Original Contribution

PROTECTIVE EFFECTS OF NATURAL BIO-ANTIOXIDANTS AND THEIR SYNTHETIC ANALOGUES IN EQUIMOLAR BINARY AND TRIPLE MIXTURES

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ABSTRACT

PURPOSE: Different effects (synergism, additivism or antagonism) of binary and triple mixtures of bio-antioxidants with alpha-tocopherol (TOH) and/or ascorbyl palmitate (AscPH) was studied. METHODS: Kinetics of bulk lipid autoxidation at 80°C was studied in absence and in presence of equimolar binary mixtures (1:1) with TOH or AscPH and of equimolar triple mixtures (1:1:1) of bio-antioxidants with TOH and AscPH at concentration of the individual components 0.1mM. Different effects was explain on the basis of the following formulae: A) For binary mixtures: Sinergism: IP_{1+2} > IP_1 + IP_2; Additivism: IP_{1+2} = IP_1 + IP_2; and Antagonism: IP_{1+2} < IP_1 + IP_2; B) For triple mixtures: Sinergism: IP_{Σ} > ΣIP_i; Additivism IP_{Σ} = ΣIP_i; and Antagonism: IP_{Σ} < ΣIP_i, where i =1, 2, 3 components. TOH content in the studied mixtures at different reaction time of lipid autoxidation was monitored by using HPLC method. RESULTS: Mechanisms of action of bio-antioxidants in binary and triple mixtures is presented. It has been proven that the synergism obtained between binary and triple mixtures is due to the significant effect of TOH regeneration. CONCLUSIONS: The practical application of results obtained is discussed.

Key words: protective effects, antioxidant mixtures, bio-antioxidants, HPLC.

INTRODUCTION

Biologically active compounds with antioxidant potential, i.e. bio-antioxidants (natural and their synthetic analogues) have a wide range of applications. They are important drugs, antibiotics, agrochemical substitutes, and food preservatives. Many of the drugs today are synthetic modifications of naturally obtained substances with both activities-biological and antioxidant. The most important known natural bio-antioxidants are flavonoids and phenolic acids. Last decades the attention of scientists was focused on design of new bio-antioxidants. The idea is to combine in one molecule by a specific synthetic way various functional fragments, responsible for biological and antioxidant activities for the individual compounds. When it is not possible it can be used so called bio-antioxidant compositions – by mixtures (double or triple) of biologically active compounds, antioxidants, synergists, which are multifunctional (1-3).

Nowadays bio-antioxidants play an important role in prevention of human diseases as components of food additives and for treatment of different diseases as monotherapy or in complex therapy with drugs. It has been found that in the first stage of the development of atherosclerosis the system works in its normal regime. The introduction of antioxidants in the affected body normalizes not only the peroxide oxidation, but also the lipid content. Monotherapy with antioxidants also is used in early stage of atherosclerosis and in oncology. In this case the antioxidants are used at high concentration. There are a lot of reports about combination therapy with drugs and antioxidants, but in this case antioxidants are mainly used as additives in the complex tumor therapy - the antioxidant is in low

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concentrations. In this respect the medical treatment of most of diseases includes formulations based on a combination of traditional drugs with targeted functionality and different antioxidants (2, 4).

For that reason, it is of importance to be studied different effects of equimolar binary and triple mixtures of some known and new bio-
antioxidants with α-tocopherol (TOH) and ascorbyl palmitate (AscPH). The aim of this study is to check which antioxidant compositions are able to ensure the highest lipid oxidation stability and different effects (synergism, additivism or antagonism) of binary and triple mixtures of bio-antioxidants with α-tocopherol (TOH) and/or ascorbyl palmitate (AscPH).

MATERIAL AND METHODS
DL-α-tocopherol (TOH), ferulic acid (FA), caffeic acid (CA), sinapic acid (SA), butylated hydroxytoluene (BHT), tret-butylhydroquinone (TBHQ) and ascorbyl palmitate (AscPH) were from from E. Merck (Germany). Quercetin (Qu), luteolin (Lu) and ritun (Ru) were isolated earlier from Carthamus lanatus L. (5), 7,8-dihydroxy-5-methyl-coumarin (Cum_5), 6,7-dihydroxy-4-methyl-coumarin (Cum_4) and 7-Hydroxy-4-methyl-coumarin (Cum_3) were synthesized from University of Delhi, India and previously reported (6). Dehydrozingerone (M_1OH), zingerone (M_2OH), dimer of dehydrozingerone D_1(OH)_2, dimer of zingerone D_2(OH)_2 and dimer of ferulic acid (DFA) was synthesized from CNR Institute of biomolecular chemistry, Italy and reported previously (7). A Shimadzu HPLC system (Shimadzu Corp., Kyoto, Japan), consisting of a LC-10AD pump, SCTL 10A system controller and SPD-M 10A photodiode array detector was employed. A detailed protocol can be seen in Ref. (8)

Lipid samples. Triacylglycerols of commercially available sunflower oil (TGSO) were cleaned from pro- and anti-oxidants by adsorption chromatography and stored under nitrogen at minus 20°C. 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. 
Lipid autoxidation. The process was carried out in a thermostatic bath at 80 °C (± 0.2 °C) 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 hydroperoxides, LOOH) concentration, i.e. the peroxide value (PV) (9). All kinetic data are expressed as the average of two independent measurements which were processed using the computer programs Origin 6.1 and Microsoft Excel-97.

Synergism, additivism, antagonism. If two or more antioxidants are added to oxidizing substrates, their combined inhibitory effect can be synergistic, additive, or antagonistic (9,10).

Synergism – the inhibiting effect of the binary and triple mixtures (IP_i) is higher than the sum of the induction periods of the individual phenolic antioxidants (IP_i), i.e. IP_i > ΣIP_i, where i = 1, 2, 3. Additivism - the inhibiting effect of the binary and triple mixtures (IP_i) is equal to the sum of the induction periods of the individual phenolic antioxidants, i.e. IP_i = ΣIP_i.
Antagonism - the inhibiting effect of the (IP_i) is lower than the sum of the induction periods of the individual phenolic antioxidants, i.e. IP_i < ΣIP_i. The percent of the synergism was calculated according to the formulae created from Frankel (4) for the binary mixtures and updated for triple mixtures by us: % Synergism = 100 [(IP_i - ΣIP_i) / ΣIP_i]. We also suggest for the first time by the similar way the calculation of % antagonism by the formulae: % Antagonism = 100 [(ΣIP_i - IP_i) / IP_i].

