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ORIGINAL PAPER

Binary combinations of natural phenolic compounds with gallic acid or with its alkyl esters: an approach to understand the antioxidant interactions

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Abstract The antioxidant activity of equimolar binary combinations of ten natural phenolic compounds (vanillic acid, vanillin, hydroquinone, caffeic acid, ellagic acid, resveratrol, genistein, kaempferol, quercetin and catechin) with gallic acid or its alkyl esters (propyl-, octyl- and dodecyl gallates) was determined measuring the scavenging 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical cation activity. Statistical significant antagonism was found for all combinations with gallic acid and for most combinations with alkyl esters. Statistical significant synergism was observed only for three combinations of dodecyl gallate with resveratrol, genistein or catechin. The antagonistic and synergistic effects were analysed from regeneration mechanisms, and most of them can be explained according to the one-electron reduction potentials of the phenolics selected. These results may facilitate the use of combinations of antioxidants, as part of the hurdle technology, which can be applied to different aspects of food preservation, in order to increase shelf life and retain nutritional quality.

Keywords Gallic acid · Alkyl gallates · Antioxidant activity · Antagonism · Synergism · Regeneration mechanisms

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Introduction

The phenolic antioxidant found in plants and beverages provide a defence against the oxidation processes caused by free radicals and reactive oxygen species (ROS). In addition, these compounds have other biological properties beneficial for health such as antiproliferative, antibacterial, anti-inflammatory and anti-allergic, and thus, they may decrease the risk of cancer, hypertension, brain dysfunction, and cardiovascular and neurodegenerative diseases [1] even though, so far, it has been not provided clinical trial-based evidences of these benefits. Many of these biological functions have been attributed to their free radical scavenging capacity and antioxidant activity.

The addition of antioxidant compounds, in particular synthetic compounds such as propyl gallate among others, is a common method to increase the oxidative stability of foods and therefore improve their quality and shelf life. Many studies indicate the need to reduce the use of synthetic antioxidants due to their potential toxicity at high dosage [2] which leads to increasing investigations on natural compounds with antioxidant properties. The use of antioxidant combinations is a part of the so-called hurdle technology, by which the antioxidant and antimicrobial activity of natural compounds are utilised to achieve target food safety and nutritional quality. Phenolic antioxidants combinations were found to increase the oxidative stability of some foods [3, 4]. Similarly, phenolics antioxidant combinations have been assayed to improve their different biological activities [5, 6]. Interaction among different antioxidants coexisting in real food matrices can be additive, synergistic or antagonistic causing desired or undesired modification of their activities and changes in food safety and shelf life [7].

The objective of this study was to investigate the effect on the total antioxidant activity of equimolar binary

combinations of eleven natural phenolics (which naturally occur in foods) and three synthetic phenolics (which have been widely used as antioxidant additives in foods) in order to establish the type of interaction and to attempt to elucidate the mechanism responsible of the antioxidant effect of the mixture. We have used the ABTS assay as it is a useful method for detecting antioxidant activity of hydrophilic, lipophilic and high-pigmented antioxidant compounds [8]. The natural phenolic antioxidants include a simple phenol (hydroquinone), a derivative of cinnamic acid (caffeic acid), derivatives of benzoic acid (vanillic acid, vanillin, gallic acid and ellagic acid), stilbenes (resveratrol) and flavonoids (genistein, kaempferol, quercetin and catechin). Among which, vanillin and gallic acid are also isolated from foods and used as flavouring agents for foods like dairy products [9]. The synthetic phenolic antioxidants used in this study were gallic acid alkyl esters (propyl-, octyl- and dodecyl gallates). They are currently permitted for uses as additives in foods like dehydrated milk [10]. Gallic acid alkyl esters are also known for their chelating capacity (to chelate transition metal ions, which can promote the damage caused by free radical in foods), and antimicrobial agents specially the octyl- and dodecyl gallates [11]. In previous works, we have reported the antibacterial activity of gallic acid against *Staphylococcus aureus* [12] as well as the antibacterial activity of octyl gallate against several strains of Gram-positive and Gram-negative bacteria [13, 14]. A phenolic compound possessing both antioxidant and antimicrobial activities would be of interest to food industry, because the presence of this compound in foods would reduce the total amount of additives required for providing these activities.

