A practical and eco-friendly method for the determination of polycyclic aromatic hydrocarbons in *açaí*-based food products by vacuum-assisted sorbent extraction coupled to gas chromatography-mass spectrometry

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Abstract

For the first time, a method for the analysis of fifteen polycyclic aromatic hydrocarbons in *açaí*-based food products (AFPs) was developed using vacuum-assisted sorbent extraction (VASE) combined with gas chromatography-mass spectrometry (GC-MS). The method requires no organic solvents and is amenable to full automation. To achieve optimal analytical extraction conditions, VASE parameters including stirring rate, extraction time, desorption temperature, desorption time, preheat time, and preheat temperature were optimized using sequential multivariate optimization. The method was validated and yielded limits of quantification below 1 μg kg^{-1} for all analytes, with recoveries ranging from 65% to 112% and good precision (≤ 11% relative standard deviation). Additionally, the greenness and practical aspects of the method were investigated using the Green Analytical Procedure Index (GAPI), eco-scale, and the Blue Applicability Grade Index (BAGI), respectively. The VASE-GC-MS approach is suitable for routine analysis and exhibits characteristics of a green analytical method. No PAHs were detected above the limits of detection in twenty-five samples of AFPs.

Keywords:

chemical contaminants; central composite rotatable design; Derringer and Suich's tool; food analysis; Placket-Burman design; VASE system

1. INTRODUCTION

Chemical contaminants have come into focus within the scientific community due to their harmful effects on human health, with food often serving as a source of these substances[1]. In this context, polycyclic aromatic hydrocarbons (PAHs) stand out as an important group of carcinogenic molecules and are most frequently detected in foods

within the chemical contaminants group [2]. PAHs may form in foods by thermal processing or be derived from environmental sources, such as contaminated water or soil [2]. In the environment, PAHs can be formed through incomplete combustion of organic carbonaceous materials or through emission processes from natural and/or anthropogenic sources [2,3].

PAHs consist of hydrogen and carbon atoms arranged in two or more fused aromatic (benzene) rings, with light PAHs consisting of up to four rings and heavy PAHs having five or more rings [2]. Their differences in chemical properties attribute specific characteristics, with them being mainly classified as semi-volatile molecules [3]. The International Agency for Research on Cancer (IARC) has classified PAHs into four groups, namely carcinogenic to humans (group 1), probably carcinogenic to humans (group 2A), possibly carcinogenic to humans (group 2B), and not classifiable as to its carcinogenicity to humans (group 3) [4]. With regards to legislation of PAHs in foodstuffs, the European Food Safety Authority (EFSA) has set maximum levels for benzo[*a*]pyrene or for the sum of four PAHs, including benzo[*a*]pyrene, benz[*a*]anthracene, benzo[*b*]fluoranthene, and chrysene[5]. The most restrictive concentration in food is 1 µg kg^{-1} and is intended for infants and young children [5]. However, for *açaí*-based food products (AFPs) or similar items, there is currently no regulation available. AFPs are derived from *Euterpe oleracea* Mart., a Brazilian native berry popularly known as *açaí* [6,7]. Recently, the AFP market has expanded mainly in the European and American markets due to its beneficial properties for human health as well as energy supplement [8]. Thermal processing and anthropogenic sources are possible pathways through which PAHs may reach *açaí*. The anthropogenic source is significant, given that *açaí* is cultivated close to highways [9], and that transportation is required by trucks until it reaches manufacturing facilities.

To investigate PAHs in foodstuffs, many studies have developed a variety of sample preparation techniques, including traditional procedures such as Soxhlet extraction, solid–liquid extraction (SLE), and solid-phase extraction (SPE), among others [10]. Additionally, methods that focus on green analytical approaches have been developed for PAH analysis and include the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method, dispersive liquid–liquid micro-extraction (DLLME), as well as other methods [10]. However, these methodologies can present some drawbacks, mainly due to the use of organic solvents (even in minimal quantities), or the impracticality of these techniques for routine analysis. From this perspective, vacuum-assisted sorbent extraction (VASE) is a promising technique that possesses green analytical characteristics due to the fact that extractions are solvent-free. VASE was first introduced in 2016 and has emerged as a new trend for vacuum extraction of semi-volatile and volatile organic compounds [11]. VASE offers practical conditions of analysis due to its easy-to-operate system that does not require high technical skills to operate. In addition, this technique works under vacuum in headspace mode (HS) and features a large volume of sorbents packed in the sorbent pens. These physico-chemical characteristics allow an exhaustive extraction of a wide range of organic compounds [12]. Thereby, offering advantages over similar techniques, such as HS-solid-phase microextraction (SPME) [12]. Additionally, VASE provides satisfactory precision results once analytes are extracted in their entirety when the system is operated near equilibrium conditions [12]. Moreover, VASE is particularly selective for compounds amenable to gas chromatography-mass spectrometry (GC-MS) analysis, such as PAH quantification in food matrices [10].

This represents the first study investigating any type of chemical contaminants in food using VASE. Within this context, a practical and eco-friendly approach based on

this technique was developed and validated in-house for the quantification of 15 PAHs in AFPs by GC-MS. Additionally, VASE parameters were optimized in an effort to identify and apply the optimal condition for the extraction of PAHs in AFPs.

2. MATERIAL AND METHODS

2.1. Samples

The commercial samples studied in this work were purchased in Brazil (n = 20) and the United States (U.S.) (*n* = 10). AFPs were classified according to their flavors, including pure *açaí* (*n* = 8 from Brazil and *n* = 7 from the U.S.), AFPs with strawberry (*n* = 4 from Brazil), AFPs with banana (*n* = 4 from Brazil), and AFPs with guaraná syrup (*n* = 4 from Brazil and *n* = 3 from the U.S.). AFPs were comprised of sorbets (pasty composition) as reported by manufacturers in the products labels. A representative blank sample (control sample), composed of AFPs with banana, strawberry, guaraná syrup, and pure *açaí* in a ratio of 1:1:1:1 (w/w/w/w), was used for optimization and in-house validation procedures. Prior to analysis, the RBS was analyzed by GC-MS to make sure of the absence of any target analyte. The samples were kept at -20 ºC ± 1 ºC until to be used for analysis. The samples were acclimatized at room temperature (21 ºC ± 2 ºC) prior to analysis.

2.2. Standards

Analytical standards (analyte mix of commercial solution) of PAHs including naphthalene, 1-methyl-naphthalene, 2-methyl-naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, and chrysene-d_{12} as internal standard (I.S.) were purchased from

Supelco (Bellefonte, PA, USA). Each analytical standard was diluted in acetonitrile to a concentration of 2000 μg mL^{-1}. A multi-analyte working solution in acetonitrile was prepared at 50 μg mL^{-1}. The standard solutions were kept at –20 °C ± 2 °C until use.

2.3. Multivariate optimization

A sequential experimental design was applied, including Plackett–Burman (PB), to identify significant factors (independent variables) and the Central Composite Rotational Design (CCRD) to determine the optimum extraction conditions for each PAH. Subsequently, Derringer and Suich's tool (D&S) was applied to determine the optimum extraction conditions for all PAHs. For the optimization experiments, the sample (10 g) was spiked with the 15 PAHs as well as the I.S. at a concentration of 0.1 ppm for each analyte.

