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Processing and Shelf Life of Cold Brew Organic Coffee

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Abstract: The cold brew method consists of soaking roasted and ground coffee beans either in cold or ambient water (4–23 °C) for up to 24 h. Using this technique, a drink with a unique sensory profile is obtained. This study was conducted to determine the shelf life of a cold brew organic coffee drink (pH~5.0) made from organic beans subjected to three roast levels: light, medium and dark. The drink was pasteurized at 90 °C/30 s, ultra-clean filled into high-density polyethylene bottles, and stored at 4 °C in the dark. Physicochemical, enzymic tests, instrumental color analysis, and microbiological and sensory assays were carried out. The product remained microbiologically stable under refrigeration for all roast levels; however, the beverage made from light roasted beans failed at the beginning of the study, in contrast to the those prepared from medium and dark roasts, which achieved 150 days of shelf life.

Keywords: stability; sensory acceptance; hurdle technology

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1. Introduction

The estimated global coffee consumption for the year 2023–2024 is 177 million 60 kg-bags, and this is driven by the consumption of specialty coffees [1–3]. Cold brew coffee (CBC) has been gaining increasing space in the market, providing health benefits that are superior to hot drinks, which is attributed to the thousands of bioactive compounds present in cold drinks [4,5]. CBC is made from roasted and ground beans soaked into water ranging from 5 to 23 °C for up to 24 h, resulting in a light, sweet flavor, floral and fruity-aroma drink [6]. CBC is also defined as a sweeter or less acidic drink when compared to the hot preparations [7,8].

CBC can be found in the market as refrigerated ready to drink (RTD), long life (shelf stable), concentrated and refrigerated infused in nitrogen, and artisanal. This diversity of products demonstrates a promising market characteristic of the product [9]. Nevertheless, there are no official protocols that regulate the preparation of the drink, such as the coffee–water ratio, infusion time and temperature [7,10]. Lachenmeier et al. [11] hold that since the artisanal drink is not subjected to heat treatment, the hygienic-sanitary practices during processing are necessary to minimize the risks of contamination.

Bellumori et al. [12] evaluated the shelf life at 20 °C of CBC subjected to pasteurization, ultraviolet radiation (UV), high hydrostatic pressure (HHP), ultra-cooling (−18 °C/60 min) and microfiltration. The authors concluded that pasteurization and HHP extended the drink's shelf life and preserved its chemical characteristics and sensory quality for 4 months; however, the ultra-cooled and UV-irradiated samples showed microbial growth after 7 days of storage, and molds and yeasts were found in the micro filtrated drink after 30 days of storage at 20 °C. Studying the influence of infusion temperature on the shelf life of CBC stored at 7 °C, Lopane et al. [13] addressed the chemical, microbiological and sensory attributes of CBC at two infusion temperatures (25 and 4 °C). The shelf life was limited by the sensory acceptance, due to off flavors such as "sour" and "overripe", after 42 days of storage.

Roasting is a crucial step in coffee processing during which the beans are subjected to a dry heat treatment whereby the contact with hot air produces desired aromas and flavors, in addition to imparting color and brittle texture, favoring grinding and extraction [14]. According to Toci et al. [15], roasting can drastically affect the coffee quality as high temperatures trigger complex chemical reactions that release numerous volatile compounds, promote dehydration and changes the microstructure of the beans; however, few studies describe the effect of roasting on CBC [15–17]. Rao et al. [18] evaluated the physicochemical parameters (pH, titratable acidity, melanoidin, total dissolved solids, chlorogenic acids, total antioxidant capacity and caffeine) of coffee at three roasting temperatures (194, 203 and 209 °C), and Yu et al. [19] studied the impact of roasting temperatures (235, 240 and 245 °C) on the volatile compounds of the drink. The authors reported that the higher the roast level, the bigger the difference between the preparation methods. Roasting requires high temperatures (>190 °C/$\geq$ 5 min) to develop the beans' sensory profile due to the complex chemical reactions. During roasting, there is a constant increase in the temperature until achieving the range of 200 to 250 °C, normally lasting from 3 to 20 min [20,21]. Numerous compounds are formed such as pyridine, furan and pyrrole derivatives from lipid and amino acid degradation; these contribute to the roasted, nutty and burnt coffee aromas [22]. Furfural and its derivatives are found in relatively higher amounts in soft-roasted coffee, but at a lower level than their longer-roasted counterparts. A limited Maillard reaction leads to insufficient production of aromatic molecules, especially volatile organic compounds with characteristics of nuts, coconut and chocolate, thus reducing the aroma quality of the coffee [7]. Lightly roasted coffees tend to have a predominantly acidic flavor, with bitter, burnt and acrid notes gradually dominating the sensory panel as the roast level increases [23]. Pan et al. [24] highlight that most studies are associated with drinks made from medium roasted beans. Also, the so-called third wave of specialty coffees is associated with drinks made from light roasted beans (<200 °C) due to their particular aroma and flavor.