Materials and methods

Chemicals

ABTS (2,2′-azino-bis[3-ethylbenzothiazoline-6-sulphonic acid]), potassium persulphate, caffeic acid, catechin, ellagic acid, genistein, hydroquinone, kaempferol, quercetin, resveratrol, Trolox, vanillin, vanillic acid, gallic acid, propyl gallate, octyl gallate and dodecyl gallate were purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals used were of analytical grade.

Antioxidant activity by ABTS assay

The modified ABTS assay at pH 4.5 was carried out as described by Ozgen et al. [15] and Zyzelewicz et al. [16], with minor modifications. The ABTS radical cations ($ABTS^{\cdot+}$) were prepared as follows: ABTS stock solution (7 mM) with 2.45 mM potassium persulphate was prepared in 20 mM sodium acetate buffer, pH 4.5, and was allowed to stand for 12–16 h at room temperature in the dark remaining stable for several weeks at 4 °C. On the day of analysis, the $ABTS^{\cdot+}$ solution was diluted with the same buffer to an absorbance of 0.7 ± 0.01 at 734 nm. For the spectrophotometric assay, one mL of the $ABTS^{\cdot+}$ solution and 10 μL of 0.2 mM of individual phenolic compounds dissolved in ethanol or equimolar binary combinations of these phenolic compounds (0.2 mM) were mixed, and the absorbance at 734 nm was registered after 6 min at 30 °C. Trolox was used as the antioxidant control, and the results were expressed as mM Trolox equivalents. The concentration of Trolox giving the same percentage reduction of absorbance at 734 nm as the 0.2 mM antioxidant solution was calculated using a Trolox standard curve (0–3.0 mM).

According to Peyrat-Maillard et al. [17], the mixture effect (ME) on the antioxidant activity of binary combination is defined as the ratio of the experimental antioxidant activity (AA) of a mixture of two compounds (A_1 and A_2) to the calculated by the sum of activity of each compound separately (AA_1 and AA_2):

$$ME = AA/AA_1 + AA_2$$

A ME value > 1 defines a synergistic effect, whereas a ME value < 1 defines an antagonistic effect and a ME value = 1 an additive effect between the implicated antioxidants.

Statistical analysis

The antioxidant activity of each phenolic compound was analysed at least 10 times alone and 10 times in the binary mixture, each performed in duplicate. The results were reported as mean ± standard deviation (SD). One-sample Student's *t* test was performed by using the SPSS 19.0 package (SPSS software available at the University of León).

Results and discussion

Firstly, we have determined the antioxidant activity of the selected phenolic compounds, at a concentration of 0.2 mM, according to their capacity to scavenge the radical cation $ABTS^{\cdot+}$. The results are shown in Table 1, where it can be seen that the esterification of the carboxylate group of gallic acid decreases the antioxidant activity as the alkyl chain length of the ester group increases, following an order: gallic acid > propyl gallate > octyl gallate > dodecyl gallate, so that the antioxidant activity of dodecyl gallate is 35% of that of the gallic acid. Lu et al. [18] analysing the scavenging efficiency of gallic acid derivatives on DPPH in ethanol absolute reported a clear decline of antioxidant

Table 1 Antioxidant activity of selected individual phenolics (0.2 mM)

Phenolics	Antioxidant activity[a] (mM Trolox equivalents)
Genistein	0.16 ± 0.06
Vanillic acid	0.22 ± 0.04
Vanillin	0.29 ± 0.04
Hydroquinone	0.34 ± 0.02
Kaempferol	0.38 ± 0.08
Caffeic acid	0.42 ± 0.10
Catechin	0.78 ± 0.09
Resveratrol	0.81 ± 0.08
Ellagic acid	0.92 ± 0.10
Quercetin	0.93 ± 0.09
Gallic acid	1.14 ± 0.18
Propyl gallate	0.85 ± 0.07
Octyl gallate	0.50 ± 0.08
Dodecyl gallate	0.40 ± 0.05

[a] The data are shown as means ± deviation ($n \geq 10$)

activity with an increase in the acyl chain length, indicating that the steric freedom is also important for the antioxidant activity. However, other authors found that the antioxidant activity of gallates against the DPPH does not depend on chain lengths [11, 19]. The antioxidant activity by the ABTS assay was carried out at pH 4.5, so that the gallic acid is mainly present (75%) in the monoanionic form (pK value ≈ 4.0). Ji et al. [20] indicated that the proton dissociation from the carboxyl group is important to understanding structure–activity relationships of gallic acid antioxidant derivatives, since the deprotonated carboxyl becomes electron-donating group, which favours radical scavenging based on H-atom-transfer and electron donation. Although the gallic acid monoanion form, present at pH 4.5, has big influence on the radical scavenging behaviour, the steric effect of alkyl chain of the gallic acid esters also appears to play an important role in the antioxidant activity of these compounds. In addition, the highest antioxidant activity of resveratrol respect to genistein (0.81 vs 0.16) highlights the importance of the conjugation of the two phenolic rings on the efficiency of the first as antioxidant.

