Article

Substituent Effects on the Radical Scavenging Activity of Isoflavonoid

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Abstract: Understanding the role of substituents is of great importance for the preparation of novel phenolic compounds with enhanced antioxidative properties. In this work, the antioxidative activity of isoflavonoid derivatives with different substituents placed at the C2 position was determined by density functional theory (DFT) calculations. The bond dissociation enthalpy (BDE), ionization potential (IP), and proton affinity (PA) related to hydrogen atom transfer (HAT), single electron transfer-proton transfer (SET-PT), and sequential proton loss electron transfer (SPLET) mechanisms were calculated. The strongest antioxidative group of isoflavonoid is not altered by the substituents. Excellent correlations were found between the BDE/IP/PA and Hammett sigma constants. Equations obtained from linear regression can be useful in the selection of suitable candidates for the synthesis of novel isoflavonoids derivatives with enhanced antioxidative properties. In the gas and benzene phases, the electron-donating substituents would enhance the antioxidative activity of isoflavonoids via weakening the BDE of 4′−OH. In water phase, they will reduce the antioxidative by strengthening the PA of 7−OH. Contrary results occur for the electron-withdrawing groups. In addition, the electronic effects of substituents on the BDE/IP/PA have also been analyzed.

Keywords: isoflavonoid; substituent effect; structure-antioxidant activity relationship; Hammett sigma constants; density functional theory; antioxidant mechanism

1. Introduction

Oxidation reactions are the main reason for deterioration in biological systems and are related to ageing and diseases such as atherosclerosis, cancer, and Alzheimer’s disease [1]. This process arises from oxidative damage of biomolecules (such as proteins, nucleic acids, unsaturated lipids, and sugars) by the presence of reactive free radicals [2].

Antioxidants are chemical compounds that can trap reactive free radicals formed during oxidative reactions. In the past decades, interest in phenolic compounds has grown greatly, due to their free radical scavenging ability and other potential health benefits [3]. Isoflavonoids are a large class of phenolic compounds, mainly found in vegetables, fruits, cereals, and bee products [4,5]. Apart from their physiological performance in plants, isoflavonoids are also vital constituents in the human diet. In recent years, growing attention to isoflavonoids can be mainly ascribed to their free radical scavenging activities, which also greatly relates to other biological activities, such as antibacterial, antiviral, anti-inflammatory and anti-ischemic activities [5]. It is reported that isoflavonoids perform their anti-inflammatory actions mainly via the ability to modulate production of free radicals by phagocytic leukocytes [6].
It is well-established that phenolic compounds commonly scavenge free radicals via three possible pathways [7–9]. They are hydrogen atom transfer (HAT), single electron transfer-proton transfer (SET-PT), and sequential proton loss electron transfer (SPLET). From the antioxidative action viewpoint, the three mechanisms have the same net result: formation of phenoxy radicals and the termination of free radicals.

The antioxidative activity of phenolic compounds depends on their capacity to resist the detrimental effects of free radicals. It is related to the structure of the compound. Among multiple structural features, the substituent effect is one of the most important factors that influences the chemical, physicochemical, and biochemical properties of phenolic compounds [10–13]. Theoretical studies on the substituent effect can be utilized in the selection and synthesis of novel compounds with enhanced antioxidative activity. There are multiple studies in the literature that focus on the number and position of functional groups in relation to the biological activity of flavonoids [14–16]. On the other hand, the substituent effect on the antioxidative activity of isoflavonoids is seldom known.

Computational methods can provide valuable information about the biological performance of phenolic compounds. Due to their low cost, short time, and high precision performing calculations on the properties of phenolic compounds, density functional theory (DFT) calculations have been widely accepted as the most applicable computational methods for studying antioxidative activity. They have been successfully used to study the antioxidative activity and structure-activity relationships of phenolic compounds [7–9,17–22]. They can reveal the effects of different structural features on the antioxidative properties of phenolic compounds. The comparisons between the experimental and theoretical data are not only able to make the controversially experimental facts clear, but also able to design novel compounds with excellent antioxidative activity. In addition, DFT can also carry out the work that is difficult to perform by experiment.

