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Comparative study of antioxidant potential of curcumin and its degradation products–vanillin, ferulic acid and dehydrozingerone

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Curcumin is one of the most intensively studied biologically active antioxidants during the last decade. Its chemical instability and rapid degradation at physiological conditions (pH ≈ 7) was stated as the most important limitation for its potential applications. Numerous approaches have been undertaken to overcome the problem with the bioavailability of curcumin, including the use of such adjuvants as piperine, liposomes, system of nanoparticles, phospholipid complexes and design of new structural analogs of curcumin. On the other hand, degradation of a compound does not necessarily lead to loss of its activity. Whether and how the degradation and oxidation pathways contribute to the biological and antioxidant activities of curcumin has also been discussed. The aim of this study was to compare the antioxidant activity of curcumin with those of its degradation products (ferulic acid, vanillin and dehydrozingerone), when added in binary and ternary mixtures. Lipid autoxidation was used for assessing the chain-breaking antioxidant efficiency and reactivity of the phenols. The results obtained showed much stronger activity of curcumin than that of all the individual compounds and their binary and triple mixtures.

Keywords: Curcumin, Vanillin, Ferulic acid, Dehydrozingerone, Antioxidant potential, Double and triple mixtures

INTRODUCTION

The use of dry extract from the rhizomes of *Curcuma longa*, i.e. turmeric, in the folk (traditional) medicine in South Asia is extremely varied and many of its therapeutic effects are proven by scientific and medicinal researches. Most of the biological properties of curcumin (**Cu**), a main pigment and active component in turmeric, are found in various *in vitro* and *in vivo* model systems and are described in detail in the review of Prasad and Aggarwal [1]. There are various mechanisms by which **Cu** exhibits its antioxidant properties depending on the conditions and the medium being used. Direct hydrogen atom transfer (HAT) from the phenolic groups is considered to be the classical mechanism of its antioxidant action in a nonpolar environment [2-4]. Litwinienko and Ingold [5] proposed the concept of the so-called SPLET-mechanism (Sequential Proton Loss Electron Transfer) by referring to the effect of the solvent. Subsequently Foti *et al.* [6] proved π-complex formation between the enolate anion of **Cu** and the picryl part of DPPH$^•$ radical.

Despite its strong chemopreventive and chemotherapeutic potential, the use of **Cu** is limited because of its poor bioavailability due to poor absorption from the gastrointestinal tract and rapid degradation [1]. Numerous approaches have been undertaken to overcome the problem with the bioavailability of **Cu**, including the use of such adjuvants as piperine, liposomes, system of nanoparticles, phospholipid complexes and the design of new structural analogs of curcumin [7]. Angelova and Antonov [8] published a theoretical study on the structure of a complex between calixarene and **Cu** (host-guest system) in water and found that both tautomeric (diketo- and keto-enol) forms of **Cu** can enter into or leave the host cavity easily.

On the other hand, degradation of **Cu** does not necessarily lead to loss of its activity. Whether and how the degradation and oxidation pathways contribute to the biological and antioxidant activities of **Cu** has also been discussed by Schneider *et al.* [9]. There are several possible degradation pathways of **Cu** (Scheme 1), some of which including cleavage of its heptadienone carbon chain. Wang *et al.* [10] described *trans*-6-(4′-hydroxy-3′-methoxyphenyl)-2,4-dioxo-5-hexenal (**TDH**) to be the major degradation product of **Cu** in phosphate buffer ensuring pH around 7.0 and at temperature 37 °C (Scheme 1).

