



Antioxidant Activity of Natural Compounds Supported on Mesoporous Silica

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Abstract

The preparation of new composites and their antioxidant properties are reported in this study. Eugenol, vanillin and cinnamaldehyde were supported on silica material, through a microwave assisted process. N₂ adsorption/desorption analysis, XRD, SAXS, TEM, FTIR and XPS were used to characterize these materials. The results proved that these compounds were successfully anchored into the channels of mesoporous silica and that the ordered mesoporous structure of inorganic material was well preserved. The antioxidant activities of composites were evaluated by the phosphomolybdenum method and results showed that they have a marked antioxidant activity better than free antioxidants.



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Introduction

Free radicals are reactive species that attack lipids, proteins and DNA. To counter this threat to their integrity, cells have involved a variety of defense systems based on antioxidant species. A high proportion of the antioxidant systems of the human body are dependent on dietary constituents. Consequently, the need to identify alternative natural for safe and natural antioxidants has increased in recent years (Ebrahimzadeh, Nabavi, & Nabavi, 2014). In this sense, a large number of

naturally compounds are important substances than possess advantageous antibacterial, antifungal and antioxidant activities.

Eugenol (4-allyl-2-methoxyphenol), a major constituent of clove oil, is a naturally occurring phenolic compound widely used in food, cosmetics, pharmaceutical and active packaging applications, due to its antimicrobial and antioxidant properties. Similar properties were observed with vanillin (4-hydroxy-3-methoxybenzaldehyde), other

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important natural compound that can be synthesized from lignin. In special, the antioxidant properties of these natural molecules are mostly given by being phenolic compounds (Ogata, Hoshi, Shimotohno, Shiro, & Toyoshige, 1997) (Horuz & Maskan, 2015).

Other natural compound, cinnamaldehyde (3-phenyl-2-propenal), obtained from the steam distillation of the oil of cinnamon bark, is used as flavoring and antimicrobial in food and cosmetics. In special, cinnamaldehyde derivatives have been studied to have antioxidant, anti-inflammatory, anti-tuberculosis and cytotoxic properties (Naveena, Muthukumar, Sen, Kumar, & Kiran, 2014).

In relation to the study of antioxidant properties, research has shown the antioxidative power of all these compounds depends, in special, electron delocalization on the aromatic nucleus (Ogata *et al.*, 1997).

On the other hand, mesoporous oxides, such as silica, are a suitable inorganic support due to its uniform wide channels that can immobilize different molecules with chemical activity. Their stability leads to better dispersion, compatibility with different substrates and subsequent functionalization (Song, Hidajat, & Kawi, 2005).

Considering the potent antioxidant activity of these molecules, in the present work, eugenol, vanillin and cinnamaldehyde, were supported into mesoporous silica and then these composites were evaluated as antioxidant. In particular, the focus was the evaluation of the antioxidant activity of these composite materials in comparison with the free molecules and 2,6-di-tert-butyl-4-methylphenol (BHT), a commercial antioxidant (Figure 1).

Materials and Methods

Chemicals

The natural antioxidant compounds, eugenol (CAS 97-53-0), vanillin (CAS 121-33-5), and cinnamaldehyde (CAS 104-55-2) were purchased from Sigma-Aldrich. All other chemicals and solvents used in these work were of analytical grade.

Synthesis of Mesoporous Silica

Mesoporous silica (SiO_2) was prepared according to Wang *et al.*, (Wang, Ji, Yin, & Liu, 2016) with some modifications (Guntero, Ferretti, Mancini, & Kneeteman, 2018). The template Pluronic P123 (4 g), as a triblock copolymer, was completely dissolved in an aqueous solution of HCl (3.1 M; 350 mL). Then, polyethylene glycol 400 (10 g) was incorporated and the solution was stirred until became clear. Tetraethyl orthosilicate (22.5 mL) was then added immediately and the resulting solution was stirred at 40 °C for 24 h. Subsequently, the solution was transferred into the microwave oven and kept at 100 °C for 12 h. The product was filtered, washed with water, and dried at 80 °C for 12 h. After drying, the mesoporous silica was calcined at 550 °C for 5 h.

Preparation of Composites

The antioxidant/ SiO_2 composites were prepared through a microwave assisted process with a concentration of 10 % wt. Briefly, 0.15 g of antioxidants were placed in a vial and 1.5 g of mesoporous silica and 18 mL of a solution of ethanol:water (50:50) were incorporated. The mixture was placed in a microwave oven and the closed system was heated at 70 °C, 1200 RPM, for 20 min. Finally, solvents were evaporated and the resulting material was dried at 80-100 °C overnight.