Understanding the role of substituents is of great importance for the preparation of novel phenolic compounds with enhanced antioxidative properties. In this paper, the effects of various substituents on reaction enthalpies related to HAT, SET-PT, and SPLET mechanisms of isoflavonoid derivations were investigated by DFT method. The 4′,5,7-trihydroxyisoflavone and 4′,5,7-trihydroxyisoflavanone (Figure 1) are selected as the model molecule. Both of them have hydroxyl groups in rings A, B, and C, and thus can be used to study the substituent influence on the antioxidative activity of different rings. Various substituents (NH₂, OMe, Me, OH, F, Cl, CHO, CF₃, CN, and NO₂) covering the electron-withdrawing groups and electron-donating groups were placed at the C2 position. To find a relationship between structural parameters and the antioxidative activity, correlations of calculated enthalpies with Hammett sigma constants of the substituents were investigated. Apart from the gas phase, benzene, and water phases were used as models to simulate environments (physiological lipids and fluids) found in many foods and body tissues.

![Figure 1](image-url). The chemical structures of 4′,5,7-trihydroxyisoflavone and 4′,5,7-trihydroxyisoflavanone.

2. Results

HAT is a one-step mechanism. SET-PT and SPLET mechanisms take place through two steps. Reaction enthalpies (bond dissociation enthalpy or BDE, ionization potential or IP, and proton affinity or PA) characterizing the first steps of three mechanisms are of great importance in evaluating the antioxidative action. Hence, the detailed analysis mainly focuses on the BDE, IP, and PA.
The Hammett sigma constant has been one of the most widely used parameters to study and interpret organic reactions and their mechanisms. In this work, the Hammett sigma constants $\sigma_m$ and $\sigma_p$ of the substituents were used. They were obtained from the ionization of organic acids in solutions and have been successfully used to predict the equilibrium and rate constants of a variety of families of reactions. The electronic effect of the substituent is mainly composed of two parts: the field/inductive effect (represented by parameter $F$) and the resonance effect (characterized by parameter $R$) [13,23,24]. In this work, the electronic effects of the substituents on the reaction enthalpies have also considered. Table 1 lists the $\sigma_m$, $\sigma_p$, $F$, and $R$ of the studied substituents [13].

**Table 1.** The Hammett sigma constants ($\sigma_m$ and $\sigma_p$) $^a$, the field/inductive parameter ($F$) $^a$ and the resonance parameter ($R$) $^a$ of the substituents.

| Substituent | $\sigma_m$ | $\sigma_p$ | $F$ | $R$ |
|-------------|------------|------------|-----|-----|
| H           | 0.00       | 0.00       | 0.00| 0.00|
| NH$_2$      | -0.16      | -0.66      | 0.08| -0.74|
| OMe         | 0.12       | -0.27      | 0.29| -0.56|
| Me          | -0.07      | -0.17      | 0.01| -0.18|
| OH          | 0.12       | -0.37      | 0.33| -0.70|
| F           | 0.34       | 0.06       | 0.45| -0.39|
| Cl          | 0.37       | 0.23       | 0.42| -0.19|
| CHO         | 0.35       | 0.42       | 0.33| 0.09 |
| CF$_3$      | 0.43       | 0.54       | 0.38| 0.16 |
| CN          | 0.56       | 0.66       | 0.51| 0.15 |
| NO$_2$      | 0.71       | 0.78       | 0.65| 0.13 |

$^a$ Data from work by Hansch, Leo, and Taft [13].

