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Effects of Total Dissolved Solids, Extraction Yield, Grinding, and Method of Preparation on Antioxidant Activity in Fermented Specialty Coffee

Matúš Várady [1,*], Jan Tauchen [2], Pavel Klouček [2] and Peter Popelka [1]

[1] Department of Food Hygiene, Technology and Safety, University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81 Košice, Slovakia
[2] Department of Food Science, Czech University of Life Sciences Prague, Kamýcká 129, 165 21 Prague, Czech Republic
* Correspondence: varadymatus@gmail.com; Tel.: +42-19-0280-7549

Abstract: The aim of this study was to determine the effect of total dissolved solids (TDS), extraction yield (EY), and grinding on total polyphenols (TP), total flavonoids (TF), and total antioxidant capacity (TAC) in a fermented specialty coffee prepared using different methods of filtration (Hario V60, Aeropress, and the French press). The concentrations of antioxidant compounds differed between the TDS treatments and the methods of preparation. The TP and TF with Hario V60 were the highest at a TDS of 1.84%. The TP with Aeropress was at its highest at a TDS of 1.82%. TAC with the French press was at its highest at a TDS of 1.58%. EY was at its highest with fine grinding (Hario V60 > French press > Aeropress at 25.91%, 21.69%, and 20.67%, respectively). French press coffees had the highest TP (p = 0.045). Hario V60 coffee had the highest TF, but the TAC of the coffees remained comparable for all methods. EY and TDS influenced TP, TF, and TAC in the coffee beverages using the finest grinding size for all methods of preparation. The finer the grind, the higher the antioxidant activity of the beverages. Measuring coffee extractions should be one of the most important processes in fermented coffee preparation.

Keywords: polyphenols; antioxidant capacity; anaerobic fermentation; fermented coffee; Hario V60; Aeropress; French press

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1. Introduction

Coffee is one of the most frequently consumed beverages in the world, and its consumption is increasing every year [1]. Coffee consumption is still affected by price, but consumers are interested in buying coffee with associated health claims [2]. Coffee production and consumption increased in the last decade with the arrival on the market of specialty coffees. Specialty coffee is made with the highest-quality green coffee beans, has a known geographical origin, uses the best postharvest treatment method (e.g., natural and washed), uses the best conditions for storing green beans, and is made using beans from the best year of the harvest [3,4]. According to the protocols of the Specialty Coffee Association of America (SCAA) and the international Q Coffee System, specialty coffee has a standardised production characterised by quality and uniqueness of origin, from the criteria used for selecting coffee plantations to brewing. The most important criterion is to achieve a cupping score of ≥80 points on a 100-point scale [5,6].

Many important variables affect the taste or quality of brewed coffee, such as water temperature, degree of roasting, bean origin, grinding, and extraction [7–10]. The quality of brewed coffee, however, is mainly influenced by two key factors: the amount of total dissolved solids (TDS), which is the weight fraction of soluble solids in the brew, and extraction yield (EY), which is the weight fraction of soluble solids removed from coffee grounds [11].

These factors indicate when the coffee is strong, weak, bitter, or underdeveloped. An ideal TDS in SCAA baristic practice is 1.15–1.35%, and EY is 18–22%.

Coffee beverages play an important role in human health due to their antioxidant properties, with chlorogenic acids especially responsible for the link between coffee consumption and lower incidences of various diseases [12–14]. The antioxidant activity of coffee depends on many properties, such as origin, variety, processing method, and roasting time [15–17]. Basic processing methods have recently been supplemented by new innovative processing methods, e.g., anaerobic fermentation and carbonic maceration. Anaerobic fermentation and carbonic maceration rapidly change the coffee fruit, resulting in a flavor much different from traditional fermentation methods.

Monitoring extraction for even better nutritional value and taste, however, is important for the preparation of good and healthy coffee. Specialty coffees have a higher total polyphenol (TP) than conventional coffees. The average loss of TP from green to dark-roasted conventional coffee is almost 93% [18]. Specialty coffees have a similar trend of lower TPs after roasting, but average losses are substantially lower [6,17]. The differences in TPs in specialty coffee beverages, however, also depend on the method of preparation (e.g., Hario V60, espresso, and pour-over) [19].