For PB analysis, some parameters of the VASE system, specifically for the 5600 sorbent pen extraction system (SPES) and of the 5800 sorbent pen desorption unit (SPDU), were optimized. The variables chosen for this step were: stirring (RPM) and extraction time (hour), both of the SPES, and desorption temperature (°C), desorption time (min), preheating time (min), and preheating temperature (°C) operated in the SPDU. All variables were evaluated at high (+1) and low (−1) levels (Table S1). To avoid exclusion of any significant variable, a significance level of 10% was established for this design [13].

The CCRD ($p < 0.05$) was performed using a 2^3 factorial design arranged with high (+1) and low (−1) levels, and axial points (−α and +α) (Table S3). Additionally, three central points (0) were explored (Table S4). Lastly, D&S was used and the mathematical models were analytically validated.

153 For this optimization, the Protimiza Experimental Design software (Protimiza
154 Experimental Design, Campinas, Brazil) was used to obtain the data from PB and CCRD,
155 and Design Expert 6.0 software (Stat-Ease, Minneapolis, USA) was used to run the
156 Derringer and Suich data.
157
158 **2.4. Vacuum-assisted sorbent extraction and desorption procedures**
159 The entire extraction procedure was based on the VASE system provided by
160 Entech Instruments (Simi Valley, CA, USA), including vials, vial caps, sorbent pens, and
161 lids (Fig. 1 a, 1 b), as well as the SPES (Fig. 1 c). PDMS GB + Tenax® model SP-HSP-
162 PDMST3560 sorbent pens were provided by Entech Instruments and used in the
163 extraction process. Extractions were carried out in 40 mL glass vials (borosilicate) (San
164 Leandro, CA, USA) containing 10 g of the sample. The sorbent pens were assembled onto
165 the vials (Fig. 1 b) and air evacuated to create the vacuum by directly connecting the SP
166 Micro QT™ to a vacuum pump for 2 min. A vacuum higher than 25 inches of Hg was
167 maintained within the vial/Pen assembly during the entire extraction period. The
168 assembled sorbent pens and vials were subsequently placed on a 5600 SPES (Fig. 1 d).
169 For extraction, the SPES was operated under optimized conditions of 16 hours and
170 39 min at 70 °C for 100 RPM. To prevent water vapor and/or condensation in the
171 extraction vial and the sorbent pen after each extraction, the vials were subjected to a
172 water management step (Fig. 1 e). In this step, the extraction vial and the sorbent pen
173 were placed in a pre-cooled block (-20 °C ± 2 °C) for 15 min. During the cool-down step,
174 the cooled block was maintained at a temperature of -2 °C ± 1 °C. The sorbent pens were
175 stored in leak-tight Silonite™-coated isolation sleeves (Fig. 1 f, 1 g) until desorption
176 (Fig.1 h). After each extraction, the sorbent pen was reconditioned, according to
177 manufacturer's operating manual, using a sorbent pen thermal conditioner – 3801 SPTC

178 (Entech Instrument, Simi Valley, CA, USA) under the following conditions: pre-purge
179 duration: 10 min; cycles: 1; duration: 60 min; and temperature: 300°C.
180
181 **2.5. Instrumentation**
182 A 7890B GC system from Agilent Technologies (Santa Clara, CA, USA)
183 equipped with a 5800 Sorbent Pen Desorption Unit (SPDU) (Figure 1h) from Entech
184 Instruments (Simi Valley, CA, USA) and interfaced to an Agilent Technologies single
185 quadrupole mass with electron ionization (EI) source (5977A) (Santa Clara, CA, USA)
186 was employed for the quantification of PAHs. The SPDU, under optimized conditions,
187 was set to preheat at 70 °C for 0.50 min. Desorption was performed at 272 °C for 1.30
188 min. The SPDU bake out was set at 260 °C for 20.30 min, followed by post-bake at 70
189 °C for 17 min. Parameters of SPDU bake out and post bake were set according to the
190 VASE system operating guidelines. SPDU was operated in split mode (10:1). A wide-
191 bore Silonite™-coated pre-column (0.6 m × 1 mm) from Entech Instruments (Simi
192 Valley, CA, USA) and an Rtx-5MS capillary column (30 m x 0.25 mm x 0.25 μm;
193 RESTEK, Bellefonte, PA, USA) were used in this analysis. Ultra-high purity helium
194 (99.999%) was employed as the carrier gas at a constant flow of 1 mL min^{-1}. The oven
195 temperature program started at 40 °C and was held for 1.0 min; ramped to 130 °C at 25
196 °C min^{-1}, then to 250 °C at 5 °C min^{-1}, and finally to 300 °C at 10 °C min^{-1}, where it was
197 held for 5 min. The total run time was 38.60 min. The temperature of the MS transfer
198 line, the ion source, and the MS quadrupole was set at 280 °C, 230 °C, and 150 °C,
199 respectively. A solvent delay of 5 min was applied, and data were acquired in the selected
200 ion monitoring (SIM) mode using the Mass Hunter Workstation software (Agilent
201 Technologies version B.07.00, Santa Clara, CA, USA). The 5800 SPDU software (Entech
202 Instruments version 1.3.0.68) was used to operate the SPDU. Analytes retention times,

quantitative and qualitative ions, and relevant chemical information for each analyte are described in Table 1.

2.6. In-house validation

In order to evaluate the method's performance, the limits of detection (LOD), limits of quantification (LOQ), linearity, precision (intra- and inter-day), matrix effects, and recovery were determined. Commission Regulation (EU) No 836/2011 [16] was used as the analytical validation guideline.

LODs and LOQs were set using a signal-to-noise ratio of 3:1 and 10:1, respectively. Linearity and matrix effects were determined using curves in water (as a solvent) and in the sample to obtain the matrix-matched calibration curve. The concentration levels used in the curves ranged from 0.8 to 100 μg kg^{-1}, and analyses were performed at seven calibration levels (0.8, 16, 33, 50, 67, 84, and 100 μg kg^{-1}).

Recovery, precision, and reproducibility were carried out at three different points, including 0.8, 50, and 100 μg kg^{-1}. The results were expressed as a percentage for recovery and relative standard deviation (%RSD) for intra- and inter-day precision.

Matrix effect was evaluated using slopes of the solvent and matrix curves [6] and it was expressed according to Equation 1:

Equation 1: $$\%\ Matrix\ effect = \left[\frac{matrix\ slope - solvent\ slope}{solvent\ slope}\right] * 100$$

2.7. Practical aspects and green assessment of the analytical method

The validated analytical method was evaluated for both its practical aspects and green analytical features. The practical aspect was assessed using the metric applied by the blue applicability grade index (BAGI) [17]. Meanwhile, for the green analytical

features, both the green analytical procedure index (GAPI) [18] and eco-scale [19] were employed.

BAGI utilizes pictograms to evaluate ten main attributes of the method, which can be depicted as white, light blue, blue, and dark blue. These colors serve as a qualitative metric for the practicality and applicability of the method parameters, with dark blue and white being the most and least desirable colors, respectively. Furthermore, each color represents a score (2.5 for white, 5.0 for light blue, 7.5 for blue, and 10 for dark blue). A score closer to 10 indicates a more 'practical' method.