### 2.1. Bond Dissociation Enthalpy

In the HAT mechanism, the BDE is an important parameter evaluating the antioxidative action. It influences the effectiveness of the H-atom transfer reaction from the phenolic compounds to the reactive radical. The lower the BDE, the stronger the antioxidative activity and the more important the role of the corresponding O$\cdots$H group in the antioxidative activity. The computed gas, benzene, and water phases of BDEs of 4$\cdots$OH, 5$\cdots$OH, and 7$\cdots$OH for the studied compounds are listed in Table 2. As can be seen in Table 2, the lowest BDEs are at 4$\cdots$OH for the investigated compounds irrespective of the studied media. Therefore, the 4$\cdots$OH is the group most likely to undergo the HAT mechanism. In addition, the strongest antioxidative group of isoflavonoids does not alter by the substituents placed at C2, irrespective of the studied media.

The Pearson correlation coefficients between the $\sigma_m/\sigma_p$ and the BDEs are drawn as histograms in Figure 2. In Figure 2, all of the $P(BDE, \sigma)$ are positive and larger than 0.8. Hence, the BDEs are positively and highly relevant with the Hammett sigma constants. The positive coefficient also characterizes how the electron-withdrawing groups in the C2 position raise the BDE, while electron-donating groups have the opposite effect. The obtained results can be interpreted to say that electron-withdrawing groups in the C2 position can stabilize the parent molecule and destabilize the radical. Hence, they increase the O$\cdots$H BDE. However, electron-donating groups in the C2 position have the opposite effect, and their presence would lead to a decrease in the O$\cdots$H BDE. Therefore, the electron-withdrawing groups placed at the C2 position would reduce the antioxidative activity of isoflavonoids, while the electron-donating groups have the opposite effect.
Table 2. O–H bond dissociation enthalpy (BDE) in kJ/mol obtained by the M062X/6-311 + G** method a.

| Gas  | Benzene | Water |
|------|---------|-------|
| H    | 362 366 | 433 428 449 391 | 364 367 428 424 413 395 \( P_{(\text{BDE}, \sigma_{m})} \) | 357 361 393 392 410 393 | \( P_{(\text{BDE}, \sigma_{p})} \) | \( P_{(\text{BDE}, \sigma_{m})} \) | 357 361 393 392 410 393 |
| NH₂  | 359 362 | 431 426 447 391 | 360 364 426 423 396 395 | 353 359 392 391 394 393 | 353 359 392 391 394 393 |
| OMe  | 337 363 | 431 428 449 391 | 359 366 427 425 406 395 | 353 361 392 393 398 394 | 353 361 392 393 398 394 |
| Me   | 363 364 | 432 427 448 391 | 365 366 427 423 408 395 | 359 360 393 391 400 393 | 359 360 393 391 400 393 |
| OH   | 359 364 | 433 429 449 392 | 361 366 427 425 406 396 | 354 362 393 393 401 395 | 354 362 393 393 401 395 |
| F    | 364 367 | 434 429 452 394 | 367 369 429 425 417 398 | 361 364 394 394 406 396 | 361 364 394 394 406 396 |
| Cl   | 365 367 | 435 429 453 394 | 368 369 431 426 428 399 | 363 364 395 395 409 396 | 363 364 395 395 409 396 |
| CHO  | 364 366 | 436 429 452 393 | 366 368 430 426 426 397 | 361 362 396 394 410 395 | 361 362 396 394 410 395 |
| CF₃  | 368 368 | 437 430 454 395 | 371 370 432 426 440 399 | 366 364 397 394 412 396 | 366 364 397 394 412 396 |
| CN   | 370 371 | 439 430 454 396 | 373 373 433 426 446 400 | 368 366 397 395 415 397 | 368 366 397 395 415 397 |
| NO₂  | 369 372 | 440 430 455 397 | 372 374 434 426 458 401 | 367 367 398 395 418 398 | 367 367 398 395 418 398 |

Figure 2. The Pearson correlation coefficients between the Hammett sigma constants (\( \sigma_{m} \) and \( \sigma_{p} \))/F/R and the BDE/ ionization potential (IP)/ proton affinity (PA) in the gas, benzene, and water phases.