Table 2 shows the mixture effect on the antioxidant activity of the binary combinations of the natural phenolic compounds studied with gallic acid or propyl-, octyl- or dodecyl gallate. The binary combinations with gallic acid and with its derivatives produce mostly antagonistic effects (ME < 1). Of the 43 possible binary combinations, 10 of them show additive effects (ME = 1) and only 3 synergistic effects (ME > 1). An analysis of variance by rows, but not indicated in Table 2 (for clarity), revealed that the values of the mixture effect (ME) of the binary combinations which are significantly equal ($p < 0.05$) show also a similar behaviour, i.e., or are equal to 1 (additive effect) or different than 1 (synergistic/antagonistic effect). Combinations with gallic acid, in all cases, lead to antagonistic effects. The higher antagonistic effects appear in the binary combinations of the vanillic acid and vanillin with gallic acid and its alkyl esters showing an 18–30% reduction in antioxidant capacity. For 6 of the natural phenolic compounds used in these binary combinations, namely hydroquinone, caffeic acid, resveratrol, genistein, quercetin and catechin, it can be said, in a general way, that the alkyl chain length transforms the observed antagonistic effects in combination with the gallic acid, in additive and, even in synergistic effects for some of

Table 2 Mixture effect (ME) on the antioxidant activity of binary combinations of selected natural phenolics with gallic acid and its alkyl esters at equimolar concentrations (0.2 mM)

Phenolics	Gallic acid	Propyl gallate	Octyl gallate	Dodecyl gallate
Vanillic acid	0.72 ± 0.04*	0.80 ± 0.07*	0.82 ± 0.13*	0.70 ± 0.12*
Vanillin	0.75 ± 0.08*	0.92 ± 0.06*	0.92 ± 0.08*	0.87 ± 0.12*
Hydroquinone	0.88 ± 0.08*	1.10 ± 0.10	0.95 ± 0.07	1.05 ± 0.06
Caffeic acid	0.90 ± 0.07*	1.04 ± 0.05	1.03 ± 0.04	0.81 ± 0.05*
Gallic acid		0.84 ± 0.05*	0.89 ± 0.06*	0.95 ± 0.05*
Ellagic acid	0.80 ± 0.06*	0.94 ± 0.03*	0.86 ± 0.15*	0.92 ± 0.10*
Resveratrol	0.90 ± 0.06*	1.03 ± 0.05	0.96 ± 0.09	1.17 ± 0.07*
Genistein	0.73 ± 0.12*	0.77 ± 0.09*	0.80 ± 0.10*	1.30 ± 0.09*
Kaempferol	0.70 ± 0.05*	0.86 ± 0.06*	0.77 ± 0.05*	0.90 ± 0.08*
Quercetin	0.87 ± 0.09*	0.86 ± 0.06*	0.87 ± 0.11*	1.06 ± 0.25
Catechin	0.95 ± 0.04*	0.99 ± 0.06	0.99 ± 0.07	1.11 ± 0.02*

ME is defined in Materials and methods. The data are presented as means ± deviation ($n \geq 10$). ME > 1 synergistic effect; ME < 1 antagonistic effect and ME = 1 additive effect

* Significant differences ($p < 0.05$) with to value 1. p values were calculated using one-sample Student's t test

Table 3 Regeneration mechanisms in the antioxidant activity of the binary combinations analysed that show antagonistic/synergistic effects

