Polycyclic aromatic hydrocarbons in soybean grains

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A B S T R A C T

During the burning of the organic material (firewood) in the drying, combustion may occur incompletely, thus generating smoke contaminants such as polycyclic aromatic hydrocarbons (PAHs) in the furnaces. This study aimed to identify possible contamination by PAHs in soybean grains from storage units in municipalities in the Midwest region that have undergone drying in a direct-fired furnace with firewood as fuel. The soybean grains were collected in different municipalities of the Midwest region of Brazil, totaling 22 samples. A survey of possible contamination of soybean grains by PAHs was carried out using High-Performance Liquid Chromatography. The experiment was carried out in a completely randomized design with three replicates, and the data were subjected to analysis of variance, with means compared by Tukey test. The sum of the groups was: PAH4 - 1.45 μg kg⁻¹ for Edéia (2), PAH8 - 2.97 μg kg⁻¹ for Catalão (19) and PAHT - 5.06 μg kg⁻¹ for Edéia (2); for benzo(a)pyrene, the sum was below 2.0 μg kg⁻¹, not exceeded by the value of 0.64 μg kg⁻¹ for Edéia (1). The values found of PAHs did not exceed the maximum limits established by the European Union, except for infant food.

1. Introduction

The technological activity in the food industry has undergone major changes not only in the processing and quality (Conceição and Almeida, 2005; Gouveia, 2006), but also in the control of the emission of pollutants of all sorts by various modes (Paulo and Serra, 2015).

Due to the high applicability of soybean grains, maintaining their integrity in the post-harvest is indispensable given the high temperatures and rainy season in the country, so it is essential to apply drying methods that ensure their final quality, hence reducing the moisture content to 12% (w.b.), recommended for safe storage (Smaniotto et al., 2014).

The Scientific Committee on Food in 2002 acted for global clarification on pathogenic agents considered highly contaminating, carriers of diseases that can lead to death. This committee recommended benzo(a)pyrene (BaP) as PAH marker, as a sufficient indicator of contamination (EFSA, 2008).

He European Food Safety Authority (EFSA), in a new evaluation, established a group of markers with four PAHs: benzo(a)pyrene (BaP), benzo(a)anthracene (BaA), benzo(b)fluoranthene (BbF) and chrysene (Chr) (PAH4), whose sum should not exceed 10.0 μg kg⁻¹ and with a maximum of 2.0 μg kg⁻¹ for BaP within this group. They had suggested a group with eight (PAH8), but the difference was not substantial compared to the first group (EFSA, 2008; CEC, 2011).

The European Union establishes limits for PAH according to the food. For crustaceans, crabs, flounders and other animals of aquatic origin, smoked or not, depending on the type, the limit is 5.0 μg kg⁻¹, and this value is also recommended for meats and smoked derivatives. For oysters the limit is up to 10.0 μg kg⁻¹, and for infant foods the restriction is 1.0 μg kg⁻¹ (EFSA, 2008).

Infant foods such as milk of vegetable origin, “soy milk”, often used due to food restriction, such as lactose allergy, can be a carrier of contamination since the raw material, soy, undergoes a drying process to maintain its integrity.

In Brazil, the grain drying process mainly uses firewood as fuel. There are other sources of heat used for drying such as gas, but the process is slow (Torres, 2006). Firewood, despite its lower cost, leads to incomplete combustion during the burning of the organic material, thus generating smoke that contains contaminants such as polycyclic aromatic hydrocarbons (PAHs), which inevitably come into contact with the grains in direct-fired furnaces (Meire et al., 2007) and can cause contamination.

Several studies in the food field about the contamination with PAHs and many

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others organic contaminants (Pérez et al., 2014; Sanchis et al., 2013, 2015) have identified the presence of these compounds at levels even higher than that established by the European legislation, which ranges between 1.0 and 10 μg kg⁻¹, depending on the type of food (EFSA, 2008). The level of contamination and/or the process within the raw material processing can influence the increase, reaching the by-products (Bettin and Franco 2005; Tfouni, 2007; Camargo et al., 2011; Azevedo et al., 2012). Even at minimum percentages of PAHs, their toxicity is high, since some PAHs are considered potentially carcinogenic and genotoxic (JARC, 2014; WHO, 2005).

The objective of the study was to identify possible contamination by PAHs in soybean grains from storage units of municipalities in the Midwest region of Brazil that have undergone drying in a direct-fired furnace with firewood as fuel.

