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Morpho-Physiological Traits and Phytochemical Compositions of *Coffea canephora* Beans from Lampung for Various Harvesting Stages and Soaking Durations

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Abstract: *Coffea canephora*, also known as Robusta, is one of the coffee species that dominates production in Indonesia. The morphology, physiology, and phytochemistry of Canephora coffee beans are affected by many factors, such as the fruit harvesting time and post-harvest processing. The maturity stage of coffee fruits at harvest time and post-harvest management will affect the various morpho-physiological and phytochemical compositions of the coffee beans. This research aimed to observe Canephora coffee beans' morpho-physiological and phytochemical compositions for various harvesting stages and soaking durations. The experiment was conducted using a completely randomized design (CRD). It tested eight treatments, a combination of harvesting times (H1 = only red fruit/ripe stage, H2 = green, yellow, red fruits/strip-picking stage) and soaking durations (S0 = 0, S1 = 12, S2 = 24, and S3 = 36 h) with four replications. The main observations were the morpho-physiological traits (size, weight, color, moisture content, bean yield) and the phytochemical compositions (TPC = total phenolic content, AA = antioxidant activity, and CC = caffeine content) of the coffee bean before and after the drying process. Based on morphological characteristics (bean size and weight before drying), the highest value came from selective harvesting or ripe-picking only without soaking duration application (H1S0). While the selective harvesting method with a 24 h soaking duration showed a high value for the color trait (L and b*), the treatment that affected the color trait after drying was the strip-picking harvesting method without soaking (a* and b*). On the other hand, the phytochemical contents had H1S0 for the highest TPC and AA values. In contrast, H1S1 or selective harvesting, with 12 h of soaking, had the highest value of CC. The optimum combination of harvesting methods and soaking duration will improve the quality of the Canephora coffee beans.

Keywords: Robusta characters; harvest phase; ripe; strip-picking; soaking duration

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1. Introduction

Robusta coffee or *Coffea canephora* is a type of coffee that dominates production in Indonesia. In 2018, Canephora produced about 81.87% of total coffee production, while the remaining 18.13% were Arabica and Liberica coffees [1]. Canephora coffee is suitably adapted to Indonesia and is considered a folk crop by farmers [2] because the species can be developed in marginal environments for coffee, such as low lands and high temperatures, which are the prevailing conditions in the country [3,4]. In Indonesia, this coffee species is mainly produced in six provinces: South Sumatera, Lampung, Bengkulu, North Sumatera, East Java, and South Sulawesi [5]. In addition to taste, Canephora coffee has many phytochemical contents, namely polyphenols, that possess antioxidants which act as natural medicines and function as anti-viral and anti-microbial agents [6–8].

In the coffee industry, bean quality is observed by morphological, physiological, and phytochemical traits. These characteristics will vary depending on their genetics and environment, cultivation technique (seedling until harvest), and post-harvest management [9]. An attempt to improve the quality of Canephora coffee can be carried out through harvesting times and post-harvest processing. The different phases of the plants, including different harvest times, will describe various morphological traits [10]. The common harvesting methods used by coffee farmers are selective (ripe fruit only) and strip-picking (mixed maturity phase) [11]. The ripe fruit of the coffee cherries has been reported as having good morpho-physiological and phytochemical contents [12], while the strip-picking method produces a variety of coffee cherries from green, yellow, and red that have deficient quality because the separated coffee cherries are not fully ripe [13].

A further problem in the degradation of Canephora coffee quality is improper post-harvest processing [14]. Generally, coffee farmers in Indonesia prefer the dry post-harvest process because the process is relatively short and simple, and the costs required are cheap [15]. Meanwhile, according to Borem et al. [16], post-harvest processing with wet methods produces coffee beans with a higher physical quality and taste than dry processing. Therefore, the post-harvest method of wet processing is recommended to improve the quality of Robusta coffee beans. One of the stages in the wet processing method is soaking. According to Sa'diyah et al. [17], the soaking duration of coffee beans can affect the coffee's morpho-physiological traits and taste. Furthermore, the soaking process of coffee beans can also affect the various phytochemical compositions of Canephora coffee [18]. This research was carried out to determine the morpho-physiological traits and phytochemical compositions of Canephora coffee beans for various harvesting stages and soaking durations.

