

Spectroscopic Studies of Natural Antioxidant-containing Food Powders Beneficial for Human Health

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Abstract

Natural antioxidant-containing food powders are rich sources of bioactive compounds that may help reduce oxidative stress and support human health. This study employed ultraviolet-visible (UV-Vis), Fourier-transform infrared (FTIR), and electron spin resonance (ESR) spectroscopy to investigate the molecular composition, functional groups, and antioxidant characteristics of selected plant-derived food powders, including acai, camu, red beet, and cacao. UV-Vis spectroscopy confirmed the presence of polyphenolic compounds, while FTIR analysis identified characteristic functional groups associated with these antioxidants. ESR spectroscopy demonstrated the presence of persistent organic radicals, consistent with the ability of antioxidant compounds to stabilize radical species. Comparative ESR analysis indicated that acai exhibited the strongest radical-stabilizing characteristics, as evidenced by its narrowest ESR linewidth (4.76 G), whereas red beet exhibited the weakest, with the broadest linewidth (8.26 G) under comparable experimental conditions. The combined spectroscopic findings provide complementary insights into the optical, structural, and paramagnetic characteristics of these antioxidant-containing food powders and support further investigation of their mechanisms of radical stabilization and potential role in mitigating oxidative damage.

Keywords: Electron Spin Resonance (ESR), Fourier transform infrared radiation (FTIR), Natural Antioxidant, Phenolic Compound, UV-Vis Spectroscopy.

1. Introduction

In the human body, systems balance together to maintain a stable internal environment through a self-regulating process called homeostasis, in which there are constant adjustments of temperature, blood sugar levels, fluid balance, and other factors; these can result from internal events, or when exposed to external fluctuations or environmental stimuli (Anwar, 2022; Pomatto & Davies, 2018).

Reactive Oxygen Species (ROS) including free radicals (atoms or molecules with unpaired electrons), and non-radical types, can be produced as biproducts of cellular metabolism and from external factors such as radiation, pollutants, and inflammatory responses. ROS have dual roles in human homeostasis, where they can exert both beneficial and detrimental effects. Low to moderate levels of free radicals help to mediate essential biological functions such as cellular signaling and homeostasis, immune defense, and redox regulation of proteins (Thannickal and Fanburg, 2000). On the other hand, excess ROS (when exceeding the antioxidant defense capacity of human body) causes oxidative stress and damage cellular components like lipids, protein, and DNA, leading to serious clinical disorders such as diabetes, cancer, dementia, depression, and death (Bardaweel et al., 2018; Jahan MS et al. 2025).

Antioxidants are molecules that neutralize and regulate the free radicals by donating free electrons to satisfy the unpaired electron condition which define free radicals, thereby neutralizing them, generally rendering them harmless and stable (Jahan et al. 2025; Nimse and Pal, 2015). The human body has a defense system comprising enzymatic and non-enzymatic antioxidants to neutralize ROS and prevent oxidative damage. The enzymatic antioxidants such as Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx) etc., are produced within the body and catalyze the reactions to eliminate ROS. On the contrary, the non-enzymatic antioxidants are partially produced within the body, such as Glutathione (GSH), Coenzyme Q10 (Ubiquinone), Alpha-Lipoic Acid (ALA) etc., while a large portion of non-enzymatic antioxidants are micronutrients such as

Vitamin C (Ascorbic Acid), Vitamin E (Tocopherols & Tocotrienols), Carotenoids (Provitamin A), Polyphenols (Plant-Based Antioxidants), Flavonoids (Polyphenolic Compound) etc., which are abundantly obtained in antioxidant-rich foods (e.g. Fruits, vegetables etc.) (Munteanu & Apetrei, 2021). Such micronutrients have the ability to donate electrons and neutralize reactive species such as free radicals and non-radical ROS. However, under certain conditions, such as exposure to natural light or environmental radiation, the antioxidant compounds can undergo chemical changes, including bond breakage, which can lead to the formation of stable antioxidant radicals. Although the antioxidant properties of these beneficial micronutrients have been reported in previous studies (Zeb, 2020; Winiarska-Mieczan et al., 2023), investigations focusing on their antioxidant capacity in relation to free radical behavior remain limited.

