Evaluation of phenolic constituents and antioxidant potential of green coffee beans from Planalto de Conquista (Bahia) by Multivariate Analysis

Avaliação de constituintes fenólicos e potencial antioxidante de grãos de café verde do Planalto de Conquista (Bahia) por meio de Análise Multivariada

Evaluación de los constituyentes fenólicos y del potencial antioxidante de los granos de café verde de la Meseta de Conquista (Bahía) mediante análisis multivariante

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Abstract
The chemical composition and bioactivity of green coffee is influenced by both intrinsic and extrinsic factors, and it is a rich source of bioactive compounds, including phenolic compounds, alkaloids, and many compounds with antioxidant and anti-inflammatory properties. These characteristics have sparked numerous studies on its chemical composition and, consequently, have been attributed numerous advantages and disadvantages for human health. This study aimed to quantify the total phenolic constituents and analyze the in vitro antioxidant activity of green coffee from the Conquista Plateau region, Bahia, Brazil, and separate them according to their antioxidant potential. The total phenolic content (TPC) was determined and the evaluation of the in vitro antioxidant potential was investigated by the DPPH radical scavenging methods and the β-carotene:linoleic acid (BCLA) system co-oxidation method. Principal component analysis (PCA) was applied to reveal which samples showed higher and lower antioxidant potential. The results showed that the samples presented moderate content of total phenolic constituents 27.02 mg. g^{-1} of sample, low primary antioxidant activity with the average value of 65.17 EC_{50} mg. g^{-1} sample and moderate secondary antioxidant activity with 44% protection from oxidation of the β-carotene molecules present, moderately inhibiting the conditions favorable to oxidation that were inserted during the analysis. The principal components analysis proved efficient in the separation and classification of the green coffee samples, allowing the observation of the dispersion and heterogeneity among the samples, demonstrating different composition and bioactivity profiles.
Keywords: Food analysis; Bioactive compounds; Quality control; Chemometrics.

Resumo
A composição química e bioatividade do café verde é influenciada por fatores intrínsecos e extrínsecos, sendo ele uma uma rica fonte de compostos bioativos, incluindo compostos fenólicos, alcalóides e muitos compostos com propriedades antioxidantes e anti-inflamatórias. Essas características têm despertado numerosos estudos sobre sua composição química e, consequentemente, têm sido atribuídas inúmeras vantagens e desvantagens para a saúde humana. Esse estudo teve como objetivo quantificar os constituintes fenólicos totais e analisar a atividade antioxidante in vitro do café verde da região do Planalto de Conquista, Bahia, Brasil, e separá-las de acordo o potencial antioxidante. Determinou-se o teor de fenólicos totais (CFT) e a avaliação do potencial antioxidante in vitro foi investigada pelos métodos de captação do radical DPPH e pelo método da co-oxidação do sistema β-

caroteno:ácido linoleico (BCAL). A análise de componentes principais (ACP) foi aplicada para revelar quais amostras apresentaram maior e menor potencial antioxidante. Os resultados mostraram que as amostras apresentaram moderado teor de constituintes fenólicos totais 27,02 mg. g^{-1} de amostra, baixa atividade antioxidante primária com o valor médio de 65,17 CE50 mg. g^{-1} de amostra e moderada atividade antioxidante secundária com 44% de proteção da oxidação das moléculas de β-caroteno presentes, inibindo moderadamente as condições favoráveis à oxidação que foram inseridas durante a análise. A análise de componentes principais mostrou-se eficiente na separação e classificação das amostras de café verde, possibilitando observar a dispersão e heterogeneidade entre as amostras, demonstrando diferentes perfis de composição e bioatividade.

Palavras-chave: Análise de alimentos; Compostos bioativos; Controle de qualidade; Quimiometria.