Statistical analysis of IP determination. The standard deviation (SD) for different mean values of IP was (in h): IP=2.0, SD=0.2; IP=5.0, SD=0.3; IP=15.0, SD=1.0; IP=25, SD=1.5; IP=50.0, SD=3.0. The SD of PV determination (in meq/kg), according to the modified iodometric method for different mean values of PV, was: PV=12.0, SD=1.0; PV=30.0, SD=2.0; PV=70.0, SD=5.0; PV=150.0, SD=10; PV=250.0, SD=20. The R_A and R_C were quite constant varying by less than 2%.

RESULTS AND DISCUSSION
Protective effects of binary mixtures of flavonoids
Flavonoids are the well-known natural bio-
antioxidants, because they are powerful antioxidants and possess various biological activities. A lot of studies of scientists are focused on the structure-activity relationship of these interesting compounds.
Figure 1. Structures of flavonoids: quercetine (Qu), luteoline (Lu) and rutine (Ru)

Figure 1 presents the structures of selected flavonoids under study. In order to check the possible synergism between two flavonoid aglicones Qu and Lu binary mixtures with equimolar concentrations (0.1mM) were prepared and their effect on the kinetics of lipid autoxidation was studied (Figure 2 and Table 1). The mixture of Qu+Lu showed almost 7-fold higher inhibiting effect than Lu and 1.4-fold higher inhibiting effect than Qu. The mixture of flavonoid aglycone and glyceride Qu+Ru showed a lower oxidation stability of lipid substrate than of Qu alone, however, almost 3-fold higher effect than Ru. Evidently, the addition of Ru to Qu not only didn’t improve the antioxidant efficiency of Qu but even led to decrease of the antioxidant potential of the aglycon. Consequently, these both mixtures of Qu+Lu and Qu+Ru show no synergism; furthermore they showed an antagonism between flavonoids. These results manifested that these flavonoid aglycons (Qu and Lu) and glycoside (Ru) must be used as individual antioxidants, not in mixtures (5).

Figure 2. Kinetic curves of lipid hydroperoxides accumulation during TGSO autoxidation at 80°C in presence of 0.1mM Luteolin (Lu), Rutin (Ru), Quercetin (Qu) and of their equimolar (1:1) binary mixtures (Lu+Qu) and (Qu+Ru)

As we can see from the Figure 3 and Table 1 the equimolar binary mixtures of flavonoids and TOH demonstrated a synergism between the components: 47% for Qu+TOH, 19% for Lu+TOH and 87% for Ru+TOH.

It is found that the lipid oxidation stability (presented as protection factor, PF in presence of flavonoids studied) decreases in the order: PF: 22.8 (Qu+TOH) > 19.2 (Ru+TOH) > 15.1 (Lu+TOH) > 8.1 (TOH) > 7.6 (Qu) > 2.1 (Ru) > 1.7 (Lu). New mechanism of action of flavonoids and tocopherol in binary mixtures is presented.
**Table 1.** Inhibiting efficiency of various binary mixtures of two (1 and 2) antioxidants in equimolar concentrations (0.1mM) and 1:1 ratio, 80°C, during TGSO autoxidation ($IP_c=1.7±0.2$ h, $R_c=1.2 \times 10^6$ M/s)

