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Original article ■

HPLC-DAD Study of Gallic Acid Autoxidation in Alkaline Aqueous Solutions and the Influence of Mg(II) Ion

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SUMMARY

High performance liquid chromatography with diode array detection (HPLC -DAD) was used to study the autoxidation of gallic acid in alkaline aqueous solutions and the influence of Mg(II) ion on this process. At pH 10, one main autoxidation product was determined, and based on the data obtained in this study its structure was proposed to some kind of gallic acid dimer. Among the minor products, the compound with characteristics similar to ellagic acid was detected. Chromatographic analysis of gallic acid autoxidation at pH 8.5 in the presence of Mg(II) ion indicated that reaction rate decreased. At the same time the mechanism of reaction(s) also changed, which was indicated by the formation of different autoxidation products. These findings may provide better insight into various actions of gallic acid in biological systems since Mg(II) ions are ubiquitous in all living cells.

***Key words*: autoxidation, gallic acid, HPLC, Mg(II) ion**

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INTRODUCTION

Gallic acid (GA, 3,4,5-trihydroxy-benzoic acid) is a polyhydroxyphenolic compound widely distributed in various plants, fruits and foods, where it is present either in free form or, more commonly, as an ingredient of tannins (1). GA and its derivates were found to be strong antioxidants which are able to scavenge reactive oxygen species (ROS), e.g., superoxide anions, hydrogen peroxide, hydroxyl radicals and hypochlorous acid (2, 3). GA derivatives have also been found in many phytomedicines with a number of biological and pharmacological activities like inducing apoptosis of cancer cells (4, 5), inhibiting squalene epoxidase and interfering the signal pathways involving Ca(II) and oxygen free radicals (6, 7). Autoxidation and oxidation of GA have been studied for many years due to its presence in various foods and its activity in living cells (mainly as antioxidant). Special attention was given to the autoxidation of GA in alkaline aqueous solutions because of its possible transformations during the food processing and because it can serve as a model for similar reactions in various foods and beverages (8, 9).

In this paper, we employed high performance liquid chromatography with diode array detection (HPLC-DAD) to study the autoxidation of GA in alkaline aqueous solutions and the influence of Mg(II) ion on this process.

MATERIAL AND METHODS

Reagents and chemicals

All chemicals used in this study were of analytical (p.a.) grade. GA (Fluka, Germany) was used without additional purification, because its purity was proved to be satisfactory by HPLC. Double distilled, air saturated water was used and GA stock solution with the concentration of 0.1 mol dm^{-3} was prepared just prior to the use by dissolving exactly weighted amount of GA. Tris, sodium carbonate and $MgCl_2$ were purchased from Fluka, Germany. For the pH adjustment, NaOH and HCl solutions (0.01 mol dm^{-3}) were used. Concentration of Mg(II) ion stock solution was 0.4 mol dm^{-3} and it was prepared by dissolving the exactly weighted amount of $MgCl_2$ in appropriate water volume.

Experimental procedure

All autoxidation experiments were performed by mixing equal volumes of GA solution and buffer solutions in an open glass put on a magnetic stirrer in order to achieve a constant saturation of solution with oxygen from air. The concentration of GA in all working solutions was 5 mmol dm^{-3}. Autoxidation process at pH 10 was performed in carbonate buffer. Autoxidation of GA at pH 8.5 in the presence of Mg(II) was performed in Tris buffer. The concentration of Mg(II) ion in working solution was 0.2 mol dm^{-3}, and it was prepared by mixing equal volumes of Mg(II) ion stock solution and Tris buffer. Autoxidation of GA was accompanied by the appearance of intense green color which became stable after approximately 30 minutes in both systems investigated in this study. All HPLC runs were performed after 45 min of autoxidation process.

Instrumentation

Agilent Technologies 1200 series liquid chromatography system equipped with an autosampler and diode array detector was used. The column used was a C18 reversed phase Zorbax SB-18 (4.6×150 mm, particle size 3.5 μm). Mobile phase eventually adopted for this study was methanol/water/orthophosphoric acid (20:79.9:0.1) and the flow rate was 1.0 mL/min. The column was operated at 30°C and the sample injection volume was 5 μL.