Resumen

La composición química y la bioactividad del café verde están influidas por factores intrínsecos y extrínsecos. El grano de café es una rica fuente de compuestos bioactivos, como los compuestos fenólicos, los alcaloides y muchos compuestos con propiedades antioxidantes y antiinflamatorias. Estas características han suscitado numerosos estudios sobre su composición química y, en consecuencia, se han atribuido numerosas ventajas e inconvenientes para la salud humana. Este estudio tuvo como objetivo cuantificar los constituyentes fenólicos totales y analizar la actividad antioxidante in vitro del café verde de la región de la Meseta de Conquista, Bahía, Brasil, y separarlos según su potencial antioxidante. Se determinó el contenido fenólico total (CFT) y se investigó la evaluación del potencial antioxidante in vitro por los métodos de barrido de radicales DPPH y por el método de co-oxidación del sistema β-caroteno: ácido linoleico (BCLA). Se aplicó el análisis de componentes principales (PCA) para revelar qué muestras mostraban un mayor y menor potencial antioxidante. Los resultados mostraron que las muestras presentaron un contenido moderado de constituyentes fenólicos totales 27,02 mg. g^{-1} de muestra, una baja actividad antioxidante primaria con el valor medio de 65,17 EC50 mg. g^{-1} de la muestra y una actividad antioxidante secundaria moderada con 44% de protección frente a la oxidación de las moléculas de β-caroteno presentes, inhibiendo moderadamente las condiciones favorables a la oxidación que se insertaron durante el análisis. El análisis de componentes principales fue eficiente en la separación y clasificación de las muestras de café verde, permitiendo observar la dispersión y heterogeneidad entre las muestras, demostrando diferentes perfiles de composición y bioactividad.

Palabras clave: Análisis de alimentos; Compuestos bioactivos; Control de calidad; Quimiometría.

1. Introduction

The coffee bean is a unique product of high economic and historical importance since the days of the café au lait policy. According to Kulapichitr et al. (2022), it is the second most economically important commodity, second only to oil. Green coffee, also called raw coffee, characterized by the unroasted seeds of the fruit, has beneficial properties that contribute to an increased interest in incorporating it into various food products (Świeca et al., 2017). When roasted, it becomes one of the most desired natural non-alcoholic beverages worldwide and this has significantly increased its popularity in recent years due to its pleasant taste sensation and its attractive beneficial health effects (Cloete et al., 2019; Dong et al., 2019).

World coffee production was approximately 10.2 million tons in 2018/2019 (ICO, 2019), thus showing the economic impact of this product in the world. Brazil leads the ranking of coffee production and export, with the state of Minas Gerais holding 51.7% of national production, followed by Espírito Santo (21.2%), São Paulo and Bahia (6.6%) (Brainer, 2020). The chemical composition of green coffee beans is influenced by intrinsic and extrinsic factors in a comprehensive way, such as its species, variety, soil condition, altitude, climate, cultivation and processing methods, varying according to the region (Lucci, Pacetti & Frega, 2015). It consists of lipids, proteins, glycides, minerals, besides several components such as trigonelline acids and caffeine that contribute to the prevention of the development of diseases.

Green coffee beans are rich dietary sources of bioactive compounds, particularly those derived from hydroxycinnamic acid. Phenolic compounds are common secondary metabolites in higher plants that perform vigorous antioxidant activity and generally defend against pests, pathogens, and ultraviolet radiation (Farah & Donangel, 2006; Somporn et al., 2011). Phenolic compounds, in addition to being reported as contributors to the characteristic flavor and aroma of coffee beverages, are known and important due to the physiological and pharmacological properties they confer to human health, such as antioxidant activity. Green coffee beans contain several phenolic compounds that exhibit antioxidant capacity, such as chlorogenic, caffeic, ferulic, and coumaric acids (Abrahão, 2010; Liang and Kitts, 2016). Chlorogenic acid is the most abundant phenolic acid in the

beans, present mainly in the form of 5-O-caffeoylquinic acid, which consists of caffeic acid esterified with quinic acid (Moon et al., 2009; Moreira et al., 2015).

Currently, it is considered a functional beverage due to being a rich source of bioactive compounds, including phenolic compounds, alkaloids, and other novel compounds with antioxidant and anti-inflammatory properties (Paiva et al., 2019; Dong et al., 2021; Esquivel & Jiménez, 2012). Due to the functionality of these substantial bioactive compounds present in its composition, several researchers have pointed out that regular coffee consumption may reduce the risk of occurrence of some chronic diseases, including Parkinson's, type 2 diabetes, cardiovascular and autoimmune diseases, as well as certain types of cancer (Harumi Kondo, 2012; Król et al., 2020).

