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# Biogenic Amine Profile in Unripe Arabica Coffee Beans Processed According to Dry and Wet Methods

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**ABSTRACT:** Immature coffee fruit processing contributes to a high amount of defective beans, which determines a significant amount of low-quality coffee sold in the Brazilian internal market. Unripe bean processing was tested, taking the levels of bioactive amines as criteria for evaluating the extent of fermentation and establishing the differences between processing methods. The beans were processed by the dry method after being mechanically depulped immediately after harvest or after a 12 h resting period in a dry pile or immersed in water. Seven bioactive amines were quantified: putrescine, spermine, spermidine, serotonin, cadaverine, histamine, and tyramine, with global amounts ranging from 71.8 to 80.3 mg/kg. The levels of spermine and spermidine were lower in the unripe depulped coffee than in the natural coffee. The specific conditions of dry and wet processing also influenced cadaverine levels, and histamine was reduced in unripe depulped coffee. A resting period of 12 h does not induce significant alteration on the beans and can be improved if performed in water. These results confirm that peeling immature coffee can decrease fermentation processes while providing more uniform drying, thus reducing the number of defects and potentially increasing beverage quality.

**KEYWORDS:** *unripe coffee, biogenic amines, postharvest treatment, coffee processing*

## INTRODUCTION

Coffee is a natural beverage, with several compounds that promote human health and performance. However, some compounds present in the grains may interfere with its quality and safety. Given the current trade barriers and restrictions on food marketing, concerns are increasing about coffee quality and safety. Meanwhile, consumers themselves are also becoming more selective.

One of the biggest problems in the Brazilian coffee production chain is the high amount of defective beans, which contributes to a significant amount of low-quality coffee sold in the internal market. The presence of defects dramatically affects coffee quality, and these defects can arise from problems that occur during harvesting, processing, or drying. In particular, "green" defects are associated with the presence of immature fruits, which lead to increased stringency. Green defects have been described as an important factor for coffee classification.[1]

Coffee quality can be improved by selecting only ripe fruits during collection. Nevertheless, most producers use the strip-picking method, which involves collecting all cherries at once with reduced economic costs. Subsequent removal of the immature cherries is required if a high-quality coffee is to be produced. It is most common for all beans to be processed and dried together, after which the defective beans are separated from the high-quality beans by mechanical sorting. The defective beans are blended with other low-quality beans and sold in the internal market, which minimizes the associated economic losses. More effective methods are needed to reduce the amount of defective beans in the final product, to decrease the economic losses that stem from low-quality beans, and to increase the quality of the coffee produced.

The key difference between the classical dry and wet processing methods is pulp removal: in the wet method, the fruit pulp is separated from the seeds before drying by a combination of mechanical depulping and fermentation, whereas in the dry method this occurs only after a prolonged period of drying of the entire cherries. The semidry method, or "pulped natural" method, is an intermediate process that has recently seen increased use in Brazil, with positive outcomes. In this method, the cherries are depulped and the seeds dried while still surrounded by part of the mucilage, and the fermentation step is omitted.[2] Processing of unripe coffee beans by the wet or semidry methods is seen as a potential way to minimize the negative impacts of defective green beans. The drying step after pulp removal is more uniform and results in fewer black, green, and sour defects, producing improved coffee with higher commercial value.[3,4]

The quality of coffee beverages is related to the chemical composition of the roasted beans, which, in turn, is affected by the chemical composition of the green beans and by postharvest processing conditions.[5] Biogenic amines are nitrogenous organic bases that serve as important indicators of quality in a wide variety of food products. They are present in small concentrations in coffee beans, with putrescine