We hypothesised that coffees processed by anaerobic fermentation with a different TDS and EY could greatly affect the antioxidant activities in filtered specialty coffee beverages. Our aim was to determine the effect of TDS, EY, and grinding on TP, total flavonoids (TF), and TAC in a specialty fermented coffee prepared by three filtering methods (Hario V60, Aeropress, and the French press).

2. Materials and Methods

2.1. Coffee Samples

Samples of 100% *Coffee arabica* beans (Father's Coffee Roastery, Ostrava, Czech Republic) were obtained from the Cajamarca coffee area, Huabal District, in Peru. The beans were of the Catuaí variety harvested in 2020. The coffee was processed using anaerobic fermentation, where coffee is fermented in an environment without oxygen (Figure 1). The ripe cherries were harvested, washed, and then spread out on a terrace, where they were dried in the sun for 20–25 d. When this process was complete, the cherries were sealed for 24–48 h inside barrels filled with water with a one-way exit on the top. As the naturally incurring yeast interacts with the sugars of the coffee fruit, carbon dioxide is released. When oxygen is removed, yeast is forced to consume the sugars in the fruits to produce energy. This chemical reaction releases enzymes and can completely change the chemical composition and, ultimately, the flavor of the coffee.

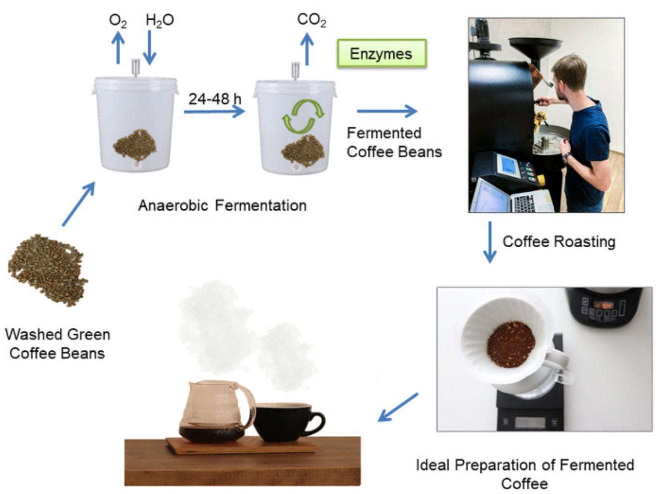

Figure 1. Life cycle of fermented coffee. Washed green coffee beans from the farm undergo anaerobic fermentation. The cherries were sealed for 24–48 h inside barrels filled with water with a one- way exit on the top. As the naturally incurring yeast interacts with the sugars of the coffee fruit, carbon

dioxide is released. When oxygen is removed, yeast is forced to consume the sugars in the fruits to produce energy. This chemical reaction releases enzymes and can completely change the chemical composition and, ultimately, the flavor of the coffee. The packaged green coffee is sent to the roastery where it is roasted lightly to preserve the original taste profile. After roasting and packaging, the coffee is finally ground and ideally prepared by various filtered methods for the most nutritious cup of coffee.

2.2. Coffee Roasting

The green beans were roasted in batches of 0.25 kg in a Probatone 5 gas roaster (Probat, Emmerich am Rhein, Germany) at a final temperature of 215 °C for 10 min, and the development time was 1.5 min. The temperature increased over time and depended on the amount of gas supplied. The temperature was measured by a probe inside the baking drum. The roasting curves are shown in Figure 2.