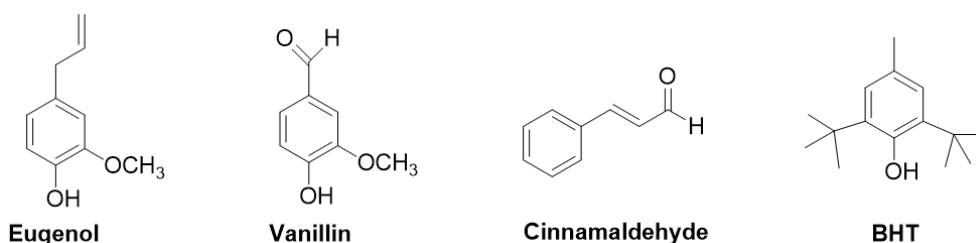


Fig. 1: Chemical structure of compounds

Characterization of Materials

The textural properties of composites were evaluated by physisorption of nitrogen at $-196\text{ }^{\circ}\text{C}$ on a NOVA-1000 Quantachrome. Prior to testing, the samples were treated at $100\text{ }^{\circ}\text{C}$ in the degassing port of the adsorption analyzer. Specific surface areas were evaluated using the Brunauer, Emmett and Teller (BET) method, while pore size distributions were calculated using the Barret-Joyner-Halenda (BJH) algorithm on the adsorption branches of the isotherms. The crystallinity of samples was identified by X-ray diffraction (XRD) using a Shimadzu XD-1 diffractometer and Ni-filtered $\text{Cu-K}\alpha$ radiation. Small-angle X-ray scattering analyses (SAXS) were conducted using a XEUS10 diffractometer (XENOCs). Transmission electron microscopy (TEM) images were taken with a JEM-2100 Plus microscope. FTIR tests were performed on a Shimadzu FTIR Prestige-21 spectrophotometer in the region from 4000 to 1000 cm^{-1} , mixed the samples (1% wt) with KBr and then pressed. X-ray photoelectron spectroscopy (XPS) studies were performed in a multi-technique system (SPECS) equipped with a hemispherical PHOIBOS 150 analyzer.

Evaluation of Antioxidant Activity

Antioxidant activity of materials was determined using phosphomolybdenum method (Alam & Bristi, 2013) and compared with BHT antioxidant and free active molecules. The samples were dissolved in a mix DMSO-ethanol (1:99) at 100 mg/mL of antioxidant. The samples were sonicated for about 5 min and then they were filtered and diluted with the mix of solvent to obtain solutions of known concentration. Such procedure was carried out with antioxidants free, composites and silica. An aliquot

of 1 mL of sample solution was mixture with 9 mL of solution of reagent composed by 28 mM sodium phosphate, 4 mM ammonium molybdate and 0.6 M sulfuric acid. Then, the solutions were incubated at $95\text{ }^{\circ}\text{C}$ for 120 min. After the samples had cooled to $25\text{ }^{\circ}\text{C}$ and the absorbance of solutions were measured at 695 nm against a blank. The control solution, contained 1 mL of reagent solution and the appropriate volume of solvent, was incubated under the same conditions as the rest of the samples. Antioxidant activity was expressed as inhibition (I) calculated by the equation: $I\text{ (\%)} = [1 - (A_s - A_{s120}) / (A_c - A_{c120})] * 100\%$, where A_s is initial absorbance, A_{s120} is the absorbance of the sample at 120 min, A_c is initial absorbance of control and A_{c120} is the absorbance of control at 120 min. The absorbance measurements were carried out on a Perkin Elmer Lambda 20 spectrophotometer.

Results and Discussion

The antioxidant compounds were checked by spectroscopic studies, confirmed the structures and purity of the compounds. N_2 adsorption/desorption isotherms of materials were determined in order to study their textural properties. All samples showed N_2 adsorption/desorption curves (not shown here) corresponding to type IV isotherms as is typically observed with mesoporous material (Song *et al.*, 2005). From the adsorption isotherms were calculated the surface area, the pore volume and the pore diameter of these materials whose results are shown in Table 1. The encapsulation of antioxidant molecules into silica caused a reduction of specific surface area and the pore volume. The values of average pore diameter changed by the incorporation of antioxidant on these material without modify the mesoporosity of materials (Stanzione *et al.*, 2017).