As for the GAPI, it is a qualitative tool that identifies the weakest and strongest points in analytical procedures regarding green analytical chemistry attributes. This tool uses green, orange, and red colors to represent low, medium, and high environmental impact, respectively. The aforementioned methods complement each other and have been utilized to assess whether the analytical methodology meets the minimal requirements for routine analysis with low environmental impact. Lastly, eco-scale was used to assess each analytical parameter that should comply with ideal green analysis and has a maximum score of 100. Each non-compliance criterion reduces this score,thereby decreasing the greenness of the analytical methodology.

3. RESULTS AND DISCUSSIONS

3.1. Multivariate optimization

Many studies have used univariate optimization in an effort to identify the optimal extraction conditions for PAHs in foodstuffs [20–22]. In the general context of chemical analysis, especially in the realm of green chemistry, this type of optimization is not desirable as it increases the number of experiments, and leads to higher consumption of organic solvents, longer operation times, and larger volumes of waste generated [23,24].

252 Thus, it is crucial to pursue alternative extraction conditions that maximize analytical
253 cost-effectiveness. Additionally, univariate optimization has statistical limitations, as it
254 does not allow evaluation of interactions between variables. This drawback reduces the
255 capability to obtain optimal extraction data for the target analytes [23]. In such
256 circumstances, multivariate optimization approach, including PB, DCCR, and D&S,
257 emerge as a set of tools to overcome these issues [23,24].
258
259 *3.1.1. Plackett-Burman design*
260 All optimization experiments were performed randomly to avoid any biases and
261 enhance the internal validity of the experiment. PB design was conducted using 15
262 experiments (Table S2) to investigate effects of the independent variables on the
263 determination of PAHs by VASE-GC-MS.
264 The European Commission set maximum levels only for BaP or for the sum of 4
265 PAHs, including *Ba*A, Cry, B*b*F, and B*a*P [5], which are the largest PAHs investigated
266 in this work. These compounds also require specific conditions (reported in the PB results
267 below) for volatilization into sorbent pens when compared with smaller ones. For this
268 reason, the PB results were evaluated only for these compounds. In this context, the
269 following variables were found to significantly affect these priority PAHs were:
270 extraction time, desorption temperature, and desorption time, as shown in Figure 2.
271 Regarding variable extraction time, it exhibited a significant positive effect on the
272 extraction of B*a*P which means that an increase in extraction time is associated with a
273 better extraction capability of B*a*P. This PAH possesses the highest molecular weight
274 among those analyzed in this study. Furthermore, it has the highest hydrophobicity (Log
275 K*ow* of 6.13) [25], making it more susceptible to exhibiting a positive correlation with

276 the extraction time in VASE [12]. A similar result was reported by Oliveira et al. [26] for
277 high molecular weight compounds evaluated using a PB design and headspace extraction.
278 Additionally, a significant positive effect was observed for the desorption
279 temperature of B*a*P and B*b*F. As these compounds are the molecules with highest
280 molecular weight among those analyzed in this work, higher temperatures are required to
281 desorb them from PEN. In the VASE system, the desorption temperature can reach up to
282 300º C. A similar result was reported in another study [12] that analyzed UV filters by
283 VASE coupled to GC-MS where it was observed that the highest peak areas were
284 achieved for analytes analyzed at higher desorption temperatures [12]. This effect may
285 occur because the mass-transfer coefficient from the sorbent pen to SPDU increases with
286 the rise in temperature [27], thereby weakening the affinity between analytes and PEN,
287 resulting in increased analyte desorption effects [12].
288 The desorption temperature exhibited a significant positive effect for Cry and
289 B*b*F. This experimental effect suggests that higher desorption time can result in improved
290 peak areas, consequently enhancing the limits of detection. Overall, high molecular
291 weight analytes may require longer desorption time so that desorption is more exhaustive
292 [28]. A similar result was reported by Trujillo-Rodríguez, where most of the analytes
293 provided better analytical performance when longer desorption times were used [12].
294 Variables that presented significant effects on priority PAHs were selected for
295 CCRD. In summary, the stirring rate for the 5600 SPES, preheating time and preheating
296 temperature of the 5800 SPDU did not have significant effect in the extraction of these
297 PAHs. Therefore, these variables were fixed at 100 RPM, 70 °C, and 0.50 min for stirring,
298 preheating temperature, and preheating time, respectively. The extraction temperature
299 was not optimized in any design because previous experiments were carried out at
300 temperatures lower than 70 ºC, and the largest PAHs were not able to be volatilized. Thus,

301 the extraction temperature was fixed at the maximum working temperature of SPES
302 (70°C).
303
304 *3.1.2. Central composite rotatable design (CCRD) and Derringer and Suich's tool*
305 Based on PB data, CCRD was performed (see Table S3). For CCRD, a design
306 with 14 assays and a central point performed in triplicate was carried out, providing a
307 total of 17 experiments. The assays and their respective independent variables studied for
308 the extraction of PAHs in AFPs are presented in Table S4. Additionally, the statistical
309 model coefficients and p-values obtained from CCRD are provided in Table S5. The
310 models showed coefficients of determination ranging from 0.8349 to 0.9771, representing
311 satisfactory relationships between the response and independent variables. PAHs yielded
312 linear, quadratic, and two-factor interaction models, which can affect the extraction.
313 Considering the diverse responses, the D&S tool was applied to identify the best
314 simultaneous global condition for the extraction of all analytes, providing predicted
315 responses based on the CCRD data. Experimental data should be within the prediction
316 interval provided by D&S, and if the responses are acceptable, the method can be
317 validated under the previously proposed condition. For this, the D&S tool proposed the
318 following optimal condition (levels based on CCRD) to be validated: extraction time in
319 the 5600 SPES (0.21), desorption temperature (-0.67) and desorption time of the 5800
320 SPDU (-1.68), which corresponds to 16 hours 39 minutes, 272 °C, and 1.30 min for each
321 variable, respectively. As observed in Table S6, all experimental values were within the
322 predicted range, making the proposed condition suitable for the subsequent analytical
323 validation step. The proposed conditions by D&S showed a desirability for maximizing
324 extraction of 0.623 out of 1.0. As observed in Figure 3, optimization was finally able to
325 increase the extraction of PAHs, ranging from 9 to 496%, demonstrating that the design

employed in the analysis achieved satisfactory efficiency. The percentage values were based on peak area when comparing results prior and after optimization. The level -1 of PB design (Table S1) was used to analyze the results before optimization.

3.2. In-house validation and occurrence

The condition identified by multivariate optimization was employed for analytical validation. Following recommendations of the European Commission (EC)[16], all flasks/vials used for the analyses were rinsed with acetone and hexane to avoid any risk of contamination. The method performance characteristics are provided in Table 2.

The LODs and LOQs were determined using a representative blank sample, as described in Section 2.1. Limits at the parts per billion level were achieved, with the LOD ranging from 0.003 to 0.30 μg kg^{-1}, and the LOQ ranging from 0.01 to 1.0 μg kg^{-1}. In addition, the method was developed based on the performance criteria for the analysis of four PAHs (B*a*A, Cry, B*b*F, and B*a*P) established by the EC [16]. These four PAHs are considered the most suitable markers for these contaminants in foodstuffs [16]. The values achieved in this work meet the requirements set by the EC, which establishes LOD and LOQ values of ≤ 0.30 μg kg^{-1} and ≤ 0.90 μg kg^{-1} for these four PAHs [16].