<table>
<thead>
<tr>
<th>Binary mixtures</th>
<th>$IP_{1+2}$ h</th>
<th>$IP_1$ h</th>
<th>$IP_2$ h</th>
<th>Synergism/Additivevism/Antagonism</th>
<th>%Syn</th>
<th>%Ant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qu (1) + Lu (2) (Qu+Lu)</td>
<td>7.5±0.8</td>
<td>9.9±0.9</td>
<td>2.2±0.2</td>
<td>Ant $IP_{1+2}&lt;(IP_1+IP_2)$</td>
<td>61%</td>
<td></td>
</tr>
<tr>
<td>Qu (1) + Ru (2) (Qu+Ru)</td>
<td>8.3±0.8</td>
<td>9.9±0.9</td>
<td>2.7±0.2</td>
<td>Ant $IP_{1+2}&lt;(IP_1+IP_2)$</td>
<td>52%</td>
<td></td>
</tr>
<tr>
<td>Qu (1) + TOH (2) (Qu+TOH)</td>
<td>29.7±1.5</td>
<td>9.9±0.9</td>
<td>10.5±0.9</td>
<td>Sin $IP_{1+2}&gt;(IP_1+IP_2)$</td>
<td>46%</td>
<td></td>
</tr>
<tr>
<td>Lu (1) + TOH (2) (Lu+TOH)</td>
<td>15.1±0.9</td>
<td>2.4±0.2</td>
<td>10.5±0.9</td>
<td>Sin $IP_{1+2}&gt;(IP_1+IP_2)$</td>
<td>19%</td>
<td></td>
</tr>
<tr>
<td>Ru (1) + TOH (2) (Ru+TOH)</td>
<td>24.9±1.5</td>
<td>2.7±0.2</td>
<td>10.5±0.9</td>
<td>Sin $IP_{1+2}&gt;(IP_1+IP_2)$</td>
<td>87%</td>
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<tr>
<td>CA (1) + TOH (2) (CA+TOH)</td>
<td>20.4±1.5</td>
<td>9.8±0.9</td>
<td>10.5±0.9</td>
<td>Add $IP_{1+2}=(IP_1+IP_2)$</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>SA (1) + TOH (2) (SA+TOH)</td>
<td>16.1±0.9</td>
<td>5.3±0.5</td>
<td>10.5±0.9</td>
<td>Sin $IP_{1+2}&gt;(IP_1+IP_2)$</td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td>BHT (1) + TOH (2) (BHT + TOH)</td>
<td>21.5±1.5</td>
<td>7.5±0.5</td>
<td>10.5±0.9</td>
<td>Sin $IP_{1+2}&gt;(IP_1+IP_2)$</td>
<td>19%</td>
<td></td>
</tr>
<tr>
<td>TBHQ (1) + TOH (2) (TBHQ + TOH)</td>
<td>26.1±1.5</td>
<td>7.9±0.5</td>
<td>10.5±0.9</td>
<td>Sin $IP_{1+2}&gt;(IP_1+IP_2)$</td>
<td>44%</td>
<td></td>
</tr>
<tr>
<td>Cum_2 (1) + TOH (2) (Cum_2+TOH)</td>
<td>12.7±0.9</td>
<td>7.1±0.5</td>
<td>10.5±0.9</td>
<td>Ant $IP_{1+2}&lt;(IP_1+IP_2)$</td>
<td>39%</td>
<td></td>
</tr>
<tr>
<td>Cum_1 (1) + TOH (2) (Cum_1+TOH)</td>
<td>14.2±0.9</td>
<td>2.0±0.2</td>
<td>10.5±0.9</td>
<td>Sin $IP_{1+2}&gt;(IP_1+IP_2)$</td>
<td>14%</td>
<td></td>
</tr>
<tr>
<td>Cum_3 (1) + TOH (2) (Cum_3+TOH)</td>
<td>11.8±0.9</td>
<td>1.5±0.2</td>
<td>10.5±0.9</td>
<td>Add $IP_{1+2}=(IP_1+IP_2)$</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>SA (1) + TOH (2) (SA+TOH)*</td>
<td>45.0±1.0</td>
<td>8.5±0.5</td>
<td>21.0±1.5</td>
<td>Sin $IP_{1+2}&gt;(IP_1+IP_2)$</td>
<td>52%</td>
<td></td>
</tr>
<tr>
<td>M_1OH (1) + TOH (2) (M_1OH+TOH)</td>
<td>14.8±0.8</td>
<td>1.3±0.2</td>
<td>10.5±0.9</td>
<td>Sin $IP_{1+2}&gt;(IP_1+IP_2)$</td>
<td>25%</td>
<td></td>
</tr>
<tr>
<td>D_1(OH)_2 (1) + TOH (2) (D_1(OH)_2+TOH)</td>
<td>13.2±0.8</td>
<td>3.2±0.2</td>
<td>10.5±0.9</td>
<td>Add $IP_{1+2}=(IP_1+IP_2)$</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>M_2OH (1) + TOH (2) (M_2OH+TOH)</td>
<td>10.3±0.8</td>
<td>1.3±0.2</td>
<td>10.5±0.9</td>
<td>Ant $IP_{1+2}&lt;(IP_1+IP_2)$</td>
<td>15%</td>
<td></td>
</tr>
<tr>
<td>D_2(OH)_2 (1) + TOH (2) (D_2(OH)_2+TOH)</td>
<td>10.9±0.8</td>
<td>2.0±0.2</td>
<td>10.5±0.9</td>
<td>Ant $IP_{1+2}&lt;(IP_1+IP_2)$</td>
<td>15%</td>
<td></td>
</tr>
<tr>
<td>FA (1) + TOH (2) (FA+TOH)</td>
<td>18.5±0.8</td>
<td>1.9±0.3</td>
<td>10.5±0.9</td>
<td>Sin $IP_{1+2}&gt;(IP_1+IP_2)$</td>
<td>49%</td>
<td></td>
</tr>
<tr>
<td>DFA (1) + TOH (2) (DFA+TOH)</td>
<td>21.5±0.8</td>
<td>2.0±0.3</td>
<td>10.5±0.9</td>
<td>Sin $IP_{1+2}&gt;(IP_1+IP_2)$</td>
<td>71%</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 3.** Kinetic curves of lipid hydroperoxides accumulation during TGSO autoxidation at 80°C in presence of 0.1mM Luteolin (Lu), Rutin (Ru), Quercetin (Qu), α-Tocopherol (TOH) and of their equimolar (1:1) binary mixtures (Lu+TOH), (Qu+TOH) and (Ru+TOH)
For that reason the following reactions of binary mixtures of flavonoids (for example quercetin) Qu(OH)₂, α-tocopherol, TOH) and of their radicals - tocopheryl radical (TO•) and semiquinone radicals Qu(OH)O• with lipid peroxide radical (LO₂•) and reactions of homo- and cross-recombination and disproportionation reactions, responsible to different effects observed are presented:

- **Reactions of Quercetin (Qu(OH)₂ and its radicals**
  
  \[
  Qu(OH)_2 + LO_2^* \rightarrow Qu(OH)O^* + LOOH (H atom transfer)
  \]
  
  \[
  Qu(OH)^* + LO_2^* \rightarrow Qu(OH)O-OOL (cross-recombination reaction with quinolide peroxides formation)
  \]
  
  \[
  Qu(OH)O-OOL + LO_2^* \rightarrow Qu(OH)O-OOL + LOOH (H atom transfer)
  \]
  
  \[
  Qu(OH)^* + LO_2^* \rightarrow Qu(OH)O-OOL (cross-recombination reaction with quinolide peroxides formation)
  \]
  
  \[
  2Qu(OH)^* \rightarrow Qu(OH)_2 + QuO_2 (homo-disproportionation reaction with Qu(OH)_2 regeneration and ortho-quinone formation QuO_2)
  \]

- **Reactions of α-Tocopherol (TOH) and its phenoxyl radical**
  
  \[
  TOH + LO_2^* \rightarrow TO^* + LOOH (H atom transfer)
  \]
  
  \[
  TO^* + LO_2^* \rightarrow TO-OOL (cross-recombination reaction with quinolide peroxides formation)
  \]
  
  \[
  2TO^* \rightarrow TOH + (homo-disproportionation reaction with TOH regeneration and tocopheryl-methylene-quinone formation T=O)
  \]

- **Reactions between Qu(OH)₂ and TOH and between their radicals**
  
  \[
  Qu(OH)^* + TOH \rightarrow Qu(OH)_2 + TO^* (H atom transfer with Qu(OH)_2 regeneration)
  \]
  
  \[
  TO^* + Qu(OH)_2 \rightarrow TOH + Qu(OH)^* (H atom transfer with regeneration of TOH)
  \]
  
  \[
  TO^* + Qu(OH)O^* \rightarrow TOH + QuO_2 (cross-disproportionation reaction with regeneration of TOH and ortho-quinone formation)
  \]
  
  \[
  TO^* + Qu(OH)O^* \rightarrow Qu(OH)_2 + T=O (cross-disproportionation reaction with regeneration of Qu(OH)_2 and tocopheryl-methylene-quinone formation T=O)
  \]
  
  \[
  TO^* + Qu(OH)O^* \rightarrow products (cross-recombination without regeneration)
  \]

**Scheme 1.** The reaction mechanism of action of binary mixtures of quercetin and α-tocopherol

H-atom transfer and cross-disproportionation reactions are responsible for the synergism obtained; however, reaction of cross-recombination is responsible to the antagonism observed. Table 2 presents results about differences in O-H bond dissociation enthalpies (BDE) of Qu, Lu and Ru and TOH, which are the suitable theoretical descriptor for explanation (and probably for prediction) of the effects of binary mixtures. It is seen that in case when ΔBDE > 0 it is observed a synergism, and in case of ΔBDE < 0 an antagonism, respectively.