The present study is aimed to detect the presence of antioxidant compounds, the chemical components of antioxidant molecules and the ability of antioxidant compounds to donate the electron inside some natural antioxidants-containing food powders such as acai, camu, red beet and cacao, while the antioxidant effect of these food powders to the free radicals are of further interest.

2. Molecular Structure of Natural Antioxidants

Natural antioxidant-containing food powders are composed primarily of polyphenols, carotenoids, and other naturally occurring organic molecules containing aromatic rings, conjugated π -electron systems, and oxygen-containing functional groups (Dini & Grumetto, 2022). These molecular architectures determine their electronic structure, vibrational characteristics, and free radical stabilization behavior, making them suitable systems for spectroscopic investigation. The electronic properties of these organic functional materials arise from the delocalization of π electrons within aromatic rings and conjugated molecular frameworks. Electron delocalization in conjugated π -electron systems stabilizes radical intermediates through resonance, influencing the electronic transitions, vibrational modes, and ESR characteristics of the materials (Xiao, 2022; Pirker et al., 2009). The radical stabilization behavior of polyphenolic molecular structures is governed primarily by Hydrogen Atom Transfer (HAT) and Single Electron Transfer (SET) mechanisms (Bhuyan & Handique, 2022). During HAT, a hydrogen atom is transferred from a phenolic hydroxyl group to a radical species, producing a resonance-stabilized phenoxyl radical. In the SET mechanism, electron donation generates an oxidized polyphenol radical cation whose unpaired electron is delocalized over the aromatic π -electron system. These charge-transfer processes modify the distribution of unpaired electron density and directly influence the ESR spectral characteristics of the materials. The molecular structures of representative polyphenolic compounds from plant-based food powders (acai, camu, red beet, and cacao) are shown in Figure 1, and their electron-transfer and radical stabilization mechanisms associated with conjugated π -electron systems and functional groups are illustrated in Figure 2.

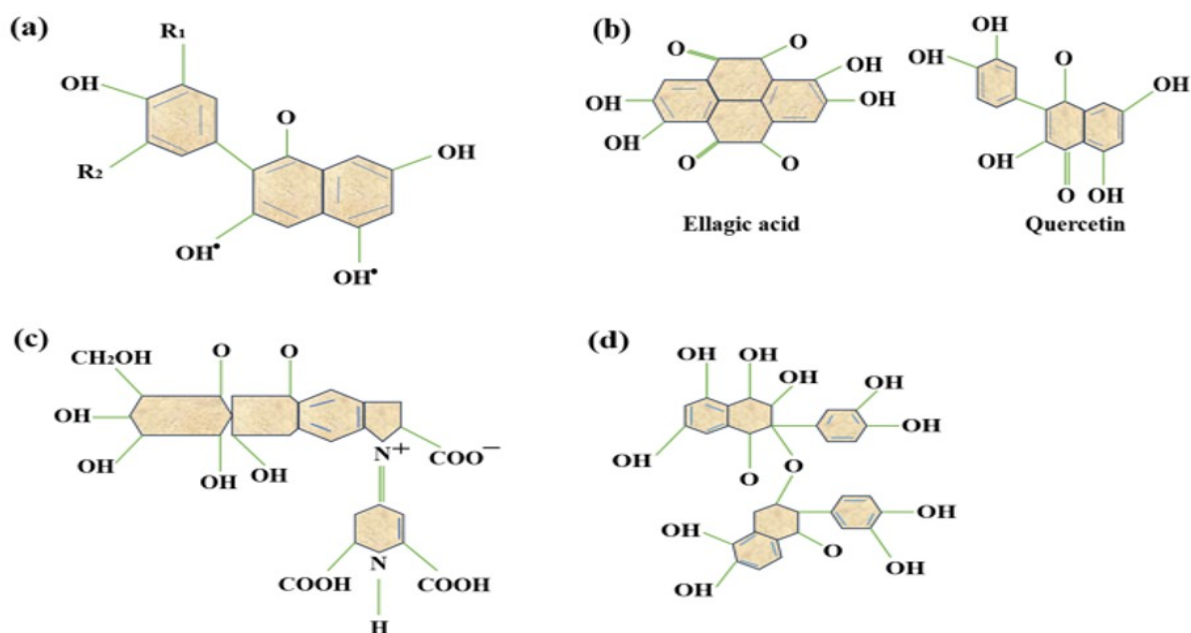


Figure 1. Molecular structure of (a) Acai, (b) Camu, (c) Red beet and (d) Cacao antioxidants