With regard to linearity, a range was developed from 0.80 to 100 μg kg^{-1}. The obtained recovery results fall within a range of 65-112%, which is considered acceptable for analytes at concentrations ≤ 1 μg kg^{-1} or for the four PAHs defined by the EC, where the acceptable range is from -50% to +20% [16,29]. Repeatability (intra-day precision) and within-laboratory reproducibility (inter-day precision) yielded results ranging from 1 to 8% RSD and from 4 to 11% RSD, respectively. The achieved precision results comply with those required by the EC, which stipulate that for concentrations < 100 μg kg^{-1}, the

result should be as low as possible [30]. Additionally, for the specific four PAHs, precision must be ≤ 22 RSD% [16].

Matrix effects provided results ranging from 15 to 60%, indicating signal enhancement [31]. However, this phenomenon was overcome using a matrix-matched calibration curve, which can compensate for any effect caused by the matrix. Similar to this work, a positive effect on the PAH signal was observed when analyzing other food matrices. For instance, results between 21 and 79% were observed for a method applied to infant formula [32]. For baby food, positive effects ranging from 4.3 to 75.4% were reported [33]. In a method developed for soft drinks, Caldeirão et al. observed matrix effects ranging up to 743.4% [34]. The signal enhancement observed in these works can be correlated with interferents that may absorb strongly to the column and GC inlet, thereby blocking active sites [35].

The validated method was applied in the evaluation of 15 PAHs in twenty-five samples of *açaí*-based products. However, there was no occurrence of these compounds in any of the studied samples. It was expected that PAHs could be present in the sample, as *açaí* berries can become contaminated through the sorption of these contaminants from the soil, air, and water. Additionally, thermal processing methods to which AFPs are subjected could be a source of contamination with these substances [10].

3.3. Vacuum-assisted sorbent extraction

The VASE system is suitable for a wide range of compounds with respect to molecular weight. Upon reviewing the literature, a few studies that employed similar techniques to VASE for the analysis of PAHs, such as HS-SPME or stir bar sorptive extraction (SBSE), have shown some analytical limitations. For instance, HS-SPME has drawback in that large PAHs are unable to be volatilized at atmospheric pressure where diffusion rates are suppressed. Studies that have analyzed PAHs using HS-SPME have

focused on PAHs with molecular weights up to Pyr (202 g mol^{-1}) [36–39]. Thus, these studies were unable to analyze the four PAHs considered as markers of contamination in foodstuffs [16]. Even Maleki and coworkers, who utilized vacuum-assisted HS-SPME, did not analyze PAHs with a molecular weight higher than 202 g mol^{-1}. In the case of SBSE, this technique often requires additional steps involving certain chemicals [40,41]. Additionally, SBSE necessitates further studies to overcome certain disadvantages that limit its widespread use in chromatography [42].

As observed in Figure S1, the VASE system provided advantages in extracting compounds with varying boiling points when compared to other techniques featuring similar characteristics. For the PAHs analyzed in this work, which have molecular weights ranging from 128 to 252 g mol^{-1}, VASE emerges as technique with superior features in the extraction of multiple PAHs. The VASE procedure, aligned with the selected ion monitoring mode used in GC-MS, made it possible to obtain an interferent-free chromatogram without the need for a clean-up step, as shown in Figure 4.

3.4. Practical aspects and green assessment of the analytical method

Methods today considered for green analytical chemistry, as well as designed for practical routine analysis, are more sought after and represent a trend in analytical and food chemistry [43]. For this reason, the VASE system coupled to GC-MS was investigated for the determination of PAHs in AFPs to assess if it was able to achieve these characteristics and consequently be classified as a green and practical method. To compare with the proposed VASE-GC-MS method in this study, the QuEChERS method – an environmentally friendly procedure commonly used for PAHs analysis – was selected [44]. It is important to note that the QuEChERS method used for evaluating the comparison effect in this study is based on that developed by Singh and Agarwal [44].

401 Regarding the practicality and applicability of the VASE-GC-MS method, BAGI
402 tools recommend that the method achieve at least 60 out of 100 points, with scores closer
403 to 100 indicating excellent method performance. As shown in the center of the asteroid
404 pictogram (Fig. 5 A), an overall score of 72.5 was obtained, surpassing the recommended
405 score by this index. In addition, the ten parts (except the central part) of the pictogram
406 represent specific parameter conditions, including: 1) the type of analysis; 2) the number
407 of analytes that are simultaneously determined; 3) the analytical technique and required
408 analytical instrumentation; 4) the number of samples that can be simultaneously treated;
409 5) sample preparation; 6) the number of samples that can be analyzed per hour; 7) the
410 type of reagents and materials used in the analytical method; 8) the requirement for
411 preconcentration; 9) the degree of automation, and 10) the amount of sample.
412 The only section that showed a white hue (a hue that should be avoided) was due
413 to the method's ability to analyze only ≤1 sample per hour. Besides that, this condition
414 was employed because it was necessary to apply optimized conditions to extract all
415 analytes with different molecular weights ranging from 128 to 252 g mol^{-1}. An option to
416 improve this drawback could be to focus the study on either low or high molecular weight
417 PAHs. However, to comply with recognized guidelines, there is a growing necessity to
418 work with simultaneous detection of multiple compounds over extended times, as ideal
419 analytical methods require more comprehensive analysis [43].
420 As shown in Fig. 5 B, the proposed QuEChERS method revealed 4 out of 10 white
421 sections and a score lower than that reported in the procedure developed in this work.
422 Furthermore, the VASE-GC-MS method, due to the performance it achieved, can be
423 classified as a method capable of demonstrating practicality and applicability, making it
424 attractive for routine analysis. BAGI is a complementary tool to metrics that evaluate the
425 greenness of the analytical method. In this context, the GAPI tool was applied to

investigate the green character of VASE-GC-MS, in addition to investigating QuEChERS [44] as a method of comparison. In summary, GAPI is divided into 5 groups (Fig. 5 C and 5 D), and each has parameters to be analyzed. Additionally, each parameter receives a number that indicates the part of the pictogram where it is located. This information is detailed as: (i) sample handling (collection (1), preservation (2), transport (3), and storage (4); (ii) type of method (direct or indirect (5)); (iii) sample preparation (scale of extraction (6), solvents/reagents (7), additional treatments (8)); (iv) reagents and solvents (amount (9), health hazard (10), and safety hazard (11)); and (v) instrumentation (energy (12), occupational hazard (13), waste (14), waste treatment (15)). Among these, only one section of the pictogram dealing with sample handling (collection) and instrumentation (energy) parameters was identified as red for the VASE-GC-MS procedure (Fig. 5 C). As reported by Nascimento et al. [24], some parameters, such as collection, become very challenging to overcome since this analysis must be performed in the laboratory making it impossible to carry it out on-site, thereby representing lower environmental (green) impact. Due to the necessity of volatilizing large PAHs over long periods of time into the PEN, consumption of more electrical energy is necessary, imparting this parameter a red classification. When investigating the QuEChERS method by GAPI (Fig. 5D), it was possible to observe that the pictogram did not exhibit any parameters classified as being of low environmental impact. Furthermore, the analytical eco-scale (see Table 3) proposed by Gałuszka et al. [19] was employed to assess the analytical parameters that do not comply with green analysis.

