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**MULTIVARIATE OPTIMIZATION OF RESIDUAL CAFFEINE EXTRACTION
FROM DECAFFEINATED COFFEE**

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ABSTRACT

This study evaluated techniques of extraction of caffeine from decaffeinated coffee samples for application in quality control of industrial decaffeination processes. The extraction was studied using two methods, an aqueous one and another through liquid-liquid partition with chloroform.

The objective was to extract the maximum amount of caffeine with the minimum of interference from the matrix and with good repeatability of extraction. After comparing the aqueous extraction and extraction with chloroform, a 23 factorial design was performed to optimize the liquid-liquid extraction. The parameters analyzed in the factorial design were the solvent:sample ratio, extraction time, and filtration or not after extraction. The optimum extraction point was defined using the amount of caffeine extracted as the response factor. Caffeine levels were quantified by capillary electrophoresis according to an analytical method previously optimized and validated.

The best extraction condition was achieved through liquid-liquid partition with chloroform, using 30 mL of solvent, 7 min of agitation, and without filtration. This condition showed good repeatability (2.8%, $n = 7$), recovery of 96.7-107.4%, and removal of interfering matrix. The method was applied to samples of decaffeinated roasted and ground coffee, and instant coffee.

Keywords: factorial design optimization; caffeine extraction; capillary electrophoresis 3

RESUMO

Este estudo avaliou técnicas de extração de cafeína a partir de amostras de café descafeinado, para aplicação no controle de qualidade dos processos industriais de descafeinização. A extração foi estudada utilizando dois métodos, um aquoso e outro através de partição líquido-líquido com clorofórmio. O objetivo foi extrair a quantidade máxima de cafeína com o mínimo de interferência da matriz e boa repetibilidade de extração. Após comparar a extração aquosa e a extração com clorofórmio, um planejamento fatorial 23 foi executado para otimizar a extração líquido-líquido. Os parâmetros analisados no planejamento fatorial foram a relação solvente:amostra, o tempo de extração e a presença ou ausência de filtração 285 após a extração. O

ponto ótimo de extração foi definido usando a quantidade de cafeína extraída como resposta. Os teores de cafeína foram quantificados por eletroforese capilar. A melhor condição de extração foi obtida através da partição líquido-líquido com clorofórmio, usando 30 mL de solvente, agitação durante 7 minutos, sem filtração. Esta condição demonstrou boa repetibilidade (2,8%, $n = 7$), recuperação entre 96,7 e 107,4% e remoção dos interferentes da matriz. O método foi aplicado em amostras comerciais de café descafeinado torrado e moído e de café descafeinado instantâneo.

Palavras-chave: otimização por planejamento fatorial; extração de cafeína; eletroforese capilar

1. Introduction

Caffeine (1,3,7-trimethylxanthine) is an alkaloid from the xanthine group, that occurs in coffee, tea, mate, cocoa products, and cola beverages. The caffeine content of Arabica and Robusta roasted beans is 1% and 2%, respectively. Other substances from this group, such as theobromine and theophylline, with molecular structures very similar to that of caffeine, are also present [1,2].

Caffeine is a mild central nervous system stimulant. At sufficiently high doses, it may produce flushing, chills, agitation, irritability, loss of appetite, weakness, and tremor. In cases of overdose, hypertension, tachycardia, vomiting, fever, delusions, hallucinations, arrhythmia, cardiac arrest, coma, and even death have been reported. Its principal mechanism of action is antagonism related to adenosine, which has inhibitory functions (caffeine has the potential to occupy adenosine receptor sites) [3]. It should be noted that caffeine also has beneficial effects. Epidemiological and experimental studies have shown positive effects of regular coffee drinking on various health aspects, such as psychoactivity (alertness and mood change), neurological conditions (infant hyperactivity, Parkinson's disease), metabolic disorders (diabetes), gonad and liver functions [3].

For many consumers, the answer to supposed or true adverse effects of caffeine ingestion is to consume decaffeinated coffee [4]. Even so, the established idea that levels of caffeine typically found in decaffeinated coffee have no behavioral effects has been revised. On the basis of studies developed by Haskell et al.

