scientific reports

OPEN

Deciphering antioxidant interactions via data mining and RDKit

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Minimizing the oxidation of lipids remains one of the most important challenges to extend the shelf-life of food products and reduce food waste. While most consumer products contain antioxidants, the most efficient strategy is to incorporate combinations of two or more compounds, boosting the total antioxidant capacity. Unfortunately, the reasons for observing synergistic / antagonistic / additive effects in food samples are still unclear, and it is common to observe very different responses even for similar mixtures. Aiming to identify chemical features that can be correlated with specific responses, this report presents an analysis of 1243 mixtures of antioxidants reported in the literature. The analysis focuses on the most commonly reported compounds and mixtures and considers how various chemical descriptors (number of atoms, number of heavy atoms, number of heteroatoms, number of carbon atoms, number of oxygen atoms, number of nitrogen atoms, number of chloride atoms, polar surface area, molecular weight, number of aromatic rings, logP, and hydrogen bond counts) affect the response. Out of those, our analysis showed that hydrogen bonding plays an important role in determining how antioxidants interact, potentially affecting the overall behavior of mixtures. Far from drawing a universal conclusion about one particular mechanism; this article provides an overview of what has worked so far, delving into the possible chemical variables behind those interactions.

Keywords Artificial Intelligence, Antioxidants, Synergism, Bibliometric analysis

Minimizing the oxidation of food products remains one of the most important scientific challenges in the field. While most food ingredients can be oxidized[1], the process can particularly affect unsaturated fats that, when exposed to oxygen (and heat[2], light, metals[3], etc.) and produce a variety of compounds with negative organoleptic profiles. In general, the oxidation process starts with the generation of reactive oxygen species (ROS), which can then propagate in a fast ($\sim 10^9$ M^{-1} s^{-1}) chain reaction, leading to the repeated abstraction of hydrogens by peroxyl radicals[4,5]. In turn, these radicals act as the chain carriers of the rapid-progressing reaction by attacking new lipid molecules[6,7]. It is also important to note that the oxidation of lipids can occur not only during manufacturing but also during storage, leading to food rancidity and a significant deterioration in the quality of these foods[8].

A variety of antioxidants can be used to minimize the effects of oxidative processes. Based on their mechanism, they can be classified as primary[9] (react slowly with lipids but rapidly with radicals[5], subsequently stopping the oxidative chain reaction[10]) or secondary[11,12] antioxidants (inhibit the initial free radical reactions that lead to oxidation). As noted by Prenzler[13], these antioxidants deactivate the deleterious action of the reactive radicals by transferring hydrogen atoms (HAT), single electrons (SET), or both. Additional information related to the reaction mechanisms of these antioxidants can be found elsewhere[14,15]. Antioxidants can also be classified based on their origin as synthetic (for example, butylated hydroxyanisole, BHA; *tert*-butylhydroquinone, TBHQ; propyl gallate, PG; or butylated hydroxytoluene, BHT)[16,17] or natural (for example carotenoids, ascorbic acid, caffeic acid, lycopene[18], or flavonoids)[19–23]. While all these molecules can be found in many consumer products[24], the use of antioxidants is carefully considered to balance stability, efficacy, flavor, and costs[25]. In addition, and due to their potential toxicity, most antioxidants have strict limits and require specific analytical methodologies[16,26]. For example, the US-FDA limits the total amount of TBHQ added to < 0.02% of the oil or fat content of the food, including the essential (volatile) oil content of the food[27]. In order to optimize the overall antioxidant capacity, combinations of two or more compounds are often used[2,28,29]. Although practical and widely accepted, the reasons for observing synergistic / antagonistic / additive effects are still unclear and it is common to observe very different responses and mechanisms even for similar mixtures[30–32]. In addition, many of the experimental procedures to measure antioxidant behavior do not target the same stages of the reaction

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(limiting the correlation between methods)[19,33], can be affected by multiple experimental conditions[34], and are often limited to binary mixtures. Moreover, the overall effectiveness of antioxidant mixtures is often deducted from a single method (at least three different in vitro antioxidant methods are recommended to determine antioxidant behavior[35]) and is then reported as antioxidant capacity[36] or antioxidant activity[37,38]. Differences between these terms are specifically discussed in the literature[15,39].

As a result, there are only few general guidelines to explain the behavior of such antioxidant combinations and making accurate predictions remains a challenging task[28,40]. Towards that goal, our group recently described an innovative approach based on machine learning[41]. While the algorithm provides a simplified way to make predictions, it does not provide a clear description of the driving forces behind those interactions or a wider perspective of the antioxidants reported in the literature.