The analytical eco-scale uses penalty points to assess the analytical steps with chemistry approaches that are not compatible with green processes, such as hazards, waste, and among others. A total score of 100 points is used for the assessment, and each penalty is subtracted from the total score. This metric is divided into three categories,

451 including a score > 75, score > 50, and score < 50, which indicates excellent green
452 analysis, acceptable green analysis, and inadequate green analysis, respectively.
453 According to the results, the method proposed in this study was classified as an excellent
454 green analysis approach. The penalties addressed for the VASE-GC-MS approach
455 stemmed from the use of GC-MS (penalty points: 2), energy due to SPES spending more
456 than >1.5 kWh per sample (penalty points: 2), and for creating waste (only sample)
457 between 1 to 10 g (penalty points: 3). Under these circumstances, the score obtained in
458 the analytical eco-scale was 93 points. On the other hand, a lower score was observed for
459 the QuEChERS approach, mainly for using organic solvent and creating a larger amount
460 of waste. Thus, a score of 79 points was obtained for this method. From the perspective
461 of the analytical eco-scale, the method proposed in this work was ratified once again, as
462 it is an approach that presents an advantage in the analysis of PAHs when compared to
463 the QuEChERS procedure.
464

465 **4. CONCLUSIONS**

466 An eco-friendly approach based on VASE was developed for the simultaneous
467 determination of 15 PAHs by GC-MS. No organic solvent was used to perform the
468 extraction of analytes for chromatographic analysis. Additionally, no clean-up step was
469 required to obtain an interferent-free chromatogram. To achieve optimal extraction
470 conditions, the extraction method was optimized using a sequential multivariate
471 optimization comprising PB, CCRD, and D&S. The method was validated in-house
472 following parameters established by the European Commission. The method fulfilled the
473 requirements for consideration as a green and practical method, which are ideal conditions
474 for routine analysis. The optimization resulted in a method that meets the requirements

outlined in the validation guidelines, reaching LOQ values ranging from 0.01 to 0.8 μg kg^{-1}. No PAHs were detected in any samples investigated.

Due to the promising approach presented by the VASE-GC-MS method, it is an attractive procedure for determining and quantifying PAHs in other types of food samples. The achieved analytical parameters make this method suitable for PAH analyses in AFPs, aiming to meet the strictest criteria already established by regulatory agencies.

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Figure Captions

Figure 1. Flowchart of the vacuum-assisted sorbent extraction system. Fig 1 a: vial kit; Fig 1 b: assembled vial; Fig 1 c: sorbent pen extraction system; Fig 1 d: loaded SPES with samples; Fig 1 e: water management system; Fig 1 f: sorbent pen and sleeves; Fig 1 g sorbent pen isolation tray; and Fig 1 h: sorbent pen desorption unit.

677

678 **Figure 2**. Pareto chart of the Plackett-Burman design showing the effects of independent
679 variables on the four priority PAHs spiked in an *açaí*-based food product.

680

681 **Figure 3.** Comparison of PAH extraction efficiency from *açaí*-based food products prior
682 and after optimization. The percentages above each bar represent the enhancement in
683 extraction after optimization for each PAH based on peak areas.

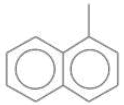
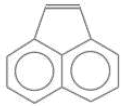
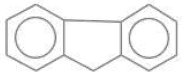
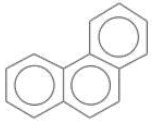
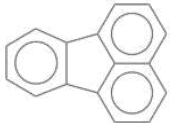
684

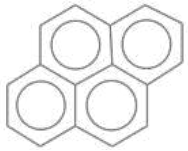
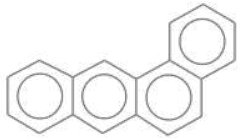
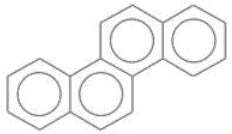
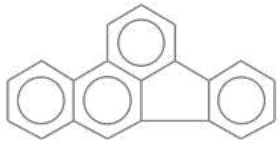
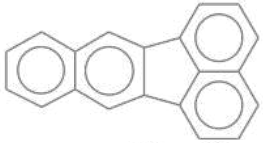
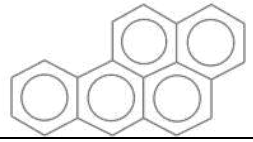
685 **Figure 4**. Representative chromatogram for PAHs spiked in *açaí*-based food product and
686 analyzed by VASE coupled to GC-MS.

687

688 **Figure 5.** Results obtained from the blue applicability grade index (BAGI) (Fig. 5 A and
689 5B) and green analytical procedure index (GAPI) (Fig. 5 C and 5 D) applied for the
690 extraction of PAHs from food matrices. Fig. 5 A and Fig. 5 C represent data obtained
691 from this method, while Fig. 5 B and Fig. 5 D represent data obtained from the
692 QuEChERS method developed by Singh and Agarwal [44].

693 **Table 1.** Analytes and their abbreviations, chemical structures, toxicity, USEPA classification, molecular weight (MW), and GC–MS parameters
694 of 15 polycyclic aromatic hydrocarbons (PAHs).

Number	PAH	Abbreviation	t_R (min)	Chemical structure [a]	Toxicity (IARC) [b]	USEPA classification [c]	MW (g mol^{-1}) [d]	SIM (*m/z*) [b]
1	Naphthalene	Nap	6.712		2B	Yes	128.1705	**128**, 127, 129
2	1-methyl-naphthalene	1-MN	8.001		-	No	142.1971	**142**, 141, 115
3	2-methyl-naphthalene	2-MN	8.242		-	No	142.1971	**142**, 141, 115
4	Acenaphthylene	Acy	10.354		-	Yes	152.1919	**152**, 151, 76
5	Acenaphthene	Ace	10.943		3	Yes	154.2078	**153**, 154, 152
6	Fluorene	Fle	12.681		3	Yes	166.2185	**166**, 165, 167
7	Phenanthrene	Phe	16.408		3	Yes	178.2292	**178**, 176, 179
8	Anthracene	Ant	16.587		2B	Yes	178.2292	**178**, 176, 179
9	Fluoranthene	Fla	21.611		3	Yes	202.2506	**202**, 203, 200

10	Pyrene	Pyr	22.558		3	Yes	202.2506	**202**, 203, 200
11	Benz[*a*]anthracene	BaA	28.131		2B	Yes	228.2879	**228**, 226, 229
12	Chrysene	Cry	28.306		2B	Yes	228.2879	**228**, 226, 229
13	Benzo[*b*]fluoranthene	BbF	32.135		2B	Yes	252.3093	**252**, 253, 250
14	Benzo[*k*]fluoranthene	BkF	32.209		2B	Yes	252.3093	**252**, 253, 250
15	Benzo[*a*]pyrene	BaP	33.010		1	Yes	252.3093	**252**, 253, 250

[a] NIST Chemistry WebBook [14].

[b] Toxicity based on the International Agency for Research on Cancer (IARC) [4]. group 1: carcinogenic to humans; group 2B: possibly carcinogenic to humans; and group 3: not classifiable as to its carcinogenicity to humans.

[c] priority PAHs for United States Environmental Protection Agency (USEPA) [15].

[d] Selected ion monitoring (SIM); ions used for quantification in bold.