Table 1: Textural parameters of composites

Sample	Specific Surface Area (m^2/g)	Pore Volume (cm^3/g)	Average Pore Diameter(\AA)
SiO_2	578	1.68	116
V/ SiO_2	405	0.90	89
E/ SiO_2	384	0.92	96
C/ SiO_2	362	0.71	78

[E: eugenol; V: vanillin; C: cinnamaldehyde]

XRD was performed to determine qualitatively the presence of a new crystalline species on the silice structure in the composites before of supporting of antioxidant compounds. The X-ray diffractograms obtained for the composites, Figure 2, were similar to the patterns of silica, which indicates that the structure of the samples was preserved after embedding of antioxidant. In all samples, only the characteristic amorphous peak at 23° was observed.

By analysis of small-angle XRD (SAXS) was demonstrated the presence of uniform mesoporosity. The SAXS patterns for the samples (not shown here) exhibit characteristic peaks at 2° between 0.5° and 2.0° , indicating the presence of ordered straight

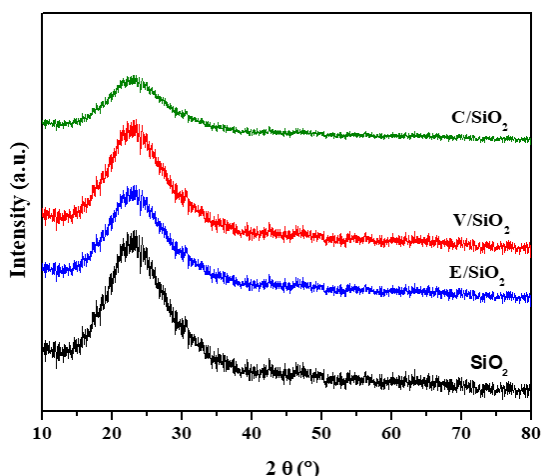


Fig. 2: XRD diffractograms of composites.
[E: eugenol; V: vanillin; C: cinnamaldehyde]

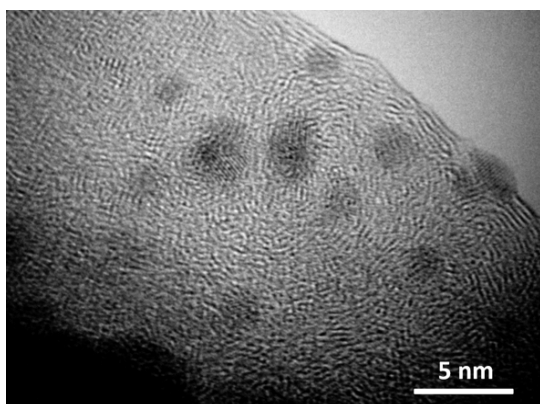


Fig. 3: TEM images of vanillin/SiO₂

uniform mesoporous (Niesz, Yang, & Somorjai, 2005). For the samples of composites, the present of antioxidant compounds on silica gives rise to a decrease in the diffracted intensity, likely attributed to the reduction of scattering contrast ascribed to the presence of the loaded molecules. TEM micrograph images, Figure 3, showed that these materials exhibit ordered structures (Li *et al.*, 2007).

The analysis of FTIR spectra of materials indicating the strong interaction between antioxidant compounds present into pores of mesoporous oxides with surface hydroxyl groups of silica. Furthermore, XPS spectra also provide evidence of this electrostatic interaction.

To evaluate of synthesized materials as composites with antioxidant activity, the phosphomolybdenum method was used. Silica showed no antioxidant activity. On the other hand, the other samples presented antioxidant properties. Regardless of the concentration of the free compound, antioxidant potency decreased in the order: BHT > vanillin > eugenol > cinnamaldehyde. The inhibitory effect of vanillin and eugenol are higher than that of cinnamaldehyde (Ogata, Hoshi, Shiro, & Toyoshige, 2000) (Tai, Sawano, Yazama, & Ito, 2011) (Suryanti, Wibowo, Khotijah, & Andalucki, 2018). Active molecules embedding in mesoporous silica presented higher antioxidant activity than that of free molecules. This was attributed to the formation

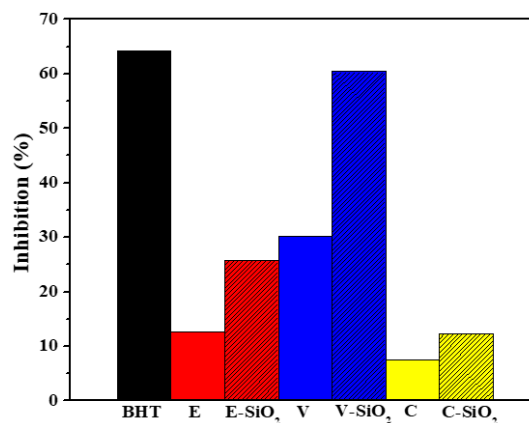


Fig. 4: Antioxidant activity of samples by phosphomolybdenum method.
[E: eugenol; V: vanillin; C: cinnamaldehyde]

of active species of antioxidant in the molecule-support system, as a result of their interaction with mesoporous oxides.