**Protective effects of binary mixtures of cinnamic acids and standard antioxidants**

Cinnamic acids are biological precursors of coumarins and chalcones and are widely distributed in the plant kingdom. We studied the effects of equimolar binary mixtures at 0.1mM concentrations of cinnamic acids – caffeic (CA), sinapic (SA) and ferulic (FA) as well as of standard antioxidants - butylated hydroxyl toluene (BHT) and tret-butylhydroquinone (TBHQ) with tocopherol (TOH) (Figure 4).

**Figure 4.** Structures of cinnamic acids, studied: caffeic acid (CA), ferulic acid (FA) and synaptic acid (SA); and of standard antioxidants studied: butylated hydroxyl toluene (BHT) and tret-butylhydroquinone (TBHQ)

**Figure 5** presents the kinetics of lipid autoxidation in presence of studied cinnamic acids and standard antioxidants as individual compounds and in equimolar binary mixtures with TOH.
The following order of lipid oxidation stability was found:
(TBHQ+TOH) > (BHT+TOH) > (CA+TOH) > (SA+TOH) > TOH > CA > TBHQ ≥ BHT > SA

Tables 1 and 3 present the result of effects observed in these binary mixtures and the differences in BDE\(_{\text{O-H}}\) with TOH. It is seen that all compounds demonstrated synergism with TOH, only CA manifested additivism with TOH. The strongest inhibiting effects and synergism obtained in the binary mixtures (TBHQ+TOH) and (BHT+TOH) can be explained by the following mechanisms (Schemes 2&3). It is seen that both components of the mixture – BHT (or TBHQ) and TOH can be regenerated during the homo-disproportionation reactions of phenoxyl radicals from BHT (or TBHQ) and TOH formed, as well as during the reactions of cross-disproportionation between their radicals: Schemes 2, 3.

Protective effects of binary mixtures of hydroxy-coumarins

Coumarins are important class of oxygen heterocycles, widespread in plant kingdom (11). They have attracted intense interest recently due to their presence in natural sources, and to their possession of diverse pharmacological properties. 4-Methylcoumarins have been found to possess choleric, analgesic, anti-spermatogenic, anti-tubercular and diuretic properties (6). Polyphenolic coumarins are known to act as antioxidants in biological systems, but it is difficult to distinguish their antioxidant activity from the many other effects they produce in cells. In order to study the possible synergism between two phenolic antioxidants, the antioxidant efficiency and reactivity of tree binary mixtures of coumarins and TOH (Cum\(_1\)+TOH, Cum\(_2\)+TOH and Cum\(_3\)+TOH) were tested and compared. Figure 6 and Table 1 present results obtained. It is found the higher oxidation stability of the lipid substrate in presence of all binary mixtures in comparison with individual compounds.

The synergism was observed only for the binary mixture of 7,8-dihydroxy-4-methyl coumarin (Cum\(_1\)) with \(\alpha\)-tocopherol (TOH) Cum\(_1\)+TOH: IP\(_{1+2}(14.2) > IP_{\text{Cum1}}(2.0) + IP_{\text{TOH}}(10.5)\) and 14 % synergism.

In case of equimolar binary mixture of 6,7-dihydroxy-4-methyl-coumarin (Cum\(_2\)) with \(\alpha\)-tocopherol (TOH), i.e. Cum\(_2\)+TOH it was found an antagonism between two antioxidants:

IP\(_{1+2}(12.7) < IP_{\text{Cum2}}(7.1) + IP_{\text{TOH}}(10.5)\).

In case of 7-hydroxy-4-methyl-coumarin (Cum\(_3\)) with \(\alpha\)-tocopherol (TOH) Cum\(_3\)+TOH an additivism was observed: IP\(_{1+2}(11.8) = IP_{\text{Cum3}}(1.7) + IP_{\text{TOH}}(10.5)\).
Regeneration of BHT during homo-dissproporation reaction

- Butylated Hydroxytoluene (BHT)
- BHT phenoxy radical
- Tret-Butyl Methylene Quinone

Regeneration of TOH during homo-dissproporation reaction

- alpha-Tocopherol (TOH)
- alpha-Tocopheryl radical (TO·)
- Tocopherol Methylene Quinone

Regeneration of TOH and BHT by H atom transfer reaction - this reaction is reversible

- alpha-Tocopheryl radical (TO·)
- BHT
- alpha-Tocopherol (TOH)
- BHT phenoxy radical

Regeneration of the stronger antioxidant TOH by reaction of cross-dissproportionation

- alpha-Tocopheryl radical (TO·)
- BHT phenoxy radical
- alpha-Tocopherol (TOH)
- Tret-Butyl Methylene Quinone

Scheme 2. Mechanism of BHT and TOH in binary mixture with possible regeneration of BHT and TOH
Regeneration of TBHQ during homo-dissproponation reaction

Scheme 3. Mechanism of TBHQ and TOH in binary mixture with possible regeneration of TBHQ and TOH

Tables 2 and 3 present the differences in BDE$_{O,H}$ with TOH.
Table 2. Binary mixtures of bi-phenolic and polyphenolic antioxidants with TOH

<table>
<thead>
<tr>
<th>$Q(OH)_2$</th>
<th>$Q(OH)_2 + \text{TOH}$</th>
<th>$\Delta BDE_{QH}$</th>
<th>$\Delta BDE_{O-H}$</th>
<th>$\text{TOH}$ regeneration</th>
<th>Side reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA</td>
<td>Additivism</td>
<td>$\Delta BDE = 0$</td>
<td>$\text{H atom transfer}$</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>7,8-di-OH-Cum $\text{Cum}_1(OH)_2$</td>
<td>Synergism</td>
<td>$\uparrow \Delta BDE_{\text{TOH}}$</td>
<td>$\text{H atom transfer}$</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>6,7-di-OH-Cum $\text{Cum}_2(OH)_2$</td>
<td>Antagonism</td>
<td>$\downarrow \Delta BDE_{\text{TOH}}$</td>
<td>$\text{H atom transfer}$</td>
<td>Cross-recombination</td>
<td></td>
</tr>
</tbody>
</table>