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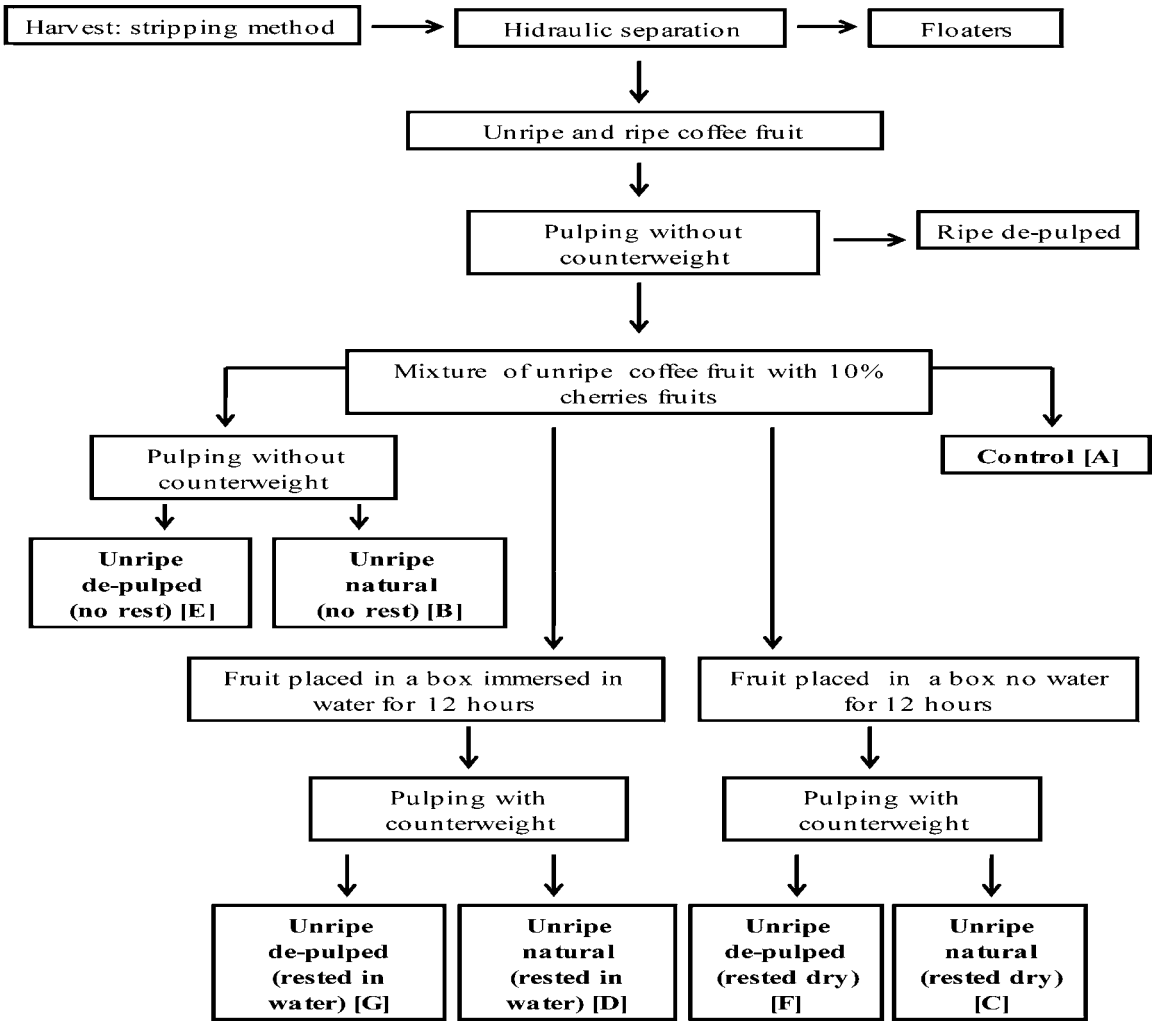


**Figure 1.** Schematic representation of coffee processing used in the present study.

reported as the main amine in Arabica beans. The presence of histamine has been reported only in coffee of lower quality, suggesting that this amine is associated with the presence of defective beans.[5]

The criteria commonly used to evaluate the quality of coffee beans include bean size, color, shape, processing method, crop year, flavor or cup quality, and presence of defects.[6] Among those, flavor (cup quality) and presence of defects (type classification) are the most important criteria employed worldwide in coffee trading. Because of the variations in processing, and also the fact that the content of amines is known to be related to the quality of food products in general, an investigation of the profiles of amines in coffee samples of different qualities, as well as the effects of processing on those compounds, is relevant.[5]

