

# Evaluation of the chemical reactivity in lignin precursors using the Fukui function

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**Abstract** The hydroxycinnamyl alcohols: *p*-coumarol, coniferol and sinapol are considered the basic units and precursors of lignins models. In this work, the specific reactivity of these molecules was studied. We investigate their intrinsic chemical reactivity in terms of the Fukui function, applying the principle of hard and soft acids and bases (HSAB) in the framework of the density functional theory (DFT). Comparisons of their nucleophilic, electrophilic and free radical reactivity show their most probably sites to form linkages among them. It is found that the most reactive sites, for reactions involving free radicals, are the carbons at the  $\beta$ -position in the *p*-coumarol and sinapol molecules, whilst the regions around the carbon-oxygen bond of the phenoxy group are the most reactive in coniferol.

**Keywords** DFT · Fukui function · Intrinsic Reactivity · Lignin · Monolignols

## Introduction

Lignin is an aromatic heteropolymer that is mainly present in the walls of secondary thickened wood cells; it derives

from simple units but contains significant complexity [1]. Typically only three hydroxycinnamyl alcohols (*p*-coumarol, coniferol and sinapol) are considered in the lignins formation. Those alcohols are named monolignols and are shown in Fig. 1.

Lignin polymerization is initiated by an oxidative enzymatic dehydrogenation of monolignols [2], which form five different resonance structures shown in Fig. 2. This leads to a large number of probable coupling reactions, yielding a disordered polymer.

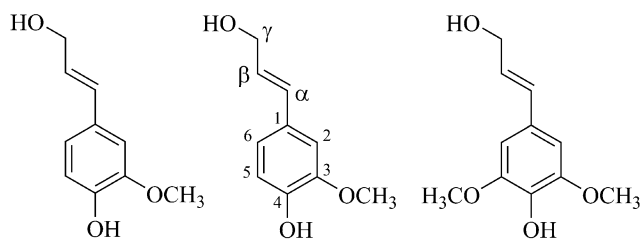
The macromolecular structure of lignins could be initiated by different combinations of interatomic linkages between two monolignols, but their relative abundance has not been established [3]. The different lignin linkages are not clearly determined, because the extraction process used to isolate the lignin modifies most of the original bonds. Furthermore, inside wood fibers the coupling reactions between monolignol units are influenced by the environment of the cell wall, leading to a racemic polymer. Lignin is often called the “cementing agent” of woody tissues [4], thus the characterization of its bonds is the first step to understand its cementing behavior and to develop new synthetic lignins. Studies about chemical modification of lignin has been developed to improve polymer-lignin compatibility [5], however the reactivity at atomistic level was not included. Synthesis and characterization of synthetic lignin has been developed to study structural aspects in the polymerization [6], again, information of the intrinsic reactivity of lignin precursors was not reported.

Relative electron densities proportionate an insight for the evaluation of the sites involved in coupling reactions. Previous results using quantum mechanical calculations have shown that phenoxy radicals have the highest  $\pi$ -electron density at the phenolic oxygen atom [7]. The high electron density on the oxygen atom promotes the formation of aryl ether linkages such as the  $\beta$ -O-4' linkages,

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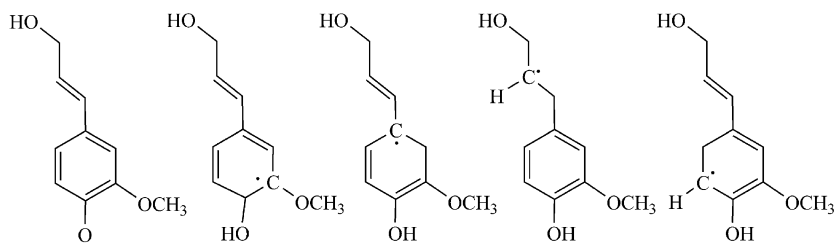