In comparing cold brew to hot coffee drinks, Córdoba et al. [8,25] concluded that the hot drink showed greater bitterness and astringency than cold brew coffee; however, no significant difference was detected in the amounts of some common compounds associated with coffee bitterness, such as caffeoylquinic acid, total acids, caffeine and trigonelline. Batali et al. [6] also compared the sensory attributes of CBC and hot-extracted coffee drinks made from beans under different roast levels and origins, and the CBC exhibited less acidic, more bitter and floral notes than the hot drink. The study herein targeted the processing and the shelf life of a cold brew coffee drink made from organic beans subjected to light, medium and dark roasts.

2. Material and Methods

To run this study, 100% Arabica blend beans were utilized; these were size-classified as "moka" and "bica corrida" drinks, certified by the Biodynamic Institute as "organic product", "Agricultura Familiar Brasil", USDA Organic, Canada Organic (Biologique Canada) and "Fair Trade", from the Cooperative of Family Farmers (COOPFAM) of Poço

Fundo and Region, located in the city of Poço Fundo/MG, Brazil (latitude: 21° 46′ 59″ south and longitude: 45° 57′ 13″ west), whose altitude is approximately 900 m.

Three roast level beans were used in the cold brew coffee: (1) light (end temperature of 195.5 °C/9 min 12 s), medium (198.3 °C/10 min 45 s) and dark (209.1 °C/12 min 27 s). To roast the beans, the Atilla 5 kg monobloc model 05 Gold 220 V roaster (Belo Horizonte/MG, Brazil was employed. The coarse grinding was conducted in compliance to Marcelina and Couto and performed in the Minoratto model mill by Carmomaq Indústria e Comércio de Peças e Máquinas Agrícolas Ltda (Espírito Santo do Pinhal/SP, Brazil) [26].

2.1. Processing

The organic cold brew coffee drink was processed in the pilot plant of the Food Engineering Department at the School of Animal Science and Food Engineering of the University of Sao Paulo. In total, 4 kg of roasted and ground coffee beans were soaked in 40 L of drinking water (Welpe, Rio Claro/SP, Brazil); this ratio (1 kg/10 L) is widely used in cold brew coffee [5,9,16–18]. Extraction was carried out by infusion at 23 °C/24 h [5,9]. The drink was then pasteurized at 90 °C/30 s in an electric plate heat exchanger (Sumá Indústria e Comércio Ltda., Campinas/SP, Brazil) with a nominal flow rate of 300 L/h and immediately cooled to 10 °C. We used 500 mL high-density polyethylene (HDPE) bottles, which were decontaminated by spraying peracetic acid at 0.05%/45 °C/10 s (Thech Desinfecção, Cotia/SP, Brazil) using a rinsing machine (Casa das Cantinas Equipamentos e Acessórios, Bento Gonçalves/RS, Brazil). The ultra-clean filling was carried out in a unidirectional horizontal air flow cabinet (ISO class 5) (Veco do Brasil, Campinas/SP, Brazil) by using a semi-automatic microprocessor gravimetric filler (model Dosaliq G54 made by Polienva Equipamentos de Envase Ltda., Sao Paulo/SP, Brazil). The finished product was kept at 4.0 ± 0.5 °C in the dark throughout the shelf life study.

2.2. Drink's Characterization

2.2.1. Physicochemical Assays

The titratable acidity, expressed as percentage of chlorogenic acid, of freshly prepared (*in natura*) and processed drink was determined according to the Association of Official Analytical Chemists method [27]. The pH was measured using a pH meter (Model k39-0014PA, Kasvi), and the soluble solids content (expressed in °Brix) through a digital refractometer (Model HI 96801, Hanna).

