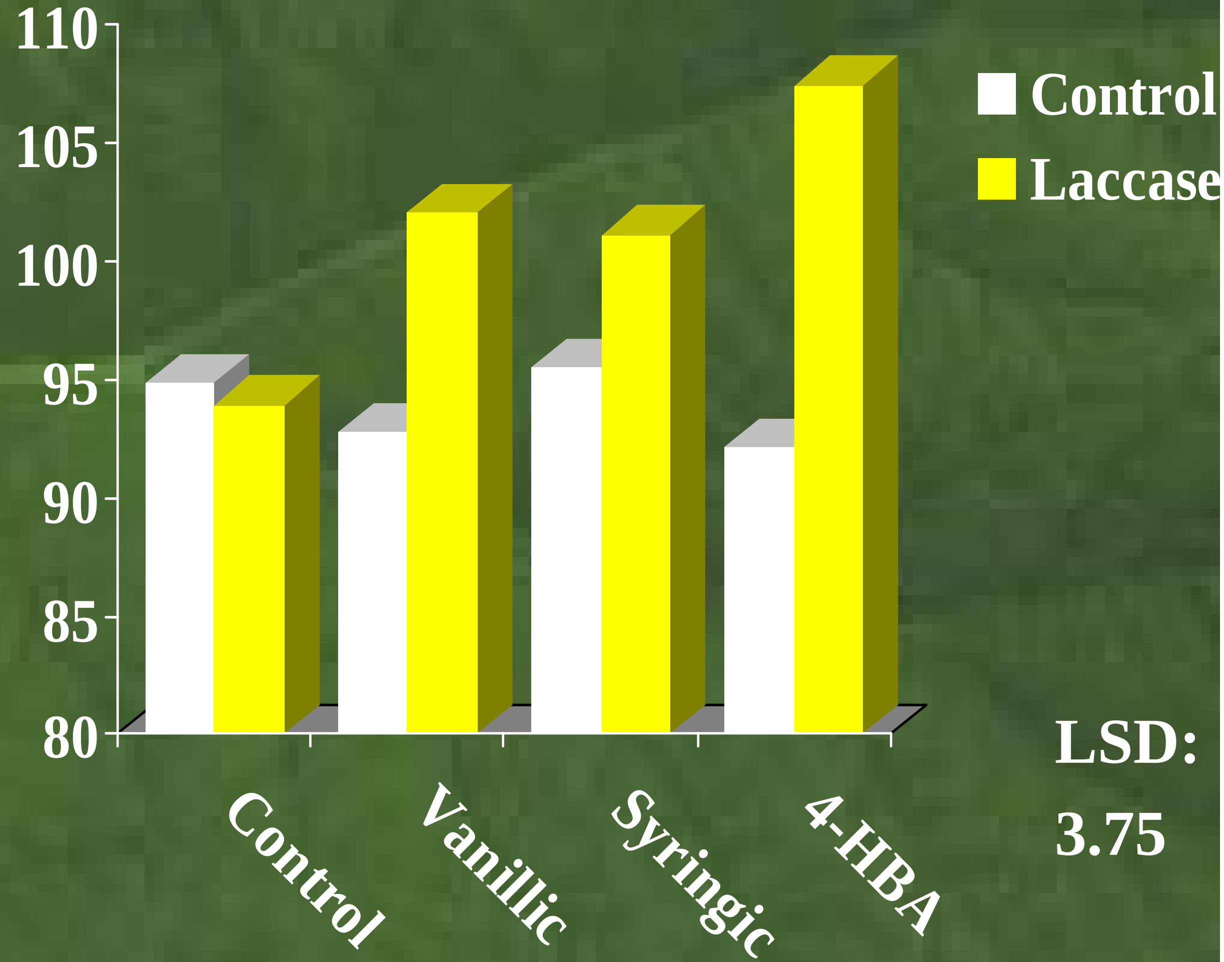
Laccase
TB:18.3

4-Hydroxybenzoic acid
TB:19.2

Laccase+4-Hydroxybenzoic acid
TB:15.3

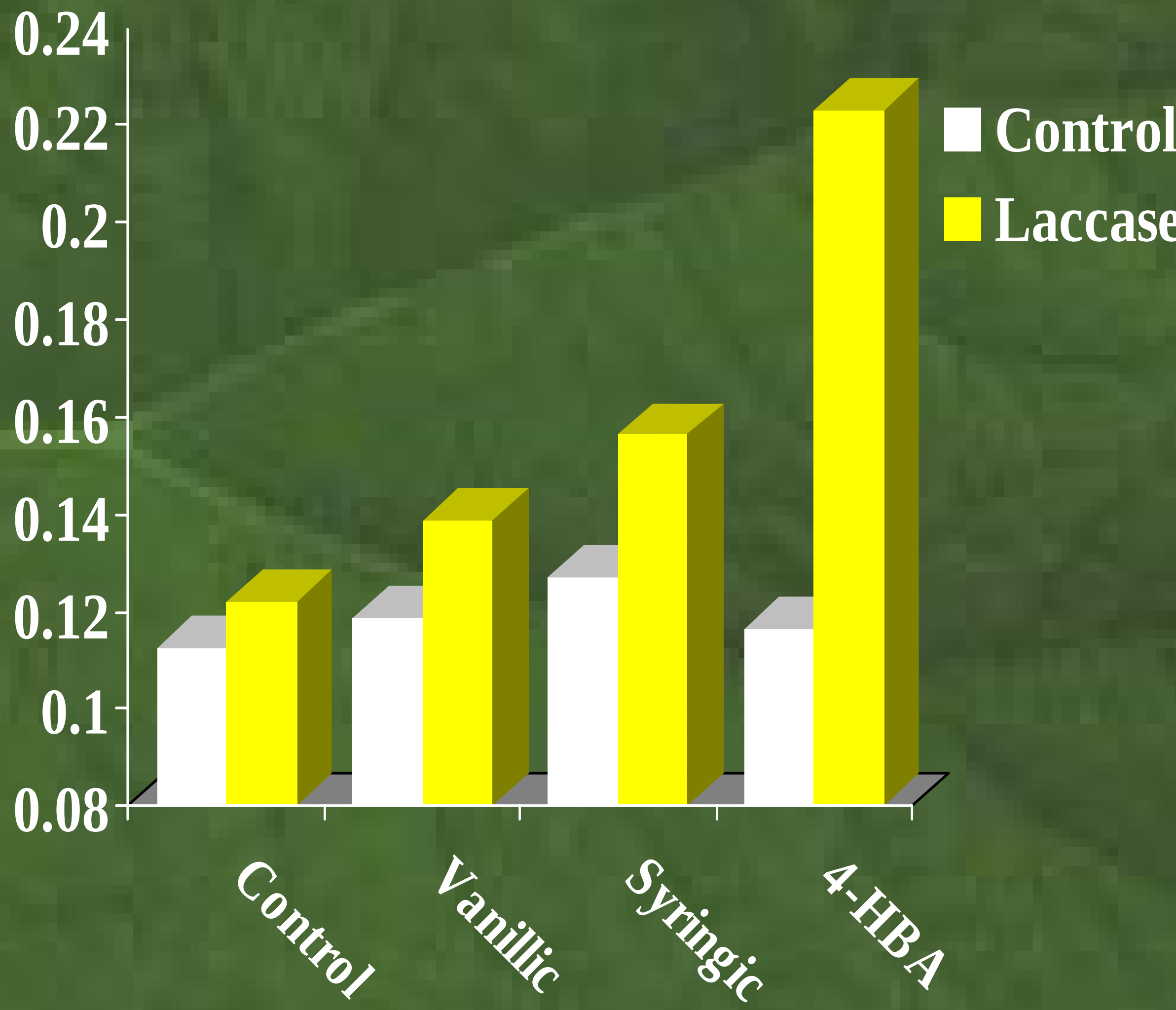
Phase 1:
Kappa Number

- Kappa increased in presence of laccase and phenolic acids
- 4-hydroxybenzoic acid (4-HBA) treatment with laccase resulted in largest kappa increase



Phase 1:
Bulk Carboxylic Acid Groups

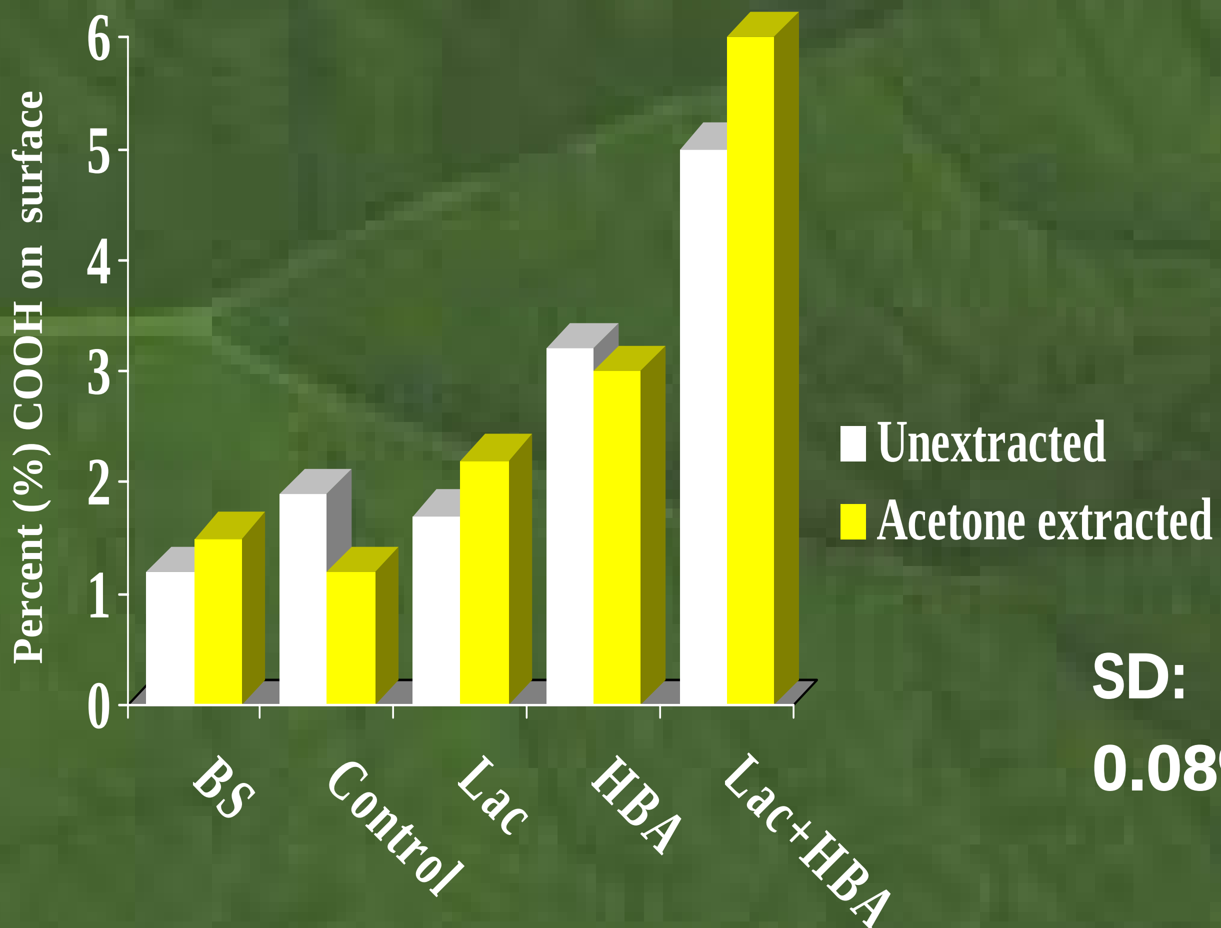
- Acid group results correlate with kappa number results
- Laccase + 4-hydroxy benzoic acid approx. doubled the bulk acid groups



LSD:
0.02 meq/g

Phase 1:
XPS Results

- Laccase and 4-hydroxybenzoic acid increased the percentage of acid groups on the surface.



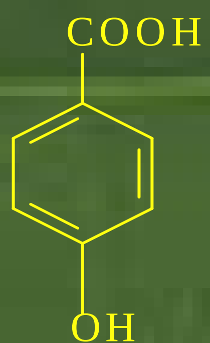
SD: 0.08%

Phase 1: Conclusions

- Laccase treatment with phenolic acids increases both kappa number and bulk carboxylic acids.
- Strong evidence for laccase facilitated coupling of phenolic acids to fiber surface.
- Laccase most effective in coupling of 4-hydroxybenzoic acid which may be due to absence of methoxyl groups on aromatic ring

Phase 2: Treatment Conditions

- Used 4-hydroxybenzoic acid to determine effect of treatment conditions
- Varied presence of oxygen, dosage of 4-hba, pulp consistency, treatment time



4-Hydroxybenzoic acid

Laccase →

→ consistency

→ 4-Hba Dosage

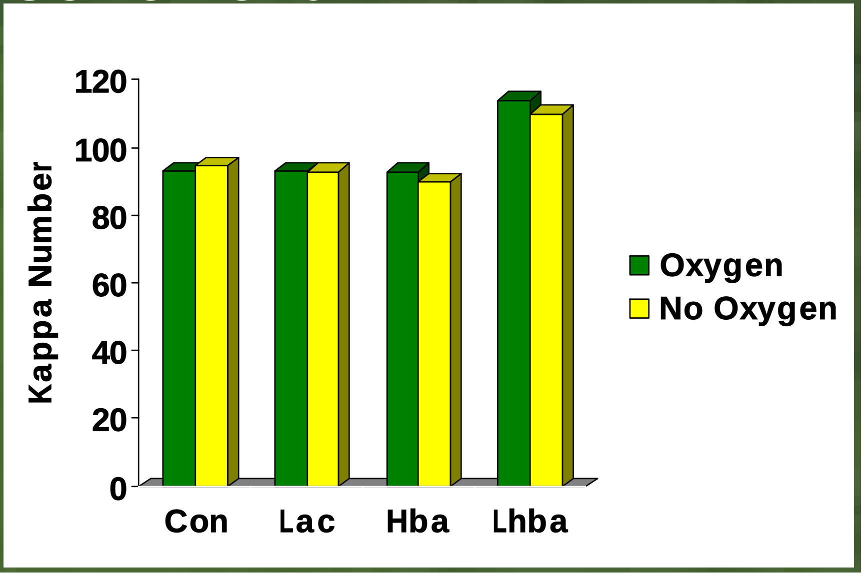
→ time

↓ Oxygen vs. No Oxygen

↓ Full 4-hba Dose vs. Periodic Addition

Phase 2:
Oxygen Pressure Vs. Ambient Conditions

- Laccase treatments for bio-delignification most effective when pressurized with O_2
- Compare to treatments performed in bags
- Treatments performed with identical conditions