**Fig. 1** Lignin precursors: *p*-coumarol, coniferol and sinapol respectively, showed in their predominant (*E*)-monolignol configuration. Typical numbering is shown over the coniferol structure

which are the most frequent type of bonds in softwood and hardwood lignins [8]. Lignin structures have been simulated using several models and classical molecular dynamics without a detailed atomic description [9–11]. Those classical studies have shown how the monomers couple and its distribution, but the results of the studies depend on the model employed. However, the results using these models do not produce relevant insights at the atomic and molecular level of the proposed models. Frontier orbital and molecular electrostatic potential calculations have been applied to study the reactivity of lignin precursors [12] through the formation of  $\beta$ -*O*-4 linkages [13, 14], but no other linkages were considered in those calculations.

In order to evaluate the most probable mechanism among several reaction pathways and products of the reaction between two chemical species, information about the specific sites interactions needs to be obtained. We apply the methodology of Fukui functions to study the specific reactivity of lignin precursors, in terms of the HSAB principle through the DFT framework. The results should predict and explain the formation of specific linkages in the monolignols, giving new perspectives to develop synthetic lignins or polymers that reproduce some of their properties. Furthermore, considering that the lignification process begins from some of the monolignols phenoxy radicals, the present study could elucidate which resonance structures predominate. Although the study for each monolignol will be performed in a closed shell configuration, the characterization of the intrinsic reactivity of lignin precursor would generate new insights in the lignification mechanism.

**Fig. 2** Phenoxy radicals produced by enzymatic oxidation of monolignols, only coniferol is illustrated



## Theoretical background

One of the most accepted criteria for reactivity is the HSAB principle. It was originally developed by Pearson [15] to describe most of the acid-base reactions through the concept of hardness, however its applicability and simplicity has been widely used to predict the nature of different chemical phenomena [16], and it has become popular among the community of chemists [17]. Many useful and important chemical concepts (i.e., electronegativity, hardness, and softness) are described naturally within the density functional theory (DFT) framework [18]. The analytical definition of global hardness [19] of a chemical species is:

$$\eta = \left( \frac{\partial^2 E}{\partial N^2} \right)_{V(r)} = \left( \frac{\partial \mu}{\partial N} \right)_{V(r)} \quad (1)$$

where  $E$  is the total energy,  $N$  is the number of electrons of the chemical species,  $\mu$  is the chemical potential, which is identified as the negative of electronegativity [20] and  $V(r)$  is the external potential, i.e., the potential over all electrons due to the fixed nuclei. The external potential determines the location of all atomic nuclei. The corresponding global softness is expressed as:

$$S = \frac{1}{\eta} = \left( \frac{\partial^2 N}{\partial E^2} \right)_{V(r)} = \left( \frac{\partial N}{\partial \mu} \right)_{V(r)} \quad (2)$$

however, the site reactivity of a chemical system cannot be studied using global descriptors. An appropriate definition [21] of local softness,  $s(r)$ , is given by:

$$s(r) = \left( \frac{\partial \rho(r)}{\partial \mu} \right)_{V(r)} \quad (3)$$

where  $\rho(r)$  is the total electronic density of a chemical species, such that

$$\int s(r) dr = S \quad (4)$$

Also local softness can be expressed by [21],

$$s(r) = \left( \frac{\partial \rho(r)}{\partial N} \right)_{V(r)} \left( \frac{\partial N}{\partial \mu} \right)_{V(r)} = \left( \frac{\partial \mu}{\partial V(r)} \right)_N S \quad (5)$$

On the other hand, the definition of the Fukui function [22] is the derivative of the electronic density with respect to number of electrons,

$$f(r) \equiv \left( \frac{\partial \rho(r)}{\partial N} \right)_{V(r)} = \left( \frac{\delta \mu}{\delta V(r)} \right)_N \quad (6)$$

Considering the nuclei position fixed, i.e., constant external potential,

$$f(r) \equiv \left( \frac{\partial \rho(r)}{\partial N} \right)_{V(r)} = \left( \frac{\partial \mu}{\partial V(r)} \right)_N \quad (7)$$