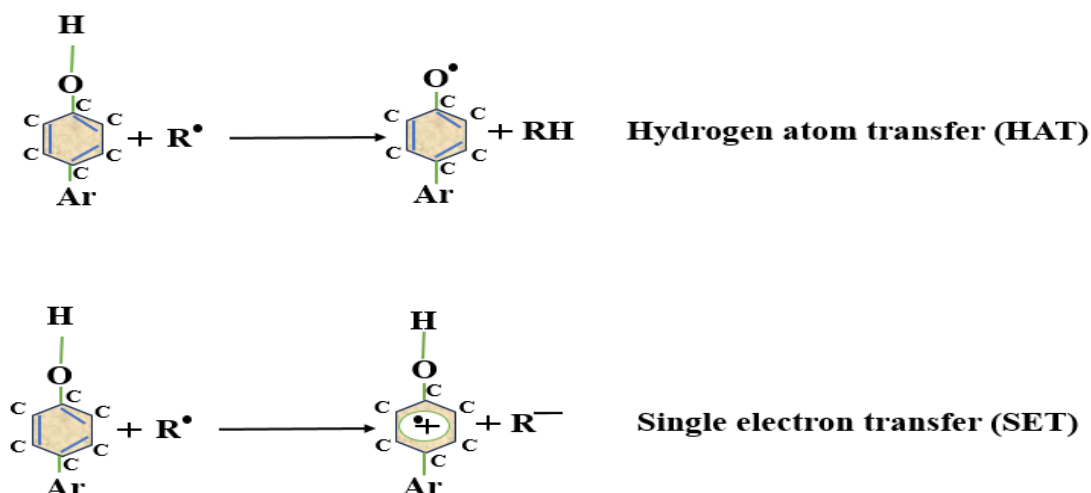


Figure 2. Reaction mechanisms of natural polyphenols as antioxidants

Depending on the number of benzene rings in the phenolic compounds and the elements that bind the rings to one another, the phenolic compounds are broadly classified into four groups: Phenolic acids, flavonoids, stilbenes, and lignans (Jahan et al., 2025; Khan et al., 2021). However, plant-based antioxidants (such as from fruits and vegetables) contain mainly polyphenols, carotenoids, and vitamins such as E and C (Xu et al., 2017).

3. Materials and Methods

3.1 Selected Materials

Commercially available food-grade powders of organic acai (*Euterpe oleracea*), organic camu (*Myrciaria dubia*), and organic cacao (*Theobroma cacao*) (Navitas Organics, USA), together with red beet (*Beta vulgaris*) powder purchased from Sprouts Farmers Market (Memphis, Tennessee, USA), were used in this study. The powders were used as received without further chemical modification. Prior to analysis, the powders were gently mixed to ensure homogeneity and directly loaded into the appropriate sample holders for UV-Vis, FTIR, and ESR measurements. All samples were stored in their original sealed containers under dry laboratory conditions at ambient temperature until characterization.

3.2 Characterization of the Samples

A comprehensive spectroscopic characterization was performed to investigate the molecular properties and antioxidant potential of natural antioxidant-containing food powders, including acai, camu, red beet, and cacao. UV-Vis spectroscopy was employed to examine the electronic absorption characteristics associated with antioxidant compounds using an Evolution 220 UV-Visible spectrometer (Thermo Scientific). The 220–400 nm wavelength region was selected as it encompasses the principal UV absorption features observed in all four powder samples, as well as the spectral region associated with absorption by aromatic and other UV-absorbing constituents, including phenolic and flavonoid components. The integrated area of absorbance over this wavelength range was calculated for each sample to provide a semi-quantitative comparison of the overall UV-absorbing response. The integrated area obtained for acai powder was used as the reference, and the values for the other samples were normalized accordingly to obtain a relative UV response index.

Fourier Transform Infrared (FTIR) spectroscopy (Nicolet iS 10, Thermo Fisher Scientific) was used to identify the characteristic functional groups and molecular structures present in the powder samples.