- No real difference between treatments performed in O_2 reactor or atmospheric

Phase 2: Factorial Design

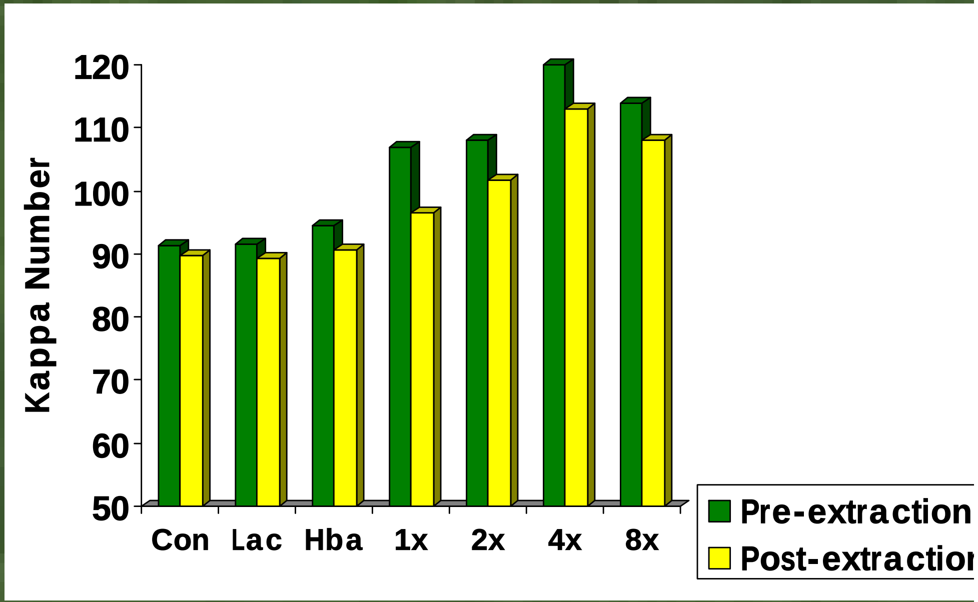
- **Three factors 3^3 factorial with kappa number as the response**
- **Variables**
  - **5, 10, & 20 % pulp consistency**
  - **0.1, 1, & 10 mmol dosage of 4-Hba**
  - **2, 4, & 8 hours treatment time**

Factor	**P-Value**
Dosage	0.000002
Consistency (CSC)	0.000005
Time	0.485361
Dosage*CSC	0.000035
Dosage*Time	0.101097
CSC*Time	0.980152

- Consistency and dosage Have the most significant effects on grafting reaction
- Consistency and dosage also have the strongest interaction

Phase 2: Periodic Addition of 4-Hba

- **Alkaline extraction removes adsorbed un-grafted material**
- **4x addition promoted polymerization to the pulp**



- Entire dose of 4-Hba added to the pulp at the beginning of the reaction vs. periodic addition of 4-Hba throughout the duration of the reaction
- To decrease 4-Hba self-condensation and increase coupling to the pulp
- Additions in two hours:
  - 1x – Entire dosage of 4-HBA
  - 2x- 1 addition per hour
  - 4x- 1 addition each 30 minutes
  - 8x- 1 addition each 15 minutes
- Treatments followed by alkaline extraction to remove adsorbed 4-Hba

Phase 2: Conclusions

- It is not necessary to perform the laccase/phenol/pulp reaction in a reactor pressurized with O_2
- A high consistency and high dosage of phenol (4-Hba) is necessary for obtaining higher amounts of phenol coupled to the pulp
- Periodic addition was effective in increasing the amount of 4-hba coupled to the pulp

Phase 3: Effects of Laccase-Grafting Treatment on Lignin

Fully Bleached Pulp → Laccase/4-Hba Treatment

Isolated Lignin → "Lignin-Impregnated Fibers"

↓

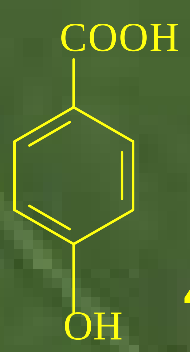
Laccase/4-hba Treatment

↓

Lignin

↓ ↓

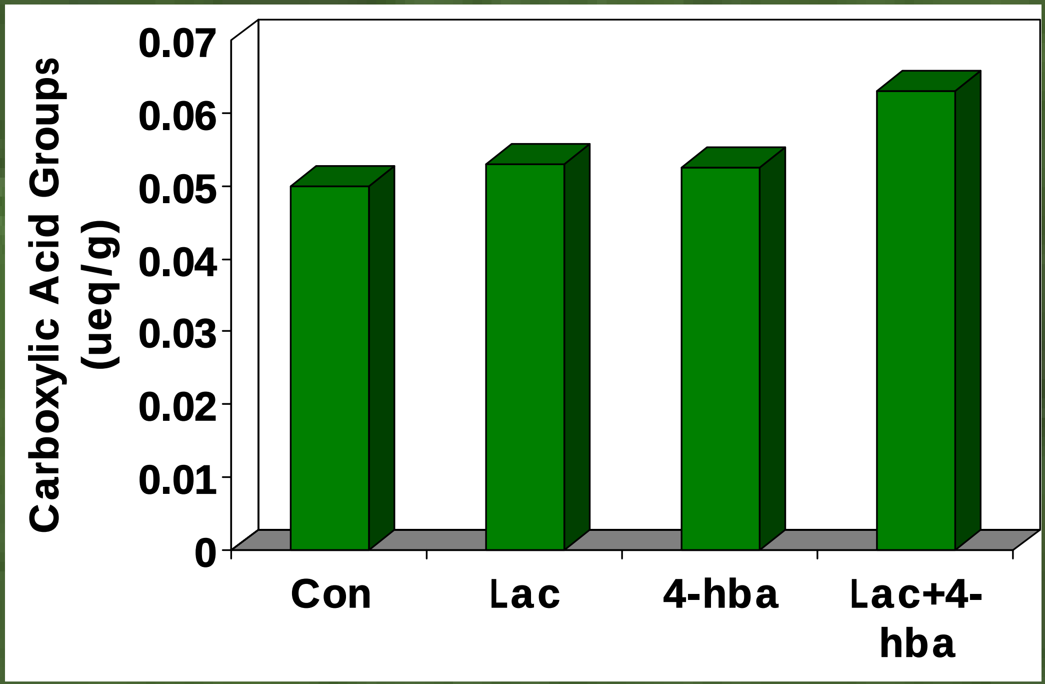
Molecular Weight | Structural Changes



4-HBA

Phase 3: Application to Fully Bleached Pulps

- Applied 4-hba with laccase
- No significant increase in acid groups when compounds applied with laccase
- Indicates pulp lignin is necessary for coupling to fibers



Phase 3:
Lignin Impregnated Fibers

Kraft (kappa 92)

Filter paper → Blender →

↓ Acetone extract

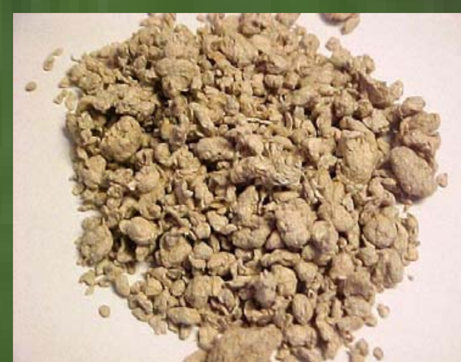
↓ Lignin extraction

- 9:1 dioxane:1N HCL
- 2 hours w/acid precipitation

Lignin+filter paper fibers
20% csc in dioxane

↓ Roto-evaporate

↓ **Lignin impregnated fibers**

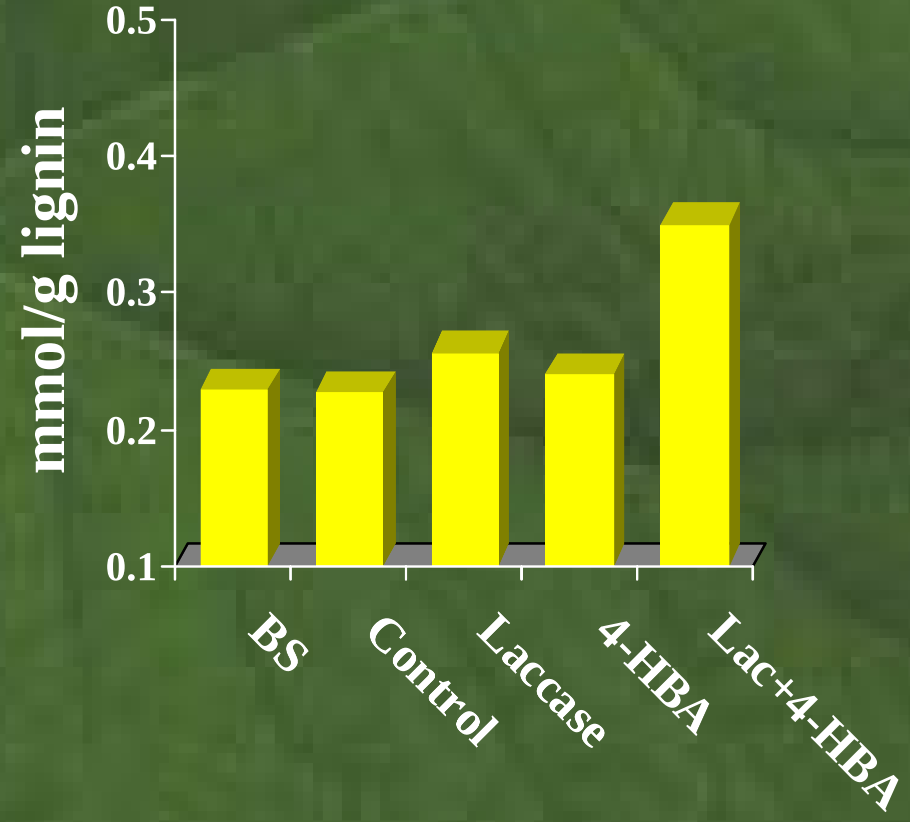


→ **React With laccase+4-Hba**

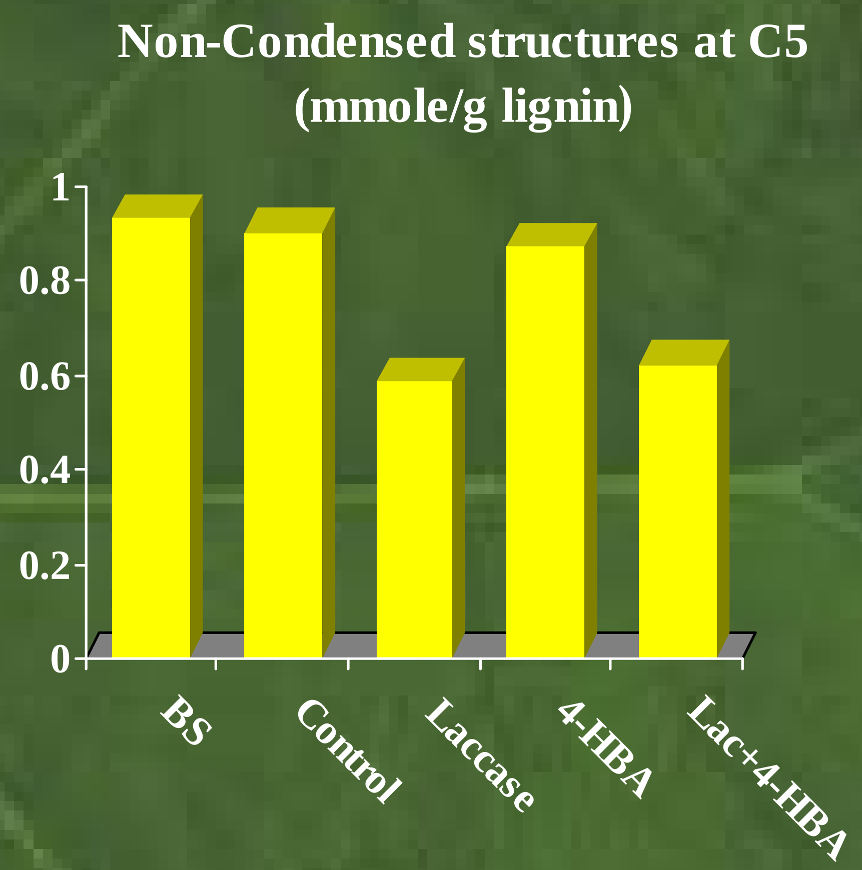
Phase 3 Lignin Impregnated Fibers :^{31}P NMR