Antioxidant combinations A_1^a/A_2^b	Combination effect	X^c (%)
Vanillic acid/gallic acid	Antagonism	58
Vanillic acid/propyl gallate	Antagonism	27
Vanillic acid/octyl gallate	Antagonism	43
Vanillic acid/dodecyl gallate	Antagonism	100
Vanillin/gallic acid	Antagonism	60
Vanillin/propyl gallate	Antagonism	7
Vanillin/octyl gallate	Antagonism	34
Vanillin/dodecyl gallate	Antagonism	82
Hydroquinone/gallic acid	Antagonism	45
Caffeic acid/gallic acid	Antagonism	43
Dodecyl gallate/caffeic acid	Antagonism	>100
Propyl gallate/gallic acid	Antagonism	>100
Octyl gallate/gallic acid	Antagonism	7.3
Dodecyl gallate/gallic acid	Antagonism	8
Ellagic acid/gallic acid	Antagonism	>100
Propyl gallate/ellagic acid	Antagonism	86
Octyl gallate/ellagic acid	Antagonism	48
Dodecyl gallate/ellagic acid	Antagonism	19
Resveratrol/gallic acid	Antagonism	>100
Resveratrol/dodecyl gallate	Synergism	46
Genistein/gallic acid	Antagonism	51
Genistein/propyl gallate	Antagonism	25
Genistein/octyl gallate	Antagonism	38
Dodecyl gallate/genistein	Synergism	71
Kaempferol/gallic acid	Antagonism	78
Kaempferol/propyl gallate	Antagonism	28
Kaempferol/octyl gallate	Antagonism	>100
Kaempferol/dodecyl gallate	Antagonism	>100
Quercetin/gallic acid	Antagonism	>100
Propyl gallate/quercetin	Antagonism	>100
Octyl gallate/quercetin	Antagonism	44
Catechin/gallic acid	Antagonism	83
Catechin/dodecyl gallate	Synergism	34

[a] A_1 is the antioxidant that acts only as a scavenger of radical $ABTS^{\cdot+}$

[b] A_2 is the antioxidant that acts as scavenger of radical $ABTS^{\cdot+}$ and as a regenerator of A_1

[c] X is the part of A_2 that regenerates A_1 calculated from Eq. (1)

them. Synergistic effects were only found in the combinations of dodecyl gallate with resveratrol, catechin and genistein, showing the last combination the biggest synergistic effect (30% increases in antioxidant activity). Probably, not only the length of the alkyl chain, but also the role that can play the structure of the partner present in the combination, contributes to change the mixture effect from antagonist to an additive effect, and finally, to synergistic.

Understanding the mechanisms underlying the functionality of combinations of antioxidant is important to their potential development. Several hypotheses have been developed to explain the effects found in binary combinations of antioxidants. Peyrat-Maillard et al. [17] described coupled reactions of regeneration between antioxidants: a synergistic effect if the less efficient antioxidant regenerates the more efficient one and an antagonistic effect if the more efficient molecule regenerates the less efficient one. In addition, other phenomena postulated to explain the interactions of antioxidants include the polarity of the interacting molecules, the reaction rates of antioxidants, the effective concentration of the antioxidants at the oxidation site and even the possibility of complex formation between antioxidants [21–23].

We have analysed the mixture effect of binary combinations of the studied phenolics to probe if they could be explained by regeneration mechanisms between antioxidants [17]. In these binary combinations, one of the antioxidants (A_2) may react either with $ABTS^{\cdot+}$ or with phenolic radical derived from the oxidation of other antioxidant (A_1) by reaction with $ABTS^{\cdot+}$, regenerating the latter antioxidant. We have considering that one mol of a phenolic antioxidant regenerates one mol of another one, and so equimolar concentrations of the two antioxidants were tested. The experimental antioxidant activity (AA) of binary combination can be expressed by the Eq. (1) from which we have deduced the fraction X of antioxidant A_2 that acts as a regenerator of antioxidant A_1:

$$\text{Experimental AA} = \text{AA}_1 + X\,\text{AA}_1 + (1 - X)\,\text{AA}_2 \qquad (1)$$

where AA_1 and AA_2 were the individual antioxidant activity of A_1 and A_2, respectively.

Table 3 shows the mixture effects of the binary combination as well as the regeneration percentage (X) of the antioxidant A_2 that acts as a regenerator of the other antioxidant A_1. The antagonistic effects found in the binary combinations of gallic acid with vanillic acid, vanillin, hydroquinone, caffeic acid, genistein, kaempferol and catechin, respectively, can be explained by the fact that any of these phenolic compounds could be regenerated by the gallic acid with higher antioxidant activity. It is worth mentioning the regeneration of antioxidant flavonoids such as catechin (83%), kaempferol (78%) and genistein (51%), as well as that of the vanillic acid and vanillin (58–60%) and to a lesser extent those of hydroquinone (45%) and caffeic acid (43%), suggesting that gallic acid seems to act better as a scavenger of the radical of these phenolic compounds than $ABTS^{\cdot+}$ radical cation. Therefore, the gallic acid seems to reduce to the radical derived from catechin > kaempferol > vanillic acid ≈ vanillin > genistein > hydroquinone ≈ caffeic acid. Antagonistic effects observed in the binary combinations of resveratrol, propyl