Electron Spin Resonance (ESR) spectroscopy was performed using an X-band Bruker EMX 300 spectrometer operating at a microwave frequency of 9.8 GHz and a magnetic field modulation frequency of 100 kHz to evaluate the free radical scavenging ability and detect stable antioxidant radicals. ESR is one of the most direct and reliable techniques for detecting and characterizing paramagnetic species. ESR measurements were carried out over a 200 G sweep width with the resonance magnetic field centered at 3510 G. The microwave power and modulation amplitude in ESR technique were optimized to obtain spectra with improved signal-to-noise ratios. Baseline correction of ESR signal was performed using the Bruker WINEPR software.

4. Result and Discussion

4.1 UV-Vis spectroscopy measurement

The UV-Vis spectra of the antioxidant samples shown in Figure 3 were obtained directly from the powder samples over the wavelength range of 200–800 nm. The integrated absorbance area over the 220–400 nm region was calculated from the spectral data to provide a semi-quantitative comparison of the relative UV-absorbing response among the samples. The integrated absorbance areas were 0.49, 2.72, 2.87, and 1.19 for the acai, camu, cacao, and red beet samples, respectively. These values were then normalized to the acai sample, which was selected as the reference, to obtain a relative UV response index. The resulting normalized values were 1.00, 5.55, 5.86, and 2.43 for acai, camu, cacao, and red beet, respectively. Cacao antioxidant powder exhibited the highest integrated UV absorbance in the 220–400 nm region, with a relative response approximately 5.86-fold higher than that of the reference acai sample. Thus, a higher integrated absorbance indicates a greater relative UV-absorbing response within the selected spectral region and may reflect differences in the relative abundance of UV-absorbing phenolic, flavonoid, and other aromatic constituents. However, in the absence of independent measurements of total phenolic content or total flavonoid content, as well as appropriate calibration standards, the integrated absorbance values were interpreted as a semi-quantitative measure of relative optical response rather than as an absolute measure of phenolic or flavonoid concentration.

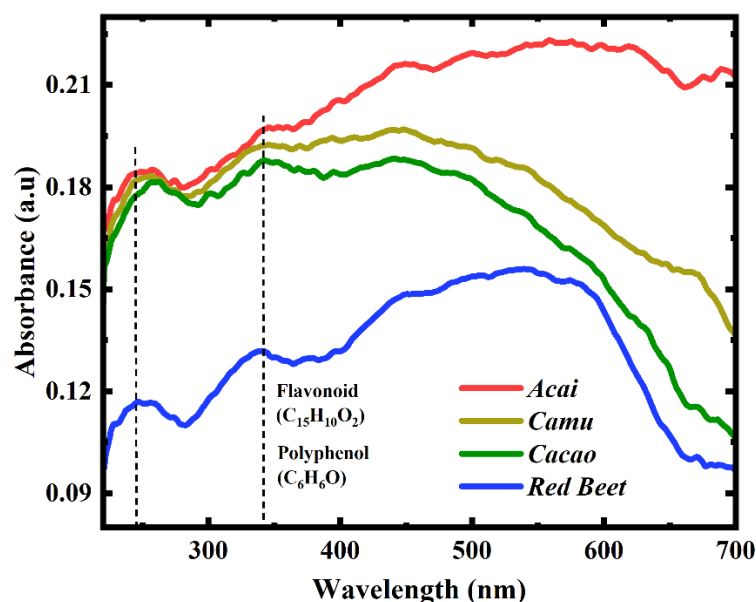


Figure 3. UV-Vis spectroscopy measurements of the selected antioxidant-containing organic powder samples of Acai, Camu, Cacao and Red beet, as received

4.2 FTIR Analysis

Fourier transform infrared radiation (FTIR) analysis was conducted to determine the functional groups and existing bonds within the antioxidant molecules. FTIR spectra (Figure 4) showed bands indicating the presence of carbon (C), hydrogen (H), and oxygen (O) from polyphenol and flavonoid compounds. The band at 908.34 cm^{-1} is attributed to the aromatic C-H out-of-plane bending vibration. The stretching of C-O-C from ester appeared at the band position of 1049.13 cm^{-1} . The band observed at 1380.04 cm^{-1} corresponds to the C-O stretching vibration. The band position at 1459.15 cm^{-1} denotes the bending of C-H of methylene on aromatic rings (Wang et al., 2014). The vibration of aromatic C=C stretching appeared at 1654.69 cm^{-1} . The bending of C=O stretching was found around 1741.48 cm^{-1} . The peaks near 2028.84 cm^{-1} and 2163.83 cm^{-1} also denote the bending stretch of C=O. The peak position around 2362.47 cm^{-1} was caused by C-H bending vibration. The strong bands observed at 2850.39 cm^{-1} and 2917.89 cm^{-1} can be attributed to the symmetric and asymmetric CH_2 and CH stretching vibration of polyphenol compounds. The band position around 3317.11 cm^{-1} is assigned to the OH stretching vibration of polyphenols (Hamimed et al., 2020).