2. Material and methods

2.1. Materials

2.1.1. Samples

The research was carried out in the Laboratory of Postharvest of Plant Products of the Federal Institute of Goiás (IF Goiano), Campus of Rio Verde – GO and in the Institute of Food Technology – ITAL, with soybean grains from different storage units in the Midwest region of Brazil, seven from Goiás state and one from Mato Grosso state. Grains with moisture contents between 16 and 21.2% on wet basis (% w.b.) were subjected to drying in direct-fired furnaces in the storage units using firewood as fuel. After drying, grain samples were sent to IF Goiano's laboratory, where they remained in a refrigerated room (+18 °C) until the time of the evaluations, which occurred from March to July 2017.

Soybean grains were collected in eight different storage units, in different days and times, totaling 22 samples. After arriving at the laboratory, the samples were identified with date and storage unit. To perform the evaluations, impurities were removed according to their size in oblong-hole sieve (3.5 × 22 mm). The samples were weighed to obtain 1.5 kg and identified with numbers from 1 to 22, (1 and 2 Edéia; 3 and 4 Mineiros; 5 Portelândia; 6 Orizona; 7, 8, 9 and 10 Jataí; 11, 12, 13, 14, 15 and 16 Morrinhos; 17 Agua Bonita (Mato Grosso)); 18, 19, 20, 21 and 22 Catalão. After identification, the samples (22 × 100 g) were kept under refrigeration until they were sent to ITAL to determine the levels of 13 PAHs: benzo(a)anthracene (BaA), benzo(b)fluoranthene (BbF), benzo(j)fluoranthene (BjF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), chrysene (Chr), dibenz(a)anthracene (DahA), dibenzo(a)pyrene (Dap), dibenz(a)pyrene (Daph), dibenzo(a)pyrene (Dalp), indeno(1,2,3-cd)pyrene (Icdp) and 5-methylchrysene (5MChr).

2.1.2. Solvents and reagents

The standards of PAHs were acquired from the brands Supelco (BaA, DahP, DahA, Dalp, DaeP, BjF), Sigma-Aldrich (Dalp, BkF, Chr, BbF, BaP, Icdp) and IRMM BCR-08IR (5-MChr). The solvents and reagents used, HPLC grade, were: hexane, N-dimethylformamide, methanol and acetonitrile (JT Baker). 0.45 μm filters (Agilent) were also used to filter the extracts before injection in the chromatograph. The water used was obtained by means of a Milli-Q purification system (Millipore Co.).

2.2. Method

2.2.1. Extraction and cleaning

5 g of sample were weighed, 50 mL of hexane were added and the mixture was placed in an ultrasound bath for 15 min and transferred to a separatory funnel. Extraction was carried out with 3 portions of dimethylformamide-water (9:1, v/v) (50, 25 and 25 mL) and, subsequently, 100 mL of 1% sodium sulfate were added to the aqueous phase, followed by a new extraction with 3 portions of hexane (50, 35 and 35 mL). The organic phase was then washed with water, dried with anhydrous sodium sulfate and evaporated in a rotary evaporator at 45 °C. The extract was cleaned using a glass column packed with silica gel ( deactivated with 15% water). The extract was eluted with hexane, collected in a round-bottom flask, concentrated in a rotary evaporator and suspended in 2 mL of acetonitrile for subsequent injection into the chromatograph.

2.2.2. Chromatographic determination

The technique used was High-Performance Liquid Chromatography with Fluorescence Detection (HPLC-FLD), using a Shimadzu chromatographic system consisting of an LC-20AT quaternary pump, DGU-20A5 online degasser, SIL-20A automatic injector (30 μL injection volume), CTO-20A column oven and RF-10AXL fluorescence detector. The compounds were separated using a C18 polymeric column (VYDAC 201 TP54 - 25 cm × 4.6 mm i.d., 5 μm, stabilized at 30 °C) and a mobile phase gradient composed of acetonitrile (A) and water (B) at a flow rate of 1 mL min⁻¹, as follows: 0–20 min - 70 to 75% of A, 20–35 min - 75 to 100% of A, 35–55 min - 100% of A, 55–60 min - 75 to 70% of A, 60–75 min - 70% of A. PAHs were detected using the following excitation and emission wavelengths (nm): BaA, Chr and 5MChr (274/414), BjF (312/507), BbF, BkF, BaP, Dalp and DahA (290/430), Icdp (300/500), DaeP (397/403) and Dalp and Dah (304/457).