Combining Eqs. (5) and (7),

$$s(r) = f(r)S \quad (8)$$

It is obvious that the local softness contains the same information as the Fukui function, i.e., local softness is a site reactivity index related to the Fukui function. Due to the discontinuity of the first derivative in Eq. (7) with respect to the number of electrons  $N$ , the following three functions can be defined in a finite difference approximation:

$$f^+(r) = \rho(r)_{N+1} - \rho(r)_N \quad (9)$$

$$f^-(r) = \rho(r)_N - \rho(r)_{N-1} \quad (10)$$

$$f^0(r) = \frac{1}{2} [\rho(r)_{N+1} - \rho(r)_{N-1}] \quad (11)$$

where  $\rho(r)_{N+1}$ ,  $\rho(r)_N$  and  $\rho(r)_{N-1}$  are the electronic densities of the system with  $N+1$ ,  $N$ , and  $N-1$  electrons, respectively, all with the ground state geometry of the  $N$  electron system. Equations (9), (10), and (11) are evaluated for nucleophilic, electrophilic and free radical attacks respectively [21]. This treatment of chemical reactivity is based on the assumption that when molecules A and B interact in order to form a product AB, a mutual perturbation of the molecular densities of both reactants occurs [23, 24]. Since the electronic density contains all information, chemical reac-

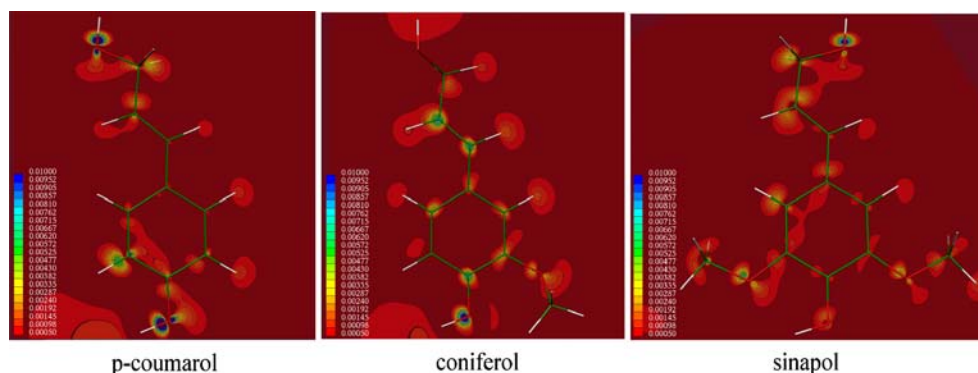
tivity should be reflected in its sensitivity to perturbations. The Fukui function is defined as the initial response in  $\rho(r)$  due to an infinitesimal perturbation in the total number of electrons  $N$ , at constant external potential  $V(r)$ . The “frozen core” approximation [25] assumes that a perturbation in the electronic density of a molecule occurs mainly in the external regions, therefore, errors in Eqs. (9), (10), and (11) should be small since the interaction of reagents begins when they approach. It is known that in a real chemical process, this condition is never satisfied, however the “frozen core” approximation is useful to predict and describe the intrinsic chemical reactivity of chemical species [26, 27].

The HSAB principle can then be applied considering the magnitude of the Fukui function in each site in the molecule, postulating that “The maxima of  $f^+(r)$  indicate regions in the molecule suitable for attack by a nucleophile, whereas regions with  $f^-(r)$  maxima are suitable for attack by an electrophile and  $f^0(r)$  maxima indicate regions suitable for radical attack”.