gallate, ellagic acid or quercetin with gallic acid cannot be explained by this process of regeneration on the basis of a 1:1 stoichiometry, since in these cases the *X* values are greater than 100%. This could be due that these phenolic compounds have an antioxidant activity similar to the gallic acid (Table 1). In addition, as already mentioned above other many processes could be involved in these interactions, including a stoichiometry higher than 1:1.

The antagonistic effects of binary combinations of the selected phenolics with the gallic acid alkyl esters in the majority of cases can be explained by regeneration mechanisms. In some combinations, the regenerator capacity of the alkyl ester diminishes and/or ceases with the increase in the length of the alkyl chain. However, for the combinations with vanillic acid and vanillin the increase in the length of the alkyl chain increases the regenerative capacity of the gallic acid alkyl ester. In this sense, it is noteworthy that the dodecyl gallate seems to act almost exclusively as a regenerator of vanillic acid and vanillin, respectively, suggesting that it may have much more affinity for the radical derivatives of vanillic acid and vanillin than for $ABTS^{\cdot+}$ radical cation. On the other hand, ellagic acid is a good regenerator of the gallic acid alkyl esters, although its regeneration capacity diminishes with the increase in the length of the alkyl chain. The synergistic effects shown in the binary combinations of resveratrol/dodecyl gallate, catechin/dodecyl gallate and dodecyl gallate/genistein (Table 3) can also be explained by a regeneration mechanism. In the first two combinations is the dodecyl gallate which should regenerate to resveratrol (46%) and the catechin (34%). In these cases, the presence of a synthetic antioxidant, as dodecyl gallate, enhances the antioxidant capacity of natural flavonoid. We believe this can be potentially important for the resveratrol as scientific evidences have highlighted its potential as therapeutic agent for cerebral and cardiovascular diseases [24]. However, it is striking that in the third combination, genistein, a natural flavonoid, regenerates in a very high percentage (71%) to the dodecyl gallate under these experimental conditions. This result shows that genistein has much more capacity to scavenge the radical of the dodecyl gallate than the $ABTS^{\cdot+}$. Gunckel et al. [25], from a carried out electrochemical study with the gallic acid and the alkyl gallates, found that the introduction of alkyl group did not greatly influence the energetics of the electron transfer, although less stable semiquinone radicals are produced. This could explain why genistein is capable of regenerate the dodecyl gallate but not gallic acid, propyl gallate and octyl gallate.

In addition, we have found that almost all the effects of the binary combinations of the phenolics tested can also be explained theoretically according to the one-electron reduction potentials (E'^{o}) of the studied phenolics. The hierarchy of electron donation is clearly based on the reduction potentials, and this allows us to predict the effect of the mixture in most binary combinations studied. Table 4 shows the E'^{o} value for the most of the phenolics used in this study with the exceptions of ellagic acid and resveratrol, for which reduction potentials could not be found. For these two cases, we have used the peak potentials (E_p) (E_p is defined as the peak oxidation potential value that reflects the redox properties of the antioxidant [26]), which could not be directly comparable. A low oxidation potential is associated with a greater facility or strength of a given molecule for the electrodonation and, thus, to act as antioxidant. Likewise, we have assumed that the gallic acid alkyl esters have values of E'^{o} very similar to the gallic acid, based on the values of half-wave potential ($E_{1/2}$) described by Gunckel et al. [25] for some alkyl esters of gallic acid.

Table 4 Electrochemical properties of some of the antioxidant phenolics used in this study

Phenolics	E'^{o} (V)[a]	E_p (V)[b]	References
Ellagic acid		0.278	Komorsky-Lovrić and Novak [31]
Quercetin	0.330		Jovanovic et al. [32]
Caffeic acid	0.534		Foley et al. [33]
Gallic acid	0.560		Jovanovic et al. [34]
Catechin	0.570		Jovanovic et al. [35]
Resveratrol		0.620	Fang and Zhou [36]
Hydroquinone	0.700		Kung and McBride [37]
Genistein	0.730		Han et al. [38]
Kaempferol	0.750		Jovanovic and Simic [39]
Vanillin	0.942		Khopde and Priyadarsini [40]
Vanillic acid	0.952		Khopde and Priyadarsini [40]