Thus, this article aims to provide a complementary assessment of the effectiveness of antioxidant mixtures of 1243 combinations reported in the literature, focusing on general trends and on the chemical descriptors of those antioxidants. As a result of the analysis, we identified not only the most commonly reported compounds and mixtures but also how various chemical descriptors affect the response. We also noted that the number of hydrogen bond donors/acceptors seems to be one of the most important variables determining which mixtures provide more synergistic effects. We believe this analysis can not only supplement existing repositories (56,666 small molecules tested for antioxidant activity)[42] but also provide initial guidelines to explain how antioxidant structure could contribute most to synergistic behavior.

Materials and methods

Antioxidant database

The process for developing and augmenting the database was recently described by our group[43]. Briefly, a chemical dataset was initially developed using information from the literature and containing examples of binary mixtures of food antioxidants displaying synergistic, additive, and antagonistic behavior (n= 1243). As a difference with other databases containing information about individual antioxidants[42,44–46], we specifically focused on intermolecular interactions between antioxidants. The classification reported in the articles (synergistic / additive / antagonistic) was preserved, despite the number of methodologies applied or the advantages and disadvantages of the methodology applied[33,47,48].

Moreover, it is important to point out that metrics associated to the magnitude of the antioxidant behavior in mixtures (e.g., Combination index – CI, and Antioxidant power) were also preserved as in the reported publications. Briefly, combination index (CI) is a well-known metric deployed in isobologram analyses aiming to quantitively quantify the interaction between different antioxidants. A CI value less than 0.85 indicates synergism (the combined effect is greater than the sum of the individual effects, a CI between 0.85 and 1.15 suggests an additive effect (the combined effect is equal to the sum of the individual effects), and a CI greater than 1.15 denotes antagonism (the combined effect is less than the sum of the individual effects). Further information concerning the calculation of this metric can be found in elsewhere[49]. Regarding antioxidant power (or total antioxidant capacity[15]), this metric computes the relative difference in response of the antioxidant mixtures (observed experimental value) compared to the sum of its individual components (maximum theoretical response). In this sense, the output value of this metric is typically converted to percentage aiming to provide a more intuitive understanding of the antioxidant effectiveness. For example, a higher percentage indicates a more pronounced antioxidant activity of the mixture compared to the sum of its individual components. Conversely, a lower percentage indicates an additive or even antagonistic effect (values lower than ~ 15%), suggesting that the mixtures display an equal (additive) or lower (antagonism) antioxidant activity compared to sum of its individual components.

To include relevant chemical information, the antioxidant database was then augmented using RDKit. During this process, all the antioxidants were converted (from their chemical name) into canonical SMILES using PubChemPy (v.1.0.4, pubchempy.readthedocs.io) and then expanded through the open source RDKit Python (v.2022.03.3, rdkit.org/docs/GettingStartedInPython.html) application programming interface (API). Out of the available options, chemical descriptors that are linked to the structure of the antioxidants (number of atoms, number of heavy atoms, number of heteroatoms, number of carbon atoms, number of oxygen atoms, number of nitrogen atoms, and number of chloride atoms) or that may influence the interactions between the antioxidants (polar surface area, molecular weight, number of aromatic rings, logP) were considered. Hydrogen bond donor count and hydrogen bond acceptor count were computed using PubChemPy, resulting in a database containing approximately 55,897 data points that provide much richer chemical information about the mixtures. An additional augmentation step was then performed to assess the frequency of specific fragments or functional groups presented in each antioxidant. More detailed information regarding those can be found in Table SI 1 (Supplementary information). Lastly, analyses presented in this manuscript were performed directly in Python using statistical data visualization libraries such as Seaborn (v. 0.12.2) and Matplotlib.

Results

Overall structure of the database

To help visualize the database more efficiently, the antioxidant combinations were grouped into three categories (using the classification reported in the corresponding papers). As it can be observed in Fig. 1A, the most commonly reported behavior corresponds to mixtures with simple additive behavior (39%), followed by antagonistic (31%) and synergistic behavior (30%). This trend is also reflected in Fig. 1B, where the frequency of the behavior is presented as a function of the antioxidant power (the most common metric in the database).

It is relevant to mention that while simple additive mixtures can be rationalized as two (or more) separate, non-interacting, chemical systems; explaining the behavior of antagonistic/synergistic mixtures of antioxidants