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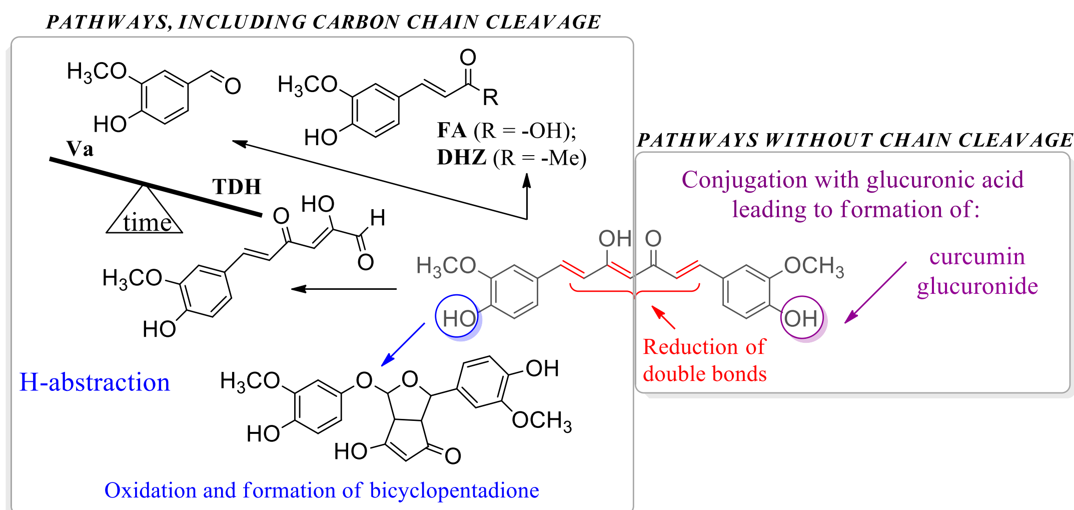

Scheme 1. Possible degradation pathways of curcumin.

Ferulic acid (**FA**), feruroylmethane or dehydrozingerone (**DHZ**) and vanillin (**Va**) are minor degradation products but it was proved that the amount of **Va** increases with incubation time at the expense of **TDH**. Despite the detailed studies of the antioxidant action of **Cu**, which has been well documented [2-13], according to Wang *et al.* [10] "it would be valuable and interesting to compare the potency of vanillin and curcumin on these aspects". Moreover, the important biological activities of **DHZ**, considered as a half **Cu** molecule [14], encourage investigations in this direction.

Considering the lipophilicity of **Cu** and the role that it exerts at the protein-lipid interface interfering on fluidity of the biological membrane [15, 16], it would be useful to study **Cu** degradation products in lipid systems.

The aim of this study was to compare the antioxidant activity of **Cu** with those of its degradation products: **FA**, **Va** and **DHZ** under bulk lipid autoxidation as individual compounds, as well as combined in binary and ternary antioxidant mixtures.

EXPERIMENTAL

All compounds used in the experimental study (Scheme 1), except **DHZ**, were purchased from Sigma-Aldrich. **DHZ** was synthesized starting from **Va** in acetone, using NaOH as the base. In the synthesis of the unsaturated compound **DHZ**, *trans*-configuration was exclusively obtained at the olefinic double bond and detected by NMR spectroscopy, as we described previously [17, 18].

Radical scavenging activity. TLC DPPH rapid test

The compounds were dissolved in acetone and spotted onto silica gel 60 F_{254} plates (E. Merck, Germany). The plates were air-dried and sprayed with 0.03% DPPH radical solution in methanol for detecting the compounds with rapid scavenging properties [19, 20]. The compounds that showed white or yellow spots onto a purple background were considered as active radical scavengers. Taking into account that the stability of DPPH radical is much higher in acetone solution than in methanol [21], the same concentration of DPPH radical in acetone was prepared and used to test the activity of the studied compounds. The effect of concentration (1mM and 10 mM), reaction time (1 min and 10 min) and solvent (acetone and methanol) were studied.

Chain-breaking antioxidant activity

Lipid samples: Triacylglycerols of commercially available sunflower oil (TGSO) were cleaned from pro- and antioxidants by adsorption chromatography and stored under nitrogen at a temperature of 20 ºC. Fatty acid composition of the lipid substrate was determined by GC analysis of the methyl esters: 10:0 (0.2%); 14:0 (0.2%); 16:0 (7.4%); 16:1 (0.3%); 18:0 (2.6%); 18:1 (29.1%); 18:2 (59.1%); 18:3 (0.7%); 20:0 (0.3%). The numbers x:y indicate the number of carbon atoms and double bonds in the fatty acid, respectively. Lipid samples containing various inhibitors were prepared directly before use. Aliquots of the antioxidant solutions in purified acetone were added to the lipid sample. Solvents were removed under a nitrogen flow. For more experimental details see reference [22].