[a] E'^{o} is the standard one-electron reduction potential

[b] E_p is the peak potential from cyclic voltammetry

The results shown in this paper suggest that the effects of the most binary combinations of phenolic compounds tested can be explained by an electron transfer mechanism, according to the experimental conditions used in this work and the theoretical data of the reduction potential values. Of the 30 antagonistic effects estimated (Table 3), 29 of them are thermodynamically possible and also they match to the experimentally obtained effect (antagonism), taking into account the E'^{o} values (Table 4). Only the antagonistic effect of caffeic acid regeneration by the gallic acid is not thermodynamically explainable. Furthermore, of the three binary combinations showing synergistic effects only the regeneration of dodecyl gallate by genistein is not thermodynamically feasible. The value of the E'^{o} only indicates that the process can be thermodynamically favourable, but its rate may be too slow to occur. So, it is possible that of the ten combinations with additive effects found (Table 2), seven of them could produce antagonistic effects and three

synergistic ones if the processes occur at sufficient rate to be detected.

The ability of gallic acid to regenerate caffeic acid may be linked to a lesser bond dissociation enthalpy (BDE) of the 4-OH bond of gallic acid (82.28 kcal mol^{-1}) versus of the 4-OH bond of the caffeic acid (86.02 kcal mol^{-1}). These BDEs values have been obtained by Leopoldini et al. [27] from density functional theory (DFT) calculation in water solvent and refer to the bond dissociation enthalpy of the most easily dehydrogenable OH group. The lower the BDE value, the higher the dissociation of the phenolic O–H bond and the transfer of the H atom to free radicals, and thus, the caffeic acid regeneration by gallic acid can be possible.

No published thermodynamic parameters in water solution to explain the regeneration of dodecyl gallate by genistein. From DFT studies, the hydrogen atom transfer (HAT) mechanism appeared as the thermodynamically preferred pathway in gas phase for several isoflavonoids including genistein [28]. However, the influence of the solvents can be important on the value of the thermodynamic parameters that even cannot follow the same trends [27]. Senthil Kumar and Kumaresan [29] in a DFT study about free radical scavenger mechanism of several isoflavonoids revealed that the thermodynamic parameters that define a single-electron transfer-proton transfer (SET-PT) and sequential proton loss-electron transfer (SPLET) are significantly lower in water than in the gas phase, as a consequence of the stabilisation of charged species in polar solvents, suggesting that the SPLET mechanism will be the thermodynamically preferred pathway in polar solvent. Recently, Lengyel et al. [30] indicated that HAT mechanism can be attributed predominantly to the B ring, while SPLET takes place preferentially in the A ring of isoflavones. Thus, in water solution genistein could regenerate dodecyl gallate by SPLET mechanism.

With all the data shown in this study, and in the experimental conditions used, it is possible to predict, in most cases the type of interaction that can occur in the binary combinations of gallic acid or its alkyl esters with natural phenolics selected using the ABTS assay. The results obtained can provide information which may be useful to prevent the use of phenolic compounds that can produce antagonistic effects in binary combinations, either when added as additives to food or if any of these phenolic compounds is present naturally in foods.

Conclusions

The antioxidant behaviour analysis, determined by ABTS assay at pH 4.5, showed statistical significant antagonism for the most of the equimolar binary combinations of natural phenolics with gallic acid or its alkyl esters. Only the combinations of dodecyl gallate with resveratrol, genistein or catechin showed statistical significant synergism. The mixture effects found were analysed in the light of regeneration mechanisms and can explain most of them according to the one-electron reduction potentials of the studied phenolics. We think that the two analyses performed represent a useful approach for understanding the interactions between antioxidant phenolics.

Compliance with ethical standards

Conflict of interest None.

Compliance with ethical requirements This article does not contain any studies with human or animal subjects.