2.2.2. Instrumental Color Analysis

The color parameters—L* (lightness), a* (red-green component) and b* (yellow-blue)—of fresh and processed drink were determined using the CIELab system in a Hunterlab Ultrascan colorimeter, Model SN7877, with an angle of 10° and illuminant parameter D65. The chroma, which indicates saturation (vivid or dull color), was calculated by Equation (1), and the °hue (color classification) by Equation (2) as described by Minolta and Pathare, Opara and Al-Said [28,29]. Additionally, the parameters L*, a* and b* were entered into the EasyRGB color calculator (https://www.easyrgb.com/en/convert.php, accessed on 8 January 2025) to obtain the color of the samples. The total color difference (TCD) between the fresh and processed samples was calculated using Equation (3). The TCD between the samples, of the same roast level, at the beginning and the end of the shelf life study was also determined.

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad (1)$$

$$^\circ hue = \arctan (b^*/a^*) \quad (2)$$

$$TCD = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2} \quad (3)$$

where:

L*: lightness (0 a 100).
a*: coordinate red (+60)/green (−60).
b*: coordinate yellow (+60)/blue (−60).
ΔL*: lightness variation.
Δa*: red/green variation.
Δb*: yellow/blue variation.

2.2.3. Determination of Polyphenol Oxidase Activity

The polyphenol oxidase (PPO) activity in both fresh and processed cold brew coffee (CBC) was determined as described by Campos et al., and adapted by Andrade [30,31]. To this end, an ultra-thermostatic bath, model MA-184 (Marconi Equipamentos para Laboratório Ltda., Piracicaba—SP, Brazil), and a spectrophotometer model SP-22 (Labmais Comércio de Equipamentos Ltda., Curitiba—PR, Brazil) were utilized. Test tubes containing 5.5 mL of phosphate buffer (0.2 M and pH 6.0) and 1.5 mL of a 0.2 M catechol solution were immersed in a water bath at 25 °C for 10 min. After the temperature stabilized, 1 mL of CBC was transferred to the test tube, and the mixture was homogenized for 10 s in a Fisatom vortex tube shaker and incubated at 25 °C for 30 min. The absorbance was immediately read at 425 nm. A sample without the CBC was used as a blank. The PPO activity was expressed as U, with one unit (U) being equivalent to a 0.001 variation in absorbance per minute.

2.3. Shelf Life Study

The pasteurized CBC was ultra-clean filled into 500 mL high-density polyethylene bottles and stored at 4 °C in the dark and was then subjected to microbiological and sensory assays to estimate its shelf life.

2.3.1. Microbiological Assays

The maximum limits set for aerobic mesophiles, psychrotrophs, and mold and yeast counts were 5, 4 and 3 logCFU/mL, respectively, based on Andrade, Matietto, Lopes and Menezes and Kunitake [31–33]. The microbiological tests were conducted according to the methodology described in the Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association [34].

In total, 1 mL of the cold brew coffee sample was pour plated for aerobic mesophile and psychrotroph counts in plate count agar (PCA). The plates were incubated at 35 °C/48 h to enumerate mesophiles, and at 7 °C/10 d, for psychrotrophs. To enumerate molds and yeasts, a 0.1 mL aliquot of the sample was spread plated onto dichloran rose bengal chloramphenicol (DRBC) medium and incubated at 25 °C/5 d. Thermotolerant coliform count was carried out by pour plating 1 mL of sample onto violet red bile lactose (VRB) agar; the plates were incubated at 45 °C/48 h.

2.3.2. Hedonic Scale Test

The coffee brew coffee's acceptance was assessed through 9-point hedonic scale tests, as described by Wichchukita and O'Mahonyc [35]. A team of one hundred untrained coffee consumer panelists evaluated the appearance, aroma, flavor and overall quality of 30 mL samples served at approximately 8 °C. The value of 5 (neither liked nor disliked) was considered as the cut-off score on a scale from 1 (disliked extremely) to 9 (liked extremely). Simultaneously, a percentage of acceptance (scores greater than 5) equal to 60, assigned to all attributes, was set as the minimum level for the sample's acceptance. This study was approved by the Research Ethics Committee of the School of Animal Science and Food Engineering of the University of Sao Paulo with the Certificate 46998721.1.0000.5422.

2.4. Statistical Analysis

The data from physicochemical, microbiological, enzymatic and sensory tests were subjected to analysis of variance (ANOVA) and Tukey's test at 5% significance, using SAS software, version 9.3, (SAS Institute, Inc., Cary, NC, USA) to compare means.

3. Results and Discussion

3.1. Physicochemical Characterization

Table 1 shows the variations in the pH, titratable acidity and soluble solids in the cold brew coffee (CBC) over the course of the shelf life study.

Table 1. Variations in the physicochemical parameters of cold brew organic coffee.