Table 2. Method performance characteristics obtained by VASE coupled to GC-MS for determination of PAHs in açaí-based food products.

	PAH	LOD (µg kg^{-1})	LOQ (µg kg^{-1})	Linearity, R^2 (range of 0.8 – 100 µg kg^{-1})	Recovery (%, n = 9)			Precision, RSD % Intra-day, n = 9 (Inter-day, n = 9)*			Matrix effect (%)
				Matrix-matched curve	0.8 µg kg^{-1}	50 µg kg^{-1}	100 µg kg^{-1}	0.8 µg kg^{-1}	50 µg kg^{-1}	100 µg kg^{-1}	
1	Naphthalene	0.003	0.01	0.9998	98	104	101	2 (9)	3 (10)	2(11)	21
2	1-methyl-naphthalene	0.003	0.01	0.9991	99	97	98	4 (5)	2 (9)	4 (6)	30
3	2-methyl-naphthalene	0.003	0.01	0.9989	110	101	102	1 (7)	1 (6)	6 (7)	28
4	Acenaphthylene	0.02	0.05	0.9987	101	95	97	2 (5)	4 (9)	2 (10)	15
5	Acenaphthene	0.02	0.05	0.9978	87	85	80	3(9)	5 (9)	1 (8)	27
6	Fluorene	0.02	0.05	0.9976	81	86	83	6 (5)	8 (8)	3 (7)	35
7	Phenanthrene	0.03	0.08	0.9972	80	85	90	4 (8)	6 (9)	4 (9)	24

8	Anthracene	0.03	0.08	0.9981	112	103	97	1 (7)	2 (6)	2 (8)	21
9	Fluoranthene	0.04	0.1	0.9977	89	97	90	5 (10)	1 (7)	6 (9)	39
10	Pyrene	0.04	0.1	0.9991	80	82	84	2 (8)	5 (7)	1 (6)	45
11	Benz[*a*]anthracene	0.1	0.3	0.9931	72	73	72	3 (10)	4 (11)	4 (10)	49
12	Chrysene	0.2	0.5	0.9964	79	80	81	6(10)	6 (6)	5 (9)	32
13	Benzo[*b*]fluoranthene	0.3	0.8	0.9891	69	75	68	4 (5)	3 (4)	6 (6)	65
14	Benzo[*k*]fluoranthene	0.3	0.8	0.9871	72	69	75	5 (8)	4 (9)	3 (7)	60
15	Benzo[*a*]pyrene	0.3	0.8	0.9896	65	70	71	2 (7)	1 (8)	2 (9)	50

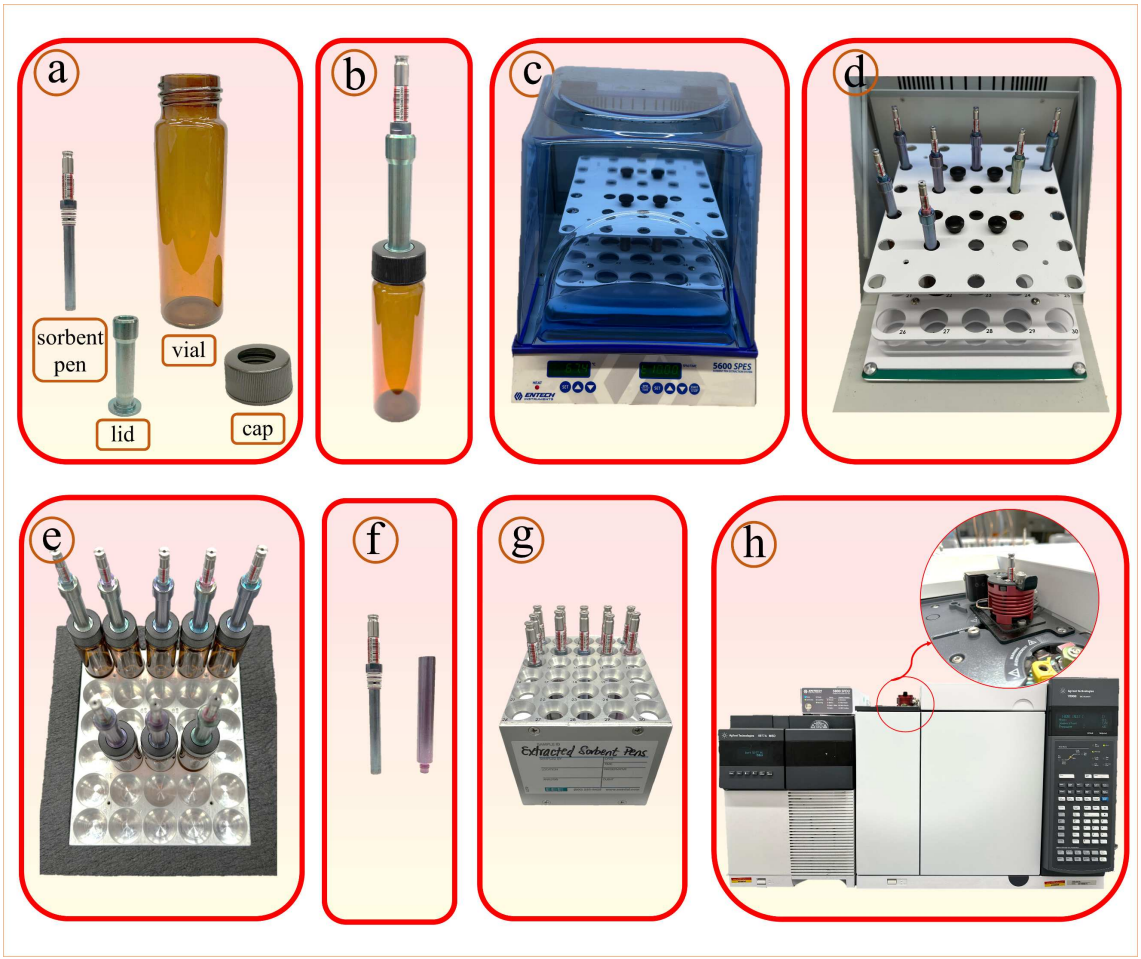
LOD: limit of detection; LOQ: limit of quantification; R^2: coefficient of determination; RSD: relative standard deviation. * Values expressed within parentheses correspond to inter-day precision.

704 **Table 3**. Analytical eco-scale scores were used to compare the VASE system and
705 QuEChERS methods for the extraction of polycyclic aromatic hydrocarbons from a food
706 matrix.

Parameters	Penalty Points	
	This method	QuEChERS* [44]
1. Reagents		
a. Acetonitrile	n/a	4
b. Acetic acid	n/a	6
c. Magnesium sulfate	n/a	1
d. Sodium acetate	n/a	0
e. Primary secondary amine sorbent	n/a	0
2. Instruments		
a. Energy		
i. VASE system	2	n/a
ii. GC-MS	2	n/a
iii. Centrifuge	n/a	1
iv. HPLC-FLD	n/a	1
b. Occupational hazard	0	3
c. Waste	3	5
Total penalty points	Σ 7	Σ 21
Analytical Eco-Scale score	93	79

707 n/a: not applicable. *Classification of QuEChERS reflects just the method developed by
708 Singh and Agarwal [44].