For O–H in 4’,5,7-trihydroxyisoflavone, the \( P_{(\text{BDE}, \sigma_{p})} \) is larger than \( P_{(\text{BDE}, \sigma_{m})} \) and exceeds 0.9. While for O–H in 4’,5,7-trihydroxyisoflavonane, the \( P_{(\text{BDE}, \sigma_{m})} \) is larger than \( P_{(\text{BDE}, \sigma_{p})} \) and are more than 0.9. Therefore, the correlation between the BDE in 4’,5,7-trihydroxyisoflavonane and \( \sigma_{p} \) is better than that between the BDE and \( \sigma_{m} \) and the BDE of O–H in the substituted 4’,5,7-trihydroxyisoflavonane can be predicted better by \( \sigma_{p} \). Meanwhile, an opposite result occurs in 4’,5,7-trihydroxyisoflavonane, and the BDE of O–H bond in substituted 4’,5,7-trihydroxyisoflavonane can be predicted better by \( \sigma_{m} \). In this work, BDE values of 4’–OH computed for the substituted isoflavonoids in the gas, benzene, and water phases are plotted against Hammett sigma constants in Figure 3. Equations obtained from the linear regression are also placed in Figure 3. They can be used to predict the BDEs of 4’–OH by the Hammett sigma constants. The hydroxyl group with the lowest BDE determines the antioxidative activity. As the 4’–OH is the strongest antioxidative hydroxyl group, thus the antioxidative activity of the substituted isoflavonoids can also be predicted by these equations.

The Pearson correlation between the F/R and the BDEs are also done and corresponding correlation coefficients are drawn as histograms in Figure 2. In Figure 2, it can also be observed that the correlation between the BDE of isoflavone and R is much better than that between the BDE of isoflavone and F. Meanwhile, a contrary result occurs for the BDE of isoflavonane. Therefore, the electronic effect of substituents at the C2 position on the BDE of 4’,5,7-trihydroxyisoflavonane is
mainly governed by the resonance effect, while that on the BDE of 4′,5,7-trihydroxyisoflavanone is mainly controlled by the field/inductive effect.

![Figure 3](image_url)

**Figure 3.** Dependence of BDE on Hammett sigma constants for 4′,5,7-trihydroxyisoflavone (A) and 4,5,7-trihydroxyisoflavone (B) in gas, benzene, and water phases.

### 2.2. Ionization Potential

In a SET-PT mechanism, the first step is electron transfer. This step is significant for the SET-PT mechanism and it is characterized by IP. The lower the IP value, the higher the electron-donating ability, and the stronger the antioxidative activity. The computed gas, benzene, and water phase IPs for the basic structure and substituted isoflavonoids are listed in Table 3. Solvent causes considerable changes in the enthalpies of the ionic species. Thus, IP in the solvent phase is significantly lower than that in the gas phase, because of the fact that the cation radical is more stable and the conjugation of the π-electrons is more delocalized in the solvent media.

|                  | Gas                   | Benzene               | Water                |
|------------------|-----------------------|-----------------------|----------------------|
|                  | 1         | 2         | 1         | 2         | 1         | 2         |
| H                | 758      | 793      | 649      | 680      | 563      | 587      |
| NH₂               | 722      | 784      | 614      | 668      | 533      | 580      |
| OMe               | 718      | 787      | 618      | 673      | 544      | 585      |
| Me                | 754      | 787      | 647      | 676      | 566      | 586      |
| OH                | 734      | 789      | 632      | 675      | 550      | 585      |
| F                 | 764      | 807      | 657      | 687      | 571      | 589      |
| Cl                | 769      | 805      | 664      | 686      | 581      | 588      |
| CHO               | 769      | 805      | 662      | 689      | 579      | 593      |
| CF₃               | 788      | 806      | 679      | 687      | 591      | 591      |
| CN                | 792      | 817      | 682      | 695      | 591      | 593      |
| NO₂               | 795      | 816      | 686      | 695      | 593      | 598      |