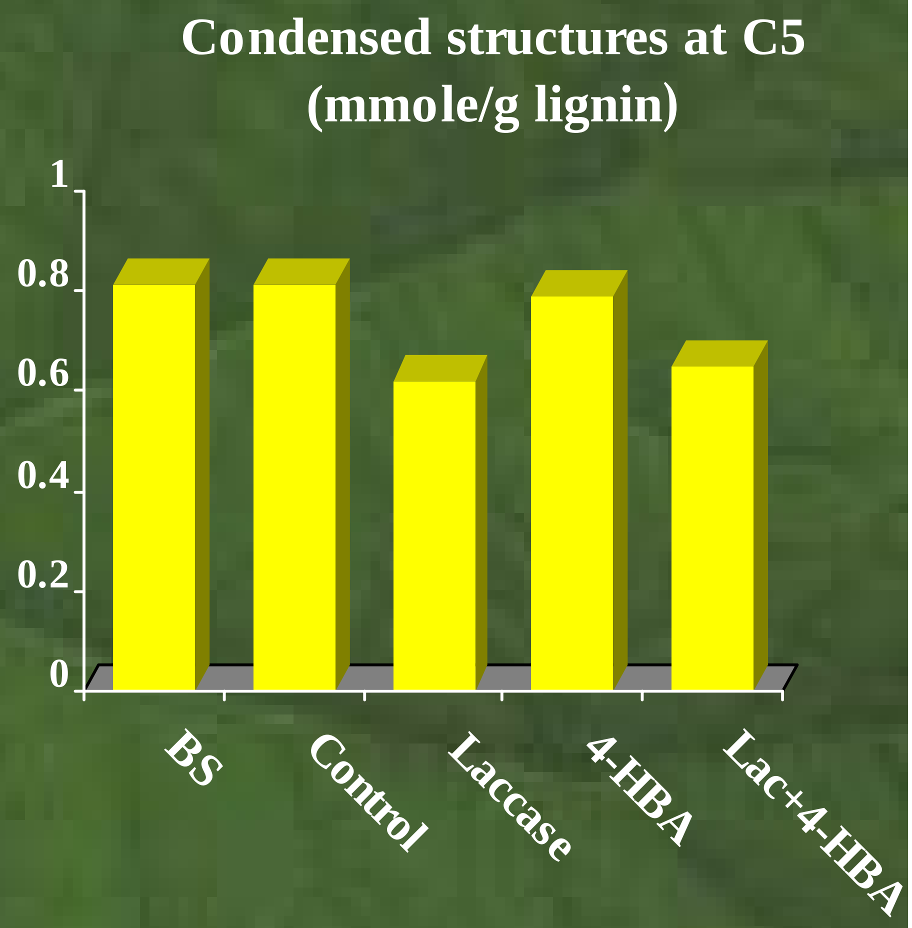
- ^{31}P NMR for quantifying hydroxyl groups on lignin
- Coupling of phenolic acids to lignin should increase carboxylic acid groups on lignin

- Carboxyllic acid groups increased indicating coupling of 4-hydroxybenzoic acid to lignin



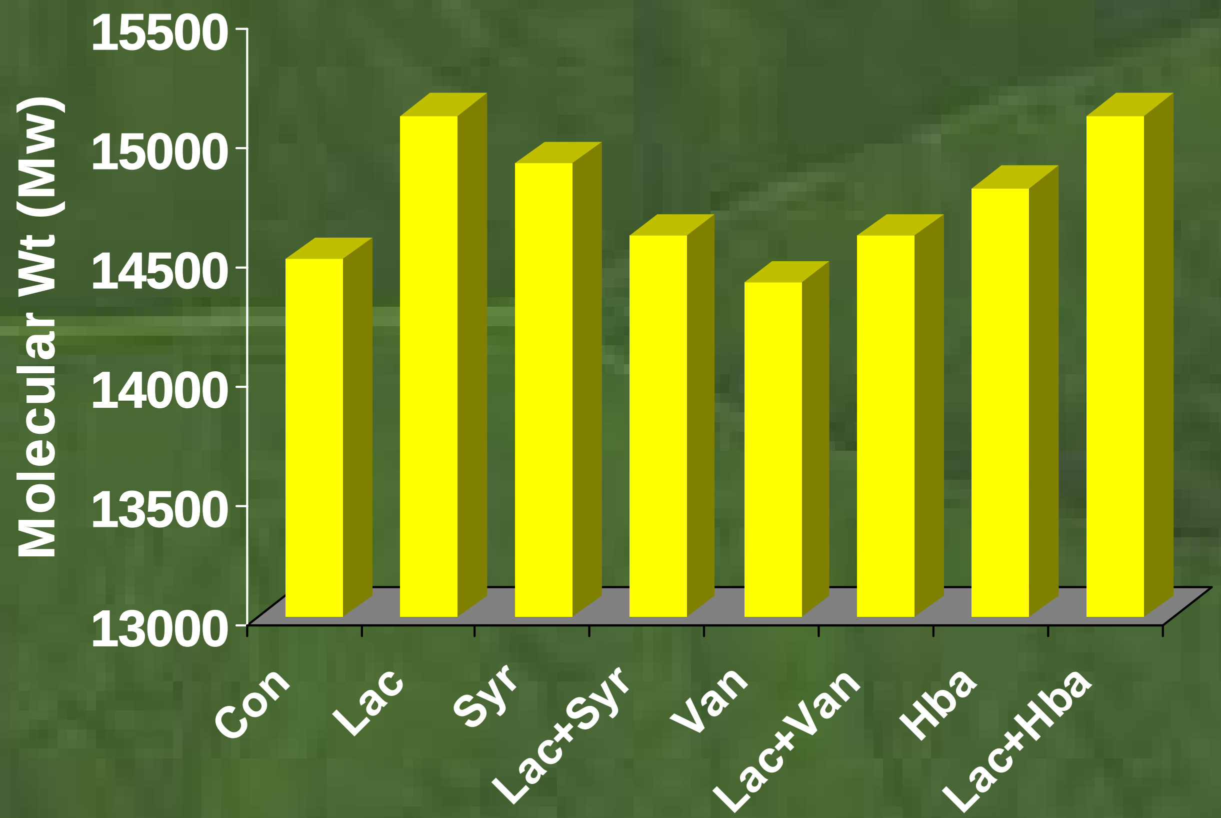
Phase 3 Lignin Impregnated Fibers :^{31}P NMR





Both non-condensed and condensed phenolic groups decreased in the presence of laccase indicating the predominant reaction was the reaction of laccase with phenols

Phase 3
Lignin Impregnated Fibers:
Molecular Weight



LSD:

1545 g/mol

Phase 3: Conclusions

- NMR data suggests increase in acid groups that indicate the attachment of 4-hydroxybenzoic acid to lignin.
- NMR data also shows that laccase decreases both the non-condensed and C5 condensed phenols during grafting.
- Sensitivity of molecular weight analysis may be insufficient to illustrate changes imparted by grafting of 4-hba to lignin

Phase 4: Pulp Surface Materials

High-kappa kraft pulp

↓

Laccase –Grafting Treatment

↓

Isolate Surface Material

↓ ↓

Molecular Weight | Carboxylic Acid Groups

Phase 4:
Isolation of Pulp Surface Material

35 g pulp Sample

Suspend at 10% Consistency

Disintegrate for 200,000 Revolutions

Filter 5x through Whatman no.41 filter paper retaining >20 um material and freeze dry

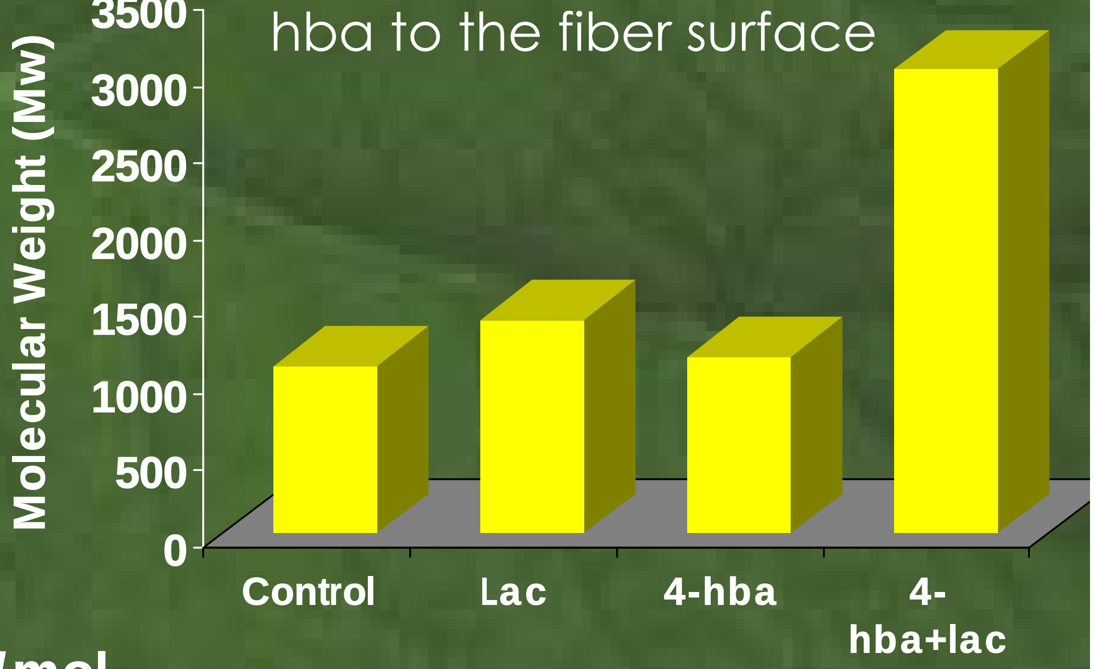
Yield of 40-50 mg
Per sample

* Heijnesson et al 1995

Phase 4:
Pulp Surface Material
Molecular Weight

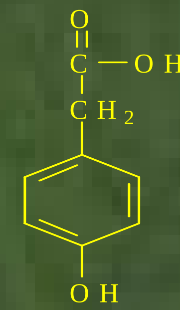
- Pulps treated with laccase and 4-hba followed by isolation of surface material
- Dissolved and acetylated with DMSO/DMF/Pyridine mixture
- Molecular weight measured by gel permeation chromatography

- Molecular weight increased with laccase+4-hba treatment
- Indicates laccase-facilitated coupling of hba to the fiber surface

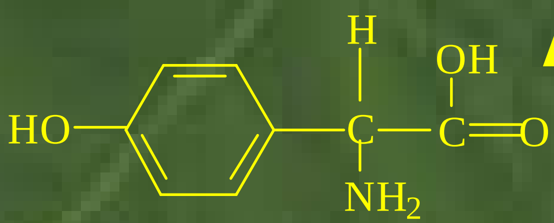


LSD: 752 g/mol

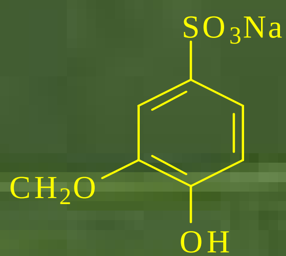
Phase 4
Pulp Surface Material:
Acid Group Titration



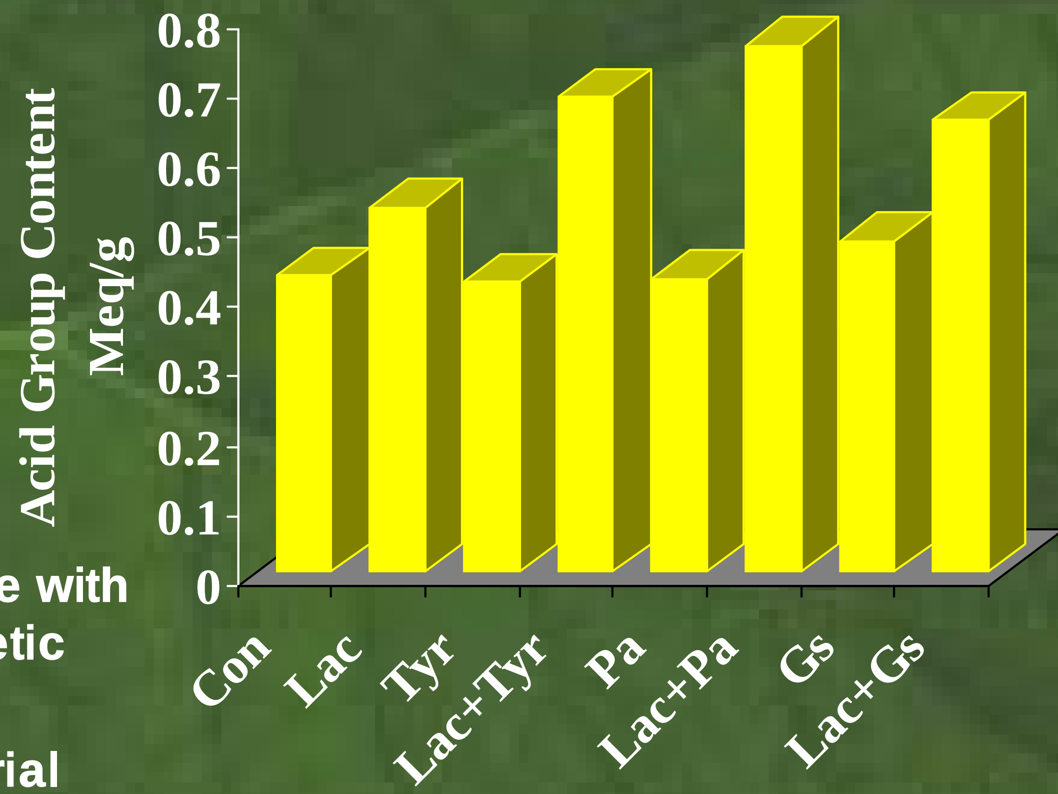
4-hydroxy phenylacetic aci



Tyrosine



Guaiacol Sulfonate



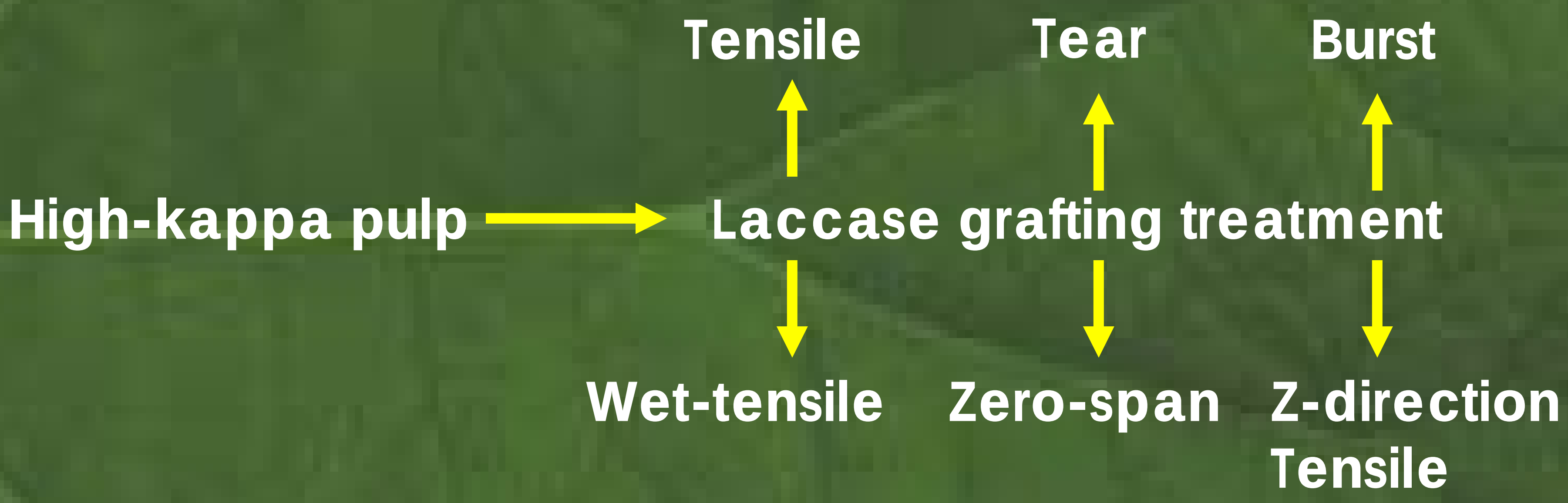
- **Pulps pre-treated with laccase with tyrosine, 4-hydroxyphenylacetic acid, and guaiacol sulfonate**
- **Acid groups on surface material increased with laccase-grafting treatments**