**FLAVONOIDS**

| Qu + Lu   | Antagonism     | $\Delta BDE < 0$ | $\text{H atom transfer}$ | Cross-recombination    |
| Qu + Ru   | Antagonism     | $\Delta BDE < 0$ | $\text{Cross-recombination}$ |
| Qu + TOH  | Synergism      | $\Delta BDE > 0$ | $\text{H atom transfer}$ | Cross-disproportion    |
| Lu + TOH  | Synergism      | $\Delta BDE > 0$ | $\text{H atom transfer}$ | Cross-disproportion    |
| Ru + TOH  | Synergism      | $\Delta BDE > 0$ | $\text{H atom transfer}$ | Cross-disproportion    |

Table 3. Binary mixtures of monophenolic antioxidants AH with TOH

<table>
<thead>
<tr>
<th>AH</th>
<th>AH+TOH</th>
<th>$\Delta BDE_{AH}$</th>
<th>AH regener.</th>
<th>TOH regeneration</th>
<th>Side reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHT</td>
<td>Synergism</td>
<td>$\uparrow \Delta BDE_{\text{TOH}}$</td>
<td>$\text{H atom transfer}$</td>
<td>No t-But</td>
<td></td>
</tr>
<tr>
<td>SA</td>
<td>Synergism</td>
<td>$\uparrow \Delta BDE_{\text{TOH}}$</td>
<td>$\text{H atom transfer}$</td>
<td>Yes +</td>
<td></td>
</tr>
<tr>
<td>FA</td>
<td>Synergism</td>
<td>$\uparrow \Delta BDE_{\text{TOH}}$</td>
<td>$\text{H atom transfer}$</td>
<td>Yes +</td>
<td></td>
</tr>
<tr>
<td>M$_1$OH</td>
<td>Synergism</td>
<td>$\uparrow \Delta BDE_{\text{TOH}}$</td>
<td>$\text{H atom transfer}$</td>
<td>Yes +</td>
<td></td>
</tr>
<tr>
<td>M$_2$OH</td>
<td>Antagonism</td>
<td>$\uparrow \Delta BDE_{\text{TOH}}$</td>
<td>$\text{H atom transfer}$</td>
<td>Yes +++</td>
<td></td>
</tr>
<tr>
<td>7-OH-Cum $\text{Cum}_3$OH</td>
<td>Additivism</td>
<td>$\uparrow \Delta BDE_{\text{TOH}}$</td>
<td>$\text{H atom transfer}$</td>
<td>Yes +++</td>
<td></td>
</tr>
</tbody>
</table>
The highest oxidation stability of TGSO in presence of all binary mixtures with α-tocopherol (TOH) may be explained taking into account that the both antioxidants may be regenerated during the oxidation process. It is known that the catecholic moiety of the coumarine molecules allows formation of semiquinone radicals. These semiquinone radicals may regenerate the initial antioxidant molecule during reaction of bimolecular recombination with homo- and cross-disproportionation of semiquinone radicals:

**Scheme 4.**

These reactions demonstrate that during lipid oxidation process initial molecules of 7,8-dihydroxy-4-methyl coumarin (Cum₁), 6,7-dihydroxy-4-methyl coumarin (Cum₂), α-tocopherol (TOH) and of their equimolar (1:1) binary mixtures (Cum₁+TOH) and (Cum₂+TOH) and tocopheryl radical (TO•) with regeneration of TOH.

Considering that 4-methylcoumarins, in contrast to many other coumarins, are not metabolized to toxic epoxide intermediates, these results indicate the possible application of these compounds as individual antioxidants and/or in complex binary mixtures.

**Protective effects of monomers and dimers**

Dehydrozingerone (M₁OH) is a half molecule of curcumin – one of the most powerful antioxidant with a wide spectrum of its biological activity (12). Ginger and curcumin are the well-known spices. Recently synthesized natural-like C2-symmetry hydroxylated biphenyls (dimers) of their corresponding monomers (dehydrozingerone, M₁OH, zingerone, M₂OH and ferulic acid, FA) were selected for this study, because of their interesting biological activity against to neuro-degenerative diseases and melanoma cancer (7).

It is seen ([Figure 7, Table 1](#)) that dimer of dehydrozingerone, D₄(OH)₂ is able to ensure the higher oxidation stability of lipid substrate, i.e. it has the better protective effect in comparison with corresponding monomer (M₁OH). TOH manifested the best protective effect on lipid autoxidation in comparison with other individual compounds. However, it is evident that AscPH didn’t show any antioxidant activity (potential).
Regeneration of dihydroxy-coumarin Cum$_1$(OH)$_2$ by cross-disproportionation reaction

\[ \text{Cum}_2$(OH)$_2$ \xrightarrow{\text{LOO}^*} \text{Cum}_1$(OH)$_2$(OH) \xrightarrow{\text{LOOH}} \text{Cum}_2$(OH)$_2$ \]

Coumarin semiquinone radical

Regeneration of Cum$_1$(OH)$_2$ by H atom transfer and cross-disproportionation reactions

\[ \text{Cum}_1$(OH)$_2$(OH) + \text{TO}^* \xrightarrow{\text{H atom transfer}} \text{Cum}_1$(OH)$_2$ \]

TOH

Regeneration of TOH by cross-disproportionation reactions and H atom transfer

\[ \text{Cum}_1$(OH)$_2$(OH) + \text{TO}^* \xrightarrow{\text{cross-disproportionation}} \text{TOH} + \text{Cum}_1$(OH)$_2$(OH) \]

Cum$_1$(OH)$_2$(OH)

Coumarin semiquinone radical

Scheme 4. Mechanism of synergistic effect of binary mixture of 7,8-dihydroxy-4-methyl-coumarin, Cum$_1$(OH)$_2$ with $\alpha$-tocopherol (TOH)
The kinetic curve in presence of AscPH is the same as for the control sample. New orders of antioxidant efficiency (as protection factor, PF) and antioxidant reactivity (as inhibition degree, ID) were found for the individual compounds:
Pf: TOH (21.2) > D1(OH)2 (13.5) > M1OH (3.5) > AscPH (1.0)
Id: TOH (29.3) = D1(OH)2 (29.3) >> M1OH (6.3) > AscPH (1.0)

The observed lower antioxidant activity of M1OH and D1(OH)2 respect to TOH is expected as a result of the higher bond dissociation enthalpy (BDE) of OH groups of M1OH and D1(OH)2 in comparison with those of TOH (∆BDEM1OH=9.6 and ∆BDED1OH2=9.0 kcal/mol, respectively) (7). Another reason for the lower antioxidant activity of M1OH and D1(OH)2 with respect to TOH can be explain with no possibility of their phenoxyl radicals to be regenerated like TOH during homo-disproportionation reaction.