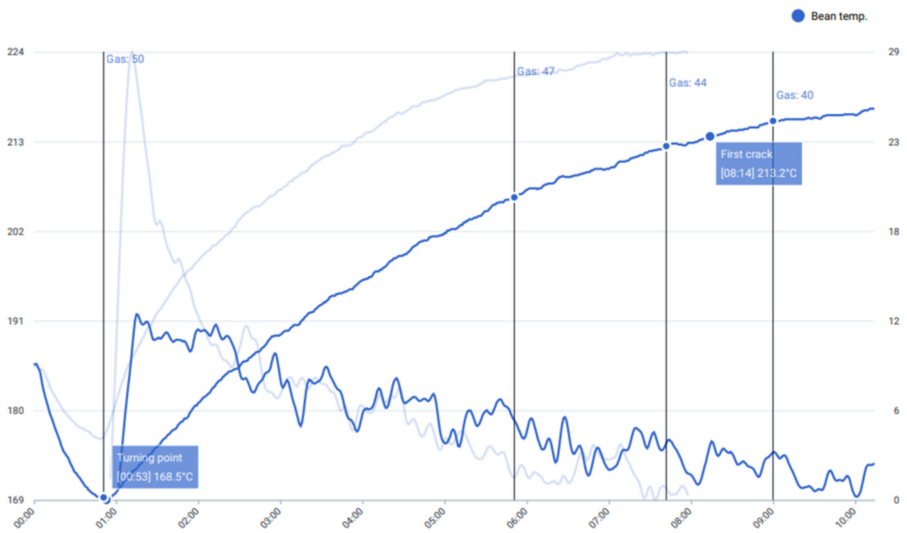

Figure 2. The roasting curves of Peruvian coffee. The green beans were roasted at a final temperature of 215 °C for 10 min, and the development time was 1.5 min. The first crack is the moment when coffee beans start to progress towards an edible state. The time after the first crack is the development time.

2.3. Coffee Grinding

The beans were ground in a Comandante C40 MK3 Nitro Blade hand coffee grinder (Comandante, Munich, Germany). We used the same grind sizes for each method of preparation: 12 clicks (fine grind), 19 clicks (medium grind), 26 clicks (medium to coarse grind), and 33 clicks (coarse grind).

2.4. Coffee Preparation

2.4.1. Hario V60 Pour-Over Method

For the Hario V60 method, a Hario filter paper (Hario, Koga-Ibaraki, Japan) was placed in a ceramic dripper and rinsed with hot water. Freshly ground beans (12 g) were added to the dripper, and water, at a temperature of 94 °C, was poured over them. An initial infusion of 30 mL of distilled water (blooming) was used to initiate the release of CO_2 from the ground beans. An additional 180 mL of water was gradually added after 30 s. The total times were: 2 min and 45 s for the fine grind, 2 min and 25 s for the medium grind, 2 min and 5 s for the medium to coarse grind, and 2 min and 10 s for the coarse grind.

2.4.2. Aeropress Immersion Method

For the Aeropress preparation, we used the classic method with one paper filter (AeroPress, Palo Alto, CA, USA). Freshly ground beans (12 g) were added to the Aeropress, and water, at a temperature of 94 °C, was poured over them. An initial infusion of 30 mL of distilled water (blooming) was used to initiate the release of CO_2 from the ground beans. An additional 180 mL of water was gradually added after 30 s. We started to push the Aeropress after 2 min, and the total time for each grind size was 2 min and 30 s.

2.4.3. French Press-Immersion Method

For the French press preparation, we used a classic French press (Bodum, Triengen, Switzerland). Freshly ground beans (12 g) were added to the French press, and water, at a temperature of 94 °C, was poured over them. An initial infusion of 30 mL of distilled water (blooming) was used to initiate the release of CO_2 from the ground beans. An additional 180 mL of water was gradually added after 30 s. The total time for each grind size was 3 min.

2.5. Total Dissolved Solids

TDSs were measured for each grind, and a digital refractometer (Atago, Tokyo, Japan) was used as the method of preparation. Each sample was measured until the temperature and TDS stabilised.

2.6. Coffee Extraction

The coffee extractions were calculated using two equations [20].

Pour-over method:

$$E\% = (TDS \times \text{weight of brewed coffee}/\text{weight of dry grounds}) + TDS$$

Immersion method:

$$E\% = (TDS \times \text{weight of brewed coffee}/\text{weight of dry grounds}) - TDS$$

2.7. Antioxidant Activity

2.7.1. Total Polyphenols

TPs were measured using a modified method for the analysis of phenols [21,22]. A sample of 100 µL (1:1000 coffee:water) was added to the wells of a 96-well microtiter plate. The range of concentrations of gallic acid (used as a reference compound) was 128–0.015625 mg/mL. Twenty-five microlitres of pure Folin–Ciocalteu reagent was then added. The reaction was initiated by adding 75 mL of 20% Na_2CO_3. The mixtures were kept in the dark at 37 °C for 2 h. The absorbance was measured at 700 nm on a Synergy H1 microplate reader (BioTek, Winooski, VT, USA). The results were expressed as gallic acid equivalents (µg GAE/mg coffee).