The presence of defects is quite relevant in establishing coffee quality, as they are associated with problems during harvesting and preprocessing operations. To increase the revenue that can be generated from immature beans, there is an ongoing effort to develop methods for processing them separately from other beans. Several processing conditions have been tested, and their effectiveness in producing higher quality beans and coffee has been evaluated, particularly by Nobre.[4] Because defective beans are known to have altered levels of bioactive amines[7] and the concentrations of such amines can depend upon the processing method,[8] the present study aims to evaluate the effect of different methods on the levels of bioactive amines in unripe coffee bean processing.

## MATERIALS AND METHODS

**Samples.** Coffee fruits (*Coffea arabica* L.) of the Topazio cultivar (2006/2007 crop) were grown at the Federal University of Lavras. Experiments were designed to evaluate the quality of immature coffee processed by dry and wet methods, with either wet or dry resting periods, as described by Nobre.[4]

**Sampling Procedures and Experiments.** Coffee cherries were collected by stripping, as is common in the region, and both ripe and unripe fruits were therefore collected. After cleaning and hydraulic separation of floaters and sinkers, the cherries were depulped without counterweight (regulation of drum pulper pressure), and two fractions were separated: the ripe pulped fraction and a fraction containing unripe coffee fruits with up to 10% fruit cherries that were not properly depulped by the machine. This mixture was used as the raw material for the assays developed in this study, and it was divided into three portions as shown in Figure 1. The first portion was used as the control (A). Another portion was stripped with regulated pressure, resulting again in two fractions: unripe (B) and unripe depulped (E). The third part of the mixture was placed in two boxes. One box was filled with water, and, after a rest period of 12 h, the fruits were depulped with regulated pressure to yield unripe (D) and unripe depulped coffee (G). The conditions in the second box were similar, but no water was added to the box, and additional fractions of unripe (C) and unripe depulped coffee (F) were produced, all in accordance


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**Table 1. Levels (Milligrams per Kilogram, Mean ± SD) of Amines in Immature Coffee Processed According to Different Methods (See Sampling Procedures and Experiments for Detail)[a]**

| | no rest | | | rested in water | | rested dry | |
|---|---|---|---|---|---|---|---|
| bioactive amine | natural (A, control) | depulped (E) | natural (B) | depulped (G) | natural (D) | depulped (F) | natural (C) |
| putrescine | 53.8 ± 2.6 | 57.8 ± 4.2 | 56.5 ± 4.0 | 59.0 ± 5.0 | 61.1 ± 4.8 | 60.8 ± 3.8 | 61.5 ± 6.5 |
| spermidine | 8.2 ± 0.8 | 6.6 ± 0.9b | 7.8 ± 0.8a | 6.7 ± 0.2 | 7.6 ± 1.3 | 6.3 ± 0.5 | 8.1 ± 1.3 |
| spermine | 7.2 ± 0.6 | 6.3 ± 0.1b | 7.1 ± 0.5a | 6.2 ± 0.5 | 7.1 ± 0.6 | 6.6 ± 0.5 | 7.2 ± 0.9 |
| serotonin | 1.5 ± 0.1 | 1.7 ± 0.1 | 1.7 ± 0.2 | 1.6 ± 0.2 | 1.8 ± 0.1 | 1.7 ± 0.1 | 1.9 ± 0.2 |
| histamine | 0.4 ± 0.0 | 0.5 ± 0.0 | 0.5 ± 0.1A | 0.7 ± 0.1 | 0.6 ± 0.2A | 0.6 ± 0.3a | 0.9 ± 0.1Bb |
| cadaverine | 0.3 ± 0.0 | 0.4 ± 0.1A | 0.4 ± 0.1 | 0.6 ± 0.1Bb | 0.5 ± 0.0a | 0.6 ± 0.1Bb | 0.4 ± 0.1a |
| tyramine | 0.4 ± 0.3 | 0.1 ± 0.0 | 0.4 ± 0.5 | 0.3 ± 0.3 | 0.5 ± 0.2 | 0.4 ± 0.3 | 0.3 ± 0.3 |
| total | 71.8 | 73.3 | 74.4 | 75.1 | 79.2 | 77.0 | 80.3 |