Tyr=tyrosine, Pa=Phenylacetic acid Gs= Guaiacol Sulfonate

Phase 4: Conclusions

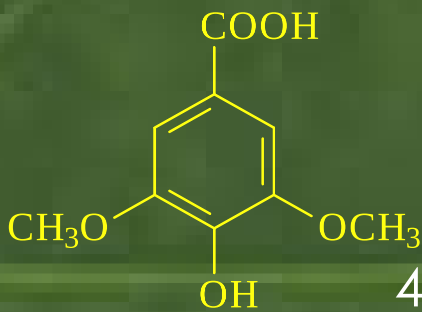
- Laccase-facilitated grafting treatments result in increases in the molecular weight and carboxylic acid groups on material isolated from the pulp fiber surface

Phase 5: Paper Physical Properties

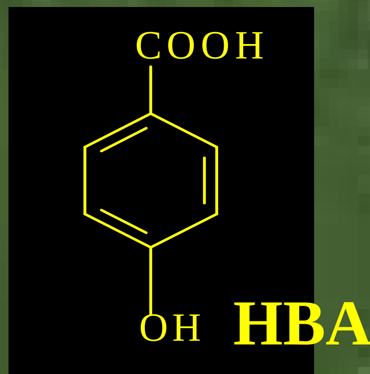


Phase 5
Compounds Studied

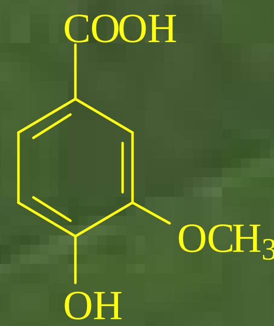
Compounds applied to high-kappa kraft pulps:



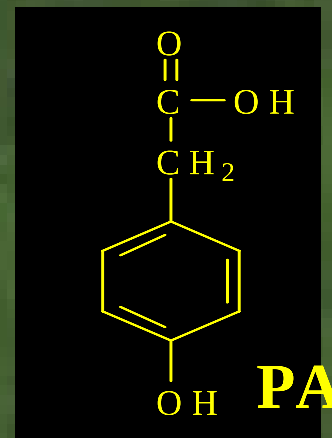
Syringic acid



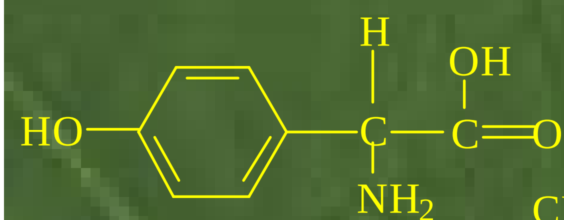
4-Hydroxybenzoic acid



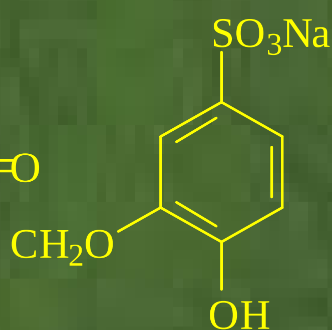
Vanillic acid



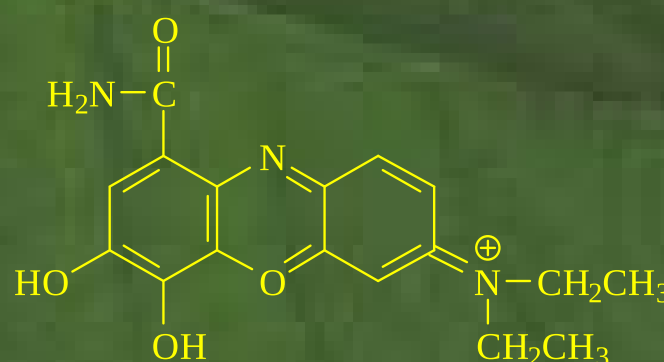
4-hydroxy phenylacetic acid



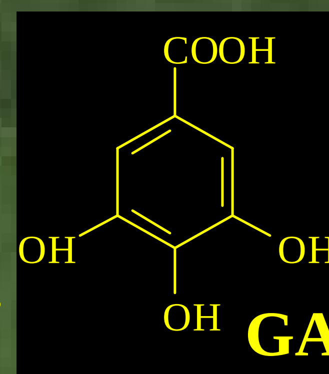
Tyrosine



Guaiacol Sulfonate

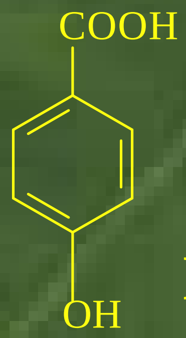


Celestine Blue

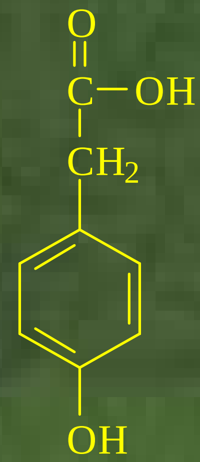


Gallic Acid

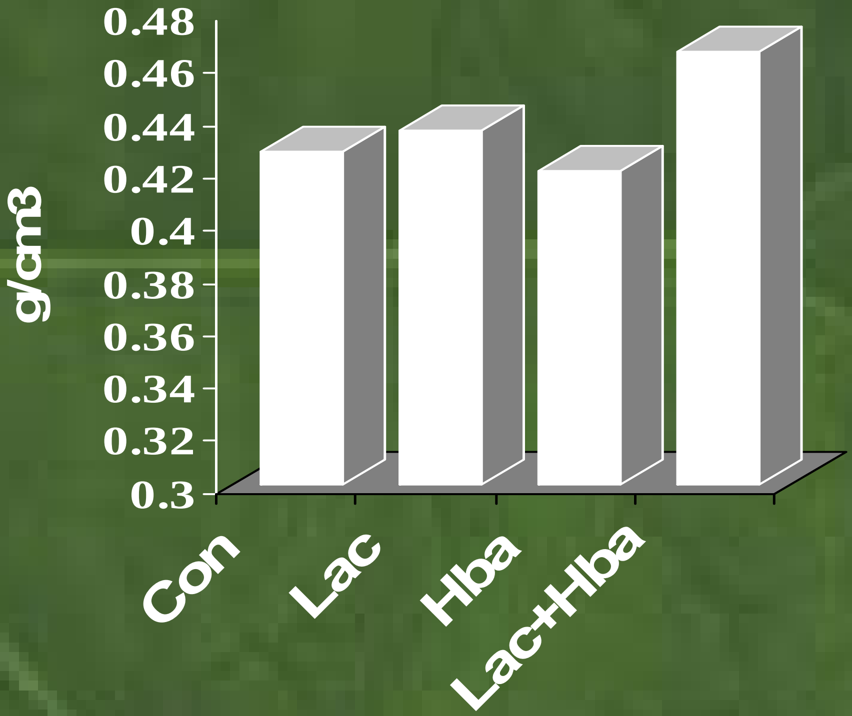
Phase 5
Paper Physical Properties: Apparent Density