Roast Level	Storage at 4 °C (Days)	pH	Titratable Acidity (% Chlorogenic Acid)	Soluble Solids (°Brix)
Light	unpasteurized	5.4 [a] ± 0.0	0.039 [a] ± 0.008	0.6 [a] ± 0.1
	1	5.3 [a] ± 0.0	0.038 [a] ± 0.008	0.6 [a] ± 0.1
	141	5.2 [a] ± 0.0	0.015 [b] ± 0.008	0.6 [a] ± 0.1
Medium	unpasteurized	5.4 [a] ± 0.0	0.036 [a] ± 0.008	1.5 [a] ± 0.1
	1	5.3 [a] ± 0.0	0.036 [a] ± 0.008	1.4 [a] ± 0.1
	141	5.1 [b] ± 0.0	0.042 [a] ± 0.008	1.4 [a] ± 0.1
Dark	unpasteurized	5.7 [a] ± 0.0	0.031 [a] ± 0.008	1.6 [a] ± 0.1
	1	5.7 [a] ± 0.0	0.031 [a] ± 0.008	1.5 [a] ± 0.1
	141	5.3 [b] ± 0.0	0.039 [a] ± 0.008	1.4 [a] ± 0.1

Mean of 3 replicates ± standard deviation. Means followed by the same exponent, in the same column within the same roast level are not different ($p > 0.05$).

With respect to the pH, differences between raw and pasteurized drinks were not detected ($p > 0.05$); however, differences ($p \leq 0.05$) between processed samples during 141 days of storage were found. The CBC made from dark roasted beans exhibited the highest pH value (5.7) for both unpasteurized and pasteurized drinks at the beginning of the shelf life study. Moon et al. [36], analyzing CBC made from beans in different roasting operations, reported that the pH values increase with the roasting intensity. A decrease in pH values over the CBC shelf life was reported by Cempaka et al. [37] and Yun et al. [36,38], due to the acid production by microbial activity.

As for the titratable acidity of light coffee, a difference ($p \leq 0.05$) was found during storage, in contrast to medium and dark coffee, where no difference ($p > 0.05$) was detected, either between raw and processed CBC or between processed CBC through 141 days of storage. Gloess et al. [39] state that the titratable acidity impacts the sensory quality of the drink. The values herein at t_0 (0.031 to 0.039%) were greater than those reported by Bellumori et al. [12], who utilize beans roasted at 200 °C/12 min (light-medium roast) and obtained values that varied from 0.025 to 0.028% chlorogenic acid. In this study, there was a decrease in titratable acidity in the drink made from light roasted beans (light CBC). As for the medium and dark CBC, the acidity increased due to the hydrolysis of the acid lactones. So et al. [40] hold that there is a negative correlation between the pH and titratable acidity due to the hydrolysis of the acid lactones.

Regarding soluble solids, no difference ($p > 0.05$) was detected, either between raw and processed CBC or between processed CBC through 141 days of storage. Siqueira and Abreu [41] claim that the soluble solids content is decisive for the drink's quality, as they contribute to a good body, and provide a sweeter taste. Also, the soluble solids content may vary with the roast level of the beans. In this study, the light roast drink showed the lowest value (0.6 °Brix), which was maintained until the end of the study. Samples made from medium and dark roast beans ranged from 1.5 to 1.4, and from 1.6 to 1.4 °Brix, respectively, which is greater than that (1.3 °Brix) reported by Pan et al. [24], who compared hot coffee drinks to CBC at different roast levels. With regard to light roast CBC, a greater value (1.2 °Brix) than that measured in this study was reported. Clearly, the roast level directly influences the soluble solids content due to the high temperatures employed over beans roasting, which affects the porosity and volume of the coffee beans when compared to roasting at lower temperatures; such changes make the extraction of compounds easier [42].

3.2. Color Parameters

Color is critical from an economic stand point since it is correlated to the drink's quality, which can directly influence the marketing of the product [43]. The "world" of color is a mixture of lightness (L*), chroma (C) and hue angle (°hue). Table 2 points out the color parameters instrumentally measured in the CBC samples.

Table 2. Color parameters of cold brew coffee.