The Pearson correlation was carried out for the IP and Hammett sigma constants. As can be seen in Figure 2, the $P_{IP,σ₀}$ is positive and larger than 0.8 in the studied phases, indicating that the IP is positively and highly relevant with the Hammett sigma constant. The positive coefficient also suggests that electron-withdrawing groups raise the IP and electron-donating groups have an opposite effect. As is known in organic chemistry, the electron-withdrawing substituents can stabilize the parent molecule and destabilize the radical and cation radical. This results in an increase in IP. However, the electron-donating substituents have an opposite effect. Their presence in the molecule leads to a decrease in IP. Therefore, the electron-donating groups at the C2 position would enhance the antioxidative activity of isoflavonoids. At the same time, electron-withdrawing groups at the C2 position would play the opposite role in a SET-PT mechanism.

In Figure 2, the $P_{IP,σ₀}$ is larger than $P_{IP,σ_m}$, and is approximate to 1. Therefore, the correlation between the IP and $σ₀$ is better than that between the IP and $σ_m$, and the IP of the substituted
isoflavonoids can be better predicted by $\sigma_p$. IP values computed for the substituted isoflavonoids in the gas, benzene, and water phases are plotted against $\sigma_p$ in Figure 4. Equations obtained from the linear regression are also placed in Figure 4. They can be used to predict the IP and antioxidative activity of the substituted isoflavonoids by the $\sigma_p$. This can be useful in the selection of suitable candidates for the synthesis of novel isoflavonoid derivatives with enhanced antioxidative properties.

**Figure 4.** Dependence of IP on Hammett sigma constants for $4',5,7$-trihydroxyisoflavone (A) and $4',5,7$-trihydroxyisoflavonone (B) in gas, benzene, and water phases.

In Figure 2, it can also be seen that the correlation between IP and $R$ is much better than that between IP and $F$. Therefore, the electronic effect of the substituents on the IP is mainly governed by the resonance effect.

### 2.3. Proton Affinity

In a SPLET mechanism, the first step is proton transfer. This step is significant for a SPLET mechanism and is characterized by PA. The PA influences the effectiveness of the proton transfer reaction. The lower the PA, the more important the role of the corresponding O−H group and the stronger the antioxidative activity. The computed gas, benzene, and water phase PAs of $4'−\text{OH}$, $5−\text{OH}$, and $7−\text{OH}$ for the basic structure and substituted isoflavonoids are listed in Table 4. As can be seen in Table 4, the lowest PAs are at $7−\text{OH}$ for the investigated compounds, irrespective of the studied media. Therefore, the $7−\text{OH}$ is the group most likely to undergo the first step of SPLET mechanism. In addition, the strongest antioxidative group of isoflavonoid is not altered by the substituents placed at C2, regardless of the studied media.

**Table 4.** Proton affinity (PA) in kJ/mol obtained by the M062X/6-311 + G** method. a

|                  | Gas        | Benzene    | Water      |
|------------------|------------|------------|------------|
|                  | 1  2  1  2 | 1  2  1  2 | 1  2  1  2 |
| $4'−\text{OH}$   | 1415  1423 | 1445  1445 | 1371  1373 |
| $5−\text{OH}$    | 1422  1334 | 1461  1443 | 1384  1371 |
| $7−\text{OH}$    | 1416  1431 | 1451  1438 | 1373  1366 |
| $4'−\text{OH}$   | 1419  1422 | 1452  1447 | 1376  1365 |
| $5−\text{OH}$    | 1434  1431 | 1445  1435 | 1368  1363 |
| $4'−\text{OH}$   | 1417  1421 | 1430  1442 | 1358  1354 |
| $5−\text{OH}$    | 1415  1420 | 1429  1420 | 1359  1352 |
| $5−\text{OH}$    | 1390  1404 | 1424  1424 | 1352  1354 |
| $4'−\text{OH}$   | 1398  1417 | 1419  1419 | 1350  1351 |
| $5−\text{OH}$    | 1384  1410 | 1411  1410 | 1342  1342 |
| $4'−\text{OH}$   | 1377  1409 | 1404  1403 | 1337  1337 |