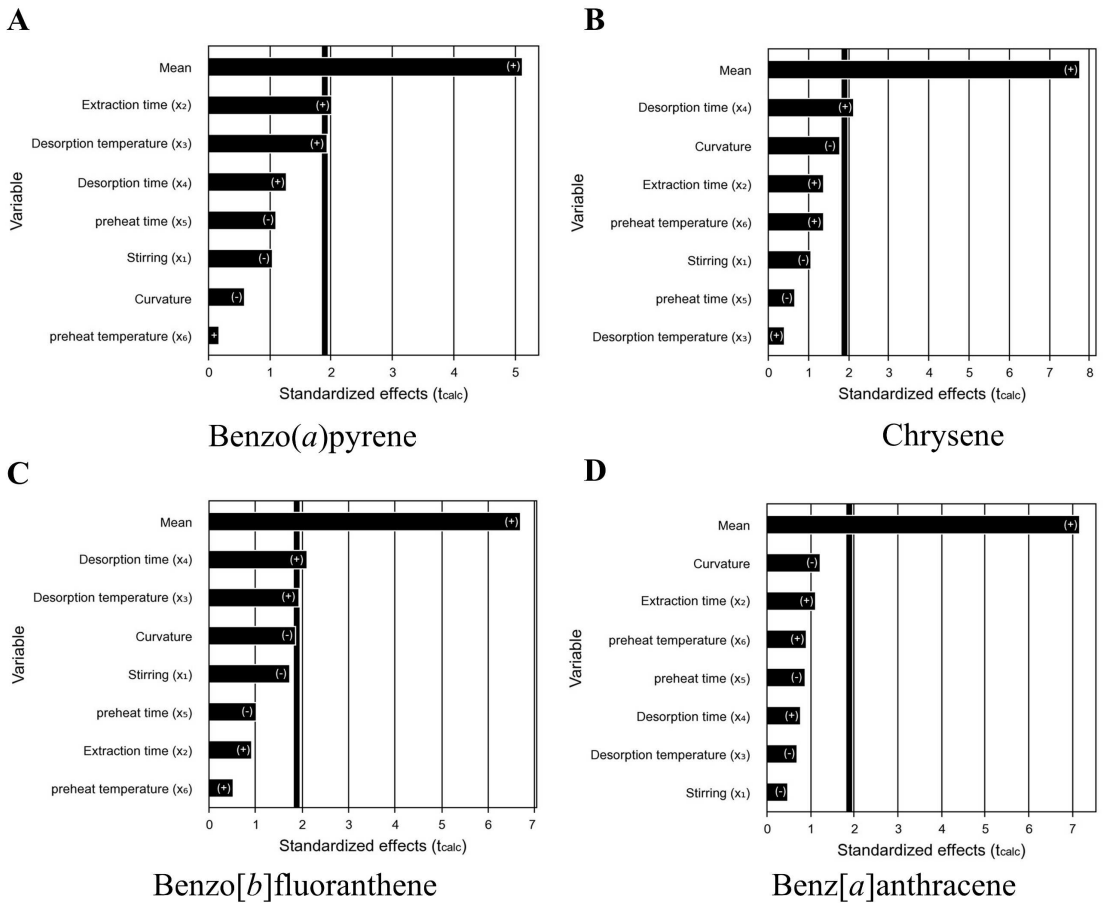
709



710

711 **Figure 1.** Flowchart of the vacuum-assisted sorbent extraction system. Fig 1 a: vial kit;
712 Fig 1 b: assembled vial; Fig 1 c: sorbent pen extraction system; Fig 1 d: loaded SPES with
713 samples; Fig 1 e: water management system; Fig 1 f: sorbent pen and sleeves; Fig 1 g
714 sorbent pen isolation tray; and Fig 1 h: sorbent pen desorption unit.

715



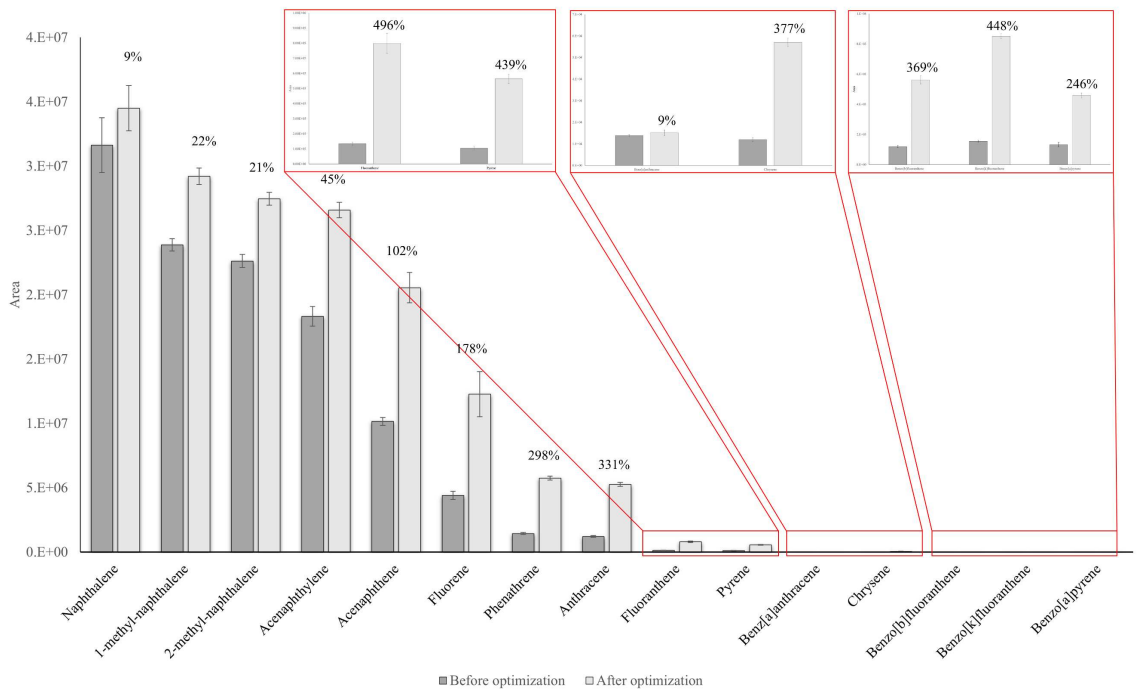
716

717 **Figure 2**. Pareto chart of the Plackett-Burman design showing the effects of independent

718 variables on the four priority PAHs spiked in an *açaí*-based food product. Positive or

719 negative signals into the bar represent positive or negative effects, respectively.

720



721

722 **Figure 3.** Comparison of PAH extraction efficiency from *açaí*-based food products prior
723 and after optimization. The percentages above each bar represent the enhancement in
724 extraction after optimization for each PAH based on peak areas.