As presented, coffee is a very popular beverage, consumed worldwide and with great antioxidant potential. For this reason, it has triggered numerous studies on its chemical composition as well as its advantages and disadvantages for human health. Due to this, the objective of this study was to quantify the total phenolic constituents and analyze the in vitro antioxidant activity of green coffee beans, and to apply the principal component analysis (PCA) to reveal the antioxidant potential of the samples.

2. Methodology

According to a study by Gunther, H. (2006), the research conducted in this study was quantitative, qualitative and experimental, since the bench analyses aimed to quantify the bioactive composition of green coffee and subsequently classify them into groups with high, moderate and low antioxidant potential

2.1 Samples preparation

Twenty-two samples of green coffee were obtained directly from different producers in the region of Planalto de Conquista, state of Bahia, Brazil, guaranteeing heterogeneity among the samples. The samples were cleaned and ground in a ball mill in the laboratory of the Universidade Estadual do Sudoeste da Bahia until a homogeneous powder with fine particle size (0.59 mm) was obtained (Oliveira et al., 2014). The powder obtained was used in the production of the extract used for further analyses.

The extracts were produced according to the methodology proposed by Costa et al. (2014), with some adaptations. Approximately 5 grams of sample were weighed in a falcon tube and distilled water was added to reach a volume of 50 mL. The mixture was vortexed for 1 minute, placed in an ultrasonic bath for 15 minutes, and centrifuged at 5000 rpm for 15 minutes, respectively. After extraction, the mixture was filtered with qualitative filter paper (80 gm-2) (Unifil) and stored at -18 ℃ until the analysis.

2.2 Content of total phenolics

The total phenolic content was determined by the Folin Ciocalteau colorimetric method, with adaptations (SINGLETON; ORTHOFER; LAMUELA-RAVENTÓS, 1998). The reaction was prepared with an aliquot of 0.5 mL of green coffee extract, 2.5 mL of 10% aqueous Folin-Ciocalteau solution, and 2.0 mL of 7.5% sodium carbonate. This mixture was placed in a 45°C water bath for 15 minutes. It was then removed and kept in the dark at room temperature for 30 minutes. The absorbance was measured in a spectrophotometer (Schimadzu Model UV-1800) at 750 nm. Using a calibration curve prepared with a known concentration of gallic acid standard solution (0.03 to 0.2 mg gallic acid. mL^{-1} of solution) under the same conditions, the content of total phenolic constituents was expressed as gallic acid equivalent (GAG) (mg GAG /g sample).

2.3 Antioxidant capacity by the DPPH method

The in vitro antioxidant activity of coffee beans was determined by the 2.2-diphenyl-1-picrylhydrazyl-DPPH method, which consists in the capture of the DPPH radical by antioxidants leading to a reduction of the compound and, consequently, a decrease in the absorbance of the solution (Brand-Williams et al., 1995). Five dilutions of the extracts (1.0; 3.0; 5.0; 10.0 and 15.0 mg green coffee. mL^{-1}) were prepared in triplicate. A 0.1 mL aliquot referring to each dilution of the extract was transferred to test tubes containing 3.9 mL of the ethanolic solution (Ethanol - absolute alcohol 99.8%) of the DPPH radical that had the initial absorbance adjusted to the range of 0.6 to 0.7 in a spectrophotometer (Schimadzu model UV-1800) at 515 nm. The tubes were incubated for 30 minutes in the dark at room temperature, and then the reduction of the DPPH free radical was measured by reading the absorbance at 515 nm. In parallel, the same procedure was performed using ethanol, which was considered as the blank. The EC50 value (extract concentration required to sequester 50% of the DPPH radical) was calculated from the linear equation of the straight line, based on the extract concentrations and their respective percentages of DPPH radical sequestration.

2.4 Antioxidant capacity by the co-oxidation method of the β-carotene:linoleic acid system

The antioxidant analysis by the co-oxidation method of the β-carotene:linoleic acid system was described by Miller (1971). Green coffee extracts were used at a concentration of 5 mg. mL^{-1}. Aliquots of 5 mL of the emulsion containing the β-carotene: linoleic acid system were transferred to tubes containing 0.5 mL of the extracts, performed in triplicate. After mixing, the tubes were homogenized and the absorbances were read immediately at 470 nm. After the first reading, the tubes were incubated in a 50°C water bath, favoring the oxidation reaction. At intervals of fifteen minutes, the reading was repeated for up to 120 min of reaction. The results were presented as percentage of oxidation inhibition. The reduction of the absorbance of the system without a sample is considered as 100% of the oxidation.