2. Materials and Methods

2.1. Location and Material Preparation

This research was conducted at the Laboratory of the Faculty of Agriculture, Universitas Padjadjaran, from January to May 2023. The material used in this research was taken from Sukamarga Village Gedong Tataan Gedong Tataan Sub District Pesawaran District Lampung Province, Indonesia (5.379891° S 105.095987° E) at an altitude of 500 m above sea level, a temperature around 29–30 °C and a humidity of 67–85%.

The *Coffea canephora* cherries were harvested using 2 methods: selective/ripe only and strip-picking (mixed maturity fruits). Six kilograms were produced for each method, with a total of 12 kg of coffee cherries. After harvesting, the coffee cherries were soaked in water for 2 h. This process aimed to sort and separate the cherries from dirt, pests, and hollow cherries that were carried away from the harvesting process. Then, the coffee cherries were peeled with a pulper machine (VKT-200, VOS Machinery Equipment Co., Ltd., Zhengzhou, China). The result of this process were coffee beans that separated from the skin. The coffee beans then entered the final soaking process, which is aimed at fermentation. Coffee beans were soaked with distilled water in a plastic bucket simultaneously based on a treatment of 0, 12, 24, and 36 h. After the soaking process, the coffee beans were washed until the pulp was removed. The final process of this research was the mechanical drying process. The oven carried out this process with a temperature of approximately 50 °C for 48 h. The main observations were morpho-physiological traits (size, weight, color, moisture content, bean yield) and phytochemical compositions (phenolic, antioxidant activity, and caffeine content).

The experiment was conducted using a completely randomized design (CRD). It tested 8 treatments (Table 1), with 4 replications.

Table 1. The treatment combinations between harvesting method and soaking duration on Canephora coffee seeds.

No	Code	Treatment
1	H1S0	Selective picking + no soaking time
2	H2S0	Control strip-picking + no soaking time
3	H1S1	Selective picking + 12 h of soaking time
4	H2S1	Strip-picking + 12 h of soaking time
5	H1S2	Selective picking + 24 h of soaking time
6	H2S2	Strip-picking + 24 h of soaking time
7	H1S3	Selective picking + 36 h of soaking time
8	H2S3	Strip-picking + 36 h of soaking time

2.2. Analysis of Morpho-Physiological and Phytochemical Composition

2.2.1. Morpho-Physiological Traits

The morphological bean traits were observed using the method of Kusolwa et al. [19] which calculated seed size using digital calipers (0–150 mm). Bean yield was calculated using the last bean weight/the initial bean weight × 100%. The seed size was measured based on LBD (length before drying), WBD (width before drying), TBD (thickness before drying), LAD (length after drying), WAD (width after drying), and TAD (thickness after drying). Seed color characters were analyzed using a Minolta Chroma Meter type CR 400. The color units selected were L*, a*, b*, or CIELAB units. L* denotes the lightness variable and a* and b* indicate the color trend while +a* indicates redness, −a* greenness, +b* yellowness, and −b* blueness trends [20].

2.2.2. Phytochemical Composition

Sample Preparation

First, the dried coffee bean was milled using a coffee grinder (Sf 3526, Sonifer, Yiwu, China). Then, the sample extracted for the determination was obtained by extracting the sample using distilled water for 24 h (25 mg/mL). The solution was filtered using Whatman filter paper No. 1 stored in an amber bottle and kept in the refrigerator at 4 °C until further use [21].

Phenolic Composition (TPC)

The TPC was determined using the Folin–Ciocalteu method [21]. The extract (0.4 mL) was mixed with Folin–Ciocalteu reagent (2 mL) and 7.5% (*w/v*) sodium carbonate (1.6 mL). The mixture was allowed to rest for 45 min in the dark, and the absorbance value was measured at 765 nm using a spectrophotometer (Cary 60, Agilent, Santa Clara, CA, USA). The TPC was calculated from the calibration curve, and gallic acid was used as the standard. The result was reported as mg of gallic acid equivalent (GAE)/g dry sample.

Antioxidant Activity (AA)

A solution consisting of AA (2.4 ± 0.02 mg) in methanol (100 mL) was prepared for DPPH radical scavenging assay [21] and was added to the methanolic DPPH (3.850 µL). The mixture was shaken vigorously and kept for 30 min in the dark at room temperature. A mixture of DPPH and methanol was used as the negative control, while ascorbic acid was used as the positive control. The absorbance value of the reaction mixture was measured at 517 nm.