a The data in bold and underline represents the lowest PAs.
The Pearson correlation coefficients between the $\sigma_m/\sigma_P$ and the PAs are drawn as histograms in Figure 2. In Figure 2, all of the $P_{(PA, \sigma)}$ are negative and the absolute value of $P_{(PA, \sigma)}$ is larger than 0.8. Hence, the PAs are negatively related to and highly relevant to the Hammett sigma constants. The negative value of the coefficient also characterizes how the electron-donating groups in the C2 position raise the PA, while electron-withdrawing groups have an opposite effect. The obtained results can be interpreted to say that electron-withdrawing groups in the C2 position can stabilize the anion and destabilize the neutral molecule. Hence, they decrease the O–H PA. However, electron-donating groups in the C2 position have an opposite effect, and their presence would lead to an increase in the O–H PA. Therefore, the electron-withdrawing groups placed at the C2 position would strengthen the antioxidative activity of isoflavonoid, while the electron-donating groups have an opposite effect.

For 4′−OH, the absolute value of $P_{(PA, \sigma_p)}$ is larger than that of $P_{(PA, \sigma_m)}$ and exceeds 0.9. Meanwhile, for 5−OH and 7−OH, the absolute value of $P_{(PA, \sigma_m)}$ is larger than that of $P_{(PA, \sigma_p)}$ and more than 0.9. Therefore, the PA(4′−OH) better correlates with $\sigma_p$ and the PA(4′−OH) in the substituted isoflavonoid can be better predicted by $\sigma_p$. At the same time, the opposite result occurs for 5−OH and 7−OH and their PA values in substituted isoflavonoid can be better predicted by $\sigma_m$. PA values of 7−OH computed for the substituted isoflavonoids in the gas, benzene, and water phases are plotted against Hammett sigma constants in Figure 5. Equations obtained from the linear regression are also placed in Figure 5. They can be used to predict the PAs of 7−OH by the Hammett sigma constants. As the 7−OH is the strongest antioxidative hydroxyl group, the antioxidative activity of the substituted isoflavonoids can also be predicted by the equations. This can be useful in the selection of suitable candidates for the synthesis of novel isoflavonoid derivatives with enhanced antioxidative properties.

The Pearson correlation between the $F/R$ and the PAs are also done and corresponding correlation coefficients are drawn as histograms in Figure 2. In Figure 2, it can be observed that the correlation between PA(4′−OH) and $R$ is much better than that between PA(4′−OH) and $F$. Meanwhile, contrary results occur for the PA(5−OH) and PA(7−OH). Therefore, the electronic effect of substituent at the C2 position on the PA(4′−OH) of isoflavonoid is mainly governed by the resonance effect, while that on the PA(5−OH) and PA(7−OH) of isoflavonoid is mainly controlled by the field/inductive effect.

![Figure 5. Dependence of PA on Hammett sigma constants for 4′,5,7-trihydroxyisoflavone (A) and 4′,5,7-trihydroxyisoflavone (B) in gas, benzene, and water phases. The black arrows indicate the corresponding vertical coordinate of the relevant line.](image)

3. Discussion

By analyzing the data in Tables 2–4, it can be deduced that the BDEs of 4′−OH (the strongest antioxidative hydroxyl group following the HAT mechanism) are significantly smaller than the IPs and the PAs of 7−OH (the strongest antioxidative hydroxyl group following the SPLET mechanism) for the studied compounds in the gas and benzene phases. Therefore, in the gas and benzene phases, the HAT represents the thermodynamically favored process for the isoflavonoids and their derivatives when undergoing the free radical scavenging progress. The high solvation enthalpies of ionic particles induce a dramatic decrease of the PAs. In water phase, the PAs of 7−OH are significantly smaller than the lowest BDEs of 4′−OH and IPs for the studied isoflavonoids and their derivatives. Thus, SPLET is
the preferred mechanism for the isoflavonoids and their derivatives when undergoing the free radical scavenging progress in water phase.