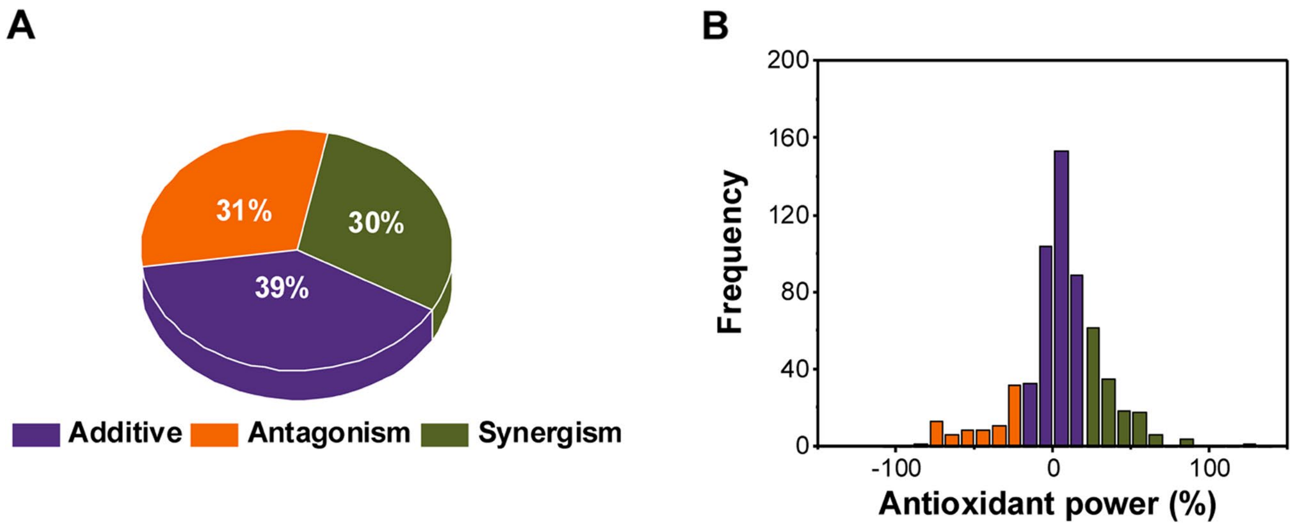

Figure 1. **A**: Distribution of articles in our database, describing additive, antagonistic, or synergistic effects (n=1243). **B**: Relative frequency of mixtures reporting overall antioxidant power (n = 622).

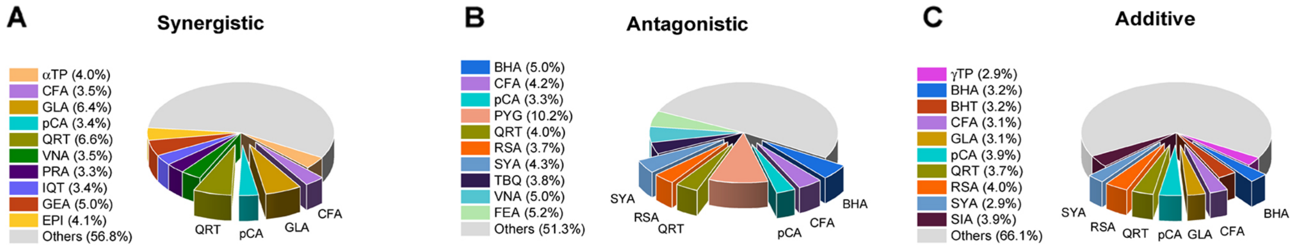

Figure 2. The 10 most prevalent compounds found in all antagonistic combinations, showing synergistic (**A**), antagonistic (**B**), or additive (**C**) behavior. Compound abbreviation: DL-alpha-Tocopherol (α-TP), Caffeic acid (CFA), Gallic acid (GLA), p-Coumaric acid (pCA), Quercetin (QRT), Vanillic acid (VNA), Protocatechuic acid (PRA), Isoquercitrin (IQT), Gentistic acid (GEA), L-Epicatechin (EPI), 2-tert-Butyl-4-methoxyphenol (BHA), Pyrogallol (PYG), Rosmarinic acid (RSA), Syringic acid (SYA), *tert*-Butylhydroquinone (TBHQ), Vanillic acid (VNA), Ferulic acid (FEA), gamma- Terpinene (γTP), Butylated Hydroxytoluene (BHT), Sinapinic acid (SIA).

requires the consideration of additional factors. Among other possible mechanisms to explain these interactions, it is worth considering the protection action of one antioxidant by means of the sacrificial oxidation of another antioxidant (also reported as regeneration or redox cycling[1,50,51]), the formation of stable intermolecular complexes between the antioxidants[19], and differences in the solubility[52] or distribution[34,40] of various antioxidants in the studied matrix. It is also important to state that these interactions are influenced by multiple factors (antioxidant type/concentration/ratio, matrix composition, pH[53], heat, kinetics, etc.), so even slight variations in experimental conditions or sample type could potentially lead to different results and interpretation of the data.

Most common components and antioxidant mixtures

Our analysis enabled identifying the most common components in mixtures reported in the literature. First, while the most frequent antioxidants in each category are listed in Fig. 2, it is interesting to note that only 6%, 13%, and 12%, of the molecules in the database are reported in manuscripts describing only synergistic[54–56], additive[54,55,57], or antagonistic[56,58] behavior (Figure SI 1). In line with this observation, we also found that about 20% of all the antioxidants in the database are present in the three categories, a clear example of both the complexity of the interactions and the importance of the compounds selected for the mixtures. Representative examples of those antioxidants include ferulic acid, syringic acid, pyrogallol, caffeic acid, quercetin, gallic acid, rosmarinic acid, vanillic acid, curcumin, TBHQ, eucalyptol, and lycopene. Additionally, 71% of all the components in the database are reported to be present in mixtures with two different categories again, highlighting the complex contribution of each individual antioxidant in the mixtures.