2.7.2. Total Flavonoids

TFs were measured by modifying the previously described method [23]. A sample of 100 µL of coffee was added to the wells of a 96-well microtiter plate and mixed with 100 µL of 10% aluminium chloride. The concentration range of quercetin (used as a reference compound) was 256–0.25 mg/mL. Afterward, the solution was incubated in the dark at room temperature for 60 min. The absorbance was read at 420 nm (Synergy H1, Biotek). The results were expressed as quercetin equivalents (µg QE/mg coffee).

2.7.3. Total Antioxidant Capacity–Radical-Scavenging Assay

The antioxidant effect of coffee was established by a slightly modified DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging assay [24]. A two-fold serial dilution of each sample was prepared in absolute methanol (100 mL) in a 96-well microtiter plate. Subsequently, 100 µL of a freshly prepared 1 mM DPPH solution in methanol was added to each well (final volume 200 mL), creating a range of concentrations of 33.333–32.552 mg/mL. Trolox was used as a positive control (range of concentration: 512–0.5 mg/mL). The mixture was kept in the dark at room temperature for 30 min. The absorbance was read at 517 nm (Synergy H1, BioTek). The results were expressed as Trolox equivalents (µg TE/mg coffee).

2.8. Statistical Analysis

All of the analyses were performed in three independent tests, each in triplicate. Results were expressed as mean ±SD (standard deviation). The acquired data were analysed using GraphPad Prism 8.3.0 (538) 2019 (GraphPad Software, Inc., San Diego, CA, USA) using an ordinary one-way ANOVA and Tukey's multiple-comparisons test. The results were considered significant at $p < 0.05$.

3. Results and Discussion

Brewed coffee has antioxidant activity, which affects the sensory profile and can be modified by various TDS and methods of preparation. The concentrations of antioxidant compounds differed between the TDS treatments and the methods of preparation (Table 1).

Table 1. Effect of TDS, grind size, and EY (%) on TP (µg GAE/mg), TF (µg QE/mg), and TAC (µg TE/mg) in specialty coffees prepared by different methods (means ± SDs, n = 7).

		Hario V60			***p***
TDS	**1.84%**	**1.62%**	**1.45%**	**1.26%**	
TP	33.3 ± 3.76 ^{c}	28.9 ± 5.49 ^{b,c}	25.3 ± 3.75 ^{a,b}	21.3 ± 3.69 ^{a}	<0.001
TF	0.73 ± 0.213 ^{b}	0.60 ± 0.198 ^{a,b}	0.50 ± 0.164 ^{a,b}	0.42 ± 0.172 ^{a}	0.015
TAC	7.72 ± 0.506 ^{a}	11.52 ± 0.558 ^{b}	10.15 ± 0.563 ^{b}	7.93 ± 0.894 ^{a}	0.002
Grind size	Fine	Medium	Medium/Coarse	Coarse	
EY	25.91	22.82	20.06	17.64	
		Aeropress			
TDS	**1.82%**	**1.44%**	**1.32%**	**1.22%**	
TP	34.9 ± 3.74 ^{b}	29.3 ± 1.93 ^{a}	28.0 ± 1.53 ^{a}	27.1 ± 1.54 ^{a}	<0.001
TF	0.45 ± 0.141	0.42 ± 0.160	0.37 ± 0.134	0.32 ± 0.143	0.200
TAC	8.85 ± 1.674	9.21 ± 1.358	9.54 ± 2.182	10.7 ± 1.311	0.565
Grind size	Fine	Medium	Medium/Coarse	Coarse	
EY	20.67	16.35	15.05	14.67	
		French press			
TDS	**1.58%**	**1.36%**	**1.17%**	**1.10%**	
TP	34.0 ± 3.98	33.6 ± 4.13	29.8 ± 4.21	29.6 ± 5.18	0.044
TF	0.56 ± 0.155 ^{b,c}	0.47 ± 0.134 ^{a,b}	0.40 ± 0.140 ^{a,b}	0.35 ± 0.155 ^{a}	0.036
TAC	39.1 ± 2.02 ^{c}	15.8 ± 1.54 ^{b}	9.35 ± 1.07 ^{a}	12.3 ± 0.52 ^{a,b}	<0.001
Grind size	Fine	Medium	Medium/Coarse	Coarse	
EY	21.69	17.16	15.84	14.44	

TDS: total dissolved solids; TP: total polyphenols; TF: total flavonoids; TAC: total antioxidant capacity; EY: extraction yield; GAE: gallic acid; QE: quercetin; TE: Trolox.; ^{a,b,c} different letters within a row indicate significant differences at $p < 0.05$.