Caffeine Content (CC)

The separation of caffeine by adsorption technique was completed according to the method described by [22] with a slight modification. The sample (15 mL) was put into a 250 mL Erlenmeyer flask and activated carbon (0.15 g) was added. The mixture was stirred briefly, allowed to stand for 5 min and then filtered using Whatman filter paper No. 1 for 10 min. The filtrate obtained was diluted to the mark with distilled water in a 100 mL

volumetric flask. The absorbance of the solution was measured using a spectrophotometer at 400 nm.

2.3. Statistical Analysis

All data are described as mean values (averages) and their standard deviations (SD) on phytochemical contents. ANOVA analysis was used to identify the variance in morphological and phytochemical parameters of coffee beans for different harvesting stages and soaking durations. The *p*-values of ≤ 0.05 were regarded as significant data, followed by Duncan's new multiple range test (DMRT). The results of the data analyses are presented in the tables.

3. Results

3.1. Morpho-Physiological Traits of Canephora Coffee at Various Harvesting Stages and Soaking Durations

Canephora coffee beans at various harvesting stages and soaking durations showed different size traits, such as LBD, WBD, TBD, LAD, WAD, and TAD (Table 2). The mixed maturity beans from the strip-picking method had the lowest values for all traits of the other harvesting method—the characteristics were significantly affected for each treatment except for TAD. The highest value of all the size characters was observed in the mature beans from the selective method with all durations of soaking times (H1S0, H1S1, H1S2, H1S3). The results illustrated that the selective harvesting method had the largest size, length, width, and thickness of Canephora coffee beans. The size of Canephora coffee beans was the feature that indicated the quality of the coffee.

Table 2. Seed size characters (before and after the drying process) of Canephora coffee at various harvesting stages and soaking durations.

Treatments	LBD (mm)	LAD (mm)	WBD (mm)	WAD (mm)	TBD (mm)	TAD (mm)
H1S0	14.55 a	12.57 a	10.65 a	9.51 a	6.83 a	5.68 a
H2S0	12.36 bc	11.71 ab	9.52 bc	8.67 bc	5.68 cd	4.97 a
H1S1	14.10 a	10.91 bc	10.43 a	8.96 ab	6.11 bc	5.26 a
H2S1	11.64 cd	9.54 d	8.90 cd	7.46 d	5.36 d	5.24 a
H1S2	13.47 ab	11.75 ab	10.15 ab	8.86 ab	6.37 ab	5.48 a
H2S2	10.93 d	10.93 bc	8.54 d	8.10 cd	5.51 cd	4.96 a
H1S3	13.46 ab	11.94 a	10.08 ab	8.89 ab	6.50 ab	5.61 a
H2S3	10.99 d	10.46 c	8.35 d	8.29 bc	5.40 d	5.54 a
CV (%)	3.59	5.50	3.58	5.91	7.91	7.91

Note: LBD (length before drying), WBD (width before drying), TBD (thickness before drying), LAD (length after drying), WAD (width after drying), TAD (thickness after drying), H1S0 (control selective picking + no soaking time), H2S0 (control strip-picking + no soaking time), H1S1 (selective picking + 12 h of soaking time), H2S1 (strip-picking + 12 h of soaking time), H1S2 (selective picking + 24 h of soaking time), H2S2 (strip-picking + 24 h of soaking time), H1S3 (selective picking + 36 h of soaking time), H2S3 (strip-picking + 36 h of soaking time). Means (±SD) in the same column followed by a common letter are not significantly different at the 95% level by DMRT ($p \leq 0.05$).

Table 3 shows the weight, moisture content, and bean yield characteristics before and after drying of Canephora coffee beans for different harvesting stages and soaking times. This table shows that the harvesting stages and soaking durations significantly affected all the traits. The mature beans from the selective method had the highest result of the BD weight (H1S0), while for the AD weight, the highest result was obtained by H1S3. The mixed-maturity beans from the strip-picking method had the lowest value of all the weight variables. Furthermore, the size and weight of Canephora coffee beans were related to each trait. This table also shows that all traits did not significantly affect the treatments except for the bean yield variable. For the BD moisture content, the highest value was obtained by H2S0, H1S1, and H2S2. Meanwhile, for the bean yield variable, the highest value was also acquired by H2S2, and H1S0 reached the lowest value.