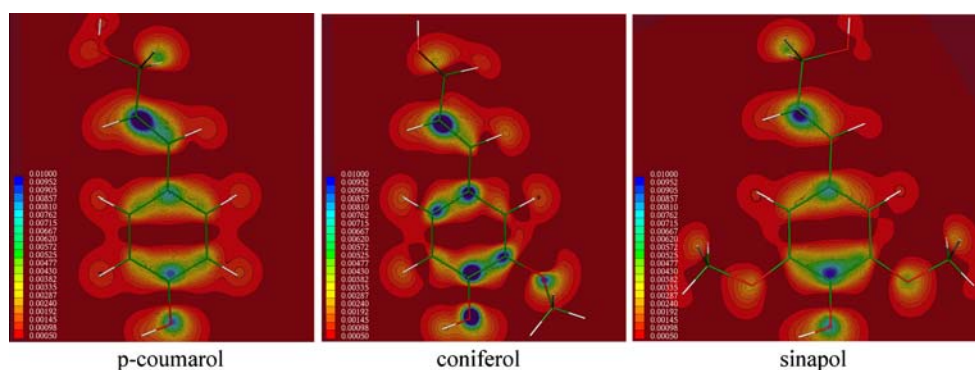
## Computational details

The studied structures were built and optimized using ab-initio calculations in the PC-GAMESS software (version 7.0) [28]. The conformational analysis to obtain the equilibrium geometry was performed using DFT calculations with the B3LYP hybrid density functional [29] (which includes the electronic correlation effect) and the 6-31G\*\* basis set was selected. The electronic densities were computed at the frozen optimized geometries, i.e., assuming a constant external potential. For each monolignol we considered their neutral, anion and cation forms with  $N$ ,  $N+1$ ,  $N-1$  electrons respectively. The electronic densities were obtained with the B3LYP functional and the 6-311++G\*\* basis set, which produces a better bond and density description. To determine the Fukui function, a three dimensional grid of electron density was considered with 100 points along each side of the cube. The grids subtractions were carried out using a

**Fig. 3** Isocontour plots for the nucleophilic form of the Fukui function  $f^+(r)$ , showing which sites are able to stabilize an uptake of electronic charge and are especially reactive toward electron-rich reactants



**Fig. 4** Isocontour plots for the *electrophilic* form of the Fukui function  $f^-(r)$ , which is associated to the softer regions of the monolignols, by giving up electronic charge, and are especially reactive toward electron-poor reactants



home developed software and the molecular visualization software MOLEKEL [30].

## Results and discussion

The (*E*)-monolignol configuration was found to be the most stable geometry for the three hydroxycinnamyl alcohols, as shown in Fig. 1. The obtained Fukui functions were calculated applying Eqs. (9) through (11), by differences of density cubes, and showed no spin contamination for the doublet open shell structures (N+1 and N-1 electron form). They were represented and visualized through bidimensional isocontour plots, selecting a plane parallel to the phenol ring which shows the maxima values of the Fukui function. The highest and lowest values are indicated in the scale by the blue and red colors, respectively.

Figure 3 shows the isocontour plots of  $f^+(r)$  for the three monolignols. The largest values of  $f^+(r)$  are located at the hydroxyl group in the vinylic chain of *p*-coumarol and sinapol, also the regions around the hydroxyl group attached to the aromatic rings of coniferol and *p*-coumarol showed large values of  $f^+(r)$ . Thus the acidity in the monolignols was associated to these sites, which are related to the softer regions of the monolignols and are able to stabilize an uptake of electronic charge. Further-

more, these sites are especially reactive toward nucleophilic reactants, like bases or electron rich moiety of a molecule.