[5], it was discovered that even low doses, such as 9 mg, it has psychoactive properties which can last for several hours.

Brazilian regulations allow at most 0.1% of residual caffeine in decaffeinated roasted and ground (powder) coffee and 0.3% in soluble coffee products [6]. Due to the low levels allowed by legislation, it is necessary to study methods for total extraction of caffeine 54 from decaffeinated coffee for an accurate analysis of decaffeinated coffee by the industry.

Caffeine in foods and beverages can be determined by various methods, such as UV spectrophotometry, gas chromatography (GC), high-performance liquid chromatography (HPLC), and capillary electrophoresis (CE), among others [7].

The investigational use of CE has increased. The main advantages of CE are its high efficiency of separation, good resolution and selectivity, fast analysis (up to 20 min), low cost of the reagents, and the need for small amounts of sample. The instrument is versatile and shows fast stabilization in the analysis conditions. It should be noted that minimum waste is generated and in most cases it does not need a specific treatment because it is constituted of simple buffers [8,9].

Among the extraction methodologies developed for hydrosoluble compound, such as caffeine, water extraction becomes an interesting alternative due to low waste generation and ease of handling during analysis. However, for some matrices, the aqueous extraction can load many interferants, which cause difficulties in repeatability or even cause erroneous quantification.

In such cases, another possibility is to evaluate the extraction with organic solvents to obtain a more selective extract. Chloroform has been found to be the most suitable solvent to extract caffeine from an aqueous solution. The reason is the high solubility of caffeine in chloroform, which is nine times higher than in water at room temperature [10]. Liquid-liquid extraction is an attractive method because it operates at atmospheric pressure, it is easy to control, and the equipment cost is low. Hu et al. [11] observed that caffeine is more soluble in chloroform when the partition is performed in basic medium.

Multivariate studies have been increasingly used to improve extraction processes with excellent results and significant reduction in numbers of tests [12-14]. In the literature researched, any studies were found about optimization of caffeine extraction in samples of decaffeinated coffee. The present study evaluated two systems for caffeine extraction from decaffeinated coffee samples (aqueous and organic system), with the multivariate optimization of the organic system. The objective was to extract as much caffeine as possible with minimal interference of the matrix and to obtain good extraction method repeatability.

2. Experimental Details

2.1. Samples and chemicals

The analysis samples consisted of commercial decaffeinated ground roasted coffee and soluble coffee purchased from local markets in Campinas, SP, Brazil.

The water used in all studies was ultrapure (18 M Ω cm) obtained from a Direct-Q 3 UV ultrapure water system (Millipore Corporation, France). Caffeine was from Sigma Aldrich (St. Louis, USA), chloroform (pro analysis grade) from Merck (Rio de Janeiro, Brazil), sodium carbonate from Synth (São Paulo, Brazil), and sodium dodecyl sulfate (SDS) from Riedel deHaën (Germany). All other reagents and solvents were of analytical grade.

All the solutions were degassed by ultrasonication (Microsonic SX-20, Arruda Ultrasons Ltda, Brazil). Running electrolytes and samples were filtered through 0.45 μ m Millex filters (Millipore Corporation, France). Running buffer was centrifuged at 5,000 rpm for 5 min (Excelsa II, model 206 BL, Fanem, SP, Brazil).

Stock solutions of caffeine (1.000 mg.L⁻¹) were maintained under refrigeration until use in preparation of standard solutions and spike analysis.

2.2. Capillary electrophoresis apparatus and operating conditions

Experiments were performed in an Agilent G1600AX (Agilent Technologies, Germany) capillary electrophoresis (CE) system equipped with a diode array detector. Separation was performed in a fused-silica capillary (Agilent Technologies, Germany) with a 48 cm total length and 50 μ m i.d.

The CE operation parameters were as follows: detection was performed at 206 nm, the capillary temperature was maintained at 25°C, and voltage was set at 15 kV. Samples were pressure-injected at the anodic side at 50 mbar for 7 s.

To achieve high reproducibility of migration times and to avoid solute adsorption, the capillary was washed between analyses with the running buffer, consisting of 50 mmol L⁻¹ sodium dodecyl sulfate (SDS) and 10 mmol L⁻¹ sodium carbonate buffer (pH 11.0) for 1 min. The quantification method was previously optimized and validated [15].