It is also important to note that QRT, PYG, CFA, GLA, and ROA account for approximately 21% (4.7% + 4.5% + 4.5% + 3.8% + 3.6%) of all the compounds present in our database ($n \approx 100$). In other words, only five compounds comprise over a fifth of the antioxidants present in the developed dataset. Further information concerning the most common antioxidants investigated in this work and their respective frequency (regardless of their classification) is presented in Table SI 2.

Aiming to investigate this observation further, we searched the literature for these compounds (in the realm of food antioxidants) using the Web of Science bibliographic platform. Interestingly, more than 32,000 results were retrieved for the five compounds (QRT = 13865, PYG = 635, CFA = 6408, GLA = 10367, and ROA = 2000), supporting their potential as critical components in this field rather than a biased analysis of the mixtures in our database. Moving forward, QRT emerges as the most frequent compound in synergistic combinations (Fig. 2A), whereas RSA and PYG are predominant in additive (Fig. 2B) and antagonistic (Fig. 2C) contexts, respectively. It is relevant to emphasize that the common presence of quercetin in synergistic antioxidant mixtures is not surprising, as this flavonoid is ubiquitously present in many fruits, vegetables, and beverages[59–63]. It is also important to mention that although the biological effects of quercetin have been extensively studied[38,64–68], translating its mechanism of action from (purely) chemical systems to food/biological applications represents a significant challenge due to the multifaceted parameters that can influence both the final antioxidant behavior in both food matrices and its biochemical effects upon ingestion[69,70]. Such biological effects were considered outside the scope of our analysis, and readers are encouraged to consult pertinent reports[14,71].

Our analysis also facilitated the identification of the most common combinations in each category, as summarized in Fig. 3. Among those, the five most commonly reported synergistic mixtures include pyrogallol/propyl gallate[72], γ-terpinene/myricetin[57,72,73], D-Tocopherol/AC[74], CUR/CAT[30,75], and CYM/CIT[30,57]. Additionally, the combinations of BHA-PYG, PYG-GLA, and TBHQ-PYG were found to be the highest percent (7.3%, see Figure SI 2) out of all of the combinations collected in the database (see Table SI 3).

Chemical properties and antioxidant behavior

To understand how the structure of one antioxidant could affect its behavior when mixed with other antioxidant(s), we performed an analysis considering several chemical descriptors that could affect intermolecular interactions. It is important to reiterate that the analysis herein described targets the interactions between antioxidants in mixtures reported in the literature, rather than the mechanism of each antioxidant with the corresponding targets[13,76]. As a first step, our analysis considered the LogP (log of the partition coefficient between water and an organic solvent), which could influence not only the interaction[19] but also the distribution of antioxidants[34]. The more negative the LogP value is, the more hydrophilic a compound tends to be. Conversely, a positive LogP value denotes a more lipophilic compound. As it can be observed in Fig. 4A, most of the antioxidants in the combinations included in our database feature positive LogP values (hydrophobic behavior), with values ranging from − 1.4 to 15.5 (see distribution on the top axes). Out of those, the most hydrophilic mixture is rutin with ascorbic acid (additive)[56] and the most hydrophobic mixtures are alpha-tocopherol with either lycopene (additive) or cis-γ, γ-carotene[77]. These findings were somewhat expected, as the reported values would support their compatibility with lipids, where prevention of rancidity is key. We also observed that the molecular weight of most antioxidants used in the studied combinations ranged from 100 to 400 Daltons, defining them as small molecules. Aiming to understand if the interplay of these variables would affect their behavior, we evaluated the dispersion (standard deviations) of these variables in each mixture, as a function of each type of antioxidant effect. As it can be observed in Fig. 4B and C, mixtures in all categories featured antioxidants with similar properties in terms of hydrophobicity (LogP) and MW. From the analysis it is also evident that neither one of these parameters can be used to predict the type of interactions a given mixture of antioxidants would have.

Aiming to further investigate what properties influence the antioxidant behavior of mixtures, we extended our analysis to other chemical descriptors related to the structure of the antioxidants, such as the percentage of oxygen atoms, the percentage of heteroatoms, and the topological surface area (Figure SI 3). Although it is noticeable that synergistic mixtures display higher average and median values across the three investigated metrics, these differences are not statistically significant and do not allow for distinguishing the type of antioxidant behavior. In this regard, one should consider that these findings are restricted to plain atoms of the antioxidant mixtures rather than their specific functional groups. For example, the average percentage of oxygen atoms in a 1:1 combination of BHA and BHT (a common synergistic combination[31,53]) is 4.7%. The calculation for this specific metric in this mixture can be achieved by analyzing each component separately, as it follows: (I)