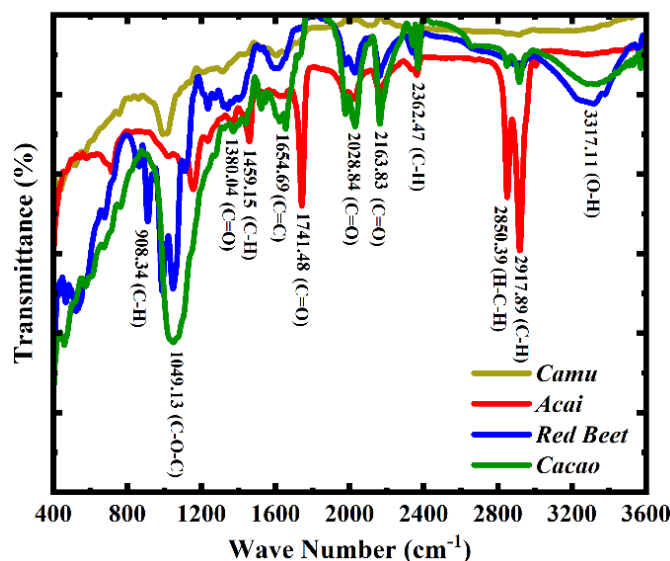


Figure 4. Results of FTIR analyses of the selected antioxidant containing organic powder samples of Camu, Acai, Red beet and Cacao as-received

4.3 Antioxidant Radical Detection Using ESR

The detection of phenolic radicals at normal temperature and environmental conditions indicates a strong antioxidant ability of the selected food powders, while the antioxidant radicals become stable, safe and unreactive radicals through the resonance of unpaired electron across the aromatic structure. The ESR tests were conducted to detect and analyze antioxidant radicals in four organic antioxidant-rich food powders acai, camu, red beet and cacao.

4.3.1 ESR Peak-to-Peak Linewidth (ΔH_{pp}) and Radical Stability

The ESR spectra of four organic antioxidant-rich powders (acai, camu, red beet, and cacao), shown in Figures 5(a–d), provide insight into the nature and magnetic environments of their associated paramagnetic species.

The ESR spectrum of the organic acai powder, shown in Figure 5(a), indicates the presence of paramagnetic species associated with antioxidant constituents, including anthocyanins and other polyphenolic compounds. These compounds can participate in radical-scavenging processes through hydrogen-atom transfer (HAT) or single-electron transfer (SET), producing antioxidant-derived radical species. Acai exhibited the narrowest peak-to-peak linewidth (ΔH_{pp}) of 4.76 G among the investigated powders. The relatively narrow linewidth suggests a comparatively homogeneous magnetic environment, with potentially less pronounced contributions from radical–radical interactions and unresolved hyperfine contributions. However, ΔH_{pp} alone does not establish the extent of electron delocalization or radical stability.

The ESR spectrum of organic camu powder, shown in Figure 5(b), indicates paramagnetic species associated with phenolic constituents, including quercetin, cyanidin-3-glucoside, ellagic acid, and ellagitannins (Jahan et al., 2025). Camu exhibited a slightly broader ΔH_{pp} of 5.18 G compared with acai, indicating a modest increase in ESR line broadening. This difference may reflect variations in the local magnetic environment and the chemical composition of the radical species present in the powder matrix. The presence of multiple phenolic constituents may contribute to differences in the distribution and local magnetic environments of paramagnetic species within the camu powder matrix.