Lipid autoxidation: The process was carried out in a thermostatic bath at (80±0.2)ºC by blowing air through the samples in special vessels. The oxidation process was monitored by withdrawing samples at measured time intervals and subjecting them to iodometric determination of the primary

products (lipid hydroxyperoxides, LOOH) concentration, i.e. the peroxide value (PV). All kinetic data were expressed as the average of two independent measurements which were processed using the computer programmes Origin 6.1 and Microsoft Excel 2010.

Determination of the main kinetic parameters of the studied compounds [22-25]: Protection factor (PF) was determined as the ratio between the induction period in the presence (IP_A) and in the absence (IP_C) of antioxidant, i.e. $PF = IP_A/IP_C$. It is a measure of antioxidant efficiency.

Inhibition degree (ID) is a measure of the antioxidant reactivity, e.g., how many times the antioxidant shortens the oxidation chain length, i.e. $ID = R_C/R_A$. The initial oxidation rates R_C in the absence and R_A in the presence of antioxidant were found from the tangent at the initial phase of the kinetic curves of hydroperoxides accumulation.

RESULTS AND DISCUSSION

Radical-scavenging activity towards DPPH•.

Rapid TLC DPPH-test is useful for preliminary selection of compounds as active or non-active towards free radicals, i.e. DPPH•. The yellowish-white spots on the purple background of DPPH• solutions in methanol and in acetone on the plates were observed (Fig. 1).

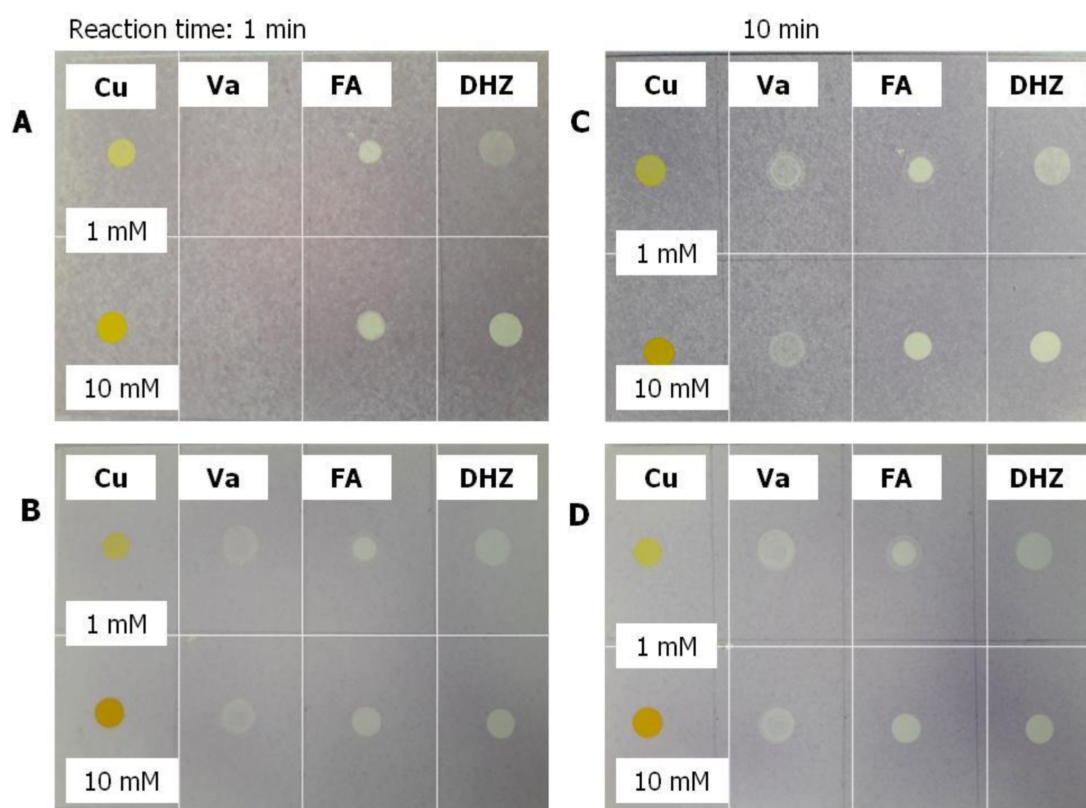

Figure 1. TLC DPPH rapid test: within 1 min after spraying the DPPH radical solution in acetone (A) and in methanol (B) and 10 min after spraying the solution of DPPH radical in acetone (C) and in methanol (D).