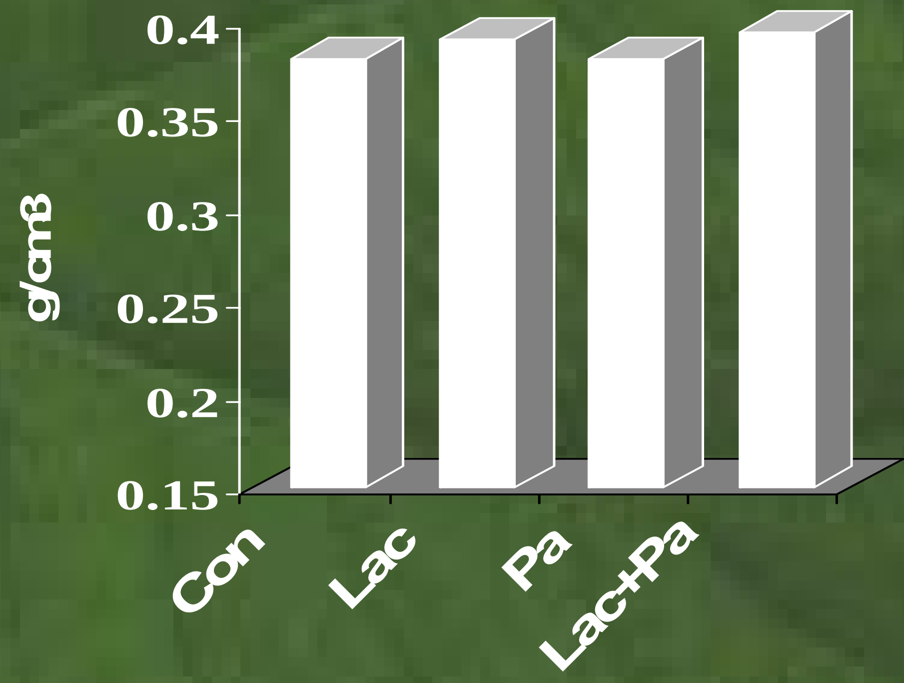


HBA



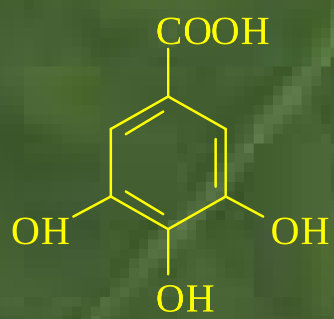
PA





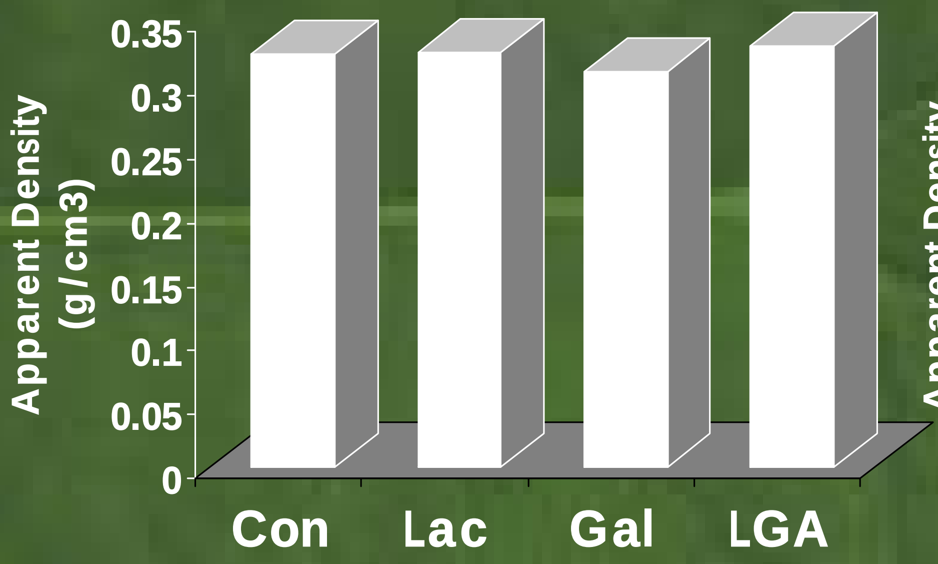
Kappa 91, Refined 2000 rev. PFI

Kappa 91, Refined 1000 rev. PFI

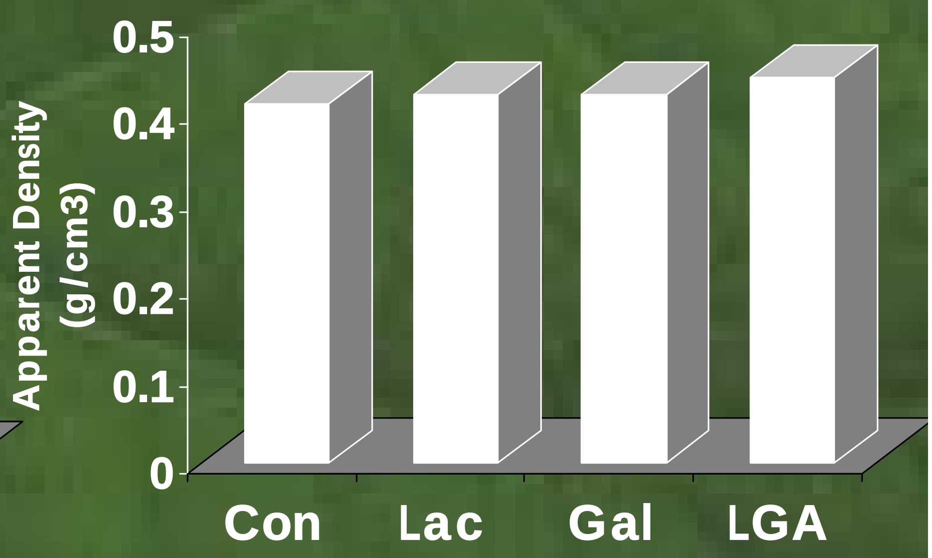


GA

Phase 5
Paper Physical Properties: Apparent Density

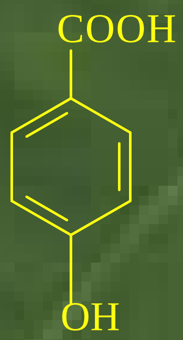


Kappa 91, No Refining

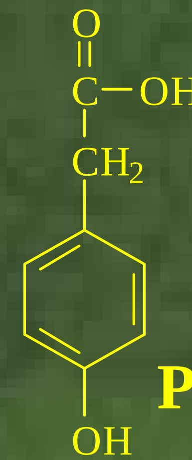


Kappa 91, Refined 2000 rev. PFI

Phase 5
Paper Physical Properties:
Burst



HBA



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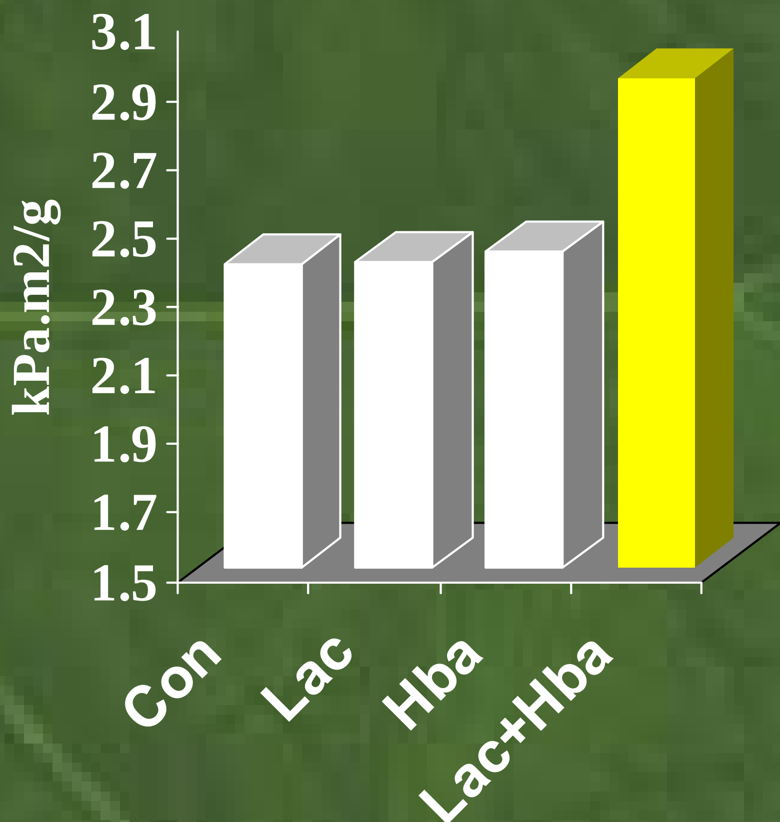
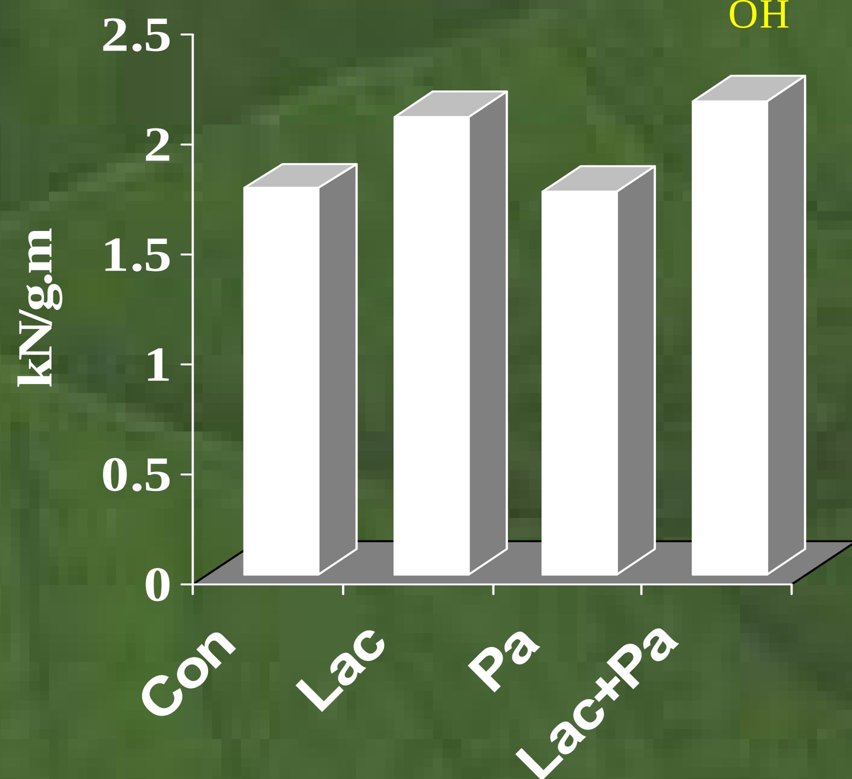
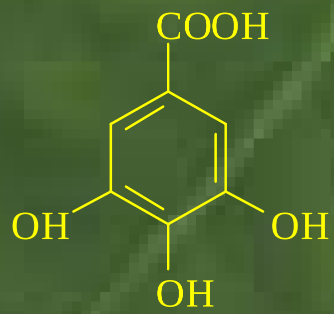


Kappa 91, Refined 2000 rev. PFI



Kappa 91, Refined 1000 rev. PFI

Phase 5
Paper Physical Properties:
Burst



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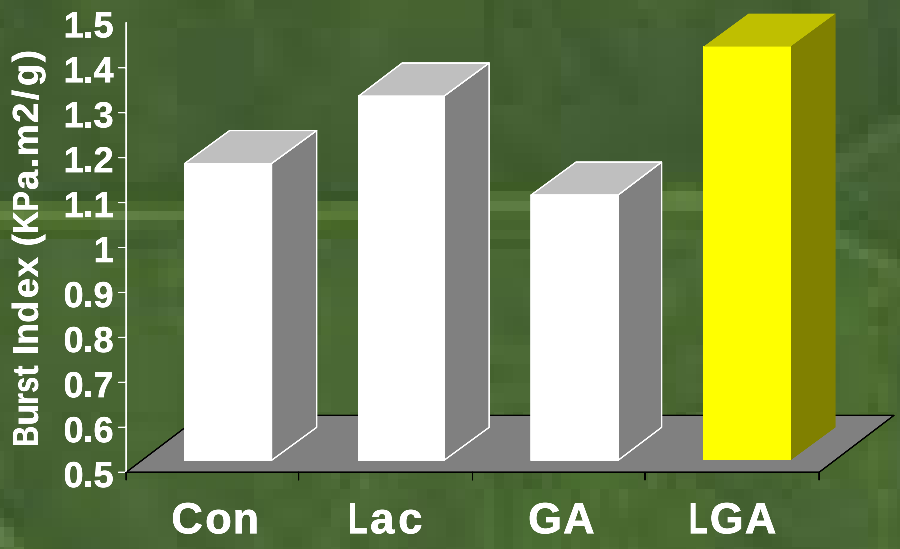
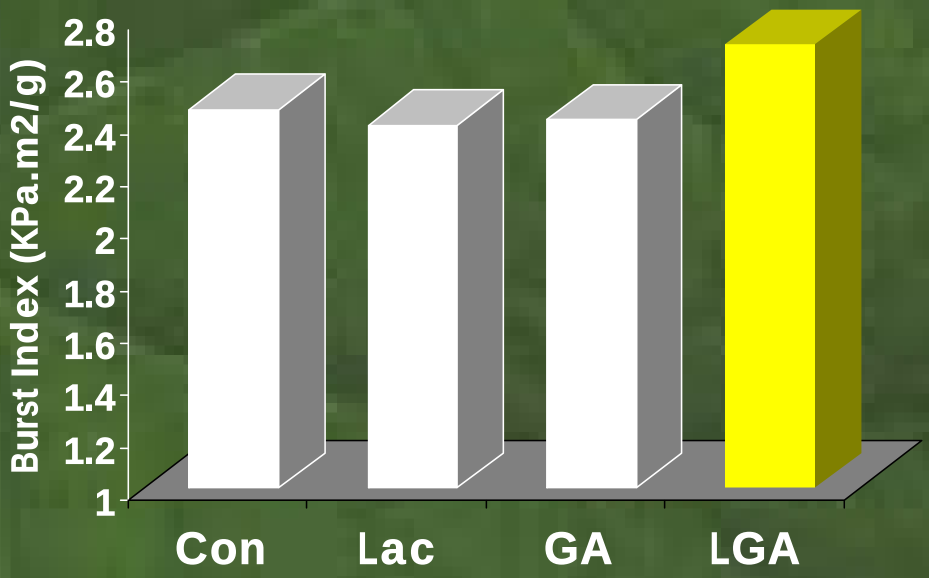
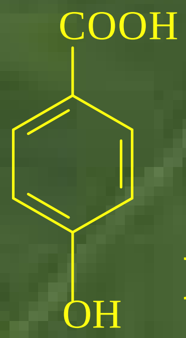




Kappa 91, No Refining

Kappa 91, Refined 2000 rev. PFI

Phase 5
Paper Physical Properties:
ZDT



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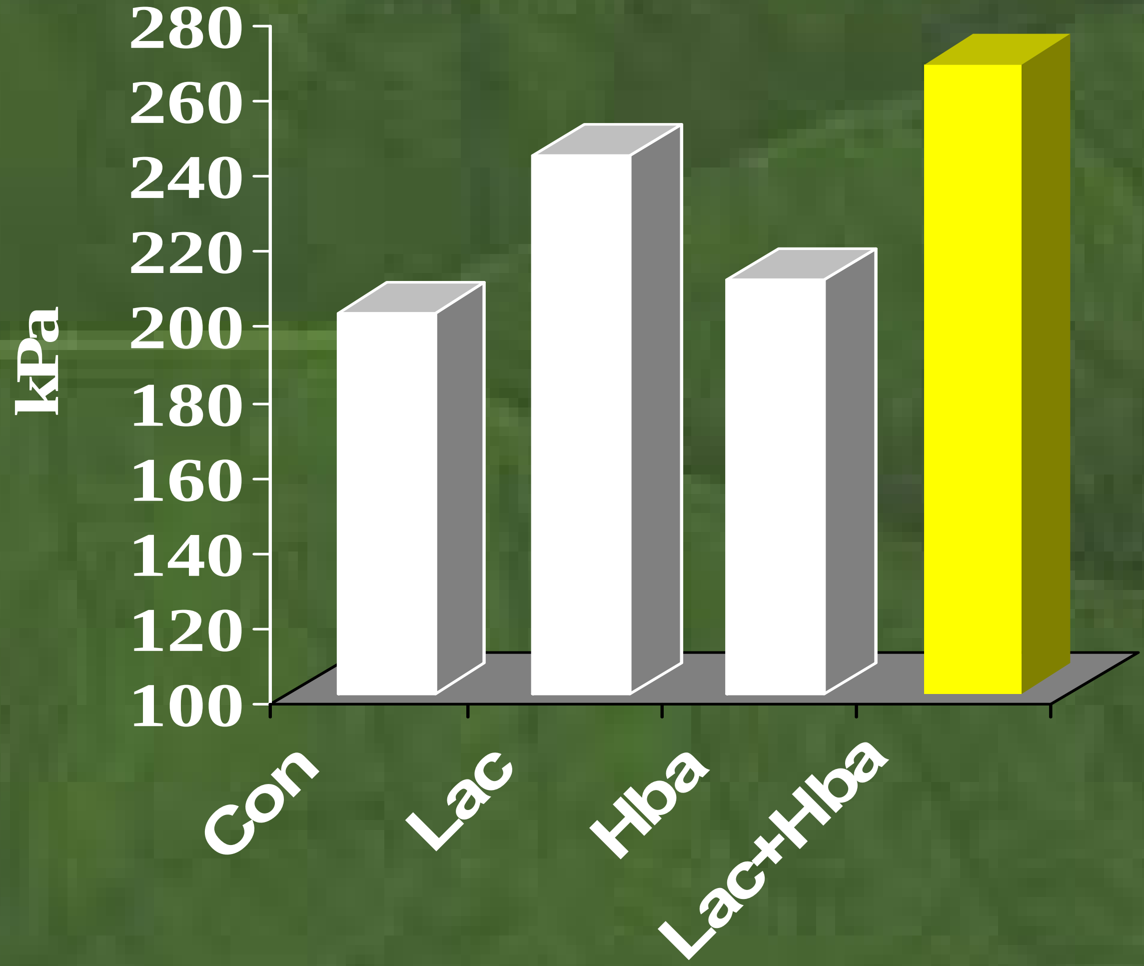
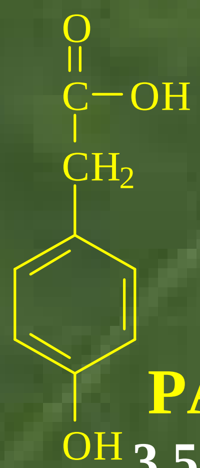


Phase 5
Paper Physical Properties: Tear Resistance



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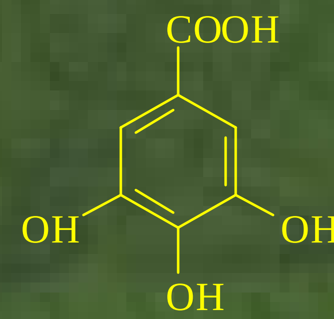