In Figure 5(c), red beet powder exhibited the broadest ΔH_{pp} of 8.26 G, indicating the greatest degree of ESR line broadening among the four samples. Red beet is rich in betalains, particularly betacyanins and betaxanthins, which possess conjugated structures containing multiple oxygen- and nitrogen-containing functional groups. These structural features can influence the distribution of spin density and the local electronic environment of the unpaired electrons. The broad linewidth may reflect contributions from radical–radical interactions, unresolved hyperfine coupling, and heterogeneity within the betalain-rich matrix. Because the measurements were performed on solid powders, differences in molecular packing, aggregation, and the spatial distribution of paramagnetic centers may also influence spin–spin interactions and contribute to the observed line broadening.

In Figure 5(d), organic cacao powder exhibited a relatively broad ΔH_{pp} of 7.63 G, which was lower than that of red beet but substantially higher than those of acai and camu. The observed broadening may reflect a comparatively heterogeneous radical environment and appreciable magnetic interactions within the polyphenol-rich matrix. Cacao contains procyanidin-rich polyphenolic compounds with conjugated aromatic structures and multiple hydroxyl groups that can provide pathways for spin delocalization in antioxidant-derived radicals. Nevertheless, the relatively broad ΔH_{pp} should not be interpreted directly as evidence of lower radical stability, because linewidth is influenced by multiple magnetic interactions and relaxation processes.

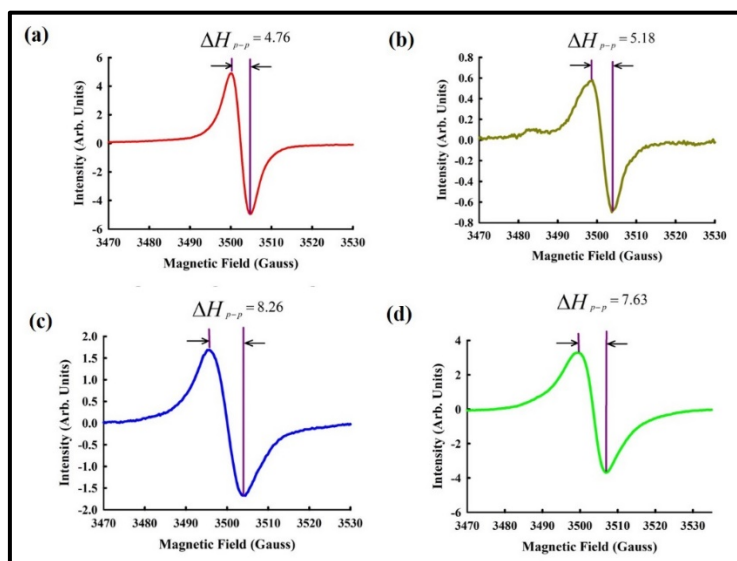


Figure 5. The ESR spectrum of (a) Acai, (b) Camu, (c) Red beet and (d) Cacao antioxidant organic powder samples as received

The ESR peak-to-peak linewidths (ΔH_{pp}) of four antioxidant-rich food powders has been reported in Table 1 within experimental error. Overall, the differences in ΔH_{pp} indicate variations in the magnetic environments of the paramagnetic species; however, linewidth alone is insufficient to establish a definitive ranking of radical stability.

Table 1. Peak to peak linewidth (ΔH_{pp}) of antioxidant-rich organic food powders

Samples	ΔH_{pp} (G)
Acai	4.76 ± 0.15308
Camu	5.18 ± 0.115518
Red beet	8.26 ± 0.13
Cacao	7.63 ± 0.235608

4.3.2 ESR g-Value and Radical Characteristics

The ESR spectra of the four antioxidant-rich organic food powders are also presented as a function of the g-factor in Figure 6. As reported in Table 2, the g-values showed only small differences among the four food powders, ranging from 2.00521 for acai to 2.00578 for cacao. Acai showed the lowest g-value, while cacao showed the highest, with red beet (2.00574) showing a value close to cacao. These small differences indicate that the unpaired electrons experience generally similar electronic environments. The slight variation in g-value may be related to differences in the conjugated molecular structures and oxygen- or nitrogen-containing functional groups of the antioxidant compounds, which can affect the distribution of spin density. The similar g-values of red beet and cacao suggest comparable local electronic environments of their dominant paramagnetic species, although their antioxidant compounds have different molecular structures. Since these are complex food powders containing

multiple compounds, the measured g-values represent the overall response of the paramagnetic species present in each sample and should not be used alone to determine radical stability or the exact degree of electron delocalization.