Roast Level	Storage at 4 °C (Days)	L*	a*	b*	Chroma	°hue	EasyRGB Color
Light	unpasteurized	68.9 ^{a} ± 6.3	8.5 ^{b} ± 4.2	50.7 ^{a} ± 7.8	51.5	80.6	
	1	61.4 ^{a} ± 5.8	11.4 ^{ab} ± 3.4	52.1 ^{a} ± 3.1	53.3	77.7	
	141	64.8 ^{a} ± 0.1	13.3 ^{a} ± 0.0	58.6 ^{a} ± 0.1	45.0	82.3	
Medium	unpasteurized	29.6 ^{a} ± 1.9	27.7 ^{a} ± 2.2	31.1 ^{a} ± 6.0	41.7	48.3	
	1	28.7 ^{a} ± 2.6	24.3 ^{a} ± 0.4	34.0 ^{a} ± 2.7	41.8	54.5	
	141	13.6 ^{b} ± 0.0	13.2 ^{b} ± 0.2	13.4 ^{b} ± 0.3	56.7	64.7	
Dark	unpasteurized	26.3 ^{a} ± 0.9	31.0 ^{a} ± 0.9	34.6 ^{a} ± 2.4	46.4	48.2	
	1	23.7 ^{ab} ± 2.5	30.5 ^{a} ± 0.8	35.4 ^{a} ± 3.2	46.7	49.3	
	141	17.5 ^{b} ± 0.0	25.1 ^{b} ± 0.1	21.1 ^{b} ± 0.1	59.5	58.6	

Mean of 3 replicates ± standard deviation. Means followed by the same exponent, in the same column within the same roast level are not different ($p > 0.05$). L* (lightness) = 0 (black), 100 (white). +a* = red. −a* = green. +b* = yellow. −b* = blue. $C^* = \left(a^{*2} + b^{*2}\right)^{\frac{1}{2}}$. $°hue = arctan\left(\frac{b^*}{a^*}\right)$.

Over the storage time (141 days), the values for lightness (L*) ranged, respectively, from 61.4 to 64.8, 28.7 to 13.6 and 23.7 to 17.5 in samples made from light, medium and dark roasts. However, the drink became significantly ($p \leq 0.05$) darker during cold storage in medium CBC only. Pasteurization (90 °C/30 s) did not affect ($p > 0.05$) the samples' lightness. The findings herein differ from those reported by Yeager et al. [44], whose values ranged from 32.5 to 37.4, 30.5 to 31.9 and 25.4 to 26.0 for coffee drinks made from light, medium and dark roasts, respectively. According to Yeager et al., the CBC's color was affected by the infusion temperature besides the roast level. Interestingly, in this study, samples turned lighter over their shelf lives, as reported by Maksimowski et al. [23], which was associated with a reduction in phenolic compounds.

As for chroma (C*), the greatest variation caused by pasteurization was found in light CBC in which the sample's color turned more vivid. Regarding medium and dark CBC, the variation was quite subtle. Conversely, C* decreased in light CBC and enhanced in medium and dark CBC throughout the shelf life study.

In terms of °hue, all samples were positioned in the first quadrant (0–90°) of the color space chromaticity diagram, ranging from red (+a*) to yellow (+b*) with a more yellowish color predominating (b* > a*). These findings are similar to those (a*= −0.6 to 1.6; b* = 0.5 to 3.3) obtained by Polanco-Estibález et al. and Cai et al. [45,46]. Yeager et al. states that reddish color drinks may have a less bitter and sweeter taste as compared to those with lower red intensities [44].

Figure 1 depicts the total color difference (TCD) between raw and processed CBC, and between samples at the beginning and the end of the shelf life study.

