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A COST-EFFECTIVE AND RAPID METHOD BASE ON HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY IN EVALUATING THE ROASTED COFFEE ADULTERATION

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ABSTRACT. Coffee is rich in phytochemicals that give its fingerprint and differentiate it from other plant based food. Due to its widespread use and high cost price, it happens that some manufacturers counterfeit it with various additions of cheaper products. This study exhibits a new approach in evaluating of roasted coffee based on high-performance thin-layer chromatography (HPTLC) method. The separation of compounds was performed on silica gel 60 F254 HPTLC plates using a mixture of chloroform – methanol – acetonitrile, 6 : 1.5 : 2.5 v/v/v as mobile phase. The images of the plate were statistically analysed in order to find the most representative featurings for each samples.

Keywords: *coffee, adulteration, HPTLC, UV-Vis spectrophotometry, chicory, soybean, wheat*

INTRODUCTION

Together with tea, coffee is the most important beverage worldwide, with great social and commercial importance. Also, from an economic point of view, coffee is the most traded and valuable product worldwide after oil [1]. Despite being consumed in large quantities, coffee is still quite expensive compared to other food products found in the market. Due to its high price, various cheaper substitutes have been added to it over the years, especially

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to the ground coffee. Theoretically, any plant material that contains sufficient soluble carbohydrates has the potential to be added to coffee [2]. The main materials with which coffee is adulterated are roasted and unroasted coffee pods, twigs, barley, soy, wheat, chicory, malt, starch, corn, maltodextrins, glucose syrup and caramelized sugar [3].

Visually, a difference cannot be made between genuine and counterfeit coffee, which is why several analytical methods have been developed in order to detect counterfeit coffee. The modern methods of evaluating fakes have replaced the old organoleptic and empirical tests and are constantly updated because food falsification is carried out constantly and new problems always appear. Thus, techniques such as mid-infrared Fourier transform spectroscopy (FT-MIR) technique [4], high-performance liquid chromatography (HPLC) [5], mass spectroscopy, gas chromatography, capillary electrophoresis [6] are used. All these techniques are sensitive and precise, but they need the sample preparation that can be take extremely large time and also, they can be performed only by trained personnel. That is why we considered that using thin-layer chromatography (TLC) can be a quick and simple alternative.

The objective of this paper is to develop a new cheap and fast method for the evaluation of the adulteration of coffee.

RESULTS AND DISCUSSION

To see the differences between the coffee samples and the chicory, soy and wheat samples, first the UV-Vis spectra of the extracts were recorded. These spectra are shown in Figure 1.

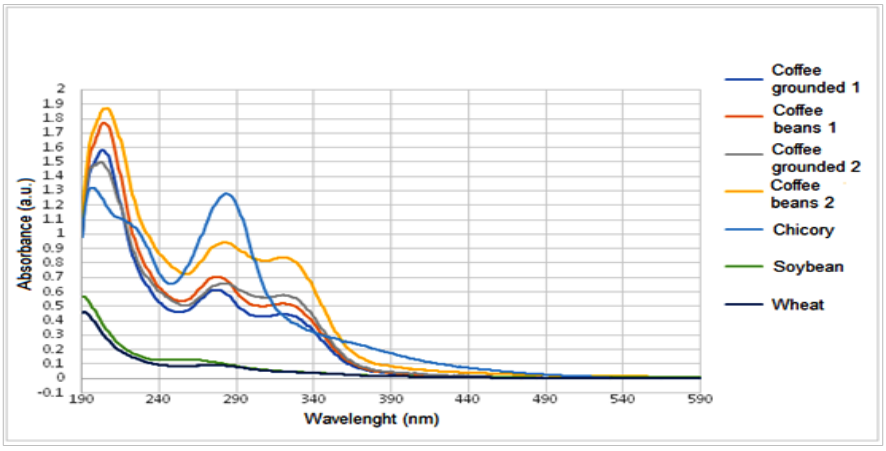

Figure 1. UV-Vis spectra of extracts.

As can be seen from figure 1, in the spectra of coffee extracts there are two absorption maxima at 275 nm and 320 nm, of which the one corresponding to the wavelength of 275 nm is that of caffeine [7]. It is found both in the case of producer 1 and producer 2 that the grounded coffee extracts contain less caffeine than those obtained from coffee beans. Also, the extracts of coffee from producer 2 contain more caffeine than those from producer 1. In the chicory extract there is a single absorption maximum at 280 nm, which is not caffeine because it is known that chicory does not contain this compound. Soy and wheat extracts show a weak absorption in UV-Vis. Taking into account the mentioned, it can be concluded that only on the basis of UV-Vis spectra it is not possible to estimate if coffee samples is or not counterfeit.

In the second step, the high-performance thin-layer chromatography (HPTLC) separation of the compounds from the extracts was attempted and also the possibility of finding specific markers. In order to optimize the separation of compounds several mobile phase systems presented in Table 1 were tested.