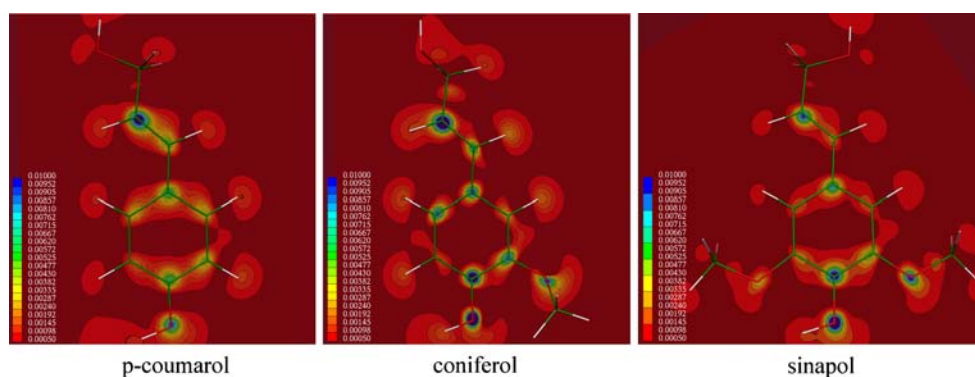
The large values associated with this reactive hydroxyl groups are probably due to the high electronegativity of the oxygen atoms and their non-bonded electron pair. However, the final interpretation should be that the most acid sites are the hydrogen atoms of the hydroxyl groups.

The isocontour plots of  $f^-(r)$ , are presented in Fig. 4. The largest values of  $f^-(r)$  are associated to regions near the  $\beta$ -C atom while the second large values of  $f^-(r)$  to regions near the 4-C atoms for all monolignols. The coniferol molecule also showed relevant large values for the 1-C, 3-C, 6-C and the hydroxyl group attached to the aromatic ring. They are associated with the softer regions of the monolignols, by giving up electronic charge, and are especially reactive toward electron-poor reactants like acids or metals.

The large value associated to the hydroxyl groups should be carefully interpreted due to the electronegativity of the oxygen, which tends to accumulate charge. Then, a maximum in the regions near the carbons should be more suitable for electrophilic attacks than the regions near the hydroxyl group.

Large values of  $f^0(r)$  are located at the regions near the atoms  $\beta$ -C, 1-C, 4-C and the hydroxyl groups attached to the aromatic ring for all monolignols, as shown in Fig. 5. For *p*-coumarol and coniferol molecules the maxima are

**Fig. 5** Isocontour plots for the *free radical* form of the Fukui function  $f^0(r)$ , showing the reactive sites toward electron-rich/poor reactants



located at the region near the atoms  $\beta$ -C, while for the sinapol molecule the maxima are located at the region near the atom 4-C. Hence,  $f^0(r)$  includes the average contribution of  $f^+(r)$  and  $f^-(r)$ , also the total contribution of the resonance structures for nucleophilic and electrophilic attack. The different behavior of the sinapol molecule is probably due to the methoxyl groups attached to the aromatic ring, which attract charge within the molecule.

The polymerization of the monolignols towards the formation of lignins, naturally develops through free radicals mechanisms, needs to be focused on the  $f^0(r)$  results. The regions with large values of the Fukui functions are the softest and reactive sites toward free radicals. From these results we expect that in the final lignin structure, a distribution of linkages will be displayed. The distribution of linkages will involve all monolignols and their carbons  $\beta$ -C, 1-C, 4-C and the oxygen of the hydroxyl group attached to the aromatic ring, showing that structures 2a, 2c and 2d in Fig. 2 can be involved. It could be predicted that the most abundant linkages will be those involving the oxygen of the hydroxyl group of the sinapol molecule and the  $\beta$ -C atoms of the *p*-coumarol and coniferol molecules, showing the interaction in their 2d and 2a phenoxy radicals. The highest values of the free radical form of the Fukui function  $f^0(r)$ , at the sites and regions described, indicate that the initial linkage between two monolignols results predominantly in the linkage  $\beta$ -O-4', which has been experimentally proven to be the most abundant linkage in lignins [31]. A previous research work [12] concerning the formation of dimers  $\beta$ -O-4' included the calculation of the intrinsic reactivity of coniferol by applying the molecular orbital calculation. It is interesting to observe that their results of reactivity for coniferol agree well with ours obtained from the HSAB principle. However, no other references concerning the calculation of the intrinsic reactivity were found for *p*-coumarol and sinapol.