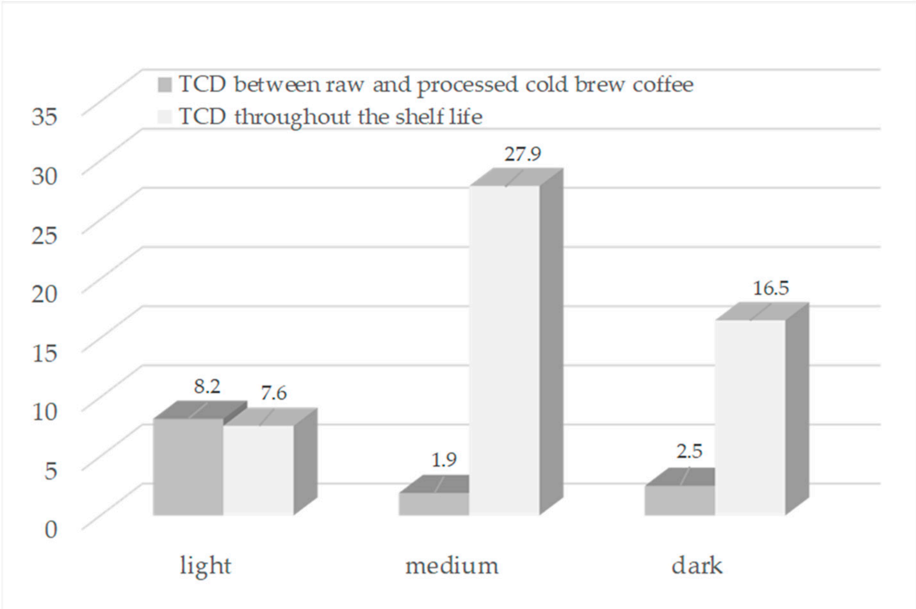

Figure 1. Total color difference (TCD) between cold brew coffee samples made from different roast level beans.

The greatest color difference (8.2), resulting from pasteurization at 95 °C/15 s, was observed in the light roast drink, and the smallest (1.9) in the medium roast. Bernat et al. [46] state that TCD less than 3 cannot be easily detected by human eyes, and values greater than 12 represent different colors; therefore, pasteurization played a noticeable impact on the light CBC's color only. Over the shelf life study, a meaningful variation was observed in all samples; the greatest difference (27.9) was found in medium roasted drink, followed by dark and light. Lopane et al. [13] discussed that change in beverages color over storage may be associated with increases in acidity and non-volatile compounds, such as melanoidins and/or chlorogenic acid.

3.3. Polyphenol Oxidase Activity

Polyphenol oxidase (PPO) is a critical enzymic system in terms of food preservation because it accelerates the oxidation and degradation of polyphenols and their derivatives. PPO is located in chloroplasts and its activation occurs during treatment that causes damage to cells as it acts under aerobic conditions triggering the onset of brown pigments [47–49]. Nevertheless, PPO has been employed as the quality indicator of coffee drinks. Mazzafera and Robinson [50], Amorim and Silva [51] reported a positive correlation between PPO activity and the quality of coffee in which a good drink that was classified as "soft" exhibited a higher PPO activity as compared to a bad drink, classified as a "Rio". Peleg et al. [52] state that PPO can promote the oxidation of catechins, which in turn reduces astringency and bitterness resulting in a smoother drink with greater sensory quality. Table 3 presents the PPO activity values determined in this study.

Table 3. Polyphenol oxidase activity in cold brew coffee.

Roast Level	Sample	PPO Acitivity (U)
Light	unpasteurized pasteurized	0.3 ± 0.0 0.4 ± 0.1
Medium	unpasteurized pasteurized	0.8 ± 0.0 1.1 ± 0.0
Dark	unpasteurized pasteurized	0.9 ± 0.0 0.9 ± 0.0

Mean values of 3 replicates ± standard deviation.

Table 3 shows that the drink made from light roasted beans had the lowest PPO activity, and the treatment at 90 °C/30 s increased its activity in the light (by 33%) and medium (38%) CBC. Such results differ from those obtained by Maksimowski et al. [23], who evaluated the effect of pasteurization (72 °C/15 s) on the shelf life of CBC made from medium roasted Brazilian beans. Using the ABTS method, an increase of 31.67 µMol/100 mL in antioxidant activity was found. Clifford e Melo et al. [23,53,54] hold that a number of factors can affect the enzymic activity, such as cultural practices, processing and storage methods. These may explain the divergences in the results. In contrast, Maksimowski et al. [23] reported that enzymic activity did not change in the dark roasted (210–230 °C) drink, which showed low antioxidant activity.

3.4. Microbiological Stability

Table 4 gathers the microbial counts in CBC at the beginning and the end of the shelf life study.

Table 4. Microbial count (logCFU/mL) in cold brew coffee.

Roast Level	Group	Fresh	Processed (Stored at 4 °C/1 d)	Processed (Stored at 4 °C/141 d)
Light	mesophiles psychrotrophs molds and yeasts coliforms (45 °C)	2.5 ± 0.2 2.7 ± 0.1 2.6 ± 0.1 1.9 ± 0.3	<1 $_{est}$	<1 $_{est}$
Medium	mesophiles psychrotrophs molds and yeasts coliforms (45 °C)	2.4 ± 0.1 1.7 ± 0.2 1.0 ± 0.1 0.3 ± 0.1	<1 $_{est}$	<1 $_{est}$
Dark	mesophiles psychrotrophs molds and yeasts coliforms (45 °C)	<1 $_{est}$	<1 $_{est}$	<1 $_{est}$

Mean values of 3 replicates ± standard deviation. <1 $_{est}$—no colony growth.