For Hario V60, TP ($p < 0.001$) and TF ($p = 0.015$) were the highest at a TDS of 1.84%, and TAC was at its highest ($p < 0.002$) at a TDS of 1.62% and 1.45%. For Aeropress, TP was at its highest at a TDS of 1.82% ($p < 0.001$), reaching 34.9 µg GAE/mg. For the French press, TF ($p = 0.036$) and TAC ($p < 0.001$) were the highest at a TDS of 1.58%. The antioxidant qualities of the fermented brewed coffee were strongly correlated with TDS and EY [25]. A few studies, however, have addressed the effect of EY on the sensory quality of coffee. Batali et al. [10] examined the role of TDS using time fractionation of drip infusions and found that attributes such as hot, sour, and smoky flavours were positively correlated with TDS, and attributes such as sweet, fruity, and floral flavours were negatively correlated with TDS. Frost et al. [26] characterised the effect of TDS and EY on the sensory quality of drip coffee, concluding that coffee with a higher TDS was more acidic and that coffee with a lower TDS was sweeter. Brewing temperatures, however, had little effects on the sensory profile of drip coffee when sensory attributes were investigated with brewing and TDS parameters [10]. TDS may, thus, decrease with increasing particle size [27,28]. Similar results were reported for the high-pressure extraction of whole beans, which increased TDS from 1.57 to 2.05%, but with minimal changes for ground samples [29]. Studies comparing the effect of TDS and EY on antioxidant activities in filtered fermented specialty coffee beverages, however, are limited.

Physicochemical characteristics, such as EY, TDS, TP, pH, and titratable acidity, are strongly influenced by the level of grinding [30]. Reducing the size of roasted beans by grinding is essential for controlling extraction and dispersion. Fine grinding in our study produced the highest extraction values, with EY in the order Hario V60 > French press > Aeropress (25.91%, 21.69%, and 20.67%, respectively). The formation of small particles by grinding to produce large surface areas is essential for the rapid release of CO_2, the reduction in the diffusion distance for soluble substances during extraction, and the improved transfer of colloidal substances to the liquid phase [31]. Fine grinding, therefore, produced the highest percentage of EY because coarser grinding reduced extractions due to the reduced contact area of the beans during extraction. Hario V60 extraction had a higher EY, even with the medium (22.82%) and medium-coarse grinds (20.06%), which implies that this method protected the coffee better during preparation before reducing the diffusion distance for soluble substances during extraction [32]. The level of grinding, however, can slightly affect the quality of coffee prepared by filtration (American), brewing (Turkish), and extraction under pressure (espresso), but these methods strongly affect antioxidant properties [33]. Fine grinding produces a large volume of ground coffee where the surfaces of the beans consist mainly of broken cells. In many brewing techniques, the extraction of soluble beans from individual beans is very useful because identifying the effect of specific ingredients on taste is possible [34]. Coffee extraction is a basic process that determines the brewing properties because water-soluble components, such as chlorogenic acids, caffeine, polyphenols, flavonoids, volatile hydrophilic compounds, and water-insoluble lipids, are extracted during this process.

We combined all measured TDSs for the given TP, TF, and TAC to determine the potential impact of the method of preparation on bioactive components. Aeropress and French press coffees had the highest TPs (p = 0.045). Hario V60 coffee had the highest TF (p = 0.004), but TAC for the coffees remained comparable for all methods (Table 2).

Table 2. Effect of method of coffee preparation on TP (µg GAE/mg), TF (µg QE/mg), and TAC (µg TE/mg) in specialty coffees (means ± SDs, n = 7).

	Method of Preparation			p
	Hario V60	**Aeropress**	**French Press**	
TP	27.2 ± 5.10 ^{a}	29.8 ± 3.49 ^{a,b}	31.7 ± 2.40 ^{b}	0.045
TF	0.562 ± 0.135 ^{b}	0.391 ± 0.059 ^{a}	0.444 ± 0.093 ^{a}	0.004
TAC	9.33 ± 1.83	9.58 ± 0.819	19.1 ± 13.59	0.298

TP, total polyphenols; TF, total flavonoids; TAC, total antioxidant capacity; GAE, gallic acid equivalent; QE, quercetin equivalent; TE, Trolox equivalent. ^{a,b} Different letters within a row indicate significant differences at $p < 0.05$.