RESULTS AND DISCUSSION

We initially studied the GA autoxidation process at pH 10 in carbonate buffer. The chromatogram presented in Figure 1 demonstrates the separation of products obtained under these conditions.

It is well-documented that autoxidation of GA in aqueous solutions proceeds at an appreciable rate with high pH values and literature data dealing with oxidation or autoxidation of GA in alkaline aqueous solutions have suggested various mechanisms with different products (8-10). Chemical structures of GA and proposed oxidation/autoxidation products are shown in Figure 2.

In the chromatogram given in Figure 1 two main peaks appeared at retention times (RT) of 1.266 and 1.450 min, and two minor peaks appeared at RT of 1.693 and 2.142 min. Peak with the RT value of 1.450 min corresponds to the GA as verified by comparison with the previously recorded chromatogram of non-oxidized GA. As for other compounds, we could draw some conclusions about their structure considering their RT values and UV-Vis spectra recorded by the DAD, which are shown in Figure 3.

Well-defined peak with RT of 1.266 min corresponds to one compound with greater polarity than GA since reverse-phase chromatographic system was used and it may correspond to GA dimer having two carboxylic groups. However, the UV-Vis spectrum of this compound (Figure 3a) cannot give conclusive answer about its structure since both C-C dimer (Figure 2c) and C-O dimer (Figure 2d) have extended π-electron delocalization which results in the appearance of new absorption bands at much higher wavelengths in comparison to GA (Figure 3b). Based on the RT value and spectral characteristics of this compound we tentatively assigned its structure to some kind of GA dimer. As for two minor peaks with RT of 1.693 and 2.142 min we might propose that the peak at 2.142 min corresponds to ellagic

acid (EA, Figure 2b) because EA/GA RT ratio fits within the range of values obtained under similar chromatographic conditions (11). In addition, UV-Vis spectrum of this compound (Figure 3c) has similar characteristics to the spectra of EA reported in literature (12). The compound with RT of 1.693 min is unknown at present and it might represent some kind of impurity.

There are literature data that diamagnetic divalent metal ions have great influence on the autoxidation process of phenolic compounds with *ortho* -OH groups at pH values close to physiological conditions (13, 14). Of all metal ions, Mg(II) has special importance since it is ubiquitous in all biological systems and we chose to study its influence on the autoxidation of GA in alkaline aqueous solution at pH 8.5 which is close to the physiological conditions in most plant and animal cells. Also, our previous spectrophotometric data showed that spectral changes observed during the autoxidation of GA at pH 8.5 in the presence of Mg(II) ion were very similar to the ones observed at pH 10 in the absence of Mg(II) ion (15). Figure 4 shows the chromatogram demonstrating the separation of products obtained during the autoxidation of GA at pH 8.5 in the presence of Mg(II) ion.

The well defined main peak in the chromatogram in Figure 4 at RT of 1.418 min originates from non-oxidized GA, and there is another poorly resolved minor peak with RT of approximately 1.315 min. Comparison of chromatograms obtained after autoxidation of GA under different conditions used in this study indicated that autoxidation at pH 8.5 in the presence of Mg(II) ion proceeded at much lower rate. This fact may be explained not only by the influence of lowering pH value which generally decreases the autoxidation rate of polyphenolic compounds (9), but also by the Mg(II) ion-caused spin stabilization of transient free radicals which are important intermediates in such autoxidation processes (13 - 15). More important, the shape of minor chromatographic peak at approximate RT of 1.315 min with the shoulder at the lower RT side indicates that this peak probably originates from at least two unresolved compounds. This means that the presence of Mg(II) ion does not only possibly influence the GA autoxidation rate but also changes the mechanism of the reaction(s).

Further research, including the optimization of conditions for chromatographic separation, is certainly needed to identify the products of GA autoxidation under various conditions, but the results of this study clearly indicate that Mg(II) ion exerts profound influence on this process. This finding is very important if we consider the ubiquitous presence of Mg(II) ion in all biological systems where the action of GA may be of some significance.