Data obtained (Figure 7 and Table 1) showed that both binary mixtures (M1OH+TOH and D1(OH)2+TOH) lead to the maximal oxidation stability of lipid substrate in comparison with the corresponding individual components. The latest is of importance for the practice, because these binary mixtures are able to ensure the best protective effect on the lipid substrate being oxidized.

Pf: D1(OH)2+TOH(35.4)> M1OH+TOH(32.7) > TOH(21.2) > D1(OH)2 (13.5) >> M1OH(3.5)
Id: D1(OH)2+TOH (29.3) = TOH (29.3) = D1(OH)2(29.3) > M1OH+TOH (25.9) >> M1OH (6.3)
**Effect of equimolar (1:1) binary mixtures of studied compounds with ascorbyl palmitate (AscPH)**

**Figure 8 and Table 1** showed the data observed for the equimolar binary mixtures of M\textsubscript{1}OH, D\textsubscript{1}(OH)\textsubscript{2}, and TOH with AscPH. New orders of antioxidant properties were obtained:

- **PF:** TOH+AscPH (31.5) >> D\textsubscript{1}(OH)\textsubscript{2}+AscPH (10.4) > M\textsubscript{1}OH + AscPH (6.2) > AscPH (1.0)
- **ID:** TOH+AscPH (29.3) >> D\textsubscript{1}(OH)\textsubscript{2}+AscPH (8.8) > M\textsubscript{1}OH+AscPH (6.8) > AscPH (1.0)

The highest antioxidant efficiency (PF) was found for binary mixture of TOH + AscPH, which is 3-fold higher than that of D\textsubscript{1}(OH)\textsubscript{2}+AscPH and 5-fold higher than that of M\textsubscript{1}OH+AscPH. D\textsubscript{1}(OH)\textsubscript{2} in binary mixture with AscPH leads to the higher oxidation stability of lipid substrate than in case of M\textsubscript{1}OH+AscPH.

The binary mixtures of studied compounds (M\textsubscript{1}OH+AscPH and D\textsubscript{1}(OH)\textsubscript{2}+AscPH) showed higher protective effect than of AscPH. The latest due to the possible regeneration of M\textsubscript{1}OH and D\textsubscript{1}(OH)\textsubscript{2} from AscPH during the oxidation process:

- \( M\textsubscript{1}O^\bullet + \text{AscPH} \rightarrow M\textsubscript{1}OH + \text{AscP}^\bullet \) reaction of H atom transfer with monomer regeneration
- \( M\textsubscript{1}O^\bullet + \text{AscP}^\bullet \rightarrow M\textsubscript{1}OH + \text{DHAP} \) (DHAP, dehydroascorbyl palmitate) cross disproportionation reaction with monomer regeneration.

Both reactions with monomer regeneration are of importance for the synergism of M\textsubscript{1}OH+AscPH mixture obtained, because M\textsubscript{1}OH is the stronger antioxidant in comparison with AscPH. Our results confirm again AscPH as synergist, but not as antioxidant during bulk lipid autoxidation. Antagonism was detected only for D\textsubscript{1}(OH)\textsubscript{2}+AscPH binary mixture that evidenced the lower level of dimer regeneration from AscPH. This result can be explained with the reverse character of H atom transfer reactions:

- \( D\textsubscript{1}(OH)O^\bullet + \text{AscPH} \Leftrightarrow D\textsubscript{1}(OH)\textsubscript{2} + \text{AscP}^\bullet \)

Probably it is a result of the possible AscPH regeneration in reaction between AscP\textsuperscript{•} and D\textsubscript{1}(OH)\textsubscript{2} and thus reduces its activity.

Another reason for antagonism obtained is the possible cross recombination reaction between AscP\textsuperscript{•} and D\textsubscript{1}(OH)\textsubscript{2} to inactive product. The latest reduced the concentration of dimer phenoxyl radical which could be regenerated by H atom transfer from AscPH.

We expected to increase significantly the efficiency of antioxidant compositions, due to the regeneration by different reactions of TOH and M\textsubscript{1}OH /or D\textsubscript{1}(OH)\textsubscript{2} during the oxidative process by preparing ternary mixtures...
M₁OH+TOH+AscPH and D₁(OH)₂+TOH+AscPH. The protective action of triple mixtures of M₁OH and D₁(OH)₂ with TOH and AscPH is presented at Figures 8 and Table 4. PF: D₁(OH)₂+TOH+AscPH (35.4) > M₁OH+TOH+AscPH (33.1) ID: D₁(OH)₂+TOH+AscPH (35.2) > M₁OH+TOH+AscPH (29.3) Triple mixture of D₁(OH)₂ with TOH and AscPH manifested also a higher antioxidant efficiency and reactivity than the corresponding triple mixture of M₁OH. However, triple mixture of M₁OH + TOH + AscPH showed similar PF as for double mixture of M₁OH + AscPH.

**Table 4. Lipid hydroperoxides concentration [LOOH] and α-tocopherol (TOH) content determined during lipid autoxidation of triglycerides of sunflower oil (TGSO) at 80°C.**

<table>
<thead>
<tr>
<th>Abbrev. of mixtures</th>
<th>Time, h</th>
<th>[LOOH] average, mM</th>
<th>TOH content, mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>(TOH)_5</td>
<td>5</td>
<td>4.9±1.1</td>
<td>0.458±0.018</td>
</tr>
<tr>
<td>(TOH)₁₀</td>
<td>10</td>
<td>11.8±1.2</td>
<td>0.452±0.018</td>
</tr>
<tr>
<td>(TOH)_₁₅</td>
<td>15</td>
<td>18.0±2.0</td>
<td>0.451±0.018</td>
</tr>
<tr>
<td>(TOH)₂₀</td>
<td>20</td>
<td>25.2±4.3</td>
<td>0.380±0.015</td>
</tr>
<tr>
<td>(TOH)_₂₅</td>
<td>25</td>
<td>33.5±5.5</td>
<td>0.258±0.010</td>
</tr>
<tr>
<td>(TOH)₃₀</td>
<td>30</td>
<td>143.0±5.0</td>
<td>0.016±0.001</td>
</tr>
</tbody>
</table>