[a]SD, standard deviation. Mean values followed by lower case letters show significant differences between pulped and natural processing by $t$ test ($p < 0.05$); mean values followed by upper case letters show significant differences between the procedures (no rest, rested dry, and rested in water) in the dry or wet processing by $t$ test and Tukey's test ($p < 0.05$).

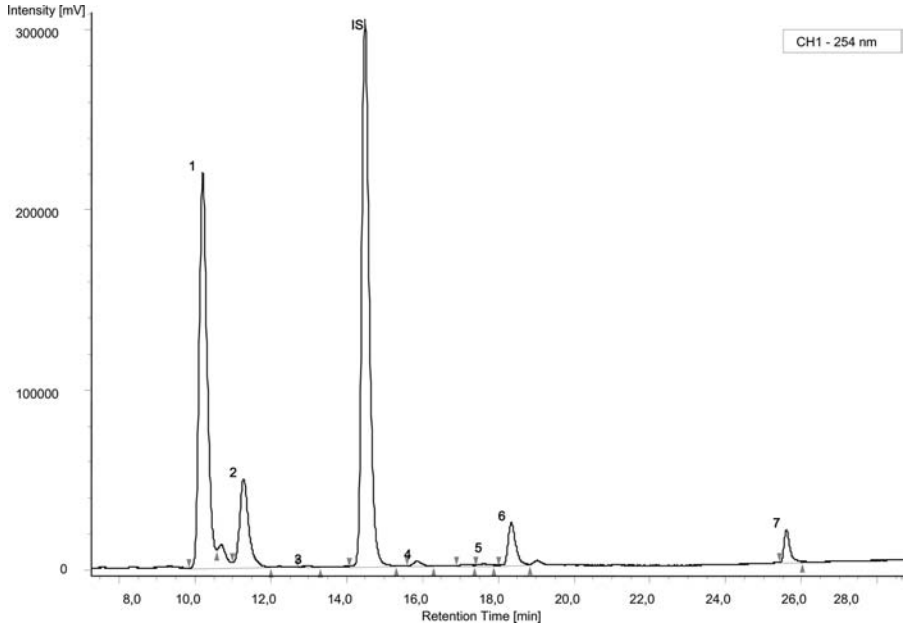


**Figure 2.** Chromatogram (254 nm) of a processed sample from treatment A (control). Peaks: 1, putrescine; 2, cadaverine; 3, histamine; IS, internal standard; 4, serotonin; 5, tyramine; 6, spermidine; 7, spermine. For experimental conditions, see Material and Methods.

with Nobre.[4] Drying of the immature cherries (A–D) was carried out by the natural method (dry method), on sun terraces in thin layers interspersed with small piles of up to 2 cm, revolving 12 times per day. Upon reaching a half-dry state, drying was conducted in piles of 15 cm, which were plowed at least 10 times per day until an 11% water content was reached. The depulped unripe coffee beans (E–G) were dried in the yard in layers of up to 2 cm, revolving 16 times per day.

**Sample Preparation.** After drying, samples were ground to a fine grain for a period of 2 min in a refrigerated mill (Technal analytical model TE 631/2). A second milling step was performed with a grinding ball mill using liquid nitrogen for 1 min, and a 0.75 mm sieve was used to isolate the smallest grains. Samples were initially preserved by freezing and then freeze-dried for extended preservation.