2.3. Extraction Optimization

Initially, studies with aqueous extraction, using 1.0 g of sample and 10 mL of water were performed. The samples were ultrasonicated 4, 7, and 30 min. Then the extracts were filtered through a 0.45 μ m cellulose membrane and injected in the CE equipment.

The aqueous extraction results were compared to the liquid-liquid extraction. An amount of 1.0 g of the decaffeinated roasted

ground coffee sample was weighed and transferred to a 125 mL separatory funnel with 10 mL of 0.2 mol L⁻¹ NaOH and 20 mL of chloroform. The system was lightly shaken for 7 min and the organic phase was collected in a 50 mL 122 flask. The aqueous phase was then washed three times with 5 mL of chloroform, always collecting the organic phase. Chloroform was evaporated from the extracts in a water bath at 60°C and the residue was resuspended in 10 mL of ultrapure water, shaken in a vortex for 2 min and filtered through a 0.45 µm Millex filter (Millipore Corporation, France). Both techniques were also evaluated based on caffeine levels extracted, the presence of interferants, and repeatability.

The liquid-liquid extraction, which showed the best results was optimized by a 23 factorial design, in which the amount of chloroform, the extraction time and the use of filter after extraction were the parameters analyzed (Table 1). All the tests were performed in duplicate. The optimum extraction point was defined using the amount of caffeine extracted as the response factor. After defining the optimum point, the extraction was tested using 40 and 50 mL of chloroform in order to compare with the factorial results. The experimental design and data analysis were performed using the Statistica version 7.0 software (Statsoft, USA).

2.4. Validation

The repeatability was determined by performing seven replicate extractions of the same decaffeinated coffee sample. In order to study the extraction efficiency in the optimal point, recovery tests were performed using decaffeinated coffee samples spiked before the extraction with caffeine standard at three levels: 5.0, 10.0, and 100.0 mg.g⁻¹. The validation was performed on samples of roasted ground coffee and instant coffee. The linearity was evaluated between 2 and 200 mg.L⁻¹ of caffeine. The statistical model was validated 146 by analysis of variance, lack of fit study, and by the model residuals.

2.5. Commercial samples

After optimization and validation of the optimal extraction condition, samples from seven commercial brands, three of roasted ground coffee and four of soluble coffee, were analyzed.

3. Results and discussion

3.1. Extraction conditions

In the aqueous extraction experiments, no significant difference in the levels of caffeine extracted was observed when employing different stirring times (4, 7, and 30 min). Quantities of 12.5 ± 0.7 mg of caffeine per 100 g of sample were extracted when agitation was performed for 30 min. A large number of interferences that caused an elevation of the baseline were observed, as shown in Figure 1A. Another important aspect in the aqueous extraction was the absence of repeatability during injection of the same sample ten consecutive times, generating a relative standard deviation (RSD) of 9% for migration time and 21% in the peak area. Time and area variations can be caused by other compounds present in the matrix, which adsorb on the active sites of the capillary, as described by other authors in studies of complex matrices [16].

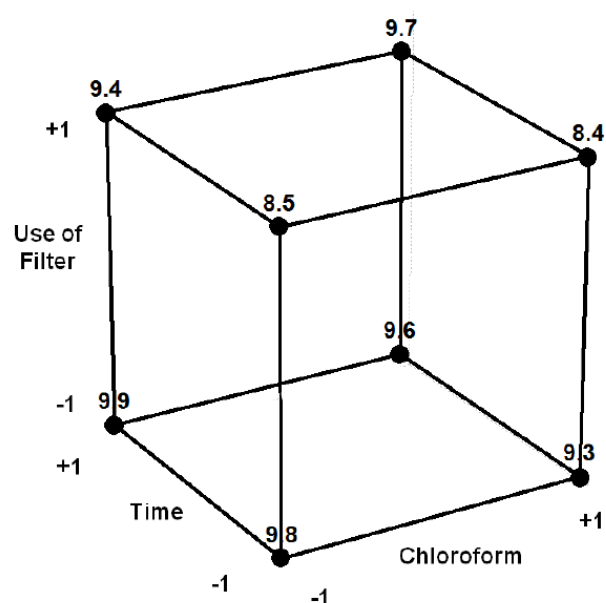


Figure 1. Electropherogram for the sample extracted using the aqueous system (A) and organic system in the optimized conditions (B). Caffeine is the peak identified with number 1.