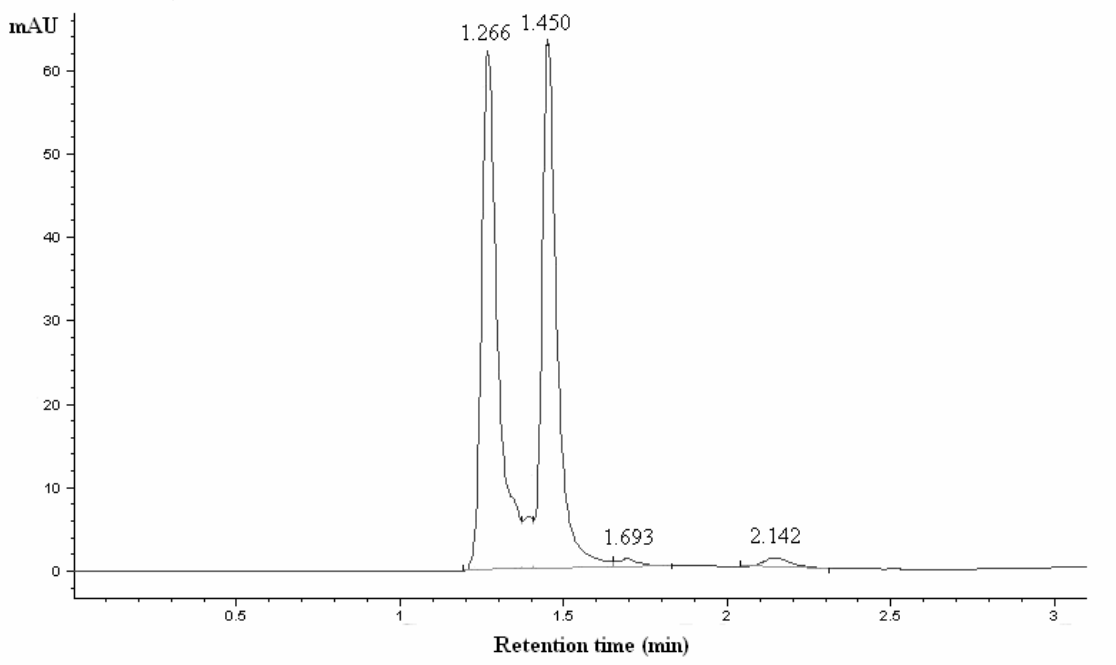

Figure 1. *HPLC chromatogram of GA autoxidation products at pH 10 (detection wavelength 258 nm)*