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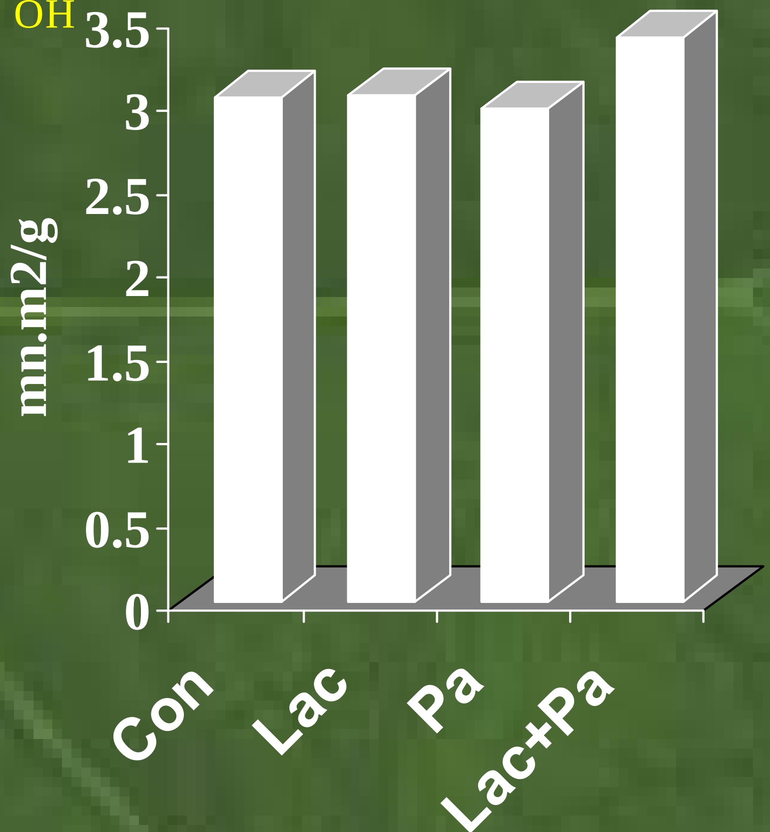
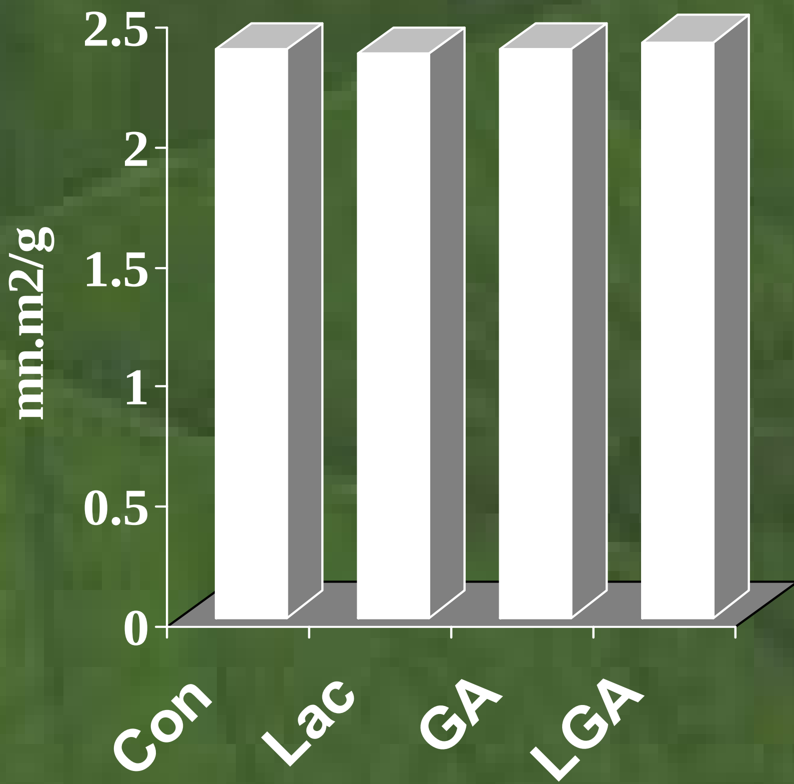
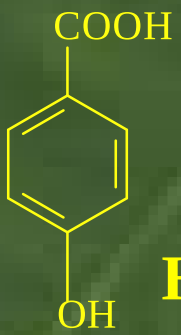


Kappa 91, Refined 1000 rev. PFI



Kappa 91, No Refining

Phase 5
Paper Physical Properties:
Zero Span



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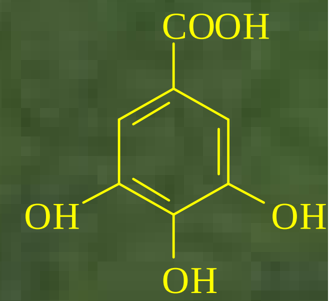

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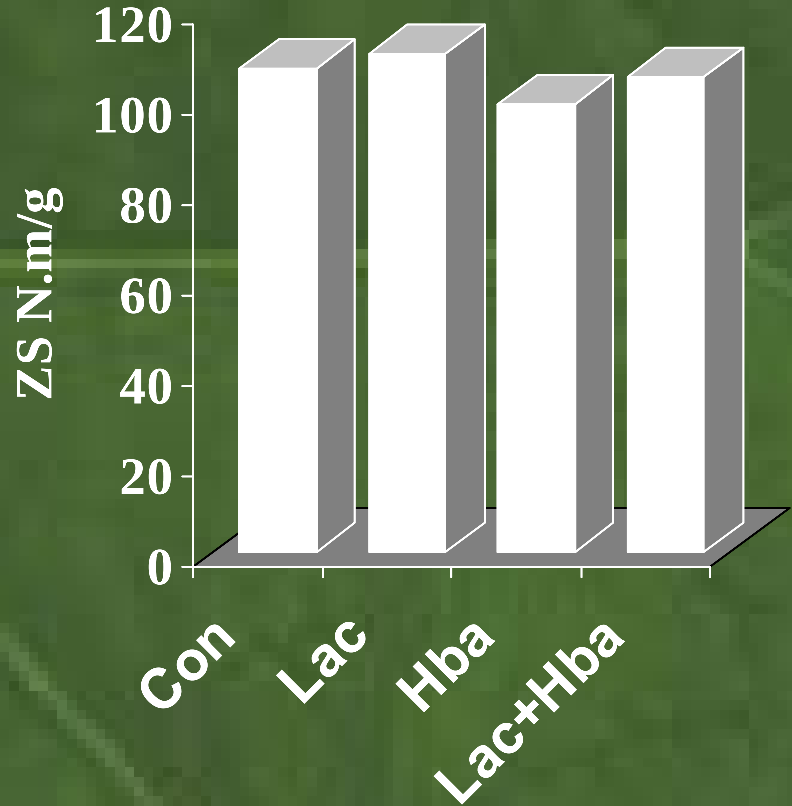
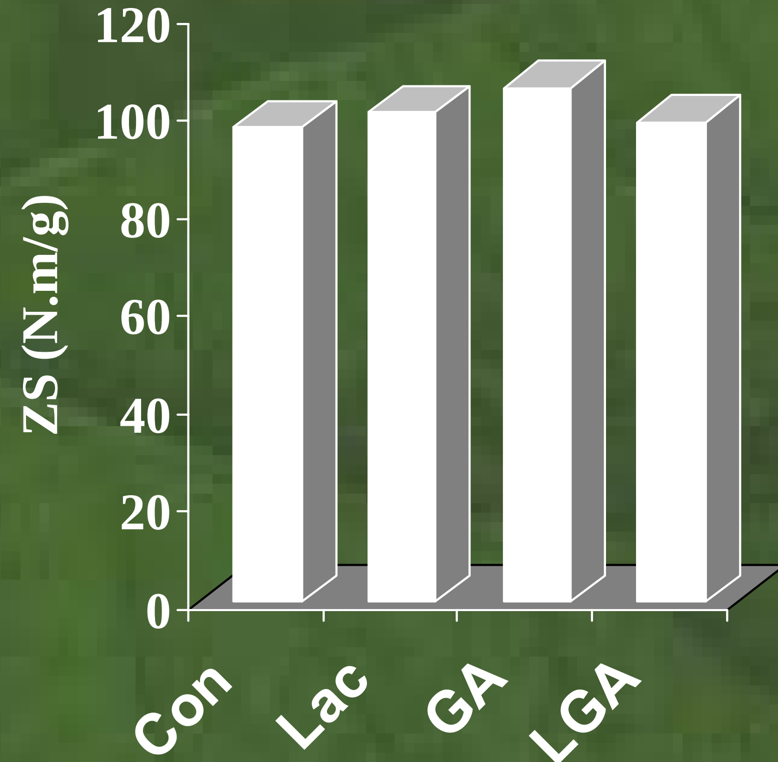


Kappa 91, Refined 2000 rev. PFI



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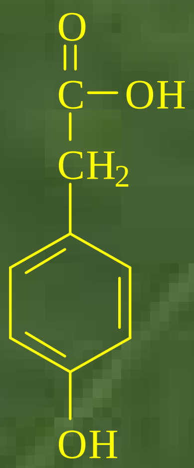
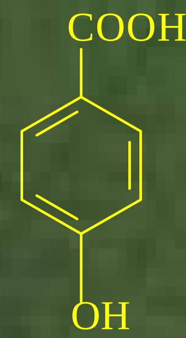


Phase 5
Paper Physical Properties: Tensile Strength



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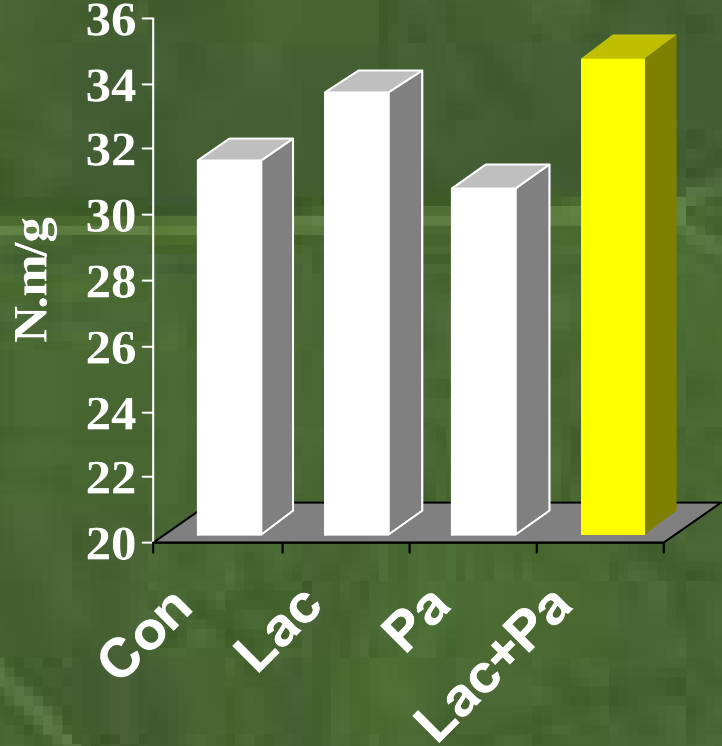

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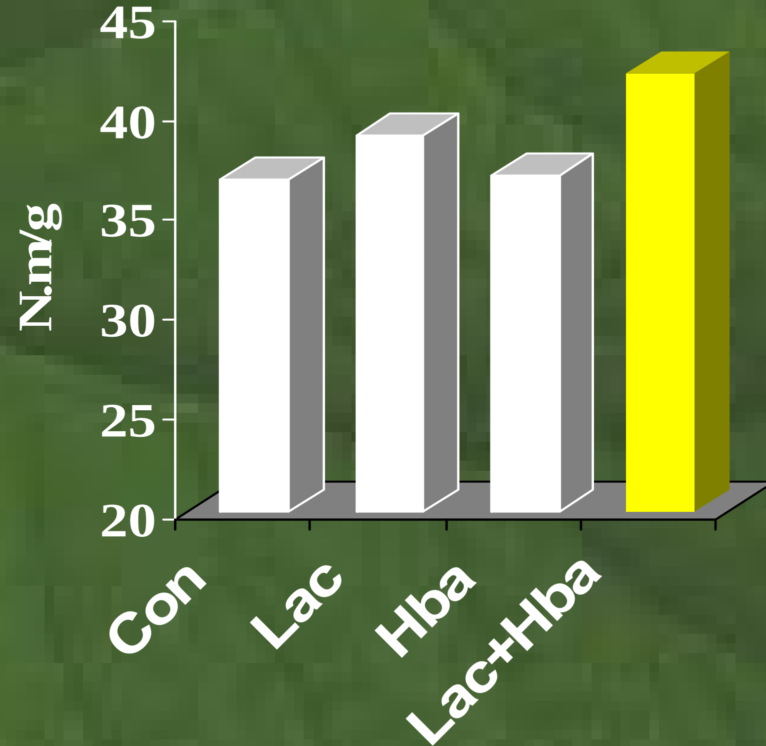
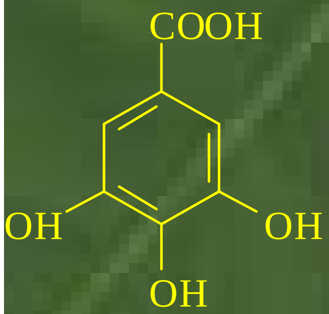


Kappa 91, Refined 1000 rev. PFI

Phase 5
Paper Physical Properties: Tensile Strength



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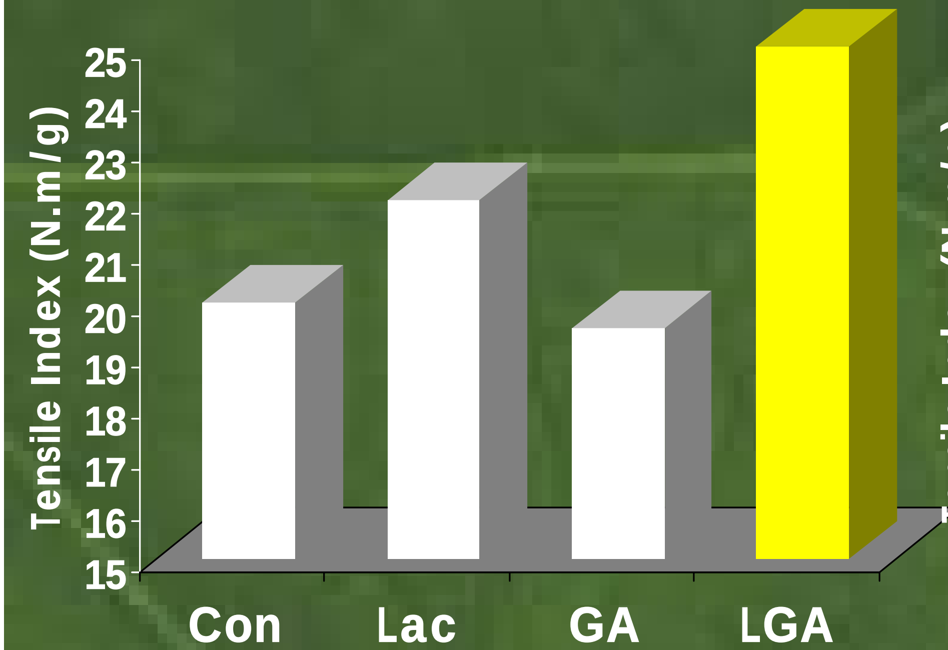