Table 3. Bean weight, moisture content, and bean yield characteristics (before and after the drying process) of Canephora coffee at various harvesting stages and soaking durations.

Treatments	BD Weight (g)	AD Weight (g)	BD Moisture Content (%)	AD Moisture Content (%)	Bean Yield (%)
H1S0	66.40 a	27.50 c	54.88 ab	4.37 a	41.42 f
H2S0	50.73 c	22.01 e	58.14 a	4.73 a	43.39 e
H1S1	59.49 b	27.89 bc	56.93 a	4.61 a	46.91 c
H2S1	44.73 e	20.57 f	56.46 ab	4.79 a	45.98 d
H1S2	59.39 b	28.25 ab	55.64 ab	2.96 a	47.57 bc
H2S2	48.45 d	24.06 d	56.66 a	4.86 a	49.66 a
H1S3	58.88 b	28.40 a	52.71 b	4.18 a	48.24 b
H2S3	48.99 d	20.93 f	55.72 ab	5.39 a	42.72 e
CV (%)	1.32	1.25	4.75	3.85	1.34

Note: BD (before drying), AD (after drying), H1S0 (control selective picking + no soaking time), H2S0 (control strip-picking + no soaking time), H1S1 (selective picking + 12 h of soaking time), H2S1 (strip-picking + 12 h of soaking time), H1S2 (selective picking + 24 h of soaking time), H2S2 (strip-picking + 24 h of soaking time), H1S3 (selective picking + 36 h of soaking time), H2S3 (strip-picking + 36 h of soaking time). Means (±SD) in the same column followed by a common letter are not significantly different at the 95% level by DMRT ($p \leq 0.05$).

Table 4 describes bean color changes in Canephora coffee before and after drying. This table also shows L*, a*, and b* values that present lightness, redness, and yellowness color content in the appearance of the beans. The L* value represents the range of lightness from white to black; the a* value illustrates the range of red to green color; and the b* value indicates the color in the blue to yellow color ranges. The traits were significantly affected by all the treatments. The L* before drying shows the H1S2 or ripe stage combined with 24 h soaking as the lightest treatment, and the darkest was H1S3 and H2H3, which were not significantly different from other treatments. On the other hand, H1S1 had more redness than H2S1 and H2S2 based on the a* value on BD because those treatments (H2S1 and H2S2) showed more yellowness according to the b* color unit on BD.

Table 4. Color characteristics (L*, a*, and b*) (before and after the drying process) of Canephora coffee at various harvesting stages and soaking durations.

Treatments	BD			AD		
	L*	a*	b*	L*	a*	b*
H1S0	45.52 ab	4.07 ab	25.65 ab	58.97 b	4.71 bc	23.76 b
H2S0	45.51 ab	4.15 ab	25.89 ab	59.10 b	8.13 a	29.18 a
H1S1	45.19 ab	4.85 a	26.76 ab	60.74 ab	5.01 b	23.47 b
H2S1	46.67 ab	1.99 b	22.25 bc	62.71 ab	3.82 bcd	22.35 b
H1S2	48.88 a	4.31 ab	28.87 a	61.51 ab	3.47 bcd	22.69 b
H2S2	45.95 ab	1.94 b	26.80 ab	62.09 ab	2.33 cd	20.49 b
H1S3	42.92 b	3.71 ab	19.55 c	64.60 a	1.51 d	21.21 b
H2S3	42.92 b	3.49 ab	22.78 bc	63.64 a	3.18 bcd	22.62 b
CV (%)	5.03	11.92	10.48	3.01	10.62	8.40

Note: BD (before drying), AD (after drying), H1S0 (control selective picking + no soaking time), H2S0 (control strip-picking + no soaking time), H1S1 (selective picking + 12 h of soaking time), H2S1 (strip-picking + 12 h of soaking time), H1S2 (selective picking + 24 h of soaking time), H2S2 (strip-picking + 24 h of soaking time), H1S3 (selective picking + 36 h of soaking time), H2S3 (strip-picking + 36 h of soaking time). Means (±SD) in the same column followed by a common letter are not significantly different at the 95% level by DMRT ($p \leq 0.05$).