References

1. Valko M, Leibfritz D, Moncola J, Cronin MTD, Mazura M, Telser J (2007) Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell B 39:44–84
2. Pokorný J (2007) Are natural antioxidants better –and safer-than synthetic antioxidants? Eur J Lipid Sci Technol 109:629–642
3. Martínez ML, Penci MC, Ixtaina V, Ribotta PD, Maestri D (2013) Effect of natural and synthetic antioxidants on the oxidative stability of walnut oil under different storage conditions. LWT Food Sci Technol 51:44–50
4. Hwang K-E, Kim H-W, Song D-H, Kim Y-J, Ham Y-K, Lee J-W, Choi Y-S, Kim Ch-J (2015) Effects of antioxidant combinations on shelf stability of irradiated chicken sausage during storage. Radiat Phys Chem 106:315–319
5. Abarikwu SO, Akiri OF, Durojaiye MA, Alabi AF (2014) Combined administration of curcumin and gallic acid inhibits gallic acid-induced suppression of steroidogenesis, sperm output, antioxidant defenses and inflammatory responsive genes. J Steroid Biochem Mol Biol 143:49–60
6. Fernández-Álvarez L, del Valle P, de Arriaga D, García-Armesto MR, Rúa J (2014) Binary combinations of BHA and other natural and synthetic phenolics: antimicrobial activity against *Staphylococcus aureus* and antioxidant capacity. Food Control 42:303–309
7. Choe E, Min DB (2009) Mechanisms of antioxidants in the oxidation of foods. Compr Rev Food Sci Food Saf 8:345–358
8. Floegel A, Kim D-O, Chung S-J, Koo SI, Chun OK (2011) Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. J Food Compost Anal 24:1043–1048
9. EFSA (European Food Safety Authority) (2010) Scientific opinion on flavouring group evaluation 20, revision 2 (FGE.20Rev2): benzyl alcohols, benzaldehydes, a related acetal, benzoic acids, and related esters from chemical groups 23 and 30. EFSA panel on food contact materials, enzymes, flavourings and processing aids. EFSA J 8:1405
10. Commission Regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No. 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives. OJ L 295 54:1–177