Effect of reaction time: Reaction time is one of the most important criteria in this measurement. We studied the activity of all compounds after 1 min and 10 min from spraying both solutions of the radical (DPPH•) - in acetone and in methanol, respectively. All compounds (**Cu**, **FA**, **Va** and **DHZ**) showed bright spots immediately after spraying with methanolic solution of DPPH• while in acetone solution, **Va** did not show a spot at both studied concentrations (1 mM and 10 mM) within the first minutes after spraying the plate. Surprisingly, after 10 min **Va** showed yellowish-white spots in acetone. The result obtained showed that **Va** exerts different activity in protic (methanol) and dipolar aprotic (acetone) solvents.

Effect of concentration: The intensity of the spots of **Cu** and its degradation products **FA** and **DHZ** was stronger at the higher concentration of 10 mM, for both reaction times and in both solutions. We did not observe any effect of concentration of **Va** immediately after spraying with acetone solution of DPPH• or even several minutes after spraying. Otherwise, the brightness of the spots of **Va** at 10 mM after 10 min was the same in both solvents (Fig. 1).

Chain-breaking antioxidant activity

Figure 2 presents the experimental results of TGSO autoxidation kinetics in presence of **Cu** and its degradation products: **FA**, **Va** and **DHZ** separately and in mixtures. The highest activity was obtained for **Cu** as individual component. In this study **Va** did not show any inhibitory activity, i.e. its behavior was analogous to that of the control sample. Usually, the lack of electron-donating group (EDG) in *ortho-* or *para*-position towards the phenolic group in the structures of some phenols, including phenol itself, is the reason for the absence of antioxidant properties. The presence of an electron-withdrawing group (EWG) in the **Va** structure, particularly in *para*-position to the phenolic OH-group, increases bond dissociation enthalpy (BDE) of the latter and also hampers the radical stabilization even though the presence of a guaiacyl unit (2-methoxyphenol) is generally considered beneficial for antioxidant activity. Our results, obtained for the antioxidant activity of **Va**, are in agreement with those obtained by other authors proving the fact that **Va** and vanillic acid do not exert high activity toward neutral radicals like DPPH$^{\bullet}$ or LOO$^{\bullet}$ in nonpolar medium [26-29]. According to Wang *et al.* [10] **Va** becomes the major degradation product with increasing the incubation time in buffer solution at 37°C. In fact, there are a number of studies in the literature reporting results for antioxidant activity of **Va** in hydrophilic or ionizing systems [29-33]. Probably, in such an environment, **Va** is involved in reactions with free radicals, different than the classical hydrogen atom transfer. The latter is assumed to be the main mechanism in autoxidation process in homogeneous lipid medium and the lack of activity of **Va** in TGSO oxidation is in accordance with what we have observed with TLC DPPH rapid test (after 1 min reaction time) in acetone (Fig. 1A).

FA and **DHZ** exerted weak or moderate antioxidant activity, as it is expected for monophenols, depending on their concentration being used [18, 34].

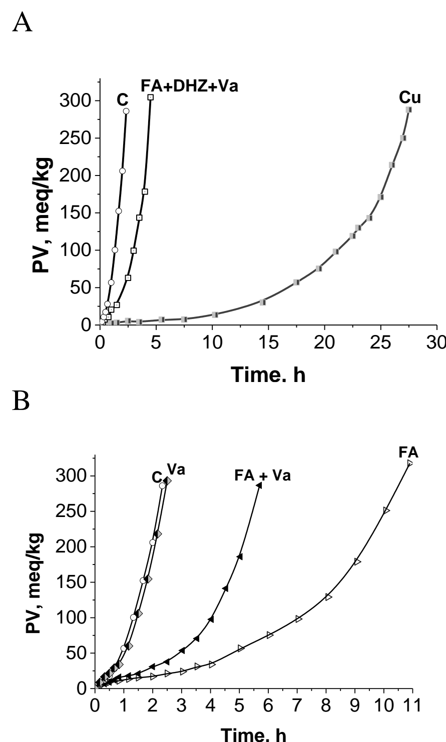

Figure 2. Kinetic curves of lipid peroxide accumulation during TGSO autoxidation at 80 ºC in absence (control, C) and in presence of studied compounds.