In contrast, the color units in the AD section describe the H1S3, and H1S3 was the highest value of L*, which means that the soaking duration of 36 h affects the light color of the beans and that those treatments were closer to light (white) colors than the others. For AD a*, the highest score was H2S0; the treatments had more redness in the bean color than the other treatments. H2S0, or mixed maturity without soaking, also had the highest value on b* after drying, meaning that this treatment has more yellowness in the beans than others.

3.2. Phytochemical Composition of Canephora Coffee at Various Harvesting Stages and Soaking Durations

Table 5 shows the phytochemical compositions that are represented by total phenolic contents (TPC), antioxidant activity (AA), and caffeine contents (CC) at various harvesting stages and soaking durations as quality parameters of Canephora coffee. The harvesting stages and soaking durations significantly affected the phytochemical content of Canephora coffee, such as TPC, AA, and CC. In contrast to TPC, the mature beans from the ripe stage without soaking had the highest result (H1S0), while the lowest result of TPC was obtained by H1S3 (ripe with 36 h of soaking). For the AA, the highest result was obtained by the selective and strip-picking method with no soaking time (H1S0, H2S0), and H2S2 acquired the lowest result. Furthermore, the mature beans from selective picking with 12 h of soaking time had the highest value on CC (H1S1), and H1S3 obtained the lowest value.

Table 5. Phytochemical compositions of Canephora coffee beans on various harvesting stages and soaking durations.

Treatments	TPC (mg GAE/g Dry Sample)	AA (mg TE/g Dry Sample)	CC (mg/100 g Dry Weight)
H1S0	17.46 ± 0.01 a	50.78 ± 0.16 a	144.80 ± 0.25 c
H2S0	15.86 ± 0.02 c	50.90 ± 0.01 a	142.18 ± 0.14 d
H1S1	14.50 ± 0.01 e	46.16 ± 0.03 d	222.26 ± 0.14 a
H2S1	17.34 ± 0.02 b	46.47 ± 0.02 c	175.15 ± 0.08 b
H1S2	13.30 ± 0.01 f	45.32 ± 0.02 e	67.32 ± 0.03 f
H2S2	14.77 ± 0.01 d	44.78 ± 0.10 f	125.76 ± 0.12 e
H1S3	3.70 ± 0.01 h	47.61 ± 0.01 b	18.30 ± 0.03 h
H2S3	12.72 ± 0.01 g	46.08 ± 0.03 d	18.81 ± 0.22 g

Note: TPC (total phenolic content), AA (antioxidant activity), CC (caffeine content), H1S0 (control selective picking + no soaking time), H2S0 (control strip-picking + no soaking time), H1S1 (selective picking + 12 h of soaking time), H2S1 (strip-picking + 12 h of soaking time), H1S2 (selective picking + 24 h of soaking time), H2S2 (strip-picking + 24 h of soaking time), H1S3 (selective picking + 36 h of soaking time), H2S3 (strip-picking + 36 h of soaking time). Means (±SD) in the same column followed by a common letter are not significantly different at the 95% level by DMRT ($p \leq 0.05$).

4. Discussion

4.1. Morpho-Physiological Traits of Canephora Coffee at Various Harvesting Stages and Soaking Duration

Morphological and physiological traits, such as LBD, WBD, TBD, LAD, WAD, TAD, BD weight, and AB weight, are essential for Canephora coffee beans' parameters. This research reported that the size and weight of Robusta coffee beans gradually increased in line with the maturity level of the coffee beans. The size and weight characteristics of the coffee beans are closely related and important to coffee quality [23]. The results showed that the selectively harvested method had the largest size and weight for all measurements. These data are supported by [24], who observed that selectively harvested coffee cherries significantly increased the size of coffee beans: the larger the coffee bean's size, the greater the resulting weight. Niwagaba and Weldon [25] explained that only red coffee cherries significantly produced a larger final bean size and weight when compared to mixed maturity (red, yellow, and green) coffee cherries. In addition, the soaking time did not affect the length of the beans. It means the fermentation process does not significantly affect changes in some of the physical properties of the coffee beans [26]. The difference in values between the before and after of the drying process was caused by the high temperature of the heating process. This was due to the shrinkage in size and weight after going through the drying process. Payel and Natarajan [27] explained that the size and dimensions of the beans change as the water content in the seeds decreases, which causes the final yield of the beans after drying to be smaller along with the absorption of the moisture content.

