# Effect of extraction techniques on the quantification of total phenolic compounds in Arabica coffee pulp

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## Introduction

Brazil maintains its position as the largest producer and exporter of green coffee in the world, contributing substantially to global demand. CONAB (Companhia Nacional do Abastecimento - National Supply Company) released a projection of approximately 58,000 bags of processed coffee for the 2024 harvest, 5.5% higher than the previous crop's production [1]. Coffee processing generates large amounts of by-products, about 30-50% of the total weight of the coffee fruit. The wet processing method involves the removal of the outer parts of the fruit through a series of steps, including pulping, fermentation, washing, and drying, which produces a by-product called pulp [2]. Arabica coffee pulp has great potential to enrich food products as it contains bioactive compounds with antioxidant potential that may provide health benefits [3]. The objective of this study was to evaluate different extraction methods of total phenolic compounds present in arabica coffee pulp flour.

## Materials and Methods

The arabica coffee pulp was obtained through a donation from a producer in the mountainous region of the state of Rio de Janeiro, from the 2023 harvest. Once collected, the pulp was dried in a ventilated oven with controlled temperature at 55 °C ± 2 °C until it reached ≤ 12% moisture. To obtain the flour, the coffee pulp was pre-ground in a conventional blender (Philco), followed by grinding in a domestic grinder (Mr.Coffe-IDS 57) and sieved with a 250 μm opening (60 mesh) tamis.

Different extraction methods were tested on the coffee pulp flour to evaluate the total phenolic compounds content. The tested extractions included: hot aqueous extraction in a water bath, where the sample was mixed with boiling distilled water (20 g/250 mL, w/v) and placed in a Dubnoff bath for 15 minutes at 100 ºC, then cooled and filtered using qualitative filter paper (Whatman/12.5 cm), and named EB [4]; and hot aqueous extraction combined with pressure, where the sample was mixed with distilled water (20 g/250 mL, w/v), placed in an autoclave for 16 minutes at 121 ºC, and subsequently filtered as mentioned above, and named EA [5]. Both extractions were also subjected to a clarification step, using Carrez I and II reagents, where the EB and EA extracts were placed in a 100 mL volumetric flask, 3 mL of each clarifying agent were added, the volume was adjusted, and the solution was left to stand for 15 minutes. Afterward, the extract was filtered again, these being called EBC – water bath with clarifiers; EAC – autoclave with clarifiers [6]. All extracts were stored in a refrigerator (2 ± 4 ºC) until subsequent analyses.

The total phenolic compounds content was determined spectrophotometrically using the Folin-Ciocalteu (FC) method, as described by Singleton & Rossi [7], and the results were expressed in mg of gallic acid (GAE) per gram of sample. The data were subjected to analysis of variance, and the extraction tests were compared at a significance level of 5% ($p \leq 0.05$). For statistically different samples, Tukey's test was used for mean comparison.

## Results and Discussion

The results of the extraction tests obtained are presented in Table 1 below.

**Table 1:** Total phenolic compound content obtained in the different extracts analyzed.

| Extraction | mg GAE/g |
|---|---|

| | |
|---|---|
| EB | 12.76±0.08[a] |
| EA | 21.10±0.17[b] |
| EBC | 3.87±0.02[c] |
| EAC | 7.22±0.28[d] |

Mean ± standard deviation; Means followed by the same letters vertically do not differ significantly from each other ($p \leq 0.05$).

The quantification of total phenolic compounds is based on the reaction between polyphenols and the FC reagent, resulting in a color change that forms a blue phosphotungstic-phosphomolybdic complex, which is read on a spectrophotometer and compared with an external calibration curve [7]. According to the results, it was possible to verify that all tested extractions differed significantly in relation to the total phenolic compound content. The EA and EB extractions showed higher phenolic content compared to the clarified extracts. This variation may have occurred because these extractions, particularly with the use of pressure, extracted other compounds besides polyphenols, which can interact with the FC reagent, such as mono- and polysaccharides, amino acids, peptides, and organic acids. This reaction may then result in a higher intensity of the blue coloration, contributing to a higher spectrophotometric reading and possibly overestimating the amount of phenolic compounds in the sample [5]. The clarifying reagents act by precipitating proteins, polysaccharides, and other substances without hydrolyzing compounds such as sugars or phenolic compounds [8]. The objective of these reagents is to rremove interferents, which could increase the efficiency of extraction and the accuracy of total phenolic quantification. Therefore, although the EBC and EAC extracts showed about 70% loss of total phenolics when compared to the extracts without clarifiers, they may provide a more accurate extraction and quantification of the total phenolic content.

## Conclusion

To optimize the extraction of total phenolic compounds, it is essential to select the most appropriate technique for the matrix under analysis. Since there is no standard method for this extraction, it is necessary to adjust the process according to the characteristics of the material being analyzed, including its structure, composition, solvent stability, and solubility of the target compound, in addition to considering the objective of the analysis and its application. It is necessary to individually identify phenolic compounds using chromatographic techniques to verify the extraction specificity of each method. It is worth noting that regardless of the technique used, arabica coffee pulp stands out for its high levels of total phenolic compounds in composition.

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