In HAT and SPLET mechanisms, the antioxidative activity of phenolic compounds is characterized by the lowest BDE and lowest PA, respectively. In the gas and benzene phases, the electron-donating substituents placed at the C2 position would enhance the antioxidative activity of isoflavonoids via a weakening of the BDE of 4′−OH, while the electron-withdrawing groups have an opposite effect. In water phase, the electron-donating substituents placed at the C2 position would weaken the antioxidative activity of isoflavonoids via an enlarging of the PA of 7−OH, while the electron-withdrawing groups have an opposite effect. Among the studied compounds, the NH2-substituted derivative is the strongest antioxidant in the gas and benzene phases, while the NO2-substituted derivative is the strongest one in water phase.

4. Materials and Methods

4.1. Computational Details

The details of HAT (Equation (1)), SET-PT (Equations (2) and (3)), and SPLET (Equations (4) and (5)) mechanisms [7–9] are as follows:

\[
\begin{align*}
R^\bullet + \text{ArOH} & \rightarrow RH + \text{ArO}^\bullet \quad (1) \\
R^\bullet + \text{ArOH} & \rightarrow R^- + \text{ArOH}^\bullet \quad (2) \\
R^- + \text{ArOH}^\bullet & \rightarrow RH + \text{ArO}^\bullet \quad (3) \\
\text{ArOH} & \rightarrow \text{ArO}^- + H^+ \quad (4) \\
\text{ArO}^- + R^\bullet & \rightarrow \text{ArO}^\bullet + R^- \quad (5)
\end{align*}
\]

In the Equations (1)–(5), \(R^\bullet, \text{ArOH}, \text{ArO}^\bullet, \text{ArOH}^\bullet\) and \(\text{ArO}^-\) represent the free radical, the flavonoid, the flavonoid radical, the flavonoid cation radical, and the flavonoid anion respectively. The reaction enthalpies related to these mechanisms are as follows:

Equation (1): BDE (bond dissociation enthalpy). The calculated equation is:

\[
\text{BDE} = H(\text{ArO}^\bullet) + H(H^\bullet) - H(\text{ArOH})
\]

Equation (2): IP (ionization potential). The calculated equation is:

\[
\text{IP} = H(\text{ArOH}^\bullet) + H(e^-) - H(\text{ArOH})
\]

Equation (3): PDE (proton dissociation enthalpy). The calculated equation is:

\[
\text{PDE} = H(\text{ArO}^\bullet) + H(H^+) - H(\text{ArOH}^\bullet)
\]

Equation (4): PA (proton affinity). The calculated equation is:

\[
\text{PA} = H(\text{ArO}^-) + H(H^+) - H(\text{ArOH})
\]

Equation (5): ETE (electron transfer enthalpy). The calculated equation is:

\[
\text{ETE} = H(\text{ArO}^\bullet) + H(e^-) - H(\text{ArO}^-)
\]

In Equations (6)–(10), the gas and solvent phases enthalpies of H\(^+\), e\(^-\), and H\(^\bullet\) were collected from the literature [25–27].
The geometries of each compound and respective radical structure were optimized using the DFT method with M062X functional and the 6-311 + G** basis set in the gas phase and solvent phase. Single point calculations were performed using the M062X/6-311 + G** method. The optimized structures were confirmed to be real minima by no imaginary frequency. The solvation effects were computed by using the solvation model based on density (SMD) solvation model. All calculations were performed using Gaussian 09 program package [28].