The fermented coffee prepared by Aeropress and the French press had the highest TPs (29.8 and 31.7 µg/GAE mg), whereas Hario V60 coffee had the highest TF, reaching 0.562 µg QE/mg. More than 70% of coffee antioxidants are extracted during the first 8 s with the espresso method, but extraction starts later after about 75 s and has higher efficiency with filtered coffees, as in our methods, especially for less polar antioxidants [35]. Organic coffees contain substantially more TPs than conventional coffees [16,36], and the content depends mainly on the origin of the beans, the roasting process, and the brewing technique [37,38].

In contrast, brewing times and water temperatures (between 86°C and 90 °C) have smaller influences on TP [7]. Of the three methods of preparation (Hario V60, espresso, and poured), Hario V60 had the highest TP in samples of specialty coffee from Ethiopia, ranging from 32.0 to 46.8 mg GAE/g [19]. In a comparison of the contents of a cup of coffee prepared by filtration (American), brewing (Turkish), and extraction under pressure (espresso), American coffee had higher TAC and TP than did espresso and Turkish coffee [33]. TP in Rwandan coffee was high when using the cold-brew method for 9 h (i.e., >7.5 mg GAE/g) and in roasted coffee using hot-water preparation (95 °C, 10 min, >7.0 mg GAE/g) [38]. Our results indicated that the brewing method had a large effect on TP and TF, consistent with the previous results [15,19,35,39], and that the hot-brewing method generally produced coffee with higher antioxidant activities [38].

Some studies have reported that TF increases with higher roasting temperatures, coffee quality, grind size, and method of storage [40,41]. TF (8.6 mg/100 mL) with light roasting (186.5 °C) was at its highest after an extraction time of 8 min compared to 4 min for organic Peruvian *C. arabica* [36]. TF depended on the method used (Hario V60, Aeropress, and the French press, at 0.562 µg QE/mg, 0.391 µg QE/mg, and 0.444 µg QE/mg, respectively). The mean TF was lower with the Aeropress method (Table 2) without a significant TDS effect (Table 1). EY was also lowest in the Aeropress method. Producing coffee that can retain its bioactive content is, therefore, extremely important for guaranteeing the quality of coffee and protecting the interests of consumers.

TAC did not differ significantly between the methods (p = 0.298), but the relatively high average SD for the French press indicated a potentially strong differential effect between TDS for the French press. TAC, however, depends on the balance of compounds formed and degraded during roasting and on coffee type and method of preparation and is strongly correlated with TP and TF [42]. Hot-brewed coffees have higher TACs, and cold infusions have lower TACs [43]. Our results indicated that Hario V60 and the French press had a large effect on the amount of antioxidant compounds and produced the highest EY, but antioxidant activities remained comparable for all methods. Much of the new coffee equipment (e.g., Hario V60, Kalita Wave, and Aeropress) has responded to changing coffee trends in the last decade. Classic espresso has been replaced in several countries by filtered coffee due to the difference in aroma and the greater sensory experience. With the advent of new coffee machines, however, questions arise about the ideal extraction of coffee using new methods. Our goal is to drink the most delicious, but also nutritious, coffee. Choosing a quality, traceable specialty coffee, and paying attention to its preparation and the parameters that may affect it, is therefore very important. This study should lead to a better understanding of coffee preparation in the coffee industry and its impact on beneficial bioactive substances. We should not forget, however, the impact of anaerobic fermentation on coffee, which is a brand-new processing method. The real question will be how the specialty coffee market, as well as the customers, will respond to the taste of fermented coffee.

4. Conclusions

The results of our study lead to the conclusion that EY and TDS affected TP, TF, and TAC in filtered fermented beverages, which also depended on the method of coffee preparation. The most nutritious beverages were prepared using the finest grind size for all methods of preparation. TAC, however, remained comparable amongst the methods. We thus conclude that the finer the grind, the higher the content of bioactive substances in the coffee beverage and that measuring coffee extraction should be one of the most important processes in coffee preparation.

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