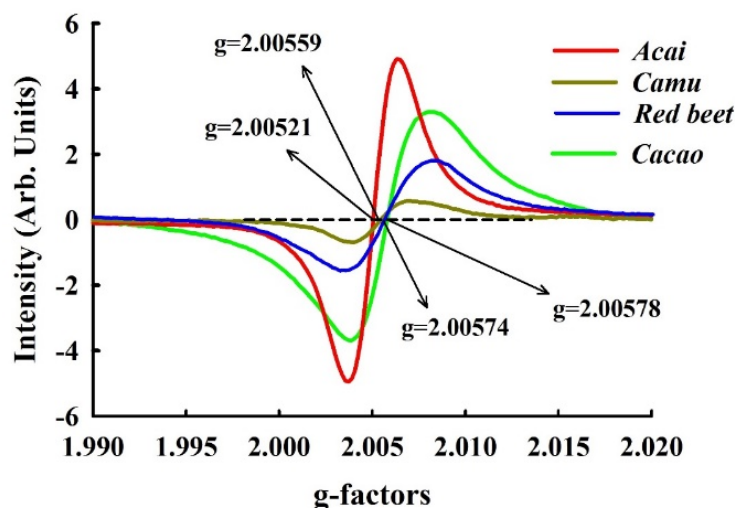


Figure 6. ESR spectra as a function of g-factors of four different antioxidant-rich organic food powders (Acai, Camu, Red beet and Cacao)

Table 2. g values of antioxidant-rich organic food powders

Samples	g-value
Acai	2.00521 ± 0.000081
Camu	2.00559 ± 0.000057
Red beet	2.00574 ± 0.000032
Cacao	2.00578 ± 0.000045

The g-values obtained in this study are comparable with those reported for other plant-based food materials. Previous ESR studies of *Beta vulgaris* (red beet) reported an organic radical signal near $g = 2.01$, which is close to the value observed for red beet in the present study $g = 2.00574$ (Raouafi et al., 2022).

ESR studies have also detected natural free radicals in vegetables, confirming the presence of paramagnetic species in plant-based foods (Lakshmi Prasuna et al., 2008).

For açai, previous EPR studies have reported radical-scavenging activity associated with its anthocyanin and polyphenolic compounds (Teixeira-Neto et al., 2012). These findings support that the observed ESR signals are associated with antioxidant-related organic paramagnetic species.

4.3.3 ESR signal intensity and relative radical concentration

The bar graph presented in Figure 7 shows the relative radical/spin concentration per unit mass of the four antioxidant-containing food powders: açai, camu, red beet, and cacao. The relative radical concentrations, estimated from the double-integrated ESR signals under comparable experimental conditions, varied substantially among the samples, following the order cacao > açai > red beet > camu. Cacao exhibited the highest concentration of ESR-detectable radical species, whereas camu showed the lowest. These differences indicate variations in the abundance of persistent paramagnetic species among the samples.

However, ESR-detectable radical concentration should not be interpreted directly as antioxidant donating capacity. ESR measures the concentration of paramagnetic species that remain detectable under the experimental conditions, which may include relatively persistent antioxidant-derived radicals. Therefore, a higher ESR signal does not

necessarily indicate greater antioxidant activity, but may instead reflect a greater abundance and/or persistence of ESR-detectable radical species.

The radical concentration should therefore be interpreted together with the ESR spectral parameters, particularly the peak-to-peak linewidth (ΔH_{pp}) and g-value. The linewidth provides information about the magnetic environment and interactions of the unpaired electron, whereas the g-value provides information about its electronic environment. A narrower linewidth may be consistent with reduced spin–spin interactions and a more homogeneous radical environment, whereas broader linewidths may reflect stronger spin–spin interactions and/or greater heterogeneity. In the present study, radical concentration did not follow the same trend as ΔH_{pp} or g-value. Acai, for example, exhibited the narrowest linewidth (4.76 G), whereas cacao showed the highest relative radical concentration with a broader linewidth (7.63 G). This difference demonstrates that radical abundance and spectral characteristics provide complementary information about the ESR-active species. Because ESR intensity, linewidth, and g-value can be influenced by molecular composition, radical structure, spin–spin interactions, and experimental conditions, these parameters should be interpreted collectively rather than as direct measures of antioxidant activity.