2.5 Statistical Analysis

The results of the antioxidant analyses were verified by analysis of variance (ANOVA) and subjected to the Tukey test for comparison of means at a 5% significance level and, subsequently, these results were subjected to Principal Component Analysis (PCA) using the Statistical Analysis System (SAS)® University Edition statistical program.

2.5.1 Principal Component Analysis (PCA)

The principal component analysis (PCA) was performed according to the methodology described by Mingotti (2005), where first an n x p matrix (rows x columns) was organized, whose rows of this data matrix correspond to the green coffee samples, while the columns correspond to the variables analyzed in each sample. The chemical properties of the green coffee extracts (TPC, DPPH and BCLA) were used as variables.

PCA was used in order to reduce the dimensions of the original variables without loss of information, since its main objective is to reduce the dimensionality of the data set while preserving the maximum information. The number of principal components was chosen using the criterion of cumulative variance evaluation, where the first PCs, accumulating percentage of variance greater than 70% and an Eigenvalue greater than one were chosen (Kulapichitr et al., 2022).

3. Results and Discussion

3.1 Total phenolic constituents and antioxidant capacity

Table 1 shows the values of the 22 green coffee samples analyzed for the content of total phenolic constituents (TPC), antioxidant activity by DPPH and protection of the β-carotene/linoleic acid co-oxidation (BCLA) system and their respective

means and standard deviations.

According to univariate analysis, the samples differ statistically among themselves ($p < 0.05$). It can be seen that there is no pattern formation among the samples with the data of phenolic constituents and antioxidant analysis. Thus, evidencing the need for the application of multivariate statistics.

Table 1. Total phenolic constituents and antioxidant potential of green coffee from Planalto de Conquista (Bahia)

Samples	TPC (mg catechin. g^{-1} of sample)	DPPH (EC$_{50}$ mg. g^{-1} of sample)	BCLA (% extract protection)
C1	21.92[u]	55.88[v]	49.24[h]
C2	21.48[v]	65.90[k]	42.80[k]
C3	37.83[b]	62.63[n]	47.56[i]
C4	31.32[c]	59.79[p]	40.55[n]
C5	38.40[a]	59.31[r]	41.35[m]
C6	25.00[q]	73.78[e]	21.38[s]
C7	27.35[j]	73.34[f]	52.67[f]
C8	22.26[t]	59.46[q]	52.56[g]
C9	26.08[o]	85.37[b]	36.50[p]
C10	26.85[l]	74.48[d]	17.23[u]
C11	25.99[p]	62.97[m]	65.15[a]
C12	27.69[i]	58.01[t]	57.37[d]
C13	26.54[m]	57.54[u]	45.24[j]
C14	30.15[f]	70.67[i]	36.11[q]
C15	27.84[h]	82.90[c]	34.78[r]
C16	24.73[r]	70.84[h]	42.13[l]
C17	26.41[n]	58.35[s]	59.31[c]
C18	28.47[g]	64.43[l]	64.80[b]
C19	30.21[e]	67.48[j]	37.76[o]
C20	27.19[k]	72.23[g]	55.23[e]
C21	30.53[d]	60.64[o]	19.03[t]
C22	23.79[s]	103.97[a]	47.56[i]
AVERAGE ± DP	27.02 ± 4.3309	65.17 ± 11.4321	44.02 ± 13.3459

[A-V] Values with different letter in a same column are significantly different within green coffees ($p < 0.05$). Source: Authors (2021).

Phenolic compounds are secondary metabolites synthesized by any part of the plant under both balanced and stressed conditions (Kraljic et al., 2015). Polyphenols are considered the most abundant antioxidants and are present in food sources such as fruits and beverages from plants, for example, fruit juices, tea, red wine, and coffee (Scalbert et al., 2005). The average value of phenolic compounds in the present study was 27.02 mg. g^{-1}, being similar to that reported by Stelmach et al. (2015) who analyzed green coffee coming from different regions of the world (Brazil, India, Nicaragua, Salvador, Ethiopia, Peru, Guatemala, Costa Rica, Colombia) and found an average content of 28.4 mg. g^{-1} of sample. The results obtained were higher than those found by Abrahão et al. (2010) who found 5.10 mg. g^{-1} in green coffee from the south of Minas Gerais state and to that reported by Murthy et al. (2012) who found 4.55 mg. g^{-1} in green coffee sample.