2.2.3. Quantification

The compounds were quantified by the external standardization method. The analytical curves were constructed from the injection of standard solutions, containing the 13 PAHs, at seven levels of acetonitrile concentration (0.30 μL L⁻¹ to 20 μL L⁻¹).

2.3. Statistical analysis

The experiment was carried out in a completely randomized design (CRD) with three replicates to obtain the means. The experimental data were subjected to analysis of variance (ANOVA) and analyzed by Tukey test at 5% significance level, in the statistical program SISVAR 5.6.

3. Results and discussion

Table 1 presents the summary of the analysis of variance for the PAHs. 13 PAHs were detected in soybean grains; the levels found for some of the compounds did not differ (BjF, Icdp, DaeP, DalP, DahP), and the coefficient of variation did not have representative values. By contrast, the others (BaP, Chr, BaA, BkF, BbF, 5MCh, DahA, DalP) differed between the analyzed samples, according to the overall mean. DalP obtained the highest CV, with average contamination level of 315.91 μg kg⁻¹, among the samples from the analyzed region.

Table 2 shows the PHAs levels detected for soybean grains. These PHAs remained below 3.0 μg kg⁻¹ and the highest average obtained was (2.20 μg kg⁻¹) in sample 2 of dibenzo(a)pyrene (Dalp). The concentration of these compounds in the drying air can be affected by several factors such as fuel temperature, pyrolyzed material, time of persistence of the molecules in the furnace and concentration of oxygen during burning process (Hutt et al., 1978; Megrath et al., 2003).

Among all the samples, Dalp differed with a value of 2.20 μg kg⁻¹, for sample 2 (Edéia), followed by 0.41 μg kg⁻¹ in sample 17 (Agua Bonita MT). The compounds BjF and Icdp remained with value of 0.40 μg kg⁻¹, except for sample 3 (Mineiros) with value of 0.27 μg kg⁻¹.

The reason for these compounds to show similarity of contamination, and low value, can be explained by their molecular weight because, except for Dalp and BjF, which have five rings, the others have six rings. The more rings, the higher the molecular weight, which reduces the solubility influenced by the octanol-water partition coefficients (log Kow), thus leading to decrease in the vapor pressure (Aparg, 1992; Netto et al., 1996; Brito et al., 2005; Tfouni et al., 2013).

Camargo et al. (2011) identified values of 1.94 μg kg⁻¹ for BaP, in commercial soy oils, close to those established by the European Union.
in general, they are considered more worrying regarding genotoxicity, greater than in the sample from Ed.

Table 1. Mean squares of the variables for benzo(a)anthracene (BaA); chrysene (Chr); 5-methylchrysene (5MChr); benzo(b)fluoranthene (BbF); benzo(k)fluoranthene (BkF); benzo(a)pyrene (BaP); dibenzo(a,h)anthracene (DahA); indeno(1,2,3-cd)pyrene (IcdP).

| SV | DF | BaA | Chr | 5MChr | BbF | BkF | BaP | DalP | DahA | IcdP |
|----|----|-----|-----|------|-----|-----|-----|------|------|------|
| Sample | 21 | 0.114** | 0.145** | 0.027** | 0.031** | 0.001** | 0.052** | 0.618** | 0.457** | 0.0025 |
| Residual | 44 | 0.02 | 0.06 | 0.004 | 0.0004 | 0.0004 | 0.0005 | 0.227 | 0.016 | 0.00 |
| Corrected total | 65 |          |          |      |      |      |      |      |      |       |
| CV (%) | 67.37 | 120.20 | 52.13 | 34.46 | 38.66 | 36.64 | 315.91 | 18.55 | 0.00 |       |
| Overall Mean | 0.21 | 0.21 | 0.12 | 0.18 | 0.05 | 0.18 | 0.15 | 0.54 | 0.40 |       |

SV: Source of Variation; DF: Degrees of freedom; (**): Significant at 5% significance level by Tukey test; NS: Not significant.