Therefore, cleaning systems between analysis with NaOH 1 mol 169 L⁻¹ and water were tested, but no improvement was observed in the variations. The baseline increases, allied with changes in migration times and areas, made it impossible to validate the method, which could lead to erroneous measurements and make the extraction system unreliable for the sample studied.

For liquid-liquid partition studies with chloroform, 12.78 ± 1.28 mg of caffeine were quantified in the same sample of roasted ground coffee used in the aqueous extraction experiments. It is important to note that there was no statistical difference between caffeine levels extracted by the two techniques. However, no baseline increase was observed in the extraction with chloroform, probably due to the removal of water-soluble interferants from the sample. The repeatability for the injection of the same sample consecutively ($n = 10$) was 5.3% (RSD) for the area, showing favorable results for this extraction system.

Based on these results, the liquid-liquid partition offered better conditions for the extraction and analysis of caffeine from decaffeinated coffee. Hence, a multivariate factorial design was performed for optimization of the extraction conditions.

Figure 2 shows the results for caffeine levels obtained in the factorial design assays. For each level, the results represent the average of two extractions. None of the studied parameters was significant at 95% of confidence by the analysis of variance (ANOVA). The linear model did not show lack of fit ($MS_{\text{lof}}/MS_{\text{spe}} = 0.005$, $< F_{\text{critic}}(1, 7) = 5.59$). This means that the two populations can be described with the same parameters and used for residuals calculation. The residuals plot distribution was normal and the regression equation was significant ($MSR/MSr = 10.933 > F_{\text{critic}}(6, 8) = 3.58$).

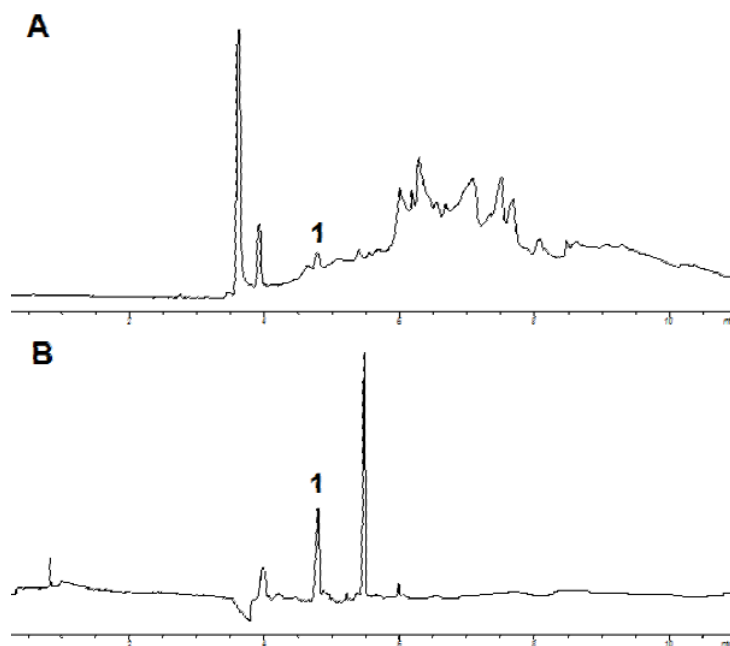


Figure 2. Amounts of caffeine (mg 100 g⁻¹) extracted in each factorial design level.

The regression coefficients of the model are in Table 2. These coefficients were not significant at 95% of confidence. However, with 90% of confidence, the variable extraction time was significant, indicating that the extraction was more effective when the extraction time was the highest (7 min). Some tests with an extraction time higher than 7 min were performed, but the formation of emulsion did not allow the liquid-liquid partition. A better repeatability was achieved in the experiments without filter, since the retention of caffeine residues in the filter was avoided.