Higher results were reported by Cheong et al. (2012), being 31.01 mg. g^{-1} of phenolic compounds in green coffee samples from Indonesia, Tailand and China. Zhu et al. (2021) verified the influence of geographic origin on the content of phenolic compounds in green coffee and found that Brazilian beans vary from 37.56 – 43.85 mg. g^{-1}, while the highest values found are from beans coming from Indonesia, ranging from 44.15 to 50.57 mg. g^{-1}. In view of this variability it can be stated that there is a great influence of the factors of the producing region on the content of phenolics in the samples of green coffee.

DPPH analysis indicates the primary antioxidant action and is one of the best known mechanisms used as a standard test to evaluate the antioxidant activity of specific compounds or extracts, due to measuring how much of a free radical can be inhibited by a given compound (Amarowicz & Pegg, 2019). The concentration values of the extracts required to inhibit 50% of DPPH radicals (EC50) were high, with the average value being 65.17 EC50 mg. g^{-1} of green coffee, demonstrating low primary antioxidant activity of the green coffee samples. These results were very similar to those found by Mehaya et al. (2020) and Kim et al. (2018) who evaluated the free radical scavenging activity of green coffee sample and obtained values in the range of 63.37 and 75.10 mg. g^{-1} green coffee, respectively.

The β-carotene/linolenic acid oxidation method indicates the secondary antioxidant action and evaluates the ability of a given substance to prevent the oxidation of β-carotene, protecting it from the free radicals generated during the peroxidation of linoleic acid, and the addition of a sample containing antioxidants will contribute to slow the decrease in absorbance of β-carotene (Sokmen et al., 2004). As for the protection potential of β-carotene, the analyzed samples showed on average 44% protection from oxidation of the β-carotene molecules present, moderately inhibiting the conditions favorable to oxidation that are inserted during the analysis, these data were similar to those found by Oliveira et al. (2019) who found 46.07-57.35% protection from oxidation of β-carotene molecules for green coffee beans from the city of Guaxupé, in the South of Minas Gerais (Brazil).

3.2 Principal Component Analysis (PCA)

Principal component analysis was applied to interpret the relationships between the antioxidant capacity data of each green coffee sample analyzed. Two principal components (PC1 and PC2) explained 79.73% of the total variance in the data set. Figure 1A presents the scatter plot of the Principal Components (PCA) of the green coffee samples from the state of Bahia according to their antioxidant capacity data.

PC1 was responsible for explaining 42.46% of the variability of the data and correlates positively with phenolic constituents and the protection of the co-oxidation of the β-carotene/linoleic acid system and negatively with the EC50 of DPPH reduction (Figure 1A), indicating that the higher the value of a sample for PC1, the higher its content of phenolic constituents and the lower its EC50 value for DPPH reduction, which is desirable for most samples. PC2 explained 37.27% of the variability in the data and correlated positively with the phenolic constituents and negatively with the EC50 for DPPH reduction and the protection from co-oxidation of the β-carotene/linoleic acid system, indicating that the lower a sample's value for PC2, the greater its protection from the β-carotene/linoleic acid system. Therefore, the best samples should have high values for PC1 and low values for PC2.