725

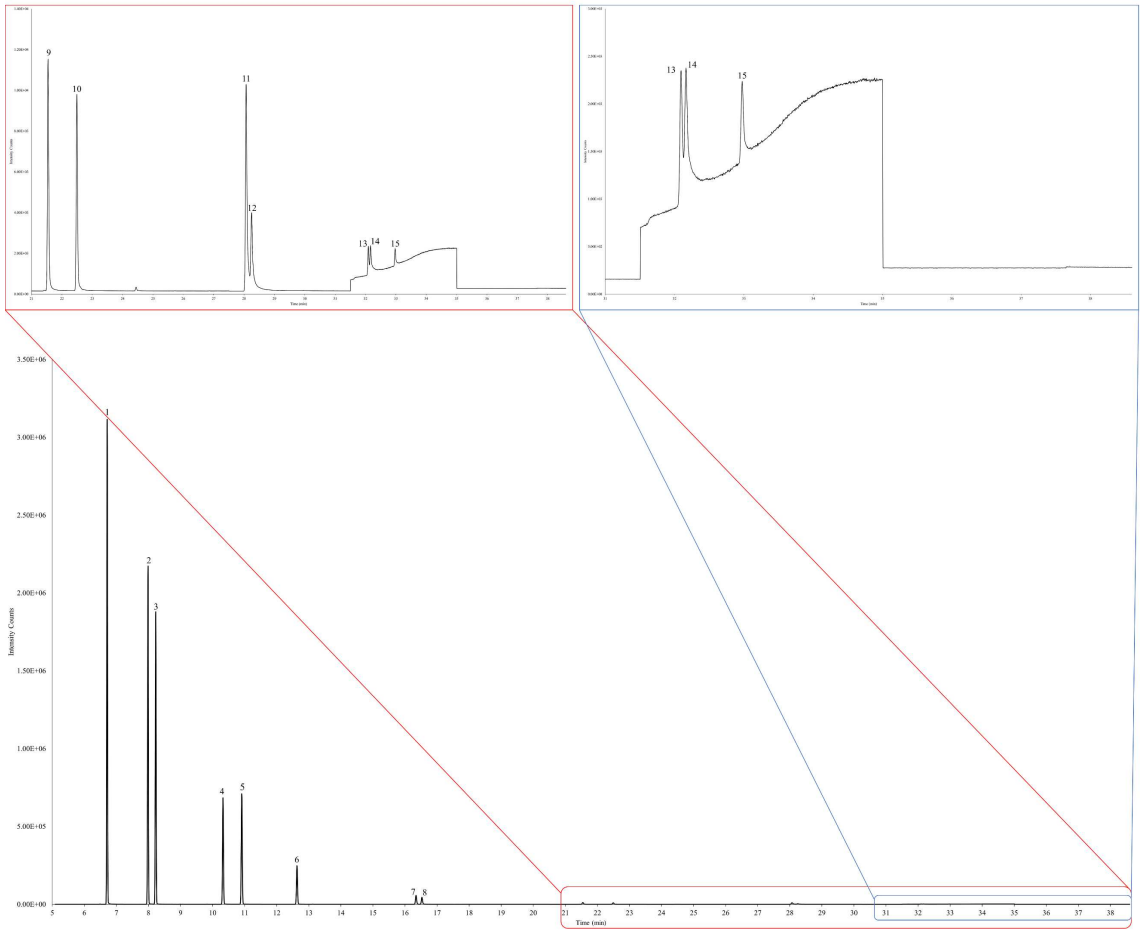
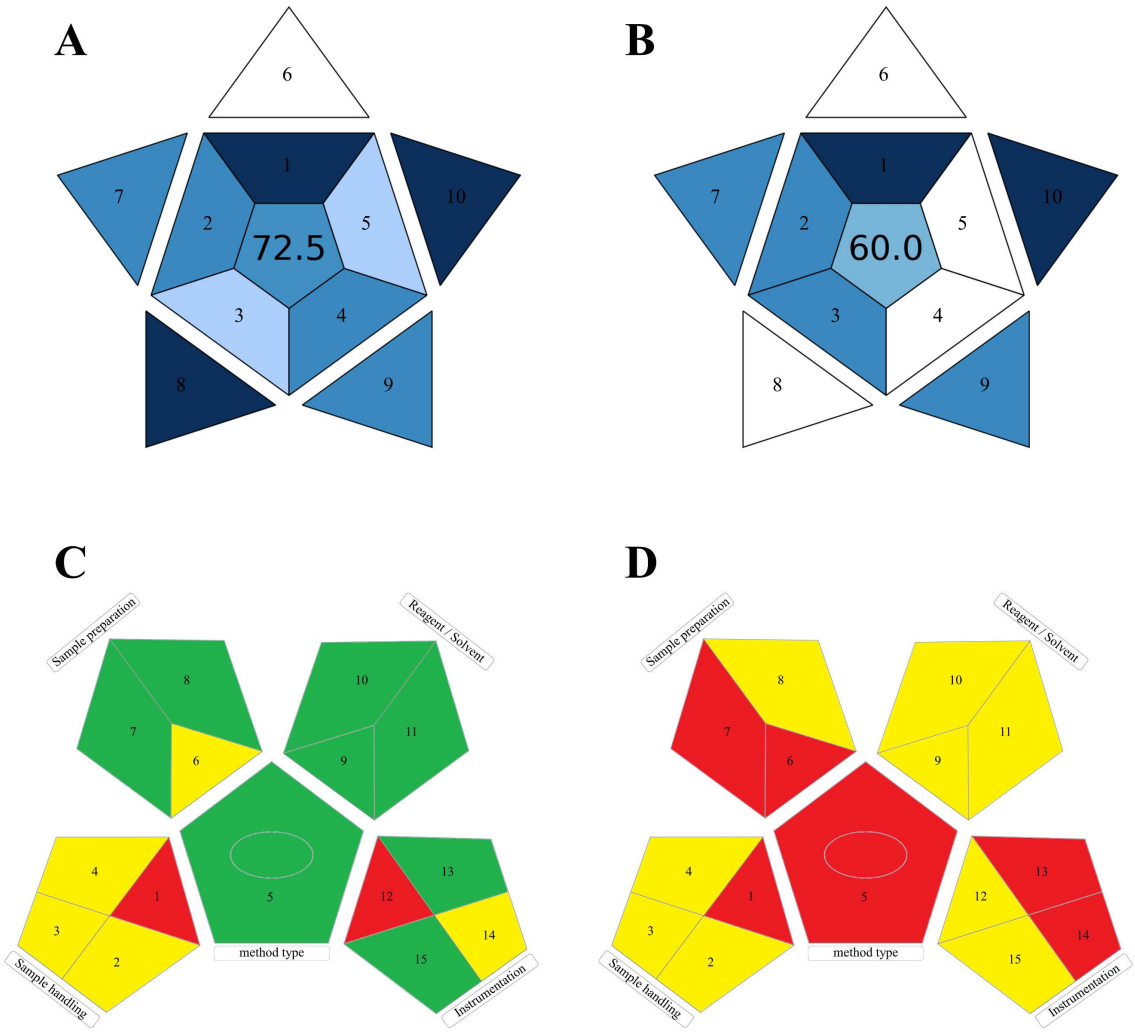


Figure 4. Representative chromatogram for PAHs spiked in *açaí*-based food product and analyzed by VASE coupled to GC-MS.



730

731 **Figure 5.** Results obtained from the blue applicability grade index (BAGI) (Fig. 5 A and
732 5B) and green analytical procedure index (GAPI) (Fig. 5 C and 5 D) applied for the
733 extraction of PAHs from food matrices. Fig. 5 A and Fig. 5 C represent data obtained
734 from this method, while Fig. 5 B and Fig. 5 D represent data obtained from the
735 QuEChERS method developed by Singh and Agarwal [44].

736