**Equimolar binary mixtures with AscPH**

<table>
<thead>
<tr>
<th>Abbrev. of mixtures</th>
<th>Time, h</th>
<th>[LOOH] average, mM</th>
<th>TOH content, mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>(TOH)+AscPH₃₅</td>
<td>5</td>
<td>2.7±2.5</td>
<td>0.466±0.019</td>
</tr>
<tr>
<td>(TOH)+AscPH₁₀</td>
<td>10</td>
<td>5.4±4.6</td>
<td>0.454±0.018</td>
</tr>
<tr>
<td>(TOH)+AscPH₁₅</td>
<td>15</td>
<td>10.2±6.4</td>
<td>0.445±0.018</td>
</tr>
<tr>
<td>(TOH)+AscPH₂₀</td>
<td>20</td>
<td>14.7±8.8</td>
<td>0.442±0.018</td>
</tr>
<tr>
<td>(TOH)+AscPH₃₀</td>
<td>30</td>
<td>28.0±12.0</td>
<td>0.440±0.018</td>
</tr>
<tr>
<td>(TOH)+AscPH₄₅</td>
<td>45</td>
<td>152.0±3.0</td>
<td>0.059±0.002</td>
</tr>
</tbody>
</table>

**Equimolar binary mixtures with TOH**

<table>
<thead>
<tr>
<th>Abbrev. of mixtures</th>
<th>Time, h</th>
<th>[LOOH] average, mM</th>
<th>TOH content, mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>(M₁OH+TOH)₅</td>
<td>5</td>
<td>6.3±1.5</td>
<td>0.466±0.019</td>
</tr>
<tr>
<td>(M₁OH+TOH)₁₀</td>
<td>10</td>
<td>11.0±2.0</td>
<td>0.449±0.018</td>
</tr>
<tr>
<td>(M₁OH+TOH)₁₅</td>
<td>15</td>
<td>16.2±1.8</td>
<td>0.442±0.018</td>
</tr>
<tr>
<td>(M₁OH+TOH)₂₀</td>
<td>20</td>
<td>21.6±2.3</td>
<td>0.366±0.015</td>
</tr>
<tr>
<td>(M₁OH+TOH)₃₀</td>
<td>30</td>
<td>39.2±1.2</td>
<td>0.288±0.012</td>
</tr>
<tr>
<td>(M₁OH+TOH)₄₅</td>
<td>45</td>
<td>86.0±20.0</td>
<td>0.047±0.002</td>
</tr>
<tr>
<td>(D₁(OH)₂+TOH)₅</td>
<td>5</td>
<td>5.7±0.9</td>
<td>0.462±0.018</td>
</tr>
<tr>
<td>(D₁(OH)₂+TOH)₁₀</td>
<td>10</td>
<td>11.3±1.3</td>
<td>0.459±0.018</td>
</tr>
<tr>
<td>(D₁(OH)₂+TOH)₁₅</td>
<td>15</td>
<td>15.7±2.6</td>
<td>0.452±0.018</td>
</tr>
<tr>
<td>(D₁(OH)₂+TOH)₂₀</td>
<td>20</td>
<td>23.0±1.0</td>
<td>0.396±0.016</td>
</tr>
<tr>
<td>(D₁(OH)₂+TOH)₃₀</td>
<td>30</td>
<td>39.4±4.5</td>
<td>0.296±0.012</td>
</tr>
<tr>
<td>(D₁(OH)₂+TOH)₄₅</td>
<td>45</td>
<td>64.7±2.3</td>
<td>0.049±0.002</td>
</tr>
</tbody>
</table>

**Equimolar triple mixtures with TOH and AscPH**

<table>
<thead>
<tr>
<th>Abbrev. of mixtures</th>
<th>Time, h</th>
<th>[LOOH] average, mM</th>
<th>TOH content, mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>(M₁OH+TOH+AscPH)₅</td>
<td>5</td>
<td>2.0±2.0</td>
<td>0.468±0.019</td>
</tr>
<tr>
<td>(M₁OH+TOH+AscPH)₁₀</td>
<td>10</td>
<td>4.8±4.8</td>
<td>0.467±0.019</td>
</tr>
<tr>
<td>(M₁OH+TOH+AscPH)₁₅</td>
<td>15</td>
<td>9.5±9.5</td>
<td>0.458±0.018</td>
</tr>
<tr>
<td>(M₁OH+TOH+AscPH)₂₀</td>
<td>20</td>
<td>13.0±7.0</td>
<td>0.449±0.018</td>
</tr>
<tr>
<td>(M₁OH+TOH+AscPH)₃₀</td>
<td>30</td>
<td>28.0±7.0</td>
<td>0.329±0.013</td>
</tr>
<tr>
<td>(M₁OH+TOH+AscPH)₄₅</td>
<td>45</td>
<td>91.0±33.0</td>
<td>0.114±0.005</td>
</tr>
<tr>
<td>(D₁(OH)₂+TOH+AscPH)₅</td>
<td>5</td>
<td>2.0±2.0</td>
<td>0.468±0.019</td>
</tr>
<tr>
<td>(D₁(OH)₂+TOH+AscPH)₁₀</td>
<td>10</td>
<td>4.8±4.8</td>
<td>0.466±0.019</td>
</tr>
<tr>
<td>(D₁(OH)₂+TOH+AscPH)₁₅</td>
<td>15</td>
<td>8.5±4.5</td>
<td>0.462±0.018</td>
</tr>
<tr>
<td>(D₁(OH)₂+TOH+AscPH)₂₀</td>
<td>20</td>
<td>12.3±6.8</td>
<td>0.459±0.018</td>
</tr>
<tr>
<td>(D₁(OH)₂+TOH+AscPH)₃₀</td>
<td>30</td>
<td>24.0±10.0</td>
<td>0.42±0.017</td>
</tr>
<tr>
<td>(D₁(OH)₂+TOH+AscPH)₄₅</td>
<td>45</td>
<td>54.0±17.0</td>
<td>0.379±0.015</td>
</tr>
</tbody>
</table>
Results, obtained about TOH content vs reaction oxidation time were separated in to the following groups:

✓ 0.420-0.470 mg/g TOH content; in this group are samples, in which the TOH content is equal to the initial TOH content. Here are TOH; [D₁(OH)$_2$+TOH]; (M$_1$OH+TOH); (TOH+AscPH); [D₁(OH)$_2$+TOH+AscPH]; and (M$_1$OH+TOH+AscPH).

It was observed that AscPH exhibited a strong effect as synergist and led to 2-fold longer period (30h), in which the initial TOH content was saved, in comparison with the individual TOH (15h). Dimer D₁(OH)$_2$ and monomer M$_1$OH in binary mixtures with TOH demonstrated the same initial high TOH content as observed when TOH was used as an individual compound. Monomer M$_1$OH in ternary mixtures with TOH and AscPH showed the high initial TOH content for a longer period (20h).