In contrast to the moisture content, the harvesting stages and soaking durations did not significantly affect all the traits. This was also reported by Alam et al. [28], who analyzed characteristics of fruit maturity levels on Canephora coffee and showed that the maturity of coffee cherries had no significant effect on moisture content variables. The BD moisture

content of Robusta coffee was in the range of 52–58%, while generally, the moisture content of Robusta coffee cherries right after harvesting was >60% [29]. Furthermore, the moisture content in Robusta coffee beans after being separated from the skin was 50–55% [30].

The harvesting stages and soaking durations influence the color characteristics of coffee beans on Canephora coffee. The bean color was reported as highly varied for the different harvesting stages (ripe and mixed maturity fruit) and soaking durations (0, 12, 24, and 36 h). All color variables obtained positive values, meaning that the Canephora coffee beans had a range of bright red and yellow colors [31]. Combining these two chromatic color parameters gives the result of a brownish-yellow color. According to Bicho et al. [32], Canephora coffee beans are brownish-yellow, while Arabica is greenish-yellow. Meanwhile, for the a (redness) value, the results showed that the harvesting method and soaking time significantly affected the variable. This value illustrated that the H2S0 treatment obtained the color closest to red for the Robusta coffee beans when compared to the other treatments [31]. Based on de Abreu et al. [33], coffee bean color indicates oxidative processes and biochemical damage, which can alter the flavor and aroma precursors of the bean and influence bean quality.

4.2. Phytochemical Composition of Canephora Coffee at Various Harvesting Stages and Soaking Durations

Canephora is a coffee species that contains many phytochemical compositions, including total phenolic contents and antioxidants, in all its parts [4]. One part of the Robusta coffee plant that contains many phytochemicals is the coffee bean. In this research, the quality traits observed were bean yield, moisture content, and phytochemical content, such as TPC, AA, and CC. Bean yield is the ratio between BD weight and AD weight. According to Alam et al. [28], bean yield value was closely related to the quality of the coffee produced: the higher the bean yield produced, the better the quality of the coffee. The mature coffee from selective harvesting with 24 h soaking time had the highest value for the bean yield variable. These data are supported by Indriati et al. [34], who observed that several factors, including the ripeness of the fruit, can cause differences in bean yield values. The research indicates that fruit maturity significantly affected the bean yield variable of Canephora coffee beans. In addition, Sa'diyah et al. [17] explained that the soaking time significantly affected the quality of Robusta coffee beans.

TPC and AA showed the highest result obtained by the control treatment without soaking time, while for CC, the highest result was obtained by a 12 h soaking time with all harvesting methods. Alam et al. [28] explained that the maturity levels of the beans can affect the content of chemical compounds, such as caffeine, phytochemicals, and other minerals. These data are supported by Purnomo [35], who observed that the optimum maturity level in coffee would form better chemical compounds than fruit harvested at green and yellow maturity levels. In addition, all the traits were significantly reduced by the soaking process. Sa'diyah et al. [17] observed that the longer the soaking process in coffee, the lower the caffeine produced. The result also showed a decrease in caffeine content. Generally, the caffeine content of Canephora or Robusta coffee was 1610 mg/100 g [36], while the indication in this research was that all treatments produced a caffeine content of less than 1610 mg/100 g with a range of 18.306–222.260 mg/100 g. This proves that the harvesting method and the soaking time reduced all the TPC, AA, and CC in Canephora coffee beans as a quality improvement.

5. Conclusions

Various harvesting stages and soaking durations influenced the Canephora coffee beans' morpho-physiological traits and phytochemical compositions. Based on the morphological characteristics (bean size and weight before drying), the highest value came from selective harvesting or ripe-picking only, without soaking duration application (H1S0), while the selective harvesting method with a 24 h soaking duration showed a high value on the color trait (L and b*). The treatment that affected the color trait after drying was the strip-picking harvesting method without soaking (a* and b*).

On the other hand, the phytochemical contents had H1S0 for the highest TPC and AA values. In contrast, H1S1, or selective harvesting, with 12 h of soaking, had the highest value of CC. The optimum combination of harvesting methods and soaking duration will improve the quality of the Canephora coffee beans.

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