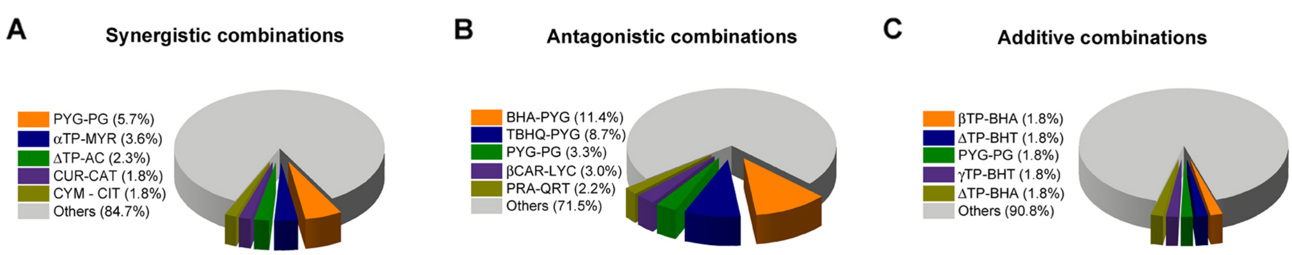

Figure 3. Five most common antioxidant combinations found in the database displaying synergistic (**A**), antagonistic (**B**), or additive interactions (**C**). Compound abbreviation: Pyrogallol (PYG), Propyl Gallate (PG), DL-α-Tocopherol (αTP), Myricitin (MYR), Δ-Tocopherol (ΔTP), Ascorbic acid (AC), Curcumin (CUR), Catechin (CAT), Cymol (CYM), Citronellal (CIT), Butylated Hydroxytoluene (BHT), *tert*-Butylhydroquinone (TBHQ), β-carotene (βCAR), Lycopene (LYC), Protocatechuic acid (PRA), β-Tocopherol (βTP), gamma-terpinene (γTP).

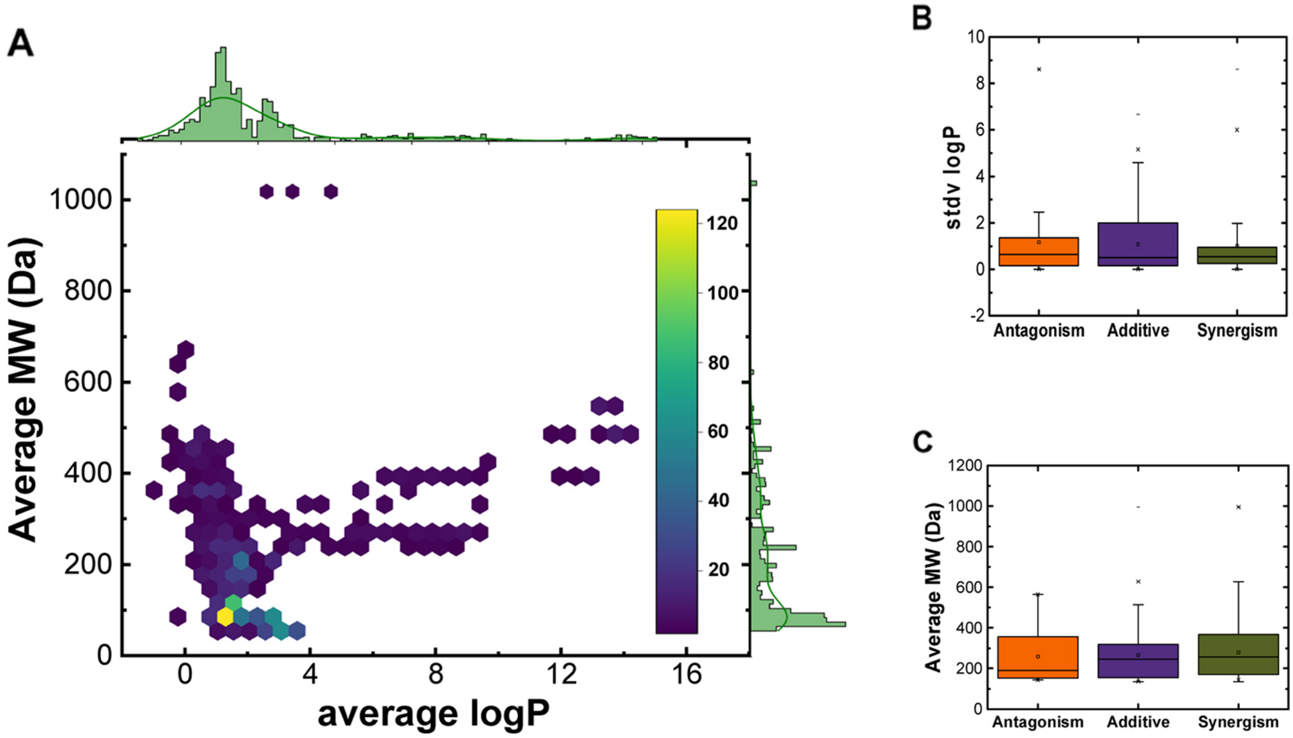

Figure 4. **A**) Distribution of the average molecular weight of the components, as a function of the average LogP of the components forming the mixtures considered in our database. **B**) Calculated variability in the LogP of antioxidants in the mixtures reported as synergistic, additive, or antagonistic. **C**) Relationship between the average molecular weight of antioxidants in the mixtures reported as synergistic, additive, or antagonistic.