**Standards and Reagents.** Aqueous standard solutions of putrescine dihydrochloride, cadaverine dihydrochloride, histamine dihydrochloride, tyramine hydrochloride, spermidine trihydrochloride, spermine tetrahydrochloride, and serotonin (Sigma Chemical Co., USA) were prepared at 10 mg/mL, stored at 5 °C, and diluted into working solutions as needed. The internal standard used was 1,7-diaminoheptane (Aldrich, USA), also at 10 mg/mL and stored at 5 °C. Trichloroacetic acid (TCA) and BEHPA (bis-2-ethylhexylphosphate) were also from Aldrich. A solution of dansyl chloride (Sigma) was prepared daily in acetone (7.5 mg/mL) and stored at −20 °C. The solution of L-proline (Sigma) was prepared at 100 mg/mL in water and kept refrigerated.

**Extraction.** The extraction process was performed as described by Casal et al.[9] Briefly, the amines were extracted with three 8 mL portions of 5% TCA, using 2 g of each coffee sample and an adequate amount of internal standard. After vortex mixing for 10 min, the supernatant was removed and the solids were further extracted in the same conditions. The separated fractions were combined, adjusted to 25 mL, and centrifuged at 4000 rpm for 10 min at room temperature. A portion of 2 mL was subjected to an ion-pair extraction with BEHPA as follows: the pH was adjusted to 7.4 with NaOH, and the volume was completed to 3 mL with phosphate buffer, pH 7.4 (0.2 M). The solution was extracted with 2 mL of BEHPA in chloroform (0.1 M) and centrifuged at 4000 rpm. The lower phase was moved to a second tube, and a back-extraction with 2 mL of 0.1 M HCl was performed.

**Derivatization.** To 400 μL of extract was added 1 mL of saturated solution of $Na_2C0_3$ (pH 11.0–11.2), followed by 1 mL of the dansyl chloride solution in acetone. After the mixture had been rapidly stirred, samples were placed for 12 min at 60 °C in a thermostatically controlled water bath, shielded from light. The extracts were cooled for 5 min on ice, and the excess dansyl chloride was consumed by


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reacting with proline solution (100 μL) for 15 min in the dark at room temperature. The extraction of biogenic amine derivatives was performed with 1.5 mL of toluene, and the organic phase was recovered after 15 min at −18 °C, evaporated under a gentle stream of nitrogen (±40 °C), and dissolved in 200 μL of acetonitrile/methanol (50:50) (with vortex). After centrifugation at 13000 rpm for 5 min, the solution was transferred to appropriate vials for use in the HPLC autosampler.

**Chromatographic Analysis.** Analyses were performed on an HPLC system with dual pumps (model PU 980), an autosampler (AS-950), and a diode array detector (DAD, model MD-910), all from Jasco (Japan). Data were analyzed in a PDA-Software Borwin Controller (JMBS, France). The column used was a reversed-phase Kromasil 100 $C_{18}$ (5 mm × 250 mm × 4.6 mm) (Teknokroma, Spain), and it was operated at room temperature. The elution was performed with a linear gradient of 0.05 M phosphoric acid and methanol/acetonitrile (1:1) at 1 mL/min with gradient elution.[9] Compounds were identified by chromatographic comparison with standards and by coelution, taking as reference published data on green coffee. Detection was performed using the DAD at 254 nm. All procedures were performed at least in duplicate, and the results were expressed as milligrams per kilogram of dried unripe coffee.

**Statistical Analysis.** The experimental design was a randomized factorial 2 × 3 design, corresponding to two processing methods (dry and wet) and three procedures for treating immature coffee (without rest, 12 h immersed in water, or 12 h in a dry pile). Four replicates were performed for each treatment. The data were subjected to an analysis of variance *F* test and compared by using the Tukey test (with 5% probability threshold) of the general linear model (GLM) procedure in the SAS software package.[10]

## RESULTS AND DISCUSSION

The different postharvest procedures performed on unripe coffee contributed to variation in bioactive amine levels. The results are detailed in Table 1, which is organized according to the type of resting period treatment (none, in water, dry) and depulping status (depulped or in shell). Seven bioactive amines were detected and quantified for all processing treatments. A typical chromatogram is presented in Figure 2, corresponding to a control sample. This analytical methodology has low quantification limits, ranging from 2 μg/kg for cadaverine to 40 μg/kg for spermidine,[9] clearly below the amounts quantified in the present study.