Table 1 presents the main kinetic parameters characterizing the TGSO autoxidation at 80 ºC in presence of the studied compounds. Then we compared the kinetic parameters of **Cu** with **FA**, **Va** and **DHZ** in equimolar ratio at 0.5 mM and 0.33 mM as binary (1:1) and ternary (1:1:1) mixtures, respectively.

Table 1. Kinetic parameters characterizing TGSO autoxidation at 80 ºC in presence of studied compounds.

Compound	Conc., M	IP_A, h	PF-	R_A, 10^{-6}M/s	ID	Activity
Curcumin (Cu)	1.0	22 ± 2.0	16.9	0.3 ± 0.02	15.7	Strong
Vanillin (Va)	1.0	1.3 ± 0.2	1.0	4.7 ± 0.5	1.0	No activity
Ferulic acid (FA)	1.0	7.5 ± 0.9	5.8	0.7 ± 0.2	12.6	Moderate
Va + FA (1:1)	0.5	4.2 ± 0.5	3.2	2.6 ± 0.3	1.8	Weak
Va + FA + DHZ (1:1:1)	0.33	3.5 ± 0.4	2.7	1.9 ± 0.3	2.5	Weak

*Kinetic parameters of **DHZ** characterizing its chain-breaking antioxidant activity was studied and described in our earlier publications [17, 18].

As an individual component **FA** inhibits the lipid oxidation process to a greater extent than in the cases when it is in mixtures. It could be seen from the results (Fig. 2, Table 1) that when **FA** is in a binary mixture with **Va** and in a ternary mixture with **DHZ** and **Va**, only weak activity was observed, much weaker than in the case of **Cu** at 1.0 mM as individual component.

However, the C2-symmetric dimer of **DHZ** prepared by us previously [18] demonstrated the same antioxidant activity as **Cu**. The latter evidence and the results obtained in the present study

encourage searching new dimeric structures in which the most important features of **Cu** or its metabolites are combined. Such compounds probably will be more active as individual components than their corresponding monomers when used in mixtures.

CONCLUSION

In this study a comparison between the antioxidant activity of **Cu** and that of its degradation products (**FA**, **Va**, and **DHZ**) at physiological conditions was assayed in bulk lipid autoxidation as individual components and in equimolar binary and ternary mixtures.

Cu showed the best antioxidant activity whereas weak activity was observed when its degradation products were studied in mixtures. **Va** did not show any activity, i.e. its behavior was analogous to that of the control sample. **FA** and **DHZ** binary mixture (1:1) showed very weak effect, weaker than that detected when both compounds were tested as individual components.

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СРАВНИТЕЛНО ИЗСЛЕДВАНЕ НА АНТИОКСИДАНТНИЯ ПОТЕНЦИАЛ НА КУРКУМИН И НЕГОВИ РАЗПАДНИ ПРОДУКТИ – ВАНИЛИН, ФЕРУЛОВА КИСЕЛИНА И ДЕХИДРОЦИНГЕРОН

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(Резюме)

Куркуминът е един от най-интензивно изследваните биологично активни антиоксиданти през последното десетилетие. Неговата нестабилност и бързо разпадане при физиологични условия (pH ≈ 7) е едно от основните ограничения, свързани с потенциалните му приложения. Предприети са редица подходи за преодоляване на проблема с бионаличността на куркумина, включително използването на пиперин, липозоми, наночастици, фосфолипидни комплекси и дизайн на нови структурни аналози на куркумин. От друга страна, разграждането на съединението не води непременно до загуба на неговата активност. Дискутира се дали и как пътищата на разграждане и окисляване допринасят за биологичните и антиоксидантните активности на куркумина. Целта на това изследване е да се сравни антиоксидантната активност на куркумина с тази на неговите продукти на разграждане (ферулова киселина, ванилин и дехидроцингерон), когато те се добавят в би- и трикомпонентни (тройни) антиоксидантни смеси. Липидното автоокисление се използва за оценка на ефективността на антиоксиданта и на реактивността на фенолите. Получените резултати показват много по-силна активност на куркумина, в сравнение с тази на което и да е от разпадните съединения и техните двойни и тройни смеси.