BHA displays 6.9% of oxygen atoms in its structure (2 oxygens atoms in a total number of 29 atoms), (II) BHT displays 2.5% of oxygen atoms in its structure (1 oxygen atom in 40 atoms), (III) The weighted % of oxygen for BHA considering its molar ratio (or contribution in the mixture) is 3.45% (6.9% x 0.5) while this value is 1.25% for BHT (2.5% x 0.5). Therefore, the overall percentage of oxygen for the proposed mixture is 4.7% (3.45% + 1.25%). Regardless of being a simple module in the data augmentation process, these findings suggest that analyzing plain atoms in antioxidant mixtures is (at best) marginally insightful when describing the type of antioxidant behavior.

To address this limitation, we investigated the correlation of the structural features of the mixtures (Table SI 2) as a function of the type of antioxidant behavior using Spearman analyses. It is worth emphasizing that the same mathematical logic previously described (weighted contribution of each property) was deployed here to calculate the overall number of fragments in each mixture. Additionally, the categorical classes of the antioxidant behavior (synergism, additive, antagonism) were substituted by their respective Antioxidant Power (AP) or Combination Index (CI) (see materials and methods section), as the implementation of Spearman's coefficient requires the use of continuous variables. Then, the weighted number of fragments for each antioxidant mixture was run against their respective AP or CI, leading to the results shown in Fig. 5.

Before advancing with our analysis, it is pivotal to understand the general patterns in the Spearman values (ρ) and how those were associated with the structural features of mixtures reported having synergistic, additive, or antagonistic behavior. In our case, a positive Spearman value indicates that the weighted structural feature (number of fragments) can directly relate to the antioxidant behavior, meaning that they increase or decrease together. Conversely, a negative Spearman value indicates an inverse relationship, meaning that when one variable increases, the other decreases. Additionally, the degree of this correlation is assessed by the module of ρ value, ranging from 0 (no correlation) to 1 (perfect monotonic correlation). In this regard, it is interesting to note that the ρ value for our analysis ranges from − 0.4 to + 0.4, indicating a weak to moderate correlation degree between the antioxidant behavior of the mixture and fragments such as phenol, bicyclic compounds, halogen groups, ester, aldehydes, methoxy groups, and aliphatic carboxylic acids (denoted as COO). Among those, it is important to point out that the number of COO fragments was inversely proportional to both AP (Fig. 5A) and CI (Fig. 5B) for additive antioxidant mixtures. We believe the negative correlation of COO fragments (reported for additive mixtures) simply implies that these groups are not determining the behavior. In other words, the presence of COO groups in antioxidants does not make the mixtures additive. More interestingly, the number of COO fragments is directly proportional to the CI in synergistic mixtures, potentially allowing the use of this metric as a chemical descriptor in computational approaches designed to predict the type of antioxidant behavior. Further analysis also reveals a direct relationship between the number of phenol fragments and the CI for synergistic mixtures, indicating that this metric increases as the number of phenolic fragments