Microbial counts in unpasteurized samples from all roast levels were relatively low, which contributed to the drink's microbiological stability. Counts remained below 1 logCFU/mL in all pasteurized samples throughout the shelf life study. These results are corroborated by Bellumori et al. [12], who evaluated the effect of pasteurization on the shelf life of CBC. Those authors reported counts lower than 1.0 logCFU/mL during 120 days of storage at ambient temperature. In this study, notwithstanding, the product was stored at 4 °C in the dark. Maksimowski et al. [23] pasteurized CBC and evaluated different storage times (90, 180 and 270 days) and temperatures (−18 °C and 25 °C). The authors found neither coliforms nor *Salmonella* spp. Mold and yeast, *Listeria monocytogenes*, *Staphylococus* *aureus* and total mesophile counts were lower than 1.0 logCFU/mL in the drink stored at 25 °C for 270 days.

De Paula and Farah [55], and Farah and De Paula [56] claim that chlorogenic acid and caffeine make up the endogenous antimicrobial system in coffee [57,58]. Additionally, in this study, the CBC's microbiological stability can be attributed by the efficient combination of hurdles, notably, (1) pasteurization (90 °C/30 s), (2) ultra-clean filling in sterilized packaging and (3) cold storage (4 °C). Microbiological assays were stopped due to the lack of samples.

3.5. Sensory Shelf Life

Figure 2 exhibits the mean scores assigned to the appearance, aroma, flavor and overall acceptance of light, medium and dark CBC throughout the shelf life study. The percentage of scores greater than 5 (percentage of acceptance) in the 9-point hedonic scale is also presented.