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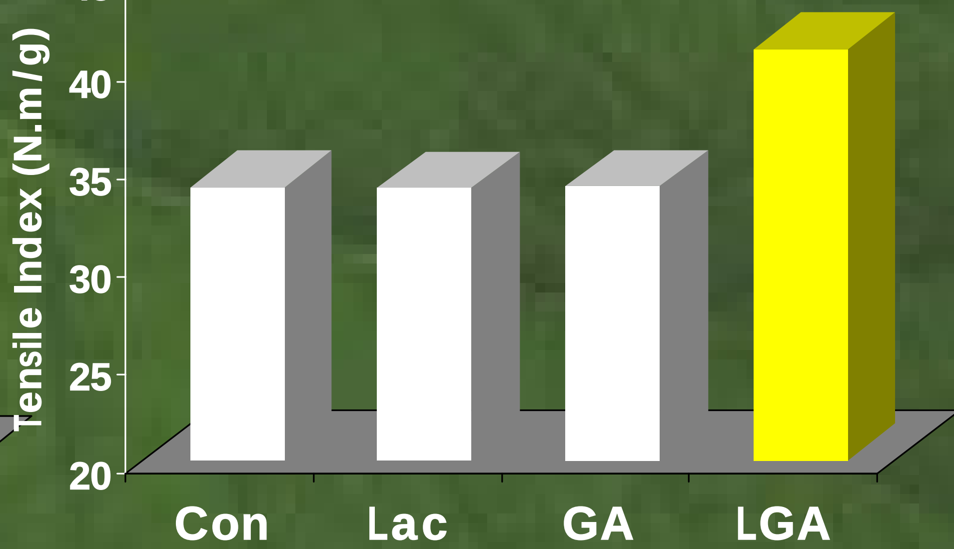
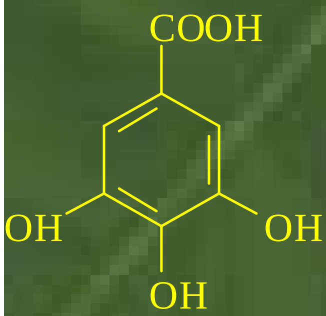


Kappa 91, Refined 2000 rev. PFI

Phase 5
Paper Physical Properties: Wet-Tensile Strength



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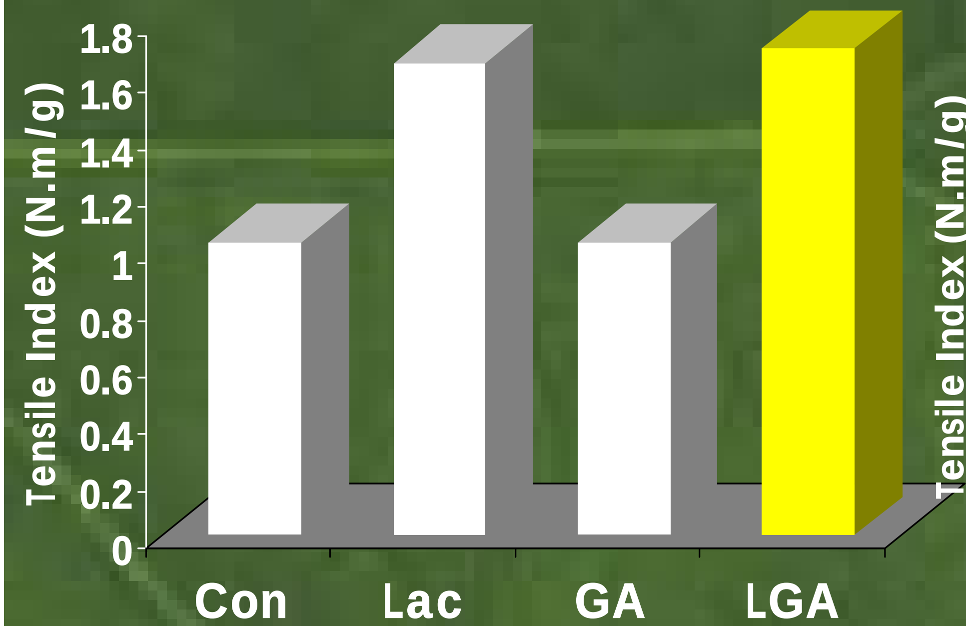
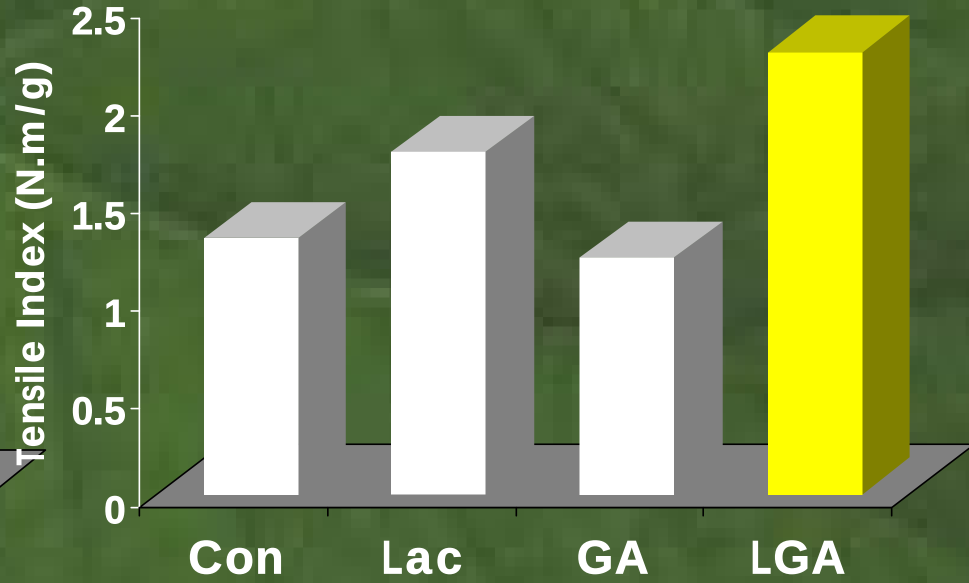


Kappa 91, No Refining



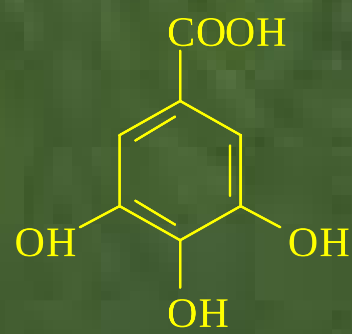
Kappa 91, Refined 2000 rev. PFI

Phase 5: Conclusions

- Addition of phenolic acids to the pulp resulted in the best performing papers during paper strength testing
- Laccase treatment with gallic acid provided the largest increases in wet and dry tensile strength of all the compounds tested
- The increases in strength with laccase/gallic acid treatment were not accompanied by changes in sheet density

Phase 6: Further Investigation

Laccase +



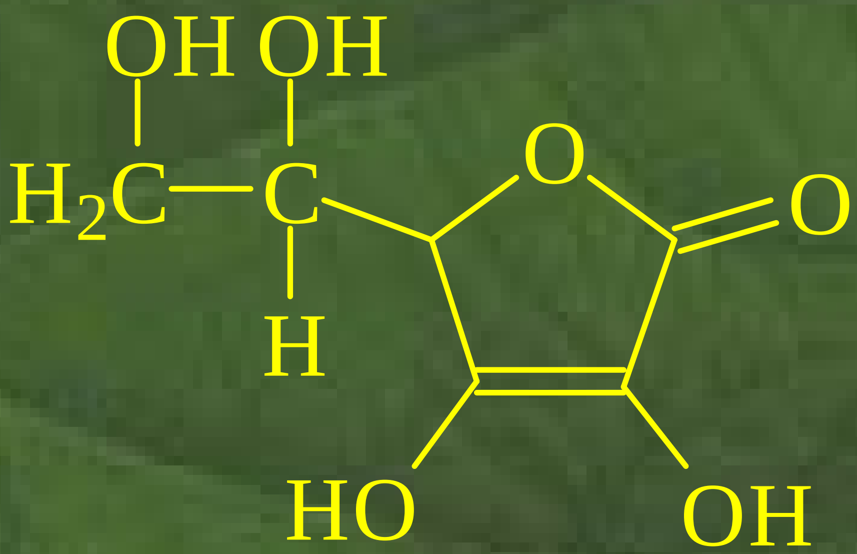
→ Radical Scavenger

Contact Angle

Vertical Wicking

Phase 6
Radical Scavenger

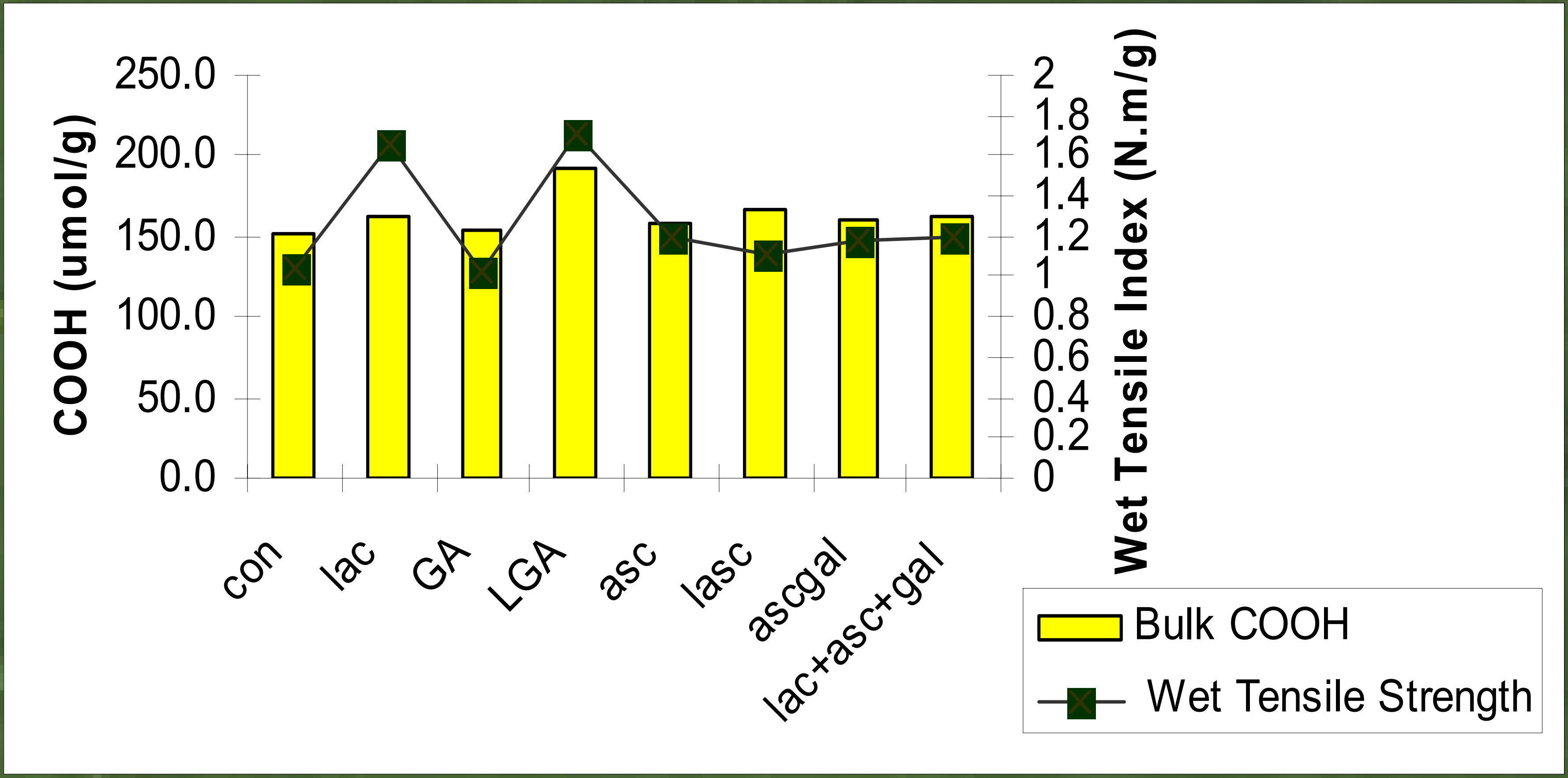
- Observe if gallic acid coupling to kraft pulp is due to radical coupling reactions
- Radical scavenger
- Added in 10:1 molar ratio to gallic acid due to laccase
- Has been shown to scavenge radicals of gallic acid in grape juice (Tulyathan 1989)
- Reduces quinones formed by laccase oxidation back to phenols (Bocks 1967)



Ascorbic Acid

Phase 6
Radical Scavenger

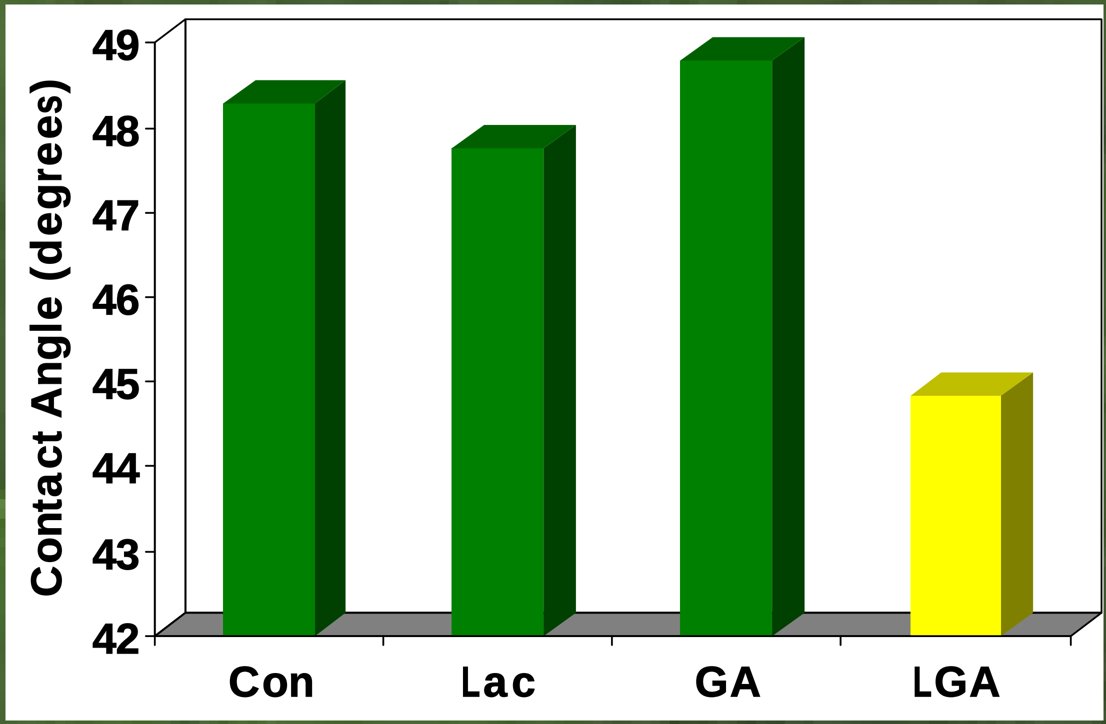
- Lac+Gal ineffective in the presence of ascorbic acid (asc)
- Wet-tensile strength of original pulp preserved with ascorbic acid