Results suggest weak interactions of electrostatic nature, such as hydrogen bond, that connect antioxidant molecules with OH groups present on the wall of mesoporous silica. These interactions do not cause chemical changes in antioxidant property of molecules supported.

Conclusions

In conclusion, organic/inorganic composites based on the mesoporous silica have been developed. Results of characterization showed that organic

molecules have been successfully supported to the channel of mesoporous silica and that the mesoporous structure has been perfectly preserved. The antioxidant activity was examined by UV-Vis spectroscopy, and the results show that antioxidant activity of composites is major of their correspondent free organic compound, which indicates that the organic/inorganic composites may be important materials for future applications in the field of packaging.

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References

1. Alam, Md. N., Bristi, N. J., & Rafiqzama Md.; Review on in vivo and in vitro methods evaluation of antioxidant activity, *Saudi Pharmaceutical Journal*, 21, 143–152, (2013).
2. Ebrahimzadeh, M. A., Nabavi, S. F., & Nabavi, S. M.; Antioxidant Activities of Methanol Extract of *Sambucus ebulus* L. Flower. *Pakistan Journal of Biological Sciences*, 12(5), 447–450, (2014).
3. Guntero, V. A., Ferretti, C. A., Mancini, P. M., & Kneeteman, M. N.; Synthesis and Encapsulation of bis-eugenol in a Mesoporous Solid Material: Enhancement of the Antioxidant Activity of a Natural Compound from Clove Oil. *Chemical Science International Journal*, 22(4), 1–10, (2018).
4. Horuz, T. I., & Maskan, M.; Effect of cinnamaldehyde on oxidative stability of several fats and oils at elevated temperatures, *Food Science and Technology*, 1, 1071725, (2015).
5. Li, L. L., Sun, H., Fang, C. J., Xu, J., Jin, J. Y., & Yan, C. H.; Optical sensors based on functionalized mesoporous silica SBA-15 for the detection of multianalytes (H^+ and Cu^{2+}) in water. *Journal of Materials Chemistry*, 17, 4492–4498, (2007).
6. Naveena, B. M., Muthukumar, M., Sen, A. R., Kumar, Y. P., & Kiran, M.; Use of cinnamaldehyde as a potential antioxidant in ground spent hen meat. *Journal of Food Processing and Preservation*, 38, 1911–1917, (2014).
7. Niesz, K., Yang, P., & Somorjai, G. A.; Sol-gel synthesis of ordered mesoporous alumina. *Chemical Communications*, 15, 1986–1987, (2005).
8. Ogata, M., Hoshi, M., Shimotohno, K., Shiro, U., & Toyoshige, E.; Antioxidant Activity of Magnolol, Honokiol, and Related Phenolic Compounds. *JAOCS*, 74(5), 557–562, (1997).
9. Ogata, M., Hoshi, M., Shiro, U., & Toyoshige, E.; Antioxidant Activity of Eugenol and Related Monomeric and Dimeric Compounds. *Chem. Pharm. Bull.*, 48(10), 1467–1469, (2000).
10. Song, S. W., Hidajat, K., & Kawi, S.; Functionalized SBA-15 Materials as Carriers for Controlled Drug Delivery: Influence of Surface Properties on Matrix - Drug Interactions. *Langmuir*, 21, 9568–9575, (2005).
11. Stanzone, M., Gargiulo, N., Caputo, D., Liguori, B., Cerruti, P., Amendola, E., Lavorgna, M., Buonocore, G. G.; Peculiarities of vanillin release from amino-functionalized mesoporous silica embedded

- into biodegradable composites. *European Polymer Journal*, 89, 88–100, (2017).
12. Suryanti, V., Wibowo, F. R., Khotijah, S., & Andalucki, N.; Antioxidant Activities of Cinnamaldehyde Derivatives. *Materials Science and Engineering*, 333, 012077, (2018).
 13. Tai, A., Sawano, T., Yazama, F., & Ito, H.; Evaluation of antioxidant activity of vanillin by using multiple antioxidant assays. *Biochimica et Biophysica Acta*, 1810, 170–177, (2011).
 14. Wang, A., Ji, Y., Yin, H., & Liu, S.; Synthesis of different-sized SBA-15 nanoparticles and their fluoride release performances from poly(methyl methacrylate) dental restorative resin. *New Journal of Chemistry*, 40, 9781–9787, (2016).