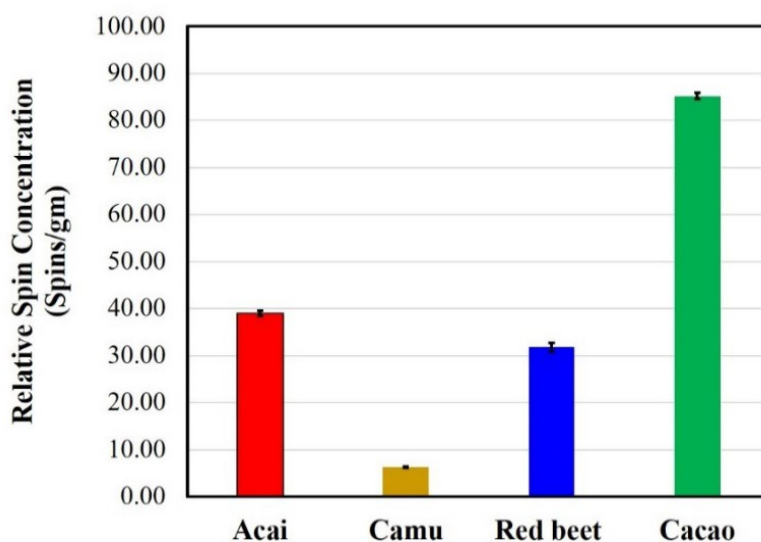


Figure 7. Free radical concentration in four different antioxidant-rich organic food powders (Acai, Camu, Red beet and Cacao)

5. Conclusion

This study characterized acai, camu, red beet, and cacao food powders using UV-Vis, FTIR, and ESR spectroscopy. UV-Vis analysis showed different relative UV-absorbing responses in the 220–400 nm region, with cacao exhibiting the highest response, followed by camu, red beet, and acai. FTIR spectra confirmed the presence of diverse oxygenated and hydrocarbon-containing functional groups, including O–H, C–O/C–O–C, C=O, aromatic C=C, and C–H groups. ESR analysis revealed substantial differences in ESR-detectable paramagnetic species, with the relative radical concentration following the order cacao > acai > red beet > camu. Acai exhibited the narrowest ESR linewidth (4.76 G), whereas red beet showed the broadest linewidth (8.26 G), while the g-values remained close to 2.00 for all samples. Based on the ESR spectral characteristics, the narrower ESR linewidth observed for acai is consistent with greater radical delocalization and stabilization, whereas the broader linewidth observed for red beet suggests comparatively lower radical stabilization. These findings demonstrate that radical abundance and ESR spectral characteristics vary independently among the powders. Overall, the combined UV-Vis, FTIR, and ESR results provide complementary information on the optical, structural, and paramagnetic properties of antioxidant-containing food powders. ESR radical concentration should therefore be interpreted as the abundance of ESR-detectable species rather than as a direct measure of antioxidant capacity.

5.1 Study Limitations

The antioxidant powders contain a complex mixture of naturally occurring compounds, and the observed UV–Vis, FTIR, and ESR responses therefore reflect the combined contributions of these constituents. The individual contribution of a specific compound or radical species cannot be determined from these measurements alone. The measured ESR g-values and ΔH_{pp} values provide information about the overall electronic and magnetic environments of the paramagnetic species but cannot independently determine their exact structures or stability. In addition, because the samples were analyzed as solid powders, differences in molecular packing and aggregation may influence the observed spectroscopic responses.

5.2 Future Research

Future studies could focus on identifying the individual antioxidant compounds and radical species responsible for the observed spectroscopic responses using complementary analytical techniques such as Liquid Chromatography–Mass Spectrometry (LC-MS) or High-Performance Liquid Chromatography (HPLC) and detailed ESR measurements. Further ESR studies using appropriate reference compounds and controlled experimental conditions could provide a better understanding of the relationship between molecular structure, spin distribution, and ESR parameters. Investigating the effects of individual antioxidants on free radical generation, scavenging, and stabilization could further clarify their specific contributions to the observed ESR responses. Studies of isolated antioxidant compounds and their solid-state behavior could also help clarify the effects of molecular packing and aggregation on radical formation and stability.

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Conflict of Interest

On behalf of all authors, there is no conflict of interest.

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