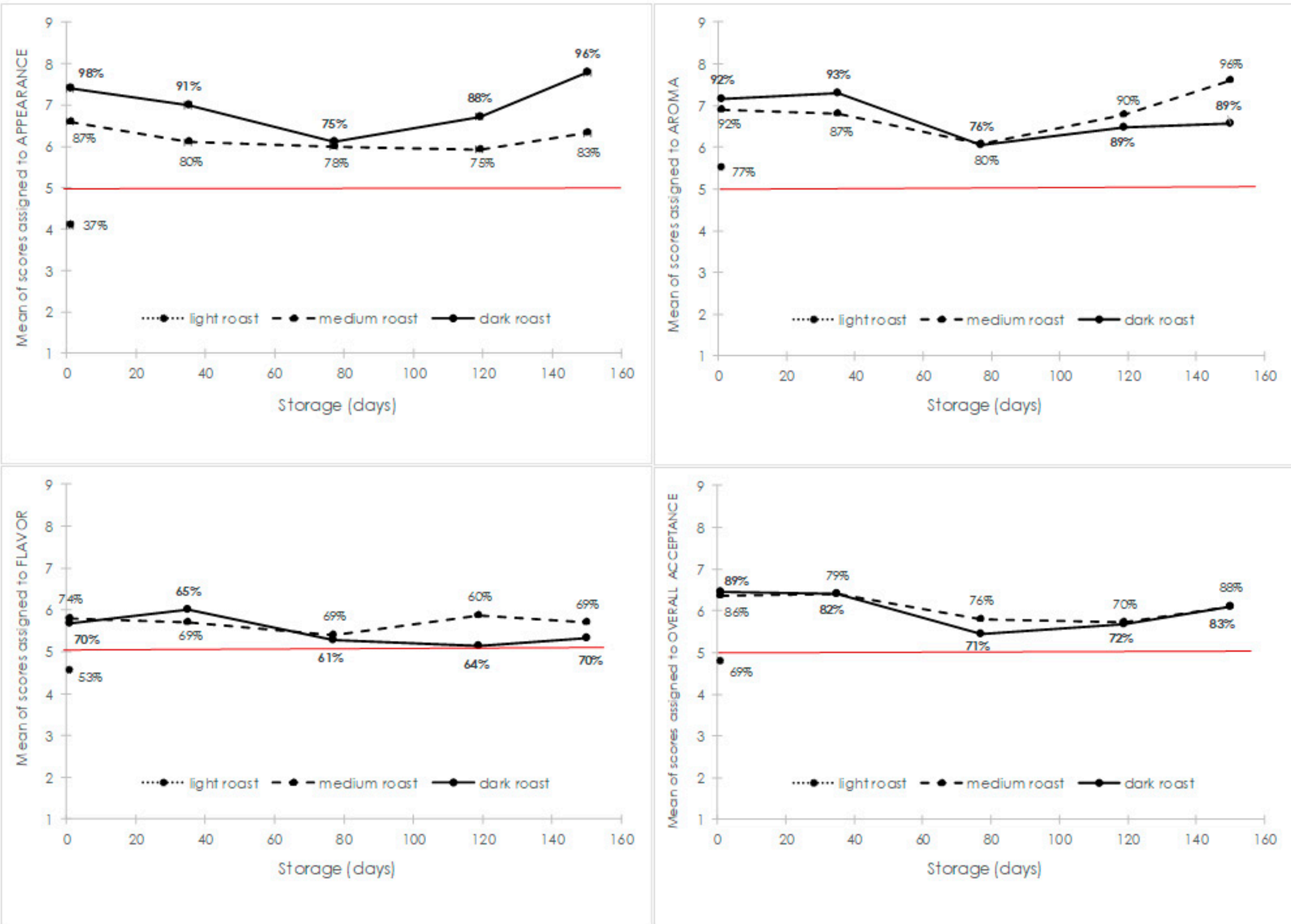

Figure 2. Mean scores and percentages of acceptance assigned to the cold brew coffee in the 9-point hedonic scale.

The CBC made from light roasted beans failed at the very beginning (t_0) of the shelf life study; the average score was lower than 5 for appearance (4.1), flavor (4.6) and overall acceptance (4.8). Additionally, the percentage of acceptance was lower than 60%. Hamilton and Lahne [54] described CBC made from light roasted beans as "sour", "astringent", "fine", "green", "fruity" and often "bad". This supports the rejection of the light CBC in this study.

In regard to medium and dark CBC, the mean scores and percentage of acceptance remained greater than 5 and 60% throughout the study conducted over a period of 150 days. This finding may be associated with greater enzymic activity (1.1 and 0.9 U). According to Mazzafera and Robinson [50], good-quality drinks have high enzymic activity. Due to the lack of samples, the study was stopped. Bellumori et al. [12] reported an unchanged flavor profile after 120 days of storage at 20 °C in pasteurized light-medium CBC.

As for the appearance, the dark CBC achieved the highest score (7.8) and percentage of acceptance (96%). Regarding the aroma and flavor, the medium CBC stood out after 150 days of cold storage. Cai et al. [59] utilized medium-dark roasted and ground beans and compared the sensory attributes of hot coffee drink and CBC. The authors found active aromatic compounds (linalool and methyl salicylate) in CBC only. Zhang et al. [59], analyzing CBC (subjected to light, medium-light, medium, medium-dark, dark and ultra-dark roasting) with an electronic tongue, concluded that the drink made from medium roasted beans showed a greater variety of aromatic compounds, especially containing linalool, 2-ethyl-3,5-dimethylpyrazine, phenylethyl aldehyde and 3-thiophenaldehyde, which were significantly different from other samples. Variations herein found in the sensory evaluation over time might be explained by the use of untrained and different panelists.

Regarding the percentage of acceptance, the light CBC was rejected in terms of appearance and flavor attributes. This finding differs from that reported by Pan et al. [24], who studied sensory attributes in different roast levels (light, medium and dark) and preparation methods (hot and cold brew coffee) with beans originating from South America (Brazil and Colombia). The authors concluded that the combination of the cold brew method with light roasted beans resulted in a drink with better sensory attributes; such divergence can be explained by the origin of the beans. Batali et al. [6] state that different cultivation sites, soil microbiology and climate patterns contribute to differences in sensory attributes. Lopane et al. [13] determined the shelf life of CBC made from beans whose roast level was classified as Gourmet 72.9 (Madison Instrument Inc., Middleton, WI, USA). The samples were stored at 7 °C, and examined at 0, 7, 14, 21 and 48 days; after 48 days, there was an increase in unpleasant taste scores such as "sour" and "overripe".

4. Conclusions

The microbial counts in the cold brew coffee (CBC) made from light, medium and dark roasted/ground beans remained very low throughout this study, highlighting the product's microbiological stability under cold storage. An increase in the polyphenol oxidase activity was found in the light and medium CBC after processing. The drink showed a difference in color after processing as well as during the shelf life study. The light CBC failed at the very beginning of the sensory study, contrasting with the medium and dark drinks which survived after 150 days of storage. The findings reported here show the microbiological efficiency of the preservation methods applied to organic CBC.

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