## Conclusions

The acid/base reactivity of monolignols through nucleophilic/electrophilic Fukui functions was characterized. Although the form  $f^0(r)$  for the free radical attack appears to be the most important descriptor due to its direct implication in the lignification mechanism, it is important to highlight the results obtained for  $f^+(r)$  and  $f^-(r)$  which show that the acidity and basicity are located in separated sites and regions of the studied monolignols. It was found that the  $\beta$ - $\beta'$  linkage between *p*-coumarol and sinapol dimers, and their mixtures, will be abundant. It is interesting to notice that lignins generated only from coniferol could produce almost

all the proposed linkages. However, the linkage 5-5' was not elucidated in this study. To our knowledge, this work is the first attempt to characterize the intrinsic chemical reactivity of monolignols in a closed shell configuration, through the Fukui function. Further studies will consider transition states and intermediaries.

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## References

- Sakakibara A, Sano Y (2001) Lignin. In: Hon DNS, Shirasi N (eds) Wood and cellulosic chemistry, chapter 4. CRC, New York, pp 109–174
- Freudenberg K (1965) Science 148:595–600
- Monties B (1989) Plant phenolics. In: Dey PM, Harborne JB (eds) Methods in plant biochemistry. Academic, New York, pp 113–157
- Higuchi T (2006) Wood Sci Technol 40:16–25
- Thielemans W, Wool RP (2005) Biomacromolecules 6:1895–1905
- John R, Yinsheng Z (1998) Tetrahedron 58:1349–1354
- Liu R, Zhou X (1993) J Phys Chem 97:9613–9617
- Glasser WG, Sarkanen S (1989) Lignin, properties and materials. American Chemical Society, Washington, DC, pp 64–113
- Jurasek L (1995) J Pulp Paper Sci 21:274–279
- Houtman CJ, Atalla RH (1995) Plant Physiol 107:977–984
- Simon JP, Eriksson LA (1995) Holzforschung 49:429–438
- Elder TJ, McKee ML, Worley SD (1988) Holzforschung 42:233–240
- Besombes S, Robert D, Utille JP, Taravel F, Mazeau K (2003) J Agric Food Chem 51:34–42
- Durbeej B, Eriksson LA (2003) Holzforschung 57:466–478
- Pearson RG (1963) J Am Chem Soc 85:3533–3539
- Beck ME (2005) J Chem Inf Model 45:273–282
- Ayers PW, Parr RG (2000) J Am Chem Soc 122:2010–2018
- Parr RG, Yang W (1989) Density functional theory of atoms and molecules. Oxford University Press, New York, pp 70–84
- Parr RG, Pearson RG (1983) J Am Chem Soc 105:7512–7516
- Parr RG, Donnelly RA, Levy M, Palke WE (1978) J Chem Phys 68:3801–3807
- Yang W, Parr RG (1985) Proc Natl Acad Sci USA 82:6723–6726
- Parr RG, Yang W (1984) J Am Chem Soc 106:4049–4050
- Chattaraj PK, Lee H, Parr RG (1991) J Am Chem Soc 113:1855–1856
- Gázquez JL (1993) Chemical hardness. In: Sen KD (ed) Structure and bonding 80:27–42
- von Barth U, Gelatt CD (1980) Phys Rev B 21:2222–2228 and references therein
- Herrera H, Nagarajan A, Morales MA, Mendez F, Jiménez HA, Zepeda LG, Tamariz J (2001) J Org Chem 66:1252–1263
- López P, Méndez F (2004) Org Lett 6:1781–1783
- Nemukhin AV, Grigorenko BL, Granovsky A (2004) Mosc Univ Chem Bull 45:75–78
- Becke AD (1993) J Chem Phys 98:5648–5652
- Portmann S, Flükiger HP, Weber J (2000) Swiss center for scientific computing, Manno Switzerland
- Sakakibara A (1980) Wood Sci Technol 14:89–100