✓ 0.340-0.420 mg/g TOH content; in this group are samples, in which the TOH content is high, but lower than the initial TOH content. Here are TOH; (TOH+M$_1$OH); [TOH+D₁(OH)$_2$]; D₁(OH)$_2$+TOH+AscPH; $\text{AscPH}_30$.

The results obtained after 20h oxidation showed that TOH content is the same for both TOH used alone and for TOH in binary mixtures of monomer M$_1$OH and dimer D₁(OH)$_2$. The same TOH content was observed in the ternary mixture of D₁(OH)$_2$+TOH+AscPH, but after 45h of lipid autoxidation. The latest result is of great significance in the search for optimal stabilization of lipid substrate.

✓ 0.250-0.340 mg/g TOH content; in this group are samples, with moderate TOH content. Here are: [D₁(OH)$_2$+TOH]; (M$_1$OH+TOH); (M$_1$OH+TOH+AscPH).

It was observed that, after 30h oxidation of lipid substrate, the same TOH content for binary mixtures of M$_1$OH and D₁(OH)$_2$ with TOH were obtained, which is equal to those of the mixture of M$_1$OH+TOH+AscPH.

✓ 0.040-0.120 mg/g TOH content; in this group samples, with the lowest TOH content. Only ternary mixture of (M$_1$OH+TOH+AscPH)$_{45}$ to this group with low content of TOH. For comparison, the ternary mixture of D₁(OH)$_2$+TOH+AscPH, after 45h oxidation reaction time, manifested much higher TOH content, equal to the initial TOH content. It must be noted that the binary mixtures of (TOH+AscPH)$_{45}$, [D₁(OH)$_2$+TOH]; and (M$_1$OH+TOH)$_{45}$ showed the same TOH content after 45h oxidation time of lipid substrate, but muchlower in comparison with the ternary mixture of M$_1$OH.

Complex mechanisms of TOH regeneration in binary and triple mixtures are presented at Figure 9 (a-d).

![Figure 9a](image-url) Complex mechanism of TOH regeneration during lipid autoxidation added as individual antioxidant (Path a) and in double mixtures with D₁(OH)$_2$ (Path b), M$_1$OH (Path c), and AscPH (Path d)
**Path a):**

a1) H-atom transfer from TOH to LO₂⁺ (the key reaction of inhibited oxidation);

a2) Homo-disproportionation of tocopheryl radicals (TO⁺) with TOH regeneration;

**Path b):**

b1) H-atom transfer from TOH to D₁₁(OH)O⁺ with regeneration of D₁₁(OH)₂⁺;

b2) H-atom transfer from D₁₁(OH)₂ to TO⁺ with regeneration of TOH;

**Path c):**

c1) H-atom transfer from M₁OH to TO⁺ with regeneration of TOH;

c2) H-atom transfer from TOH to M₁O⁺ with regeneration of M₁OH;

**Path d):**

d1) Cross-disproportionation between TO⁺ and AscP⁺ with regeneration of TOH

And DHAP – dehydroascorbypalmitate formation;

d2) H-atom transfer from AscPH to TO⁺ with regeneration of TOH.

Observed synergism of M₁OH+TOH (32.3 %) is due to the possible regeneration of TOH, as the strongest antioxidant, by reaction of H atom transfer from M₁OH to the tocopheryl radical, TO⁺.

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**Figure 9b.** Complex mechanism of possible side reactions tocopherol TOH and its tocopheryl radical TO⁺ resulting to decrease of TOH activity

**Path a):**

a1) Additional generation of free radicals (LO₂⁺) from TOH in reaction with lipid substrate (LH); (at high TOH concentration - activity of TOH decreases);

a2) TOH increases decomposition of LOOH into free radicals (side reaction, reducing activity of TOH);

**Path b):**

b1) Cross-recombination of tocopheryl radical (TO⁺) and M₁O⁺ (formation of inactive products);

b2) Homo-recombination of two tocopheryl radicals (TO⁺) to inactive products;

**Path c):**

c1) H-atom transfer from TOH to D₁₁(OH)O⁺ with regeneration of D₁₁(OH)₂⁺;

c2) Cross-disproportionation reaction between TO⁺ and D₁₁(OH)₂ with regeneration of D₁₁(OH)₂⁺;

**Path d):**

d1) Cross-disproportionation reaction between TO⁺ and M₁O⁺ with regeneration of M₁OH;

---

**Figure 9c.** Complex mechanism of synergism obtained between components in triple mixture M₁OH + TOH + AscPH

Total synergism of triple mixture 28.7%
Main reactions, responsible to the synergism obtained:
a1) H-atom transfer from TOH to LO$_2^•$ (the key reaction of inhibited oxidation)
b1) H-atom transfer from M$_1$OH to TO$^•$ with regeneration of TOH;
c1) H-atom transfer from AscPH to M$_1$O$^•$ with regeneration of M$_1$OH;
d1) H-atom transfer from AscPH to TO$^•$ with regeneration of TOH;

Additional reactions:
a2) Decomposition of LOOH by tocopheryl radical (TO$^•$) to LO$_2^•$ (decreasing TOH activity)
b2) H-atom transfer from TOH to M$_1$O$^•$ with regeneration of M$_1$OH;
c2) H-atom transfer from AscPH to M$_1$O$^•$ with regeneration of M$_1$OH;
d2) H-atom transfer from TOH to AscP$^•$ with regeneration of AscPH.

CONCLUDING REMARKS
Antioxidant activity is capacity of the compound to shorten the oxidation chain length as a result of its reaction with peroxyl radicals. For that reason we mean as antioxidant activity the chain-breaking activity of the compounds.

Synergism between individual components in binary mixtures is explained with differences in BDE$_{O-H}$ and by H atom abstraction and cross disproportionation reaction, leading to regeneration of the most powerful antioxidant. Additivism is found for antioxidants with close BDE$_{O-H}$ and similar power. Antagonism is found for antioxidants with lower BDE$_{O-H}$ than of TOH and when regeneration of antioxidants is not possible during the reaction of cross-recombination of both phenoxy radicals.

Structural characteristics of the complex system: oxidizing substrate-antioxidant must be considered. On the basis of this comparative analysis, the most effective individual antioxidants and binary mixtures were proposed for the highest and optimal lipid oxidation stability. The latest is of importance for the practice.

REFERENCES
4. V.D.Kancheva, Phenolic Antioxidants of Natural Origin – Structure Activity Relationship and their Beneficial Effect on