The presence of bioactive amines in coffee has been described by several authors. These organic compounds are usually formed by free amino acid decarboxylation as a consequence of physical−chemical and biochemical processes that take place during coffee bean processing. These fermentations can be intentionally carried out by the producer, as in the wet processing method, to remove the mucilage remains. Some fermentation can also occur during the drying steps, used in both wet and dry processing methods, although the nature of the process depends on the coffee beans: because of the lack of external layers, the wet-processed beans dry more rapidly and more uniformly, resulting in higher quality beans. In the dry method, the beans are dried still within the cherry, which results in reduced drying efficiency and consequently undesired fermentation.

In this study, immature cherries (with up to 10% ripe cherries) were processed according to the classical dry method (A−D) and the wet method, in which the cherries are mechanically depulped before being dried (E−G). Shell removal in immature beans is a step that induces intense mechanical stress and wear in the pulping equipment. To reduce peel adhesion and facilitate pulp removal, the immature cherries were left to rest for 12 h either placed in dry piles or immersed in water. A resting period is often necessary due to processing constraints, as mature beans are typically given higher priority and are depulped sooner.

Quantities of total bioactive amines ranged from 71.8 mg/kg in the control (A) to 80.3 mg/kg in treatment C. Variations can be seen as a consequence of differences in resting period, drying method, drying period, and maturity of the cherries (Table 1). All samples used in the present study were subjected to two depulping steps. Those that were not depulped in the first step were used as the initial material for this work (A), as described under Sampling Procedures and Experiments. Those that were still whole after the second depulping step (B−D) were considered the most immature. Thus, aside from the processing differences imposed by the different drying methods, these batches also differ in terms of maturity. The presence of exocarp in the natural coffee fruits delayed the beginning of the drying process for almost a week, as seen in previous studies.[12] The induction of germination due to water absorption and the stress generated by anoxia may have also contributed to changes in amine formation. After the resting period, the percentages of depulped cherries were similar for all treatments, with no benefit attributed to increasing the amount of depulped cherries.

Globally, these values are slightly lower than those reported recently by Vasconcelos et al.[7] for immature beans, and they are similar to those reported for nondefective beans by the same authors. Indeed, the immature beans analyzed in that work were selected by a sorter machine only after being processed and, thus, they were subjected to the same processing conditions as the mature and unhealthy beans. In this study, immature cherries were separated before being processed, allowing specific adjustments to be made in the drying step for each batch (i.e., each batch was either pulped or dry-processed). When the different processes are compared (dry vs wet), all naturally processed batches had increased amounts of total bioactive amines compared to those that were previously depulped, although the result was not found to be statistically significant.

Putrescine was the most abundant amine in all samples, regardless of the processing method applied, as reported in most studies on ripe Arabica coffee.[5,8,11,13] Putrescine levels varied from 53.8 mg/kg in the control sample (A) to 61.5 mg/kg in the immature cherries with a 12 h dry rest before drying (C). These values are within the range of the only reference values available in the literature for immature beans (average value of 53.9 mg/kg).[7] When the different processes are compared, an apparent increase in putrescine is observed for all treatments compared to the control method. An increase in putrescine concentration can also be observed for the samples that were processed after a 12 h resting period in comparison with the samples processed by the natural method, although the difference is not statistically significant. This increase could be caused by the increased drying time, the presence of the shell (C and D), or the immaturity of the beans. It is possible that increased enzymatic activity occurs during drying, which could promote the formation of free amino acids, particularly arginine, which can serve as precursors of putrescine.[14]