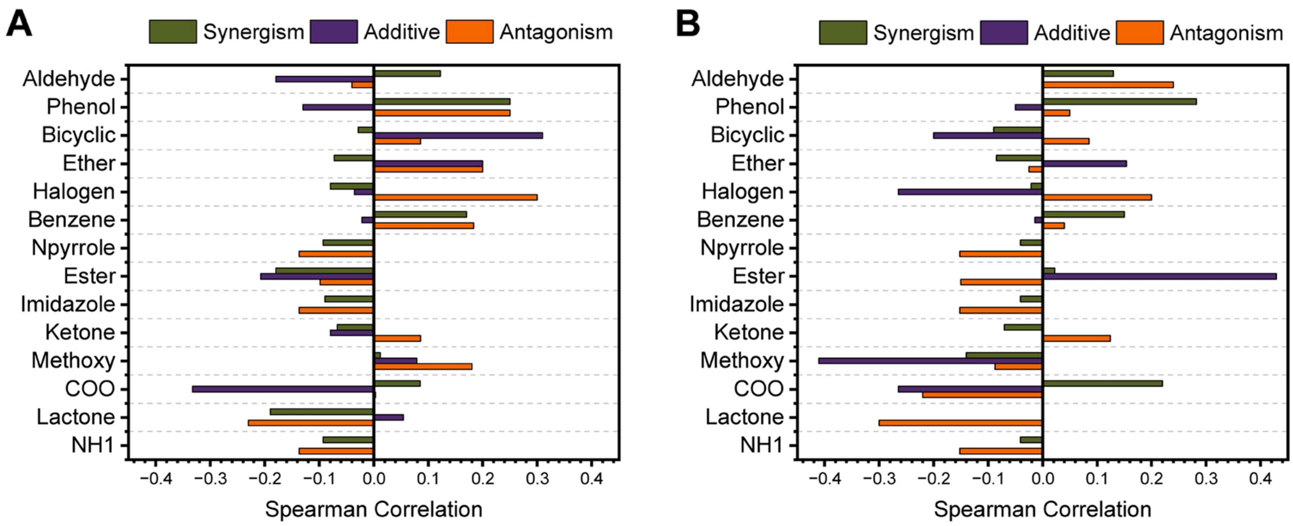

Figure 5. Spearman's correlation between structural features of antioxidant mixtures for **A**) Antioxidant Power (AP, $n = 622$) or **B**) Combination Index (CI, $n = 300$).

also increases. Despite the encouraging results, it is crucial to approach these findings with caution, as they only represent the overall behavior of mixtures included in our developed database. In other words, these correlations may not maintain the same level of relevance in different antioxidant systems and for experimental conditions not considered in our database. Finally, one might assume that the Spearman correlation analysis would yield similar results regardless of whether AP or CI is used. However, it is important to mention that the antioxidant mixtures associated with each metric do not necessarily overlap, as CI and AP are typically not reported together. As a result, the potential disparities in these analyses can be explained by the fact that the antioxidant mixtures (classified based on their CI or AP) may not share the same chemical space. Regardless, the intricate complexity of these findings reinforces the notion that the behavior of those mixtures is not only affected by experimental conditions but also by a wide variety of structural-dependent features of the antioxidants themselves[57,78–80].

Hydrogen bonding

Understanding a mechanism of action that supports, for example, synergistic behavior in antioxidant mixtures is still unclear despite several efforts from the scientific community[34,81–84]. As noted by Bayram and Decker[1], the hypothesized mechanism underlying this effect could be explained by various factors such as different antioxidant partitioning of the individual components (different solubility), regeneration of the stronger antioxidant, formation of new dimers or adducts, and the formation of stable intermolecular complexes exhibiting enhanced antioxidant capacity. Furthermore, it is important to mention that several groups have reported the potential role of hydrogen bonds[36,37,85–90] in the behavior of antioxidant mixtures, potentially serving as a metric to predict synergistic, additive, or antagonistic behavior in those systems. In this context, we calculated the weighted number of hydrogen bonds (HB_x) available between the components in each mixture, aiming to analyze this value across the three groups of antioxidant behavior. Then, these values were compared to the average number of hydrogen bonds in additive mixtures (set as reference). While representative examples of mixtures in each category are presented in Table 1, the overall analysis is summarized in Fig. 6.

As it can be observed, the synergistic antioxidant combinations displayed, on average, 50% more hydrogen bonds than the additive mixtures (used as a reference, 0). On the other hand, antagonistic combinations displayed 15% less hydrogen bonds than the baseline group (additive combinations). While these findings are in agreement with previous publications[36,88,92,93], it is relevant to point out that the formation of intermolecular hydrogen bonds can also be associated with antagonistic effects[37,87,94], suggesting that other variables/forces are prevalent in such cases. Again, these results should be carefully analyzed as H-bonding only seems to be one of the elements involved in the interactions of synergistic antioxidant mixtures. As noted by Pinney's group, solvents and matrix components can certainly compete with these interactions[95], potentially explaining why mixtures of similar compounds could behave so differently when used in different conditions[57].

Lastly, we performed a Tukey's HSD test (Table 2) to investigate the possibility of differentiating (with statistical confidence) the type of antioxidant behavior based on the number of hydrogen bonds for each studied group.

Interestingly, the Tukey's HSD results suggest that synergistic antioxidant mixtures are statistically different ($p < 0.05$) from both additive and antagonistic systems, at least in terms of hydrogen bonds. On the other hand, antagonistic mixtures cannot be statistically distinguished from additive systems ($p > 0.05$), although they display 15% fewer hydrogen bonds than the mixtures classified as additive (see Fig. 6).