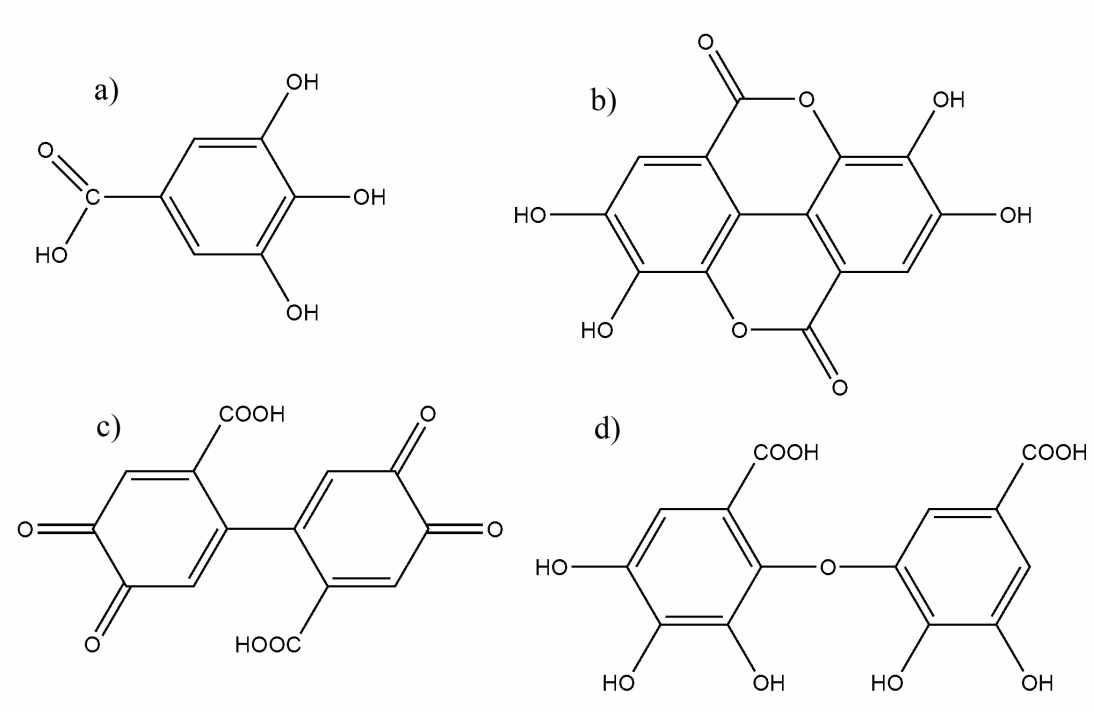

Figure 2. *Chemical structures of: a) Gallic acid (GA), b) Ellagic acid (EA), c) C-C dimer formed by the autoxidation of GA (8), d) C-O dimer formed by the oxidation of GA (10).*