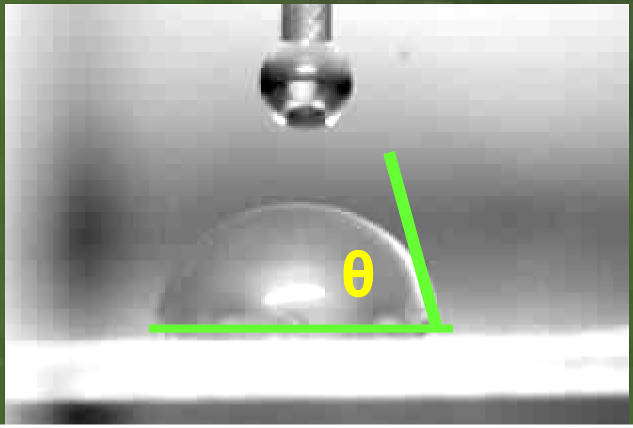


Unrefined Pulp

Phase 6
Contact Angle

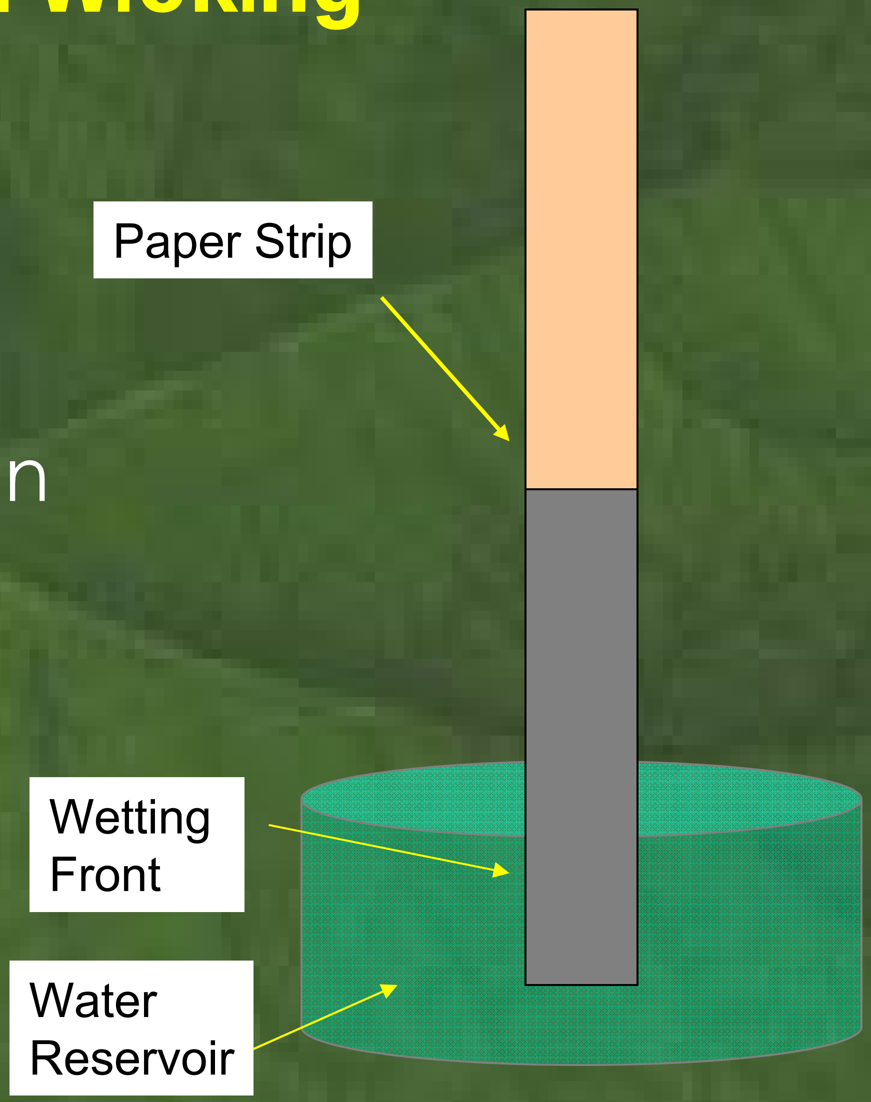
- Compatibility of sheet surface with water
- Measured with camera shooting 1 frame/0.067sec
- Slight decrease in contact angle observed with gallic acid treatment
- Higher dosages of gallic acid could not be measured since there was no drop holdout





Phase 6
Vertical Wicking

- Important for absorbent characteristics of non-woven structures
- Kinetic relationship can be used to relate structural and surface properties



Phase 6
Vertical Wicking

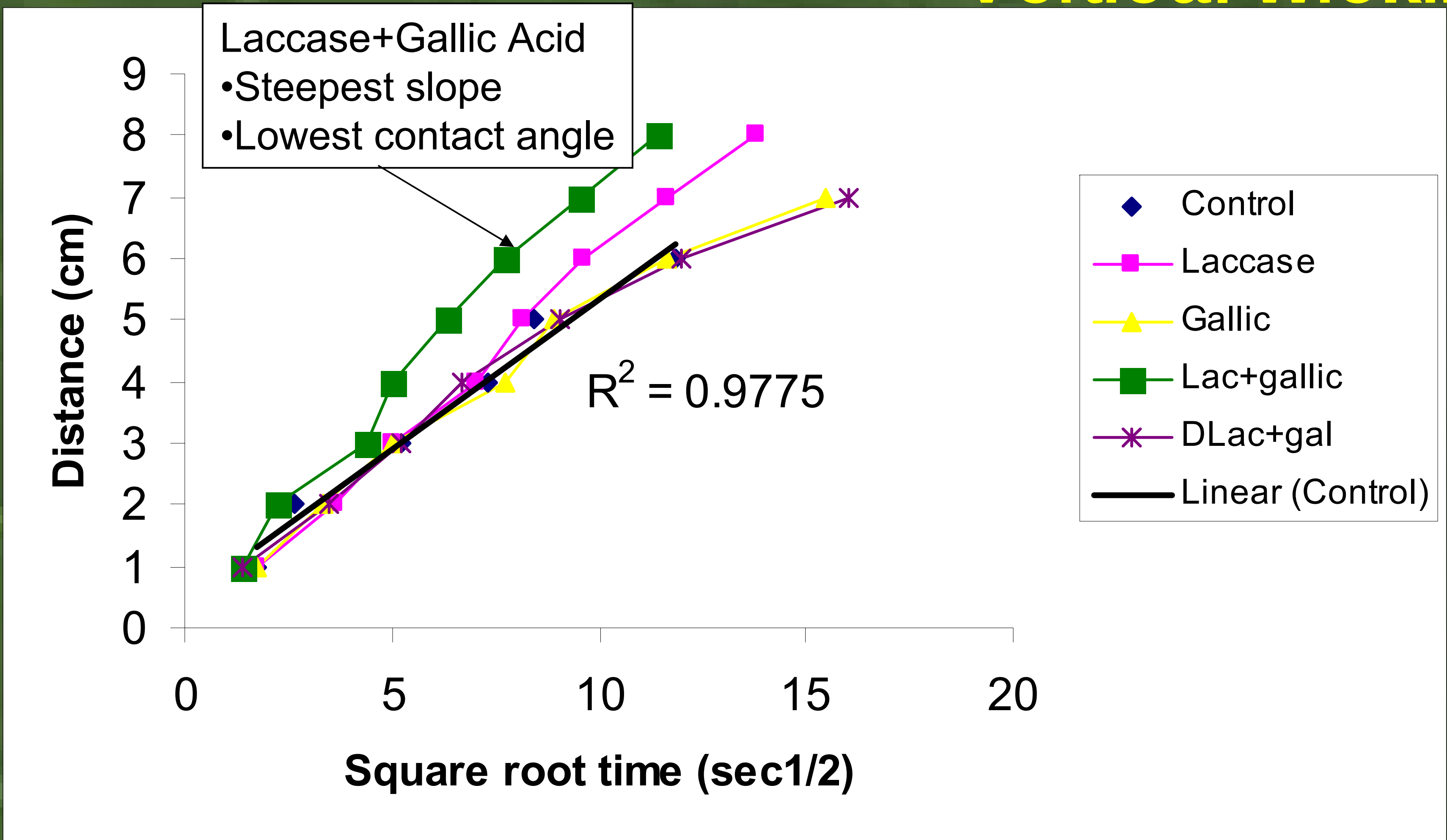
- Lucas-Washburn Wicking Equation:

$$h = \left(\frac{r\sigma \cos\theta}{2\,\tau^{2}\,\mu} \right)^{1/2} \sqrt{t} = k\sqrt{t}$$

h=distance traveled
r=capillary radii
t=time
σ=surface tension
τ=tortuosity factor
μ= viscosity

- **Square-root time should be linear to vertical distance traveled (Hodgson and Berg 1988)**
- **Increasing slope of distance vs. square root time curve should indicate enhanced liquid absorbing capability of material**
- **Increased compatibility with H_2O may indicate propensity for hydrogen bonding during paper sheet formation**

Phase 6
Vertical Wicking



Test Duration = 5 min
Sample weight =0.15g +/- 0.01g
Sample density= .27g/cm^3 +/- .01 g/cm^3
Average of 2 tests

Lac+gallic wicked 20% further and absorbed 30% more H_2O than control sample

Phase 6: Conclusions

- Ascorbic acid inhibits the laccase/gallic treatment strongly suggesting a free-radical mechanism
- Laccase/gallic acid treatment results in an increase in hydrophilicity of fibers

Thesis Conclusions

- It is possible to use laccase to couple phenolic compounds to high-kappa kraft pulps
- Coupling imparts significant changes to the pulp fiber surface
- Lignin is the main site of coupling for laccase generated phenoxy radicals
- Coupling of gallic acid to the fiber results in tremendous increases in wet/dry tensile strength coupled with an increase in hydrophilicity of fibers.
- Strength improvements may be a combination of improvement of surface characteristics and cross-linking of phenoxy radicals between fibers in the sheet

Publications and Presentations

- Chandra, R.P. and Ragauskas, A.J., *Enzyme and Microbial Technology* June 2002, Vol. 30 (7), 855-861, "Evaluating the Ability of Laccase to Couple Phenols to High-Yield Kraft Pulps With Laccase."
- Chandra, R.P., Felby, C.L. and Ragauskas, A.J., submitted to: *Journal of Wood Chemistry and Technology* "Improving Laccase-facilitated Coupling of Phenolic Acids to High-Kappa Kraft Pulps"
- Chandra, R.P. and Ragauskas, A.J., *11th International Symposium in Wood and Pulping Chemistry*, June 11, 200, Nice, France, "Sculpting the Molecular Weight of Lignin via Laccase."
- Chandra, R.P. and Ragauskas, A.J., *Tappi Pulping Conference*, November 4-7, 2001, Seattle, Washington, U.S.A. Paper accepted for presentation, " Laccase: Renegade of Fiber Modification"
- Chandra, R.P. and Ragauskas A.J., *8th International Conference on Biotechnology in the Pulp and Paper Industry*, Invited Paper and Book Chapter, pp.165-172, June 4, 2001, Helsinki, Finland "Elucidating the Effects of Laccase Treatments on the Physical Properties of High-Kappa Kraft Pulps."
- Chandra, R.P. and Ragauskas, A.J., *American Chemical Society National Meeting 2002*, Invited Speaker and Published Book Chapter for Anselme Payen Award Symposium, April 2002, Orlando, Florida, "Bio-grafting Celestine Blue to High-Kappa Kraft Pulps with Laccase"
- Chandra, R.P., Lehtonen, L.K., and Ragauskas, A.J., Accepted in *Biotechnology Progress*. "Modification of High-Yield Kraft Pulps with Laccase to Improve Strength Properties: Laccase Treatment in the Presence Gallic Acid"

Publications and Presentations

- Chandra, R.P. and Ragauskas, A.J., submitted to ***Journal of Agriculture and Food Chemistry***. "Modification of High-Yield Kraft Pulps with Laccase: Enhancing the Effects of Laccase Treatment with Xylanase"
- Chandra, R.P. and Ragauskas, A.J., ***American Chemical Society National Meeting 2002***, Invited Speaker for Anselme Payen Award Symposium, April 2002, Orlando, Florida, "Fiber Modification with Laccase: You Say You Want a Revolution?"
- Chandra, R.P., Chakar, F.S., Allison, L., Kim, D.H., Elder, T., and Ragauskas, A.J., ***10th International Conference on Biotechnology in the Pulp and Paper Industry***, June 4, 2001, Helsinki, Finland, "Delving into the Fundamental LMS Delignification of High-kappa Kraft Pulps."
- Chandra, R.P. and Ragauskas, A.J., ***American Chemical Society Pacifichem Conference 2000***, December 2000, Honolulu, Hawaii, " Parsing Laccase's Effects on Modifying Lignin."
- Chandra, R.P. , Dyer, T.J. and Ragauskas, A.J., Publication to be submitted, "The Aptitude of Laccase to Attach Compounds to Bleached Chemical Pulps "

Future Lignocellulosics

Tip of the iceberg

- Many new avenues
  - New grafting agents and substrates
  - Various application methods
  - Chemo-enzymatic opens door for novel fiber modification possibilities
    - Energy efficient
    - Environmentally friendly
    - Untapped potential

Acknowledgements

- Dr. Barry Crouse
- Novozymes
- Temple Inland and Riverwood
- IPST member Companies