Table 2. Mean levels of PAHs identified and quantified in soybean grain samples (n = 3).

| Sample | Mean (µg kg⁻¹) |
|--------|----------------|
| BaA    | Chr            |
| 0.04 ± 0.00 a | 0.04 ± 0.00 a |
| 0.21 ± 0.11 abcd | 0.04 ± 0.00 a |
| 0.22 ± 0.00 ab | 0.04 ± 0.00 a |
| 0.41 ± 0.33 bc | 0.04 ± 0.00 a |
| 0.11 ± 0.07 abc | 0.04 ± 0.00 a |
| 0.50 ± 0.01 d | 0.30 ± 0.01 bc |
| 0.51 ± 0.01 bcd | 0.17 ± 0.04 ab |
| 0.45 ± 0.08 abcd | 0.18 ± 0.06 ab |
| 0.25 ± 0.05 abcd | 0.04 ± 0.00 a |
| 0.26 ± 0.08 d | 0.04 ± 0.00 a |

Means followed by the same letters in the same column do not differ from each other by the Tukey test at 5% significance level.

BaA: benzo(a)anthracene; Chr: chrysene; 5MChr: 5-methylchrysene. BbF: benzo(b)fluoranthene. BkF: benzo(k)fluoranthene. BaP: benzo(a)pyrene; DalP: dibenzo(a,h)pyrene; DahA: dibenzo(a,h)anthracene; IcdP: indeno(1,2,3-cd)pyrene.

(2.0 µg kg⁻¹) (FAO/WHO, 2006), and total PAH reached approximately 6.0 µg kg⁻¹. These results are above those found in the present study, in which the highest level of PAHs was 3.70 µg kg⁻¹ (Catalão, 19).

It is not possible to determine the time of exposure of each sample to the smoke during drying, as it is directly linked to the initial moisture content, and whether this influenced the level of contamination. According to Silva et al. (2018), convective drying in a direct-fired furnace increases the level of PAHs contamination in soybean grains.

It is important to note that the heavier compounds, in addition to being more stable, have a higher lipophilic character, a characteristic that facilitates their absorption by the body (Rey-Salgueiro et al., 2009). Thus, in general, they are considered more worrying regarding genotoxicity, such as BaP, IcdP, DahA, BaA, BbF, BkF. Sample 11 (Morrinhos) showed significant values of BaP, DahA and IcdP.

Table 3 shows two groups suggested as markers (PAH4 and PAH8) and the sum of the PAH identified in the samples (13 PAH). It was observed that the sum of PAH (except benzo(ghi)perylen, not analyzed in the samples and added to the other seven compounds), although greater than in the first group, did not show a substantial representation of contaminants, pointing out that PAH4 (markers determined by CEC, 2011) establishes a sufficient safety margin.

All samples had the sum of PAHs greater than 1.0 µg kg⁻¹, defined as the contamination limit for infant foods (EFSA, 2008). It was observed that BaP was below 2.0 µg kg⁻¹, which is extremely important because of its carcinogenic potential. The highest value found for the sum of the 13 PAHs was 5.06 µg kg⁻¹ in the sample from Edêia (2).

These results point to a contamination level higher than that established for infant foods containing soy in their composition. For lactating infants and children, the maximum limit is 1.0 µg kg⁻¹ according to CEC (2005).

The highest concentration of PAH4 (sum of BaP, BaA, BbF and Chr) was found in sample 2, with a total of 1.45 µg kg⁻¹, but BaP resulted in half of sample 1, remaining below the value established by the European legislation (CEC, 2011).

The means comparison test (Tukey at 5% significance level) showed differences between the groups, PAH4 and PAH8, and the samples 2, 4, 5, 9, 10, 11, 12 and 6 had smaller margins. The other samples showed more accentuated differences.

The values are within the standards established by the European Union and ensure that the PAH4 offers a satisfactory margin of safety as to the quantification of these compounds in food, so it is not necessary to establish a new group of markers.
The samples from Catalão (18–22) had higher values for all PAHs identified compared to the other regions, but still for the markers PAH4 and PAH8, the sum does not pose risk because they are below the limits established by the European Union. The samples from Morrinhos (11, 12, 13, 14, 15 and 16) showed higher values, and this city was the second region in contamination. However, the total sum of PAHs was higher for the sample from Edéia (17), which can be observed in sample 1 (Edéia), in which the initial moisture content was 19.00% (w.b.) and the drying air temperature was 90 °C, being the highest temperature used among the dryers analyzed. On the other hand, sample 12 (Morrinhos) was dried at the lowest temperature, 78 °C. The sample from Portelândia had the highest initial moisture content (21.2% w.b.) before drying, but the drying air temperature was 80 °C.