During factorial design extractions, it was observed that the use of chloroform in lower levels (20 mL) was prejudicial to extraction repeatability, probably due to saturation of the organic phase by the matrix components. When 30 mL of chloroform was used, the repeatability showed better results. Higher amounts of chloroform were tested (40 mL) without significant difference in repeatability and the extracted caffeine concentration.

The best extraction condition was achieved using 30 mL of chloroform, 7 min of extraction time, and no filtration. The electropherogram of

the sample extracted in optimum conditions is shown in Figure 1B.

3.2. Validation

The repeatability was determined by performing replicated extractions ($n = 7$) of the same decaffeinated coffee sample. The RSD value for caffeine content was 2.80%.

The accuracy of extraction was evaluated by calculating the percentage 216 of recovery. The results, summarized in Table 3 show that it is not possible to detect loss of the analyte in the procedure.

The linearity of the method was evaluated between 2.0 and 200.0 mg L⁻¹, and the model was validated using ANOVA (95% confidence), as shown in Table 4. It should be noted that the model showed no lack of fit, since the p value for this parameter was equal to 0.1011 (greater than 0.05). Thus, this mathematical model can be used to evaluate the samples, as it showed an error

below the statistical limits (95% confidence). The regression was significant for the linear model (Table 4), indicating that the model can be used to quantify caffeine in the samples.

3.3. Caffeine quantification

The caffeine content was quantified in seven different samples, three of roasted ground coffee and four of soluble coffee; the results are presented in Table 5.

The caffeine levels found in samples of decaffeinated, roasted ground 237 coffee ranged between 11.6 and 20.0 mg of caffeine per 100 g of sample, while caffeine levels in soluble coffee were between 22.3 and 138.0 mg of caffeine per 100 g of sample. In all samples, the levels found were below the limits required by Brazilian regulations.

Table 1. Factorial design parameters for caffeine extraction

Variables	Studied levels	
	-1	1
Amount of chloroform	20 mL	30 mL
Extraction time	4 min	7 min
Use of filter	without filter	with filter

Table 2. Regression coefficients of the caffeine extraction model

	Regression	Standard Error	t(7)	p
Mean/Intercept	9.351	0.175	53.497	0.000
(1) Chloroform (mL)	-0.063	0.175	-0.365	0.730
(2) Extraction time (min)	0.326	0.175	1.867	0.100
(3) Use of filter	-0.323	0.175	-1.851	0.110
1 by 2	0.086	0.175	0.493	0.637
1 by 3	0.136	0.175	0.779	0.462
2 by 3	0.226	0.175	1.295	0.236

Table 3. Caffeine recovery test in decaffeinated coffee samples

Decaffeinated coffee sample	Spiking level (mg 100 g ⁻¹)	Recovery (%)
Roasted ground	Level 1	96.3
	Level 2	107.4
	Level 3	99.7
Soluble	Level 1	95.1
	Level 2	102.3
	Level 3	98.7

Table 4. Analysis of Variance (95%) of the calibration curve

Factor	Sum of Squares	Degree of Freedom	Mean Square	F	p
(1) Concentration (L)	507042.4	1	507042.4	31071.95	0.000000
Concentration (Q)	5.8	1	5.8	0.36	0.559111
Lack of Fit	182.3	5	36.5	2.23	0.101106
Pure Error	261.1	16	16.3		
Total Sum of Squares	507500.1	23			

Table 5. Caffeine quantification in seven brands of decaffeinated coffee produced in Brazil

Sample	Brand	Concentration (mg 100 g ⁻¹)	RSD* (%)
Roasted and ground coffee	1	20.0	0.1
	2	15.5	0.8
	3	11.6	2.1
Instant coffee	1	82.1	1.0
	2	22.3	0.2
	3	86.7	0.4
	4	138.0	0.7

*Relative Standard Deviation

4. Conclusions

The quantified caffeine extraction levels in aqueous solution were the same as those observed in liquid-liquid partition with chloroform. However, the aqueous extract showed high amounts of interferants and low repeatability, indicating possible adsorption of solutes in the capillary. On the other hand, solvent extraction showed lower amounts of interferants and better repeatability during the analysis, ensuring greater reliability of the analytical method. The experimental design proved to be an efficient tool for the extraction optimization, with a reduced number of experiments and, consequently, shorter analysis time.

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