11. Kubo I, Masuoka N, Ha TJ, Shimizu K, Nihei K (2010) Multifunctional antioxidant activities of alkyl gallates. TOBCJ 3:1–11
12. Gutiérrez-Larraínzar M, Rúa J, Caro I, de Castro C, de Arriaga D, García-Armesto MR, del Valle P (2012) Evaluation of antimicrobial and antioxidant activities of natural phenolic compounds against foodborne pathogens and spoilage bacteria. Food Control 26:555–563
13. Gutiérrez-Fernández J, García-Armesto MR, Álvarez-Alonso R, del Valle P, de Arriaga D, Rúa J (2013) Antimicrobial activity of binary combinations of natural and synthetic phenolic antioxidants against *Enterococcus faecalis*. J Dairy Sci 96:4912–4920
14. Gutiérrez-Larraínzar M, Rúa J, de Arriaga D, del Valle P, García-Armesto MR (2013) In vitro assessment of synthetic phenolic antioxidants for inhibition of foodborne *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas fluorescens*. Food Control 30:393–399
15. Ozgen M, Reese N, Jr Tulio AZ, Scheerens JC, Miller AR (2006) Modified 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method to measure antioxidant capacity of selected small fruits and comparison to ferric reducing antioxidant power (FRAP) and 2,2´-diphenyl-1-picrylhydrazyl (DPPH) Methods. J Agric Food Chem 54:1151–1157
16. Zyzelewicz D, Zaklos-Szyda M, Juskiewicz J, Bojczuk M, Oracz J, Budryn G, Miskiewicz K, Krysiak W, Zdunczyk Z, Jurgonski A (2016) Cocoa bean (*Theobroma cacao* L.) phenolic extracts as PTP1B inhibitors, hepatic HepG2 and pancreatic β-TC3 cell cytoprotective agents and their influence on oxidative stress in rats. Food Res Int 89:946–957
17. Peyrat-Maillard MN, Cuvelier ME, Berset C (2003) Antioxidant activity of phenolic compounds in 2,2´-azobis(2-amidinopropane) dihydrochloride (AAPH)-induced oxidation: synergistic and antagonistic effects. J Am Oil Chem Soc 80:1007–1012
18. Lu Z, Nie G, Belton PS, Tang H, Zhao B (2006) Structure-activity relationship analysis of antioxidant ability and neuroprotective effect of gallic acid derivatives. Neurochem Int 48:262–274
19. Losada-Barreiro S, Bravo-Díaz C, Paiva-Martins F, Romsted LS (2013) Maxima in antioxidant distributions and efficiencies with increasing hydrophobicity of gallic acid and its alkyl esters. The pseudophase model interpretation of the "cutoff effect". J Agric Food Chem 61:6533–6543
20. Ji H-F, Zhang H-Y, Shen L (2006) Proton dissociation is important to understanding structure-activity relationships of gallic acid antioxidants. Bioorg Med Chem Lett 16:4095–4098
21. Frankel EN, Huang SW, Kanner J, German JB (1994) Interfacial phenomena in the evaluation of antioxidants: bulk oil vs emulsions. J Agric Food Chem 42:1054–1059
22. Koga T, Terao J (1995) Phospholipids increase radical-scavenging activity of vitamin E in a bulk oil model system. J Agric Food Chem 43:1450–1454
23. Cuvelier C, Bondet V, Berset C (2000) Behaviour of phenolic antioxidants in a partitioned medium: structure-activity relationship. J Am Oil Chem Soc 77:819–823
24. He S, Yan X (2013) From resveratrol to its derivatives: new sources of natural antioxidant. Curr Med Chem 20:1005–1017
25. Gunckel S, Santander P, Cordano G, Ferreira J, Muñoz S, Nunez-Vergara LJ, Squella JA (1998) Antioxidant activity of gallates: an electrochemical study in aqueous media. Chem Biol Interact 114:45–59
26. Zhang D, Chu L, Liu Y, Wang A, Ji B, Wu W, Zhou F, Wei Y, Cheng Q, Cai S, Xie L, Jia G (2011) Analysis of the antioxidant capacities of flavonoids under different spectrophotometric assays using cyclic voltammetry and density functional theory. J Agric Food Chem 59:10277–10285
27. Leopoldini M, Marino T, Russo N, Toscano M (2004) Antioxidant properties of phenolic compounds: H-atom versus electron transfer mechanism. J Phys Chem A 108:4916–4922
28. Zhang J, Du F, Peng B, Lu R, Gao H, Zhou Z (2010) Structure, electronic properties, and radical scavenging mechanisms of daidzein, genistein, formononetin, and biochanin A: a density functional study. J Mol Struct THEOCHEM 955:1–6
29. Senthil Kumar K, Kumaresan R (2012) A DFT study on the structural, electronic properties and radical scavenging mechanisms of calycosin, glycitein, pratensein and prunetin. Comput Theor Chem 985:14–22
30. Lengyel J, Rimarčik J, Vagánek A, Klein E (2013) On the radical scavenging activity of isoflavones: thermodynamics of O–H bond cleavage. Phys Chem Chem Phys 15:10895–10903
31. Komorsky-Lovrić Ŝ, Novak I (2011) Determination of ellagic acid in strawberries, raspberries and blackberries by square-wave voltammetry. Int J Electrochem Sci 6:4638–4647
32. Jovanovic SV, Steenken S, Hara Y, Simic MG (1996) Reduction potentials of flavonoid and model phenoxyl radicals. Which ring in flavonoids is responsible for antioxidant activity? J Chem Soc Perkin Trans 2:2497–2504
33. Foley S, Navaratnam S, McGarvey DJ, Land EJ, Truscott TG, Rice-Evans CA (1999) Singlet oxygen quenching and the redox properties of hydroxycinnamic acids. Free Radic Biol Med 26:1202–1208
34. Jovanovic SV, Hara Y, Steenken S, Simic MG (1995) Antioxidant potential of gallocatechins—a pulse-radiolysis and laser photolysis study. J Am Chem Soc 117:9881–9888
35. Jovanovic SV, Steenken S, Tosic M, Marjanovic B, Simic MG (1994) Flavonoids as antioxidants. J Am Chem Soc 116:4846–4851
36. Fang J-G, Zhou B (2008) Structure-activity relationship and mechanism of the tocopherol-regenerating activity of resveratrol and its analogues. J Agric Food Chem 56:11458–11463
37. Kung KH, McBride MB (1988) Electron transfer processes between hydroquinone and iron oxides. Clay Clay Miner 36:303–309
38. Han R-M, Tian Y-X, Liu Y, Chen Ch-H, Ai X-Ch, Zhang J-P, Skibsted LH (2009) Comparison of flavonoids and isoflavonoids as antioxidants. J Agric Food Chem 57:3780–3785
39. Jovanovic SV, Simic MG (2000) Antioxidants in nutrition. Ann N Y Acad Sci 899:326–334
40. Khopde SM, Priyadarsini I (2000) Application of Marcus theory of electron transfer for the reactions between HRP compound I and II and 2,4-disubstituted phenols. Biophys Chem 88:103–109