According to Smaniotto et al. (2014), the adequate moisture content for storing soybean grains under the climatic conditions of Brazil is up to 12% (w.b.). In order to reach this moisture content, the process is directly influenced by the drying temperature and drying time, which can increase the exposure of the grains to smoke. Normally, the dryers should use air temperatures lower than 100 °C and, under these operating conditions, are not expected to generate the PAHs. However, depending on the exposure time even at lower temperatures, the samples can be contaminated during drying (Bartle, 1991).

The consequence of the higher initial moisture content in the grains, as in sample 5 (Portelândia - 21.2% w.b.), is the increase in the time of exposure of the grains to the smoke generated in the direct-fired furnace.

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**Table 3. Mean levels of PAH4, PAH8 and 13PAHs in soybean grains (n = 3).**

| Sample | Region  | Mean (µg kg⁻¹) | PAH4 | PAH8 | 13 PAHs |
|--------|---------|----------------|------|------|---------|
| 1      | Edéia   | 1.07 ± 0.22 ab | 1.88 ± 0.24 bc | 2.12 ± 0.45 abc |
| 2      | Edéia   | 1.45 ± 1.84 b | 2.05 ± 1.93 bc | 5.06 ± 4.17 c |
| 3      | Mineiros| 0.35 ± 0.11 ab | 1.15 ± 0.17 ab | 1.76 ± 0.19 ab |
| 4      | Mineiros| 0.66 ± 0.14 ab | 1.41 ± 0.20 bc | 2.14 ± 0.21 abc |
| 5      | Portelândia| 0.56 ± 0.12 ab | 1.50 ± 0.16 bc | 2.18 ± 0.25 abc |
| 6      | Orizona | 0.09 ± 0.06 ab | 1.38 ± 0.11 abc | 2.07 ± 0.37 abc |
| 7      | Jataí   | 0.65 ± 0.25 ab | 1.63 ± 0.35 bcd | 2.54 ± 0.35 abc |
| 8      | Jataí   | 0.57 ± 0.27 ab | 1.52 ± 0.41 bc | 2.16 ± 0.43 ab |
| 9      | Jataí   | 0.93 ± 0.68 ab | 1.90 ± 0.73 bcd | 2.64 ± 0.73 abc |
| 10     | Jataí   | 0.44 ± 0.15 ab | 1.31 ± 0.33 abc | 2.00 ± 0.33 ab |
| 11     | Morrinhos| 1.30 ± 0.19 ab | 2.13 ± 0.27 bcd | 2.82 ± 0.31 ab |
| 12     | Morrinhos| 0.12 ± 0.19 ab | 1.17 ± 0.28 ab | 2.19 ± 0.30 ab |
| 13     | Morrinhos| 1.34 ± 0.01 ab | 2.20 ± 0.06 bcd | 3.00 ± 0.06 bc |
| 14     | Morrinhos| 1.24 ± 0.12 ab | 2.06 ± 0.23 bcd | 2.25 ± 0.28 ab |
| 15     | Morrinhos| 0.63 ± 0.05 ab | 1.50 ± 0.14 bc | 2.79 ± 0.19 ab |
| 16     | Morrinhos| 0.67 ± 0.42 ab | 1.60 ± 0.53 bcd | 1.78 ± 0.59 bc |
| 17     | Agua Bonita| 0.32 ± 0.11 b  | 0.96 ± 0.11 ab | 2.19 ± 0.47 ab |
| 18     | Catalão | 0.80 ± 0.02 ab | 2.65 ± 0.04 cd | 3.54 ± 0.05 bc |
| 19     | Catalão | 0.85 ± 0.04 ab | 2.97 ± 0.33 d  | 3.70 ± 0.37 bc |
| 20     | Catalão | 0.77 ± 0.19 ab | 2.07 ± 0.29 bcd | 2.81 ± 0.35 abc |
| 21     | Catalão | 0.25 ± 0.05 ab | 1.91 ± 0.25 bcd | 2.51 ± 0.25 abc |
| 22     | Catalão | 0.80 ± 0.07 ab | 2.36 ± 0.22 bcd | 2.96 ± 0.22 bc |

Means followed by the same letters in the column do not differ from each other by the Tukey test at 5% significance level. PAH4: Summation of benzo(a)pyrene; benzo(a)anthracene; benzo(b)fluoranthene and chrysene; PAH8: summation of PAH4 and indeno(1.2.3-CD)pyrene; benzo(k)fluoranthene (BkF); dibenzo(a,h)anthracene; 13 HPAs: summation of the 13 PAHs analyzed.