4.2. Statistics Analysis

The Pearson correlation is based on the covariance matrix of the data. It is an effective method for studying a broad class of relationships among variables [29]. The Pearson correlation coefficient is the parameter evaluating the strength of relationship between two vectors. The calculated equation for the Pearson correlation coefficient ($P_{x,y}$) between two vectors $x$ and $y$ is:

$$P_{x,y} = \frac{\sum xy - \frac{\sum x \sum y}{N}}{\sqrt{(\sum x^2 - \frac{(\sum x^2)^2}{N})(\sum y^2 - \frac{(\sum y^2)^2}{N})}}$$

where $N$ refers to the size of the signature array. It is 11 in this work. The Pearson correlation coefficient is symmetrical: $P_{x,y} = P_{y,x}$.

The $P_{x,y}$ is among 1 to $-1$. The correlation between $x$ and $y$ is distinguished by the following criterions [29]:

1. $0.00 < |P_{x,y}| < 0.19$: very low correlation;
2. $0.20 < |P_{x,y}| < 0.39$: low correlation;
3. $0.40 < |P_{x,y}| < 0.69$: moderate correlation;
4. $0.70 < |P_{x,y}| < 0.89$: highly correlation;
5. $0.90 < |P_{x,y}| < 1.00$: very highly correlation.

The $P_{x,y}$ equal to 1 or $-1$ corresponds to data points lying exactly on a line, or to a bivariate distribution entirely supported on a line.

5. Conclusions

Understanding the role of substituents is of great importance for the preparation of novel phenolic compounds with enhanced antioxidative properties. In this paper, the effects of various substituents on reaction enthalpies related to HAT, SET-PT, and SPLET mechanisms of isoflavonoid derivations were investigated by the DFT method. The strongest antioxidative group of isoflavonoid is not altered by the substituents placed at the C2 position. Excellent correlations were found between the BDE/IP/PA and Hammett sigma constants. Equations obtained from the linear regression can be useful in the selection of suitable candidates for the synthesis of novel isoflavonoid derivatives with enhanced antioxidative properties. It is also found that:

1. The substituent effects on BDE and IP are different than those on PA. The electron-withdrawing groups enhance BDE and IP, while the electron-donating groups reduce the BDE and IP. The effect of the substituents on PA is contrary to this.
2. The electronic effect of the substituent on the BDE of isoflavone, IP, and PA of 4’−OH is mainly governed by the resonance effect, while that on the BDE of isoflavonane and PA of 5−OH and 7−OH is mainly controlled by the field/inductive effect.
3. In gas and benzene phases, the free radical scavenging progress of the investigated compounds will most likely undergo the HAT mechanism. In water phase, SPLET is the most favorable mechanism.
4. In the gas and benzene phases, the electron-donating substituents placed at the C2 position would enhance the antioxidative activity of isoflavonoids via a weakening of the BDE of 4’−OH. In water phase, they will reduce antioxidative activity by strengthening the PA of 7−OH. Contrary
results occur for the electron-withdrawing group. Moreover, the NH$_2$–substituted derivative is the strongest antioxidant in the gas and benzene phases, while the NO$_2$–substituted derivative is the strongest antioxidant in water phase.

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**Abbreviations**  
DFT Density Functional Theory  
HAT Hydrogen Atom Transfer  
SPLET Sequential Proton Loss Electron Transfer  
SET-PT Single Electron Transfer Followed by Proton Transfer  
ArOH Flavonoid  
BDE Bond Dissociation Enthalpy  
IP Ionization Potential  
PDE Proton Dissociation Enthalpy  
PA Proton Affinity  
ETE Electron Transfer Enthalpy  
$\sigma$ Hammett sigma constants  
$F$ field/inductive effect  
$R$ resonance effect  
$P$ Pearson correlation coefficient

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