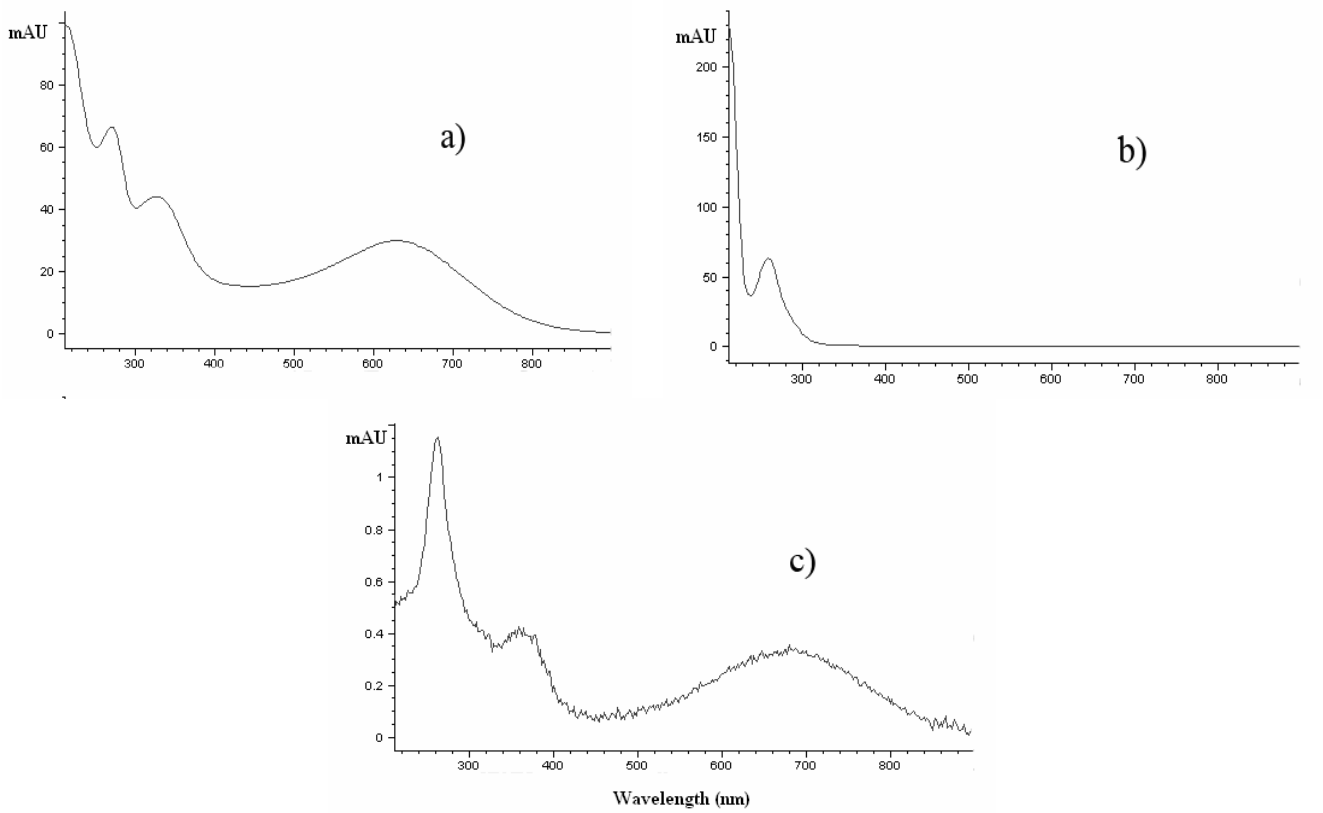

Figure 3. *UV-Vis spectra of compounds from HPLC chromatogram shown in Figure 1 at RT values: a) 1.266, b) 1.450, c) 2.142 min*

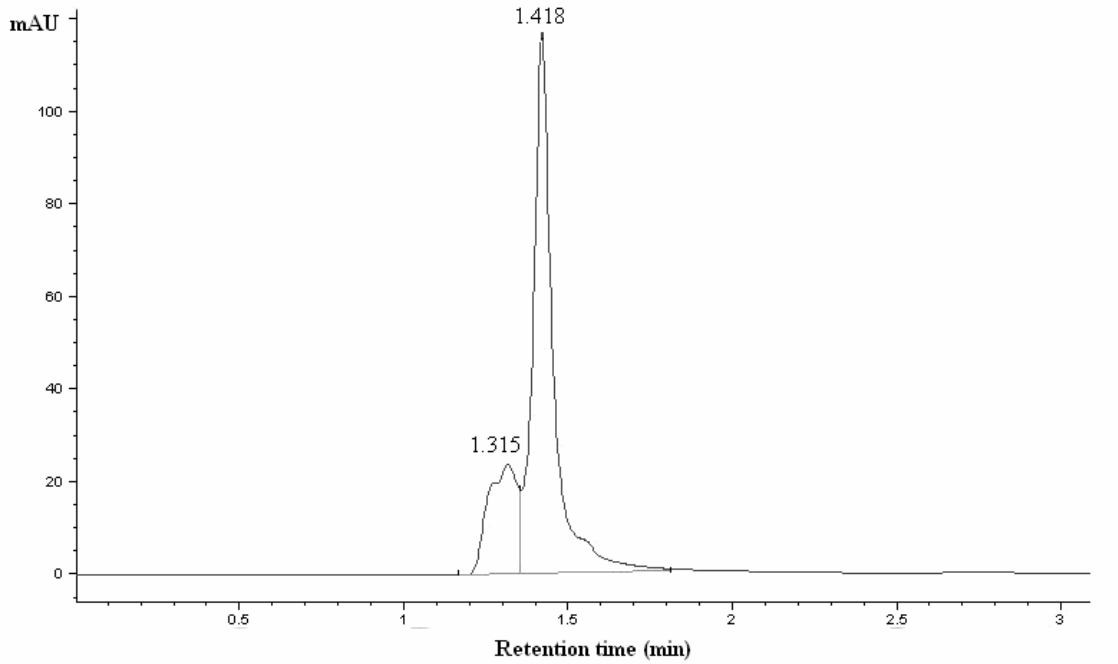

Figure 4. *HPLC chromatogram of GA autoxidation products at pH 8.5 in the presence of Mg(II) ion (detection wavelength 258 nm)*

CONCLUSION

HPLC-DAD was employed to study gallic acid autoxidation in alkaline aqueous solutions. By comparing chromatographic data obtained for this system at pH 10 and at pH 8.5 in the presence of Mg(II) ion, we concluded that Mg(II) ion influenced the autoxidation of GA in two ways. It possibly contributed to the decrease of reaction rate by the spin stabilization of intermediate free radicals and, more important, it also changed the mechanism of reaction(s), thus leading to the formation of some different autoxidation products. These findings may provide better insight into various actions of GA in biological systems, since Mg(II) ions are ubiquitous in all living cells.