Component #1	Component #2	Ratio	Reported Behavior	Assay	HB
Myricetin	α-Tocopherol	2:1	Synergistic[73]	PV, conjugated dienes	4.3
Pelargonidin-3-O-glucoside	Quercetin	2:1	Synergistic[91]	FRAP or DPPH	6.3
Epicatechin	Quercetin – 3-β-glucoside	3:1	Synergistic[91]	FRAP or DPPH	5.7
Peonidin 3-glucoside	Kaempferol	1:1	Additive[91]	FRAP or DPPH	5.5
Quercetin	Epicatechin	3:1	Nearly additive[78]	Phosphomolybdenum, FRAP, or DPPH	3.6
Syringic acid	Protocatechuic acid	1:1	Additive[79]	FRAP or ORAC method	2.5
Peonidin 3-glucoside	Pelargonidin	2:1	Antagonistic[32]	TEAC or ORAC	6.0
Citronellal	Menthol	1:1	Antagonistic[57]	DPPH	0.5
P-Cymene	Linalool	1:1	Antagonistic[57]	DPPH	0.5

Table 1. Representative examples of antioxidant mixtures, their reported behavior, the method used for their evaluation, and their calculated hydrogen bond count (HB). PV: peroxide value, FRAP: Ferric Reducing Antioxidant Power, DPPH: 2,2-diphenyl-1-picrylhydrazyl analysis, ORAC: Oxygen Radical Antioxidant Capacity, TEAC: Trolox Equivalent Antioxidant Capacity

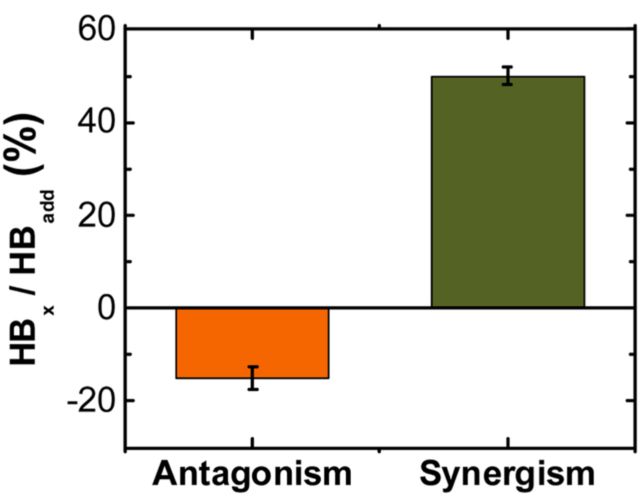

Figure 6. Percentage of number of hydrogen bonds in synergistic or antagonistic antioxidants, compared to the number of hydrogen bonds in additive mixtures. HB_x denotes the weighted number of hydrogen bonds available between the compounds for either antagonistic (HB_a) or synergistic mixtures (HB_s). Similarly, HB_{add} represents the weighted number of hydrogen bonds available between the compounds for additive mixtures. As a result, the y-axis represents the relative percentage of available hydrogen bonds compared to additive antioxidant mixtures (baseline).

Group 1	Group 2	$Mean_{diff}$	*p*-adj	Lower	Upper	Reject
Additive	Antagonism	0.46	0.26	-0.23	1.15	False
Additive	Synergism	1.73	0.01	1.00	2.47	True
Antagonism	Synergism	1.27	0.01	0.58	1.96	True

Table 2. Tukey's HSD test comparing the number of hydrogen bonds across different groups of antioxidant behavior.

Conclusions

Antioxidants play a key role in the preservation of food products by inhibiting or quenching free radical reactions of lipids. Although various antioxidant strategies can be implemented to increase shelf-life, combining antioxidants has been one of the most successful approaches to date. Aiming to understand the relation between the structure of those antioxidants and their resulting behavior, this report presents an analysis of the chemical properties of common antioxidant combinations in relation to the resulting synergistic, antagonistic, and additive effects. For that purpose, a database containing more than 1000 combinations of antioxidants was assembled and then augmented using multiple chemical descriptors. Among those, the number of hydrogen bond donors/acceptors in those antioxidants mixtures showed a general correlation with the antioxidant behavior, an aspect that, up until now, had been reported as one of the possible mechanisms[81] and only for specific systems[36,96].

In addition, we have identified a number of additional functional groups, which could complement previous attempts[79] to predict antioxidant properties, as well as explaining the possible properties that contribute the most to synergistic attributes. While our analysis highlights the importance hydrogen bonding seems to have in the resulting behavior, it is essential to state that this cannot be regarded as the only mechanism involved[1] and that the behavior of specific mixtures is often influenced by a number of additional factors including the inherent antioxidant effectiveness of each molecule, their concentration and ratios, the distribution, and the chemistry of the sample matrix.

Data availability

The datasets generated and/or analyzed during the current study are not publicly available due to confidentiality agreements managed by Clemson University Research Foundation. Sections of the dataset are available from the corresponding author upon reasonable request.

Received: 20 August 2024; Accepted: 28 October 2024
Published online: 03 January 2025

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Acknowledgements
Financial support for this project has been provided by the Department of Chemistry at Clemson University, the South Carolina Department of Agriculture ACRE Competitive Grant Program, and the USDA-NIFA (award 2024-67021-42520).

Author contributions
L.B.A wrote the main manuscript text and prepared figures. and J.T.F. assisted with the development and analysis of the database. C.D.G. contributed to the conception and design of the work, and wrote/revised the work. All authors reviewed and approved the manuscript.

Declarations

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-024-77948-9.

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