The polyamines spermidine and spermine are formed sequentially from putrescine, and these also occur naturally in fruits. They act together with putrescine in processes such as fruit development.[15,16] Changes in their concentrations can be observed in response to a variety of stresses, including mechanical damage, carbonic gas treatment, and temperature

fluctuations.[17] In this study, spermine and spermidine levels in immature coffee beans averaged 6.8 and 7.3 mg/kg, respectively. These are lower than the levels measured by Vasconcelos et al.[7] in defective immature Arabica beans (18.9 mg/kg), but they are in agreement with the mean values of 9.0 mg/kg spermine and 5.5 mg/kg spermidine found in healthy Arabica beans.[11] As discussed previously, the fact that the immature beans were separated before processing may have contributed to this significant reduction. Spermine and spermidine concentrations were higher in the natural coffees (B−D) than in the depulped ones (E−G), although this result was significant ($p < 0.05$) only for the samples processed immediately (B and E); there was greater variation in the other treatments. The increased concentrations in the naturally processed coffees may be due to the longer drying periods required for this type of coffee.

Serotonin was also detected in the processed coffee beans, but in comparatively low amounts and without observed differences among the processing methods applied. This amine, in particular, although formed from the amino acid tryptophan or from tryptamine, is also naturally present in the coffee wax, a natural protective external layer surrounding the coffee bean. Although depulped beans were dried without their external layers, no reduction in serotonin levels was observed for these batches.

The last three bioactive amines (histamine, cadaverine, and tyramine) were detected in reduced amounts, below 1 ppm. In this study, these biogenic amines had the potential of forming during the unripe coffee processing treatments. The most toxic of these compounds is histamine, being a potential cause of cutaneous, gastrointestinal, neurological, and hemodynamic symptoms.[18] Temperature is recognized as a critical factor in histamine formation[19,20] Thus, when immature fruits were piled for 12 h, the temperature increase may have favored histamine formation. Additionally, the drying time of natural coffee is longer compared to depulped coffee, mainly due to the presence of the exocarp, allowing more time for the formation of histamine in the bean. The higher amine levels in the procedure without water may be due to other factors that influence this amine, such as pH variation, temperature, oxygen levels, and the concentrations of free amino acids.[21] Still, the amounts of histamine present in the beans were of negligible health concern.

The average level of tyramine found in immature seeds was around 0.38 mg/kg. Cadaverine showed average levels of 0.49 mg/kg in immature coffee beans. Vasconcelos et al.[7] did not detect the presence of this amine in coffee associated with green defects, and the tyramine levels they found were slightly higher than the 0.20 mg/kg found in normal Arabica coffee beans.[11] In the present work, lower cadaverine levels were found in the procedures that lacked a resting step. However, when immature coffee beans remained at rest, increased concentrations were observed when depulped after 12 h of resting, both with and without water. This may be related to various physiological processes in fruit that result from stress due to the accumulation of grains in a box.

It was observed in this study that the specific conditions of unripe coffee bean processing influenced the resulting concentrations of biogenic amines, particularly spermine, spermidine, histamine, and cadaverine. Knowing that the quality of coffee beverages is related to the chemical composition of the roasted beans, which, in turn, is affected by the chemical composition of the green beans and by postharvest processing conditions, the mechanisms underlying these changes need to be investigated. The depulping of unripe beans reduces fermentation and favors uniform drying, contributing to a reduced formation of biogenic amines. However, if cherries could not be depulped immediately and had to be reserved, then the 12 h resting period was not responsible for a substantial increase in the concentrations of bioactive amine amounts. Nevertheless, a benefit could still be observed if the cherries were immersed in water. When the two processing methods are compared (natural vs wet), the total amount of bioactive amines is smaller in the latter and the amount of defective beans after processing is also reduced.[4]

Treating immature beans separately and using adjusted depulping could provide additional improvements to the quality of immature beans and reduce the levels of biogenic amines, similar to those observed in ripe coffee. The depulping step allows for a shorter and more uniform drying period, reducing the extent of fermentation and the number of defective beans found after processing and increasing the beverage quality with regard to the amine composition in the unripe coffee beans. These results indicate that the use of more appropriate techniques for processing and drying leads to a reduction in polyamine levels. Whether this reduction is associated with increased cup quality or not is an issue that deserves further attention.

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### Notes

The authors declare no competing financial interest.

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