Acknowledgments

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References

1. Niemetz R, Gross GG. Enzymology of gallotannin and ellagitannin biosynthesis. Phytochemistry 2005; 66: 2001-11.
2. Polewski K, Kniat S, Slawinska D. Gallic acid, a natural antioxidant, in aqueous and micellar environment: spectroscopic studies. Curr Top Biophys 2002; 26: 217-27.
3. Kim YJ. Antimelanogenic and antioxidant properties of gallic acid. Biol Pharm Bull 2007; 30: 1052-5.
4. Sakagami H, Satoh K, Hatano T, Yoshida T, Okuda T. Possible role of radical intensity and oxidation potential for gallic acid-induced apoptosis. Anticancer Res 1997; 17:377-80.
5. Serrano A, Papacios C, Roy G, Cespon C, Villar ML, Nocito M, Gonzalez-Porque P. Derivatives of gallic acid induce apoptosis in tumoral cell lines and inhibit lymphocyte proliferation. Arch Biochem Biophys 1998; 350: 49-54.
6. Inoue M, Sakaguchi N, Isuzugawa K, Tani H, Ogihara Y. Role of reactive oxygen species in gallic acid-induced apoptosis. Biol Pharm Bull 2000; 23: 1153-7.
7. Sakaguchi N, Inoue M, Ogihara Y. Reactive oxygen species and intracellular Ca^{2+}, common signals for apoptosis induced by gallic acid. Biochem Pharm 1998; 55: 1973-81.

8. Tulyathan V, Boulton RB and Singleton VJ. Oxygen uptake by gallic acid as a model for similar reactions in wines. J Agric Food Chem 1989; 37: 844-9.
9. Friedman M and Jürgens HS. Effect of pH on the stability of plant phenolic compounds. J Agric Food Chem 2000; 48: 2101-10.
10. Oniki T, Takahama U. Free radicals produced by the oxidation of gallic acid and catechin derivatives. J Wood Sci 2004; 50: 545-7.
11. He Z and Xia W. Analysis of phenolic compounds in Chinese olive (*Canarium album* L.) fruit by RPHPLC-DAD-ESI-MS. Food Chem 2007; 105: 1307-11.
12. Gil MI, Tomas-Barberan FA, Hess-Pierce B, Holcroft DM, Kader AA. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. J Agric Food Chem 2000; 48: 4581-9.
13. Nikolić GM, Premović PI, Nikolić RS. Spectrophotometric study of catechol oxidation by areal O_2 in alkaline aqueous solutions containing Mg(II) ions. Spectrosc Lett 1998; 31: 327-33.
14. Lebedev AV, Ivanova MV, Timoshin AA, Ruuge EK. Effect of group II metal cations on catecholate oxidation. Chem Phys Chem 2007; 8: 1863-9.
15. Nikolić GM, Veselinović A M, Nikolić RS, Mitić SS. Spectroscopic study of Mg(II) ion influence on the autoxidation of gallic acid in weakly alkaline aqueous solutions. Russ J Physi Chem A 2011; 85: 2270-3.

HPLC-DAD ISPITIVANJE AUTOOKSIDACIJE GALNE KISELINE U BAZNIM VODENIM RASTVORIMA I UTICAJ Mg(II) JONA

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Sažetak

Tečna hromatografija visokog učinka sa 'diode array' detekcijom (HPLC-DAD) je primenjena za ispitivanje autooksidacije galne kiseline u baznim vodenim rastvorima i uticaja Mg(II) jona na taj proces. Na pH 10 utvrđen je jedan glavni proizvod autooksidacije i na osnovu podataka dobijenih u ovom radu pretpostavljeno je da se radi o nekoj vrsti dimera galne kiseline. Među sporednim proizvodima detektovana je komponenta čije karakteristike odgovaraju elaginskoj kiselini. Hromatografska analiza autooksidacije galne kiseline na pH 8,5 u prisustvu Mg(II) jona pokazala je da dolazi do smanjenja brzine reakcije i do promene mehanizma reakcije, na šta ukazuje nastajanje drugačijih proizvoda autooksidacije. Dobijeni rezultati mogu da pomognu u boljem razumevanju različitih efekata galne kiseline u biološkim sistemima, jer je Mg(II) jon prisutan u svim živim ćelijama.

***Ključne reči*: autooksidacija, galna kiselina, HPLC, Mg(II) jon**