Figure 1. Scatter of Principal Components 1 and 2 of variables (A) and samples (B) of green coffee from Planalto de Conquista (Bahia) according to antioxidant activity data.
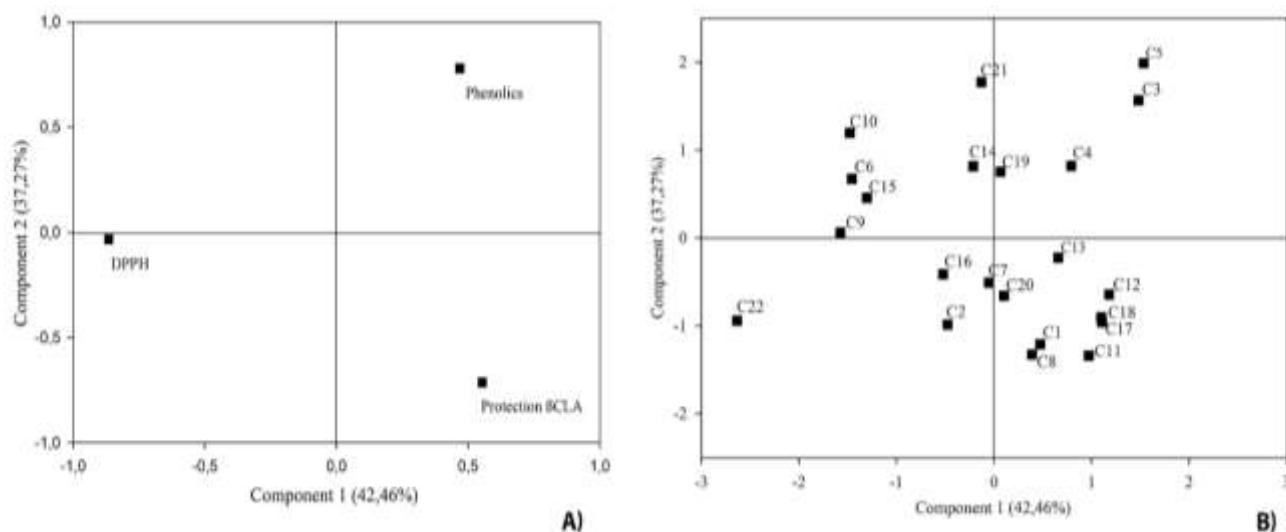
Source: Authors (2021).

According to Figure 1B, the samples located in the lower right quadrant show higher antioxidant capacity, because they have high content of phenolic constituents, low EC50 of DPPH reduction, and high percentage of protection of the β-carotene/linoleic acid system. The samples that are located in the upper right quadrant also have high content of phenolic constituents and low EC50 of DPPH reduction, but do not exert as high protection of the β-carotene/linoleic acid system as high as those mentioned above, this behavior being attributed to the memorable content of bioactive compounds present in these samples and, consequently, lower secondary antioxidant activity, moderately inhibiting free radicals generated during linoleic acid peroxidation.

Analyzing the samples in the upper left quadrant, it can be seen that they are the ones with the lowest antioxidant activity, due to their low content of phenolic constituents, high EC50 of DPPH reduction, and low percentage of protection of the β-carotene/linoleic acid system. The samples located in the lower left quadrant have low content of phenolic constituents, high EC50 of DPPH reduction, but present high percentage of protection of the β-carotene/linoleic acid system.

The green coffee samples from the region Planalto de Conquista, Bahia, Brazil, showed moderate content of phenolic constituents and moderate antioxidant activity in vitro. The principal component analysis was efficient in separating and classifying the green coffee samples according to their antioxidant potential. Samples C1, C8, C11, C12, C13, C17, C18 and C20 showed the highest antioxidant activity among the others. The differences in the profiles obtained by PCA correlate with the overall chemical composition of green coffee beans that are influenced by the particularity of each region, comprehensively involving intrinsic and extrinsic factors, such as bean species and varieties, soil condition, altitude, climate, growing methods, and processing.

Thus, the principal component analysis proved efficient in separating and classifying the green coffee samples according to their antioxidant capacity, allowing the observation of dispersion and heterogeneity among the samples, demonstrating different composition and bioactivity profiles. The study of the level of antioxidant activity in green coffee (high, moderate, and low) allows a better evaluation of its effects and, consequently, improves the understanding and effectiveness of its use as a raw material for a food or the consumption of this natural product, resulting in a healthier and more balanced diet, based on its bioactive composition. Therefore, this analysis is important because it allows complex analyses that are not possible through univariate analysis.

4. Conclusion

The green coffee bean is a rich dietary source of bioactive compounds with excellent antioxidant potential, making it a promising source of studies to investigate other compounds, as well as the numerous benefits to the consumer, with potential application in the food, pharmaceutical, and cosmetics industries.

We can evaluate the green coffee samples according to the proposed analyses and conclude that the chemical composition of the coffee beans and their antioxidant capacity depend on their variety and by the intrinsic and extrinsic conditions of their processing. From the principal component analysis it was possible to observe the formation of patterns among the samples and classify them into groups with high, moderate and low antioxidant potential, which was not possible before through univariate statistics. Thus, demonstrating the heterogeneity among the samples and their different compositional and bioactivity profiles.

Acknowledgments

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