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**Table 4. Location of municipalities where soybeans were collected.**

| Municipality | Latitude  | Longitude | Altitude (m) | Area (km²) |
|--------------|-----------|-----------|--------------|------------|
| Edéia        | 17° 20’ 18” S | 49° 55’ 53” W | 601 | 1466.3 |
| Mineiros     | 17° 34’ 10” S | 52° 33’ 04” W | 750 | 9096.4 |
| Portelândia  | 17° 21’ 27” S | 52° 40’ 28” W | 848 | 556.58 |
| Orizona      | 17° 01’ 53” S | 48° 17’ 45” W | 806 | 1979.4 |
| Jataí        | 17° 52’ 53” S | 51° 42’ 52” W | 696 | 7197.1 |
| Morrinhos    | 17° 43’ 52” S | 49° 05’ 58” W | 771 | 2855.3 |
| Agua Bonita  | 14° 3’ 19” S  | 52° 9’ 33” W  | 446 | 7537.95 |
| Catalão      | 18° 09’ 57” S | 47° 56’ 47” W | 835 | 3789.5 |
during drying. However, it was noted with samples 2 (Edéia) and 5 (Portelândia) that, although the initial moisture contents greatly differed, the drying air temperatures were the same. As the final moisture content had a difference of 2.06 ºC, it is not possible to know the time of exposure of each sample to the smoke generated in the drying. This result is consistent with a time of exposure to smoke in drying similar to that for the sample from Portelândia, since the lowest value of contamination was obtained for the sum of PAHs. According to Conde et al. (2004), the synthesis of PAHs is influenced by the following factors: type of biomass present, pressure, amount of available oxygen and, mainly, amount of heat.

4. Conclusions

Polycyclic aromatic hydrocarbons were identified and quantified in soybean grains from the Midwest region of Brazil and subjected to drying in a direct-fired furnace with firewood as fuel. The values found did not exceed the maximum limits established by the European Union, except those for infant food. In the next studies, it would be interesting to control the residence time of the grains in the dryers to assess the contamination rate.

Declarations

Author contribution statement

Rosana Maria Pereira Silva: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Osvaldo Resende: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data.

Jaqueline Ferreira Vieira Bessa, Fernanda Moralez Leme Gomes, Silvia Amélia Verdiani Tfouni: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Adrielle Borges de Almeida: Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data included in article.supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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Table 5. Moisture contents before drying (MCBD) and after drying (MCAD) and drying air temperature (DAT) of soybean samples (n = 3).

| Region     | Sample | MCBD (%) | MCAD (%) | DAT (ºC) |
|------------|--------|----------|----------|----------|
| Edéia      | 1      | 19.00    | 11.34 f  | 90.0     |
|            | 2      | 16.00    | 9.33 a   | 80.0     |
| Mineiros   | 3      | 16.00    | 11.95 h  | 80.0     |
|            | 4      | 19.70    | 12.60 j  | 80.0     |
| Portelândia| 5      | 21.20    | 12.47 i  | 80.0     |
| Orízona    | 6      | NI       | 12.43 i  | NI       |
| Jatai      | 7      | NI       | 11.76 g  | NI       |
|            | 8      | NI       | 11.78 g  | NI       |
|            | 9      | NI       | 11.47 g  | NI       |
|            | 10     | NI       | 12.38 i  | NI       |
| Morrinhos  | 11     | 18.00    | 11.07 e  | 82.0     |
|            | 12     | 16.90    | 11.58 g  | 78.0     |
|            | 13     | 18.30    | 13.09 j  | 80.0     |
|            | 14     | 17.00    | 10.87 c  | 80.0     |
|            | 15     | 16.70    | 11.07 e  | 80.0     |
|            | 16     | 17.00    | 10.71 b  | 79.0     |
| Agua Bonita| 17     | 16.60    | 13.87 l  | 80.2     |
| Catalão    | 18     | 20.00    | 11.46 g  | 85.0     |
|            | 19     | NI       | 10.96 d  | 85.0     |
|            | 20     | NI       | 11.54 g  | NI       |
|            | 21     | NI       | 11.03 e  | 85.0     |
|            | 22     | NI       | 11.33 f  | NI       |

NI: not informed; Means followed by the same letters in the same column do not differ from each other by the Tukey test at 5% significance level.
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