Table 1. The tested mobile phase systems

No.	Mobile phase solvents	Composition (v/v/v)	Reference
1	chloroform – diclormethane – *i*-propanol	4 : 2 : 1	
2	*i*-propanol – methyl-ethyl-cetone – chloroform	1 : 3 : 1	
3	*n*-hexane – acetone	37 : 13	
4	*n*-buthyl-acetate – ethyl-acetate – methanol – water	5 : 4 : 1 : 0.1	
5	toluene – ethyl-acetate	9 : 2	[8]
6	chloroform – methanol	8.5 : 1.5	[9]
7	*n*-hexan – ethyl-acetate	7 : 3	[10]
8	dichlormetane – ethyl-acetate	4 : 1	[11]
9	chloroform – ethylic ether	2 : 1	[12]
10	chloroform – methanol	4 : 1	[13]
11	ethyl-acetate – methanol	9 : 1	[14]
12	petroleum ether – acetone	9 : 1	[14]
13	*n*-hexan – ethylic ether – ethyl-acetate	1 : 1 : 1	[15]
14	toluen – ethyl-acetate – methanol	40 : 9 : 1	[16]
15	chloroform – acetonitrile	3 : 2	[17]
16	ethylic ether – petroleum ether	1 : 9	[18]
17	chloroform – methanol – acetonitrile	6 : 1.5 : 2.5	

With the exception of mobile phases 6 and 15, all systems proved unsuitable for the separation of the target compounds in order to obtain relevant information about the presence of the alleged adulterants.

Mobile phases 6 and 15 provided satisfactory separation, so a new solvent system was tested as a combination of these two mobile phases, namely system 17.

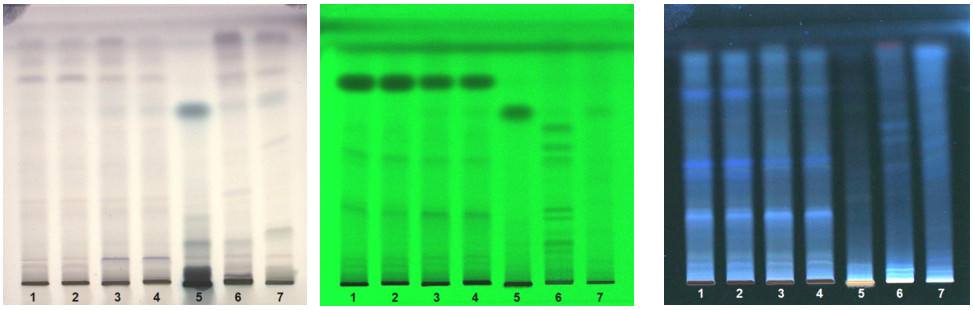

Figure 2. The image of the plate in: A - visible light after spraying with vanillin-H_2SO_4 solution; B – UV light at 254 nm and 366 nm: 1- Coffee grounded 1; 2 - Coffee beans 1; 3 - Coffee grounded 2; 4 - Coffee beans 2; 5 – Chicory; 6 – Soybean; 7 – Wheat

The only image in the visible light that provides adequate information is that of the plate sprayed with vanillin-H_2SO_4 solution (Figure 2A).

The last three purple bands from the upper part of the plate visible in the coffee samples are also observed in soy and wheat samples, indicating the presence of the same or similar compounds. Also, an intense blue-green coloured compound can be observed in the chicory sample, which is less visible in the soybean and wheat samples, but also in both coffee samples from producer 2. At R_f = 0.31 a violet-coloured compound is observed in both coffee and soy samples, at R_f = 0.15 there is a green band in chicory and soy samples, and at R_f = 0.1 a characteristic violet band is observed chicory, soy and wheat samples. However, this information is not enough for the detection of a possible counterfeit, thus the images of the plates obtained in UV light must also be analyzed in order to obtain more information.

By examining the image of the plate without derivatization under UV light at 254 nm (Figure 2B), the appearance of additional bands for soy is observed. At R_f=0.47, one compound is observed in all four coffee samples and in soy, and the compounds that appear in coffee at the bottom of the plate are also present in chicory and wheat. On the other hand, the cereal compounds supposed to belong to the coffee samples are not visible here

and therefore it can be stated that they are different from those present in coffee. The image of the plate in UV light at 366 nm confirms the information obtained from the other images.

In order to better highlight the differences between the fingerprints of the coffee, chicory, soybean and wheat samples and to reveal their significant contribution in samples characterization, the statistical analysis of the digitized images was carried out using Principal Component Analysis (PCA) with Varimax orthogonal rotation of factor axes. Thus, following this analysis, it was found that the R_f domains that explain a maximum amount of variance for the analyzed samples are: 0.01-0.07; 0.22-0.29; 0.47-0.49 for coffee; 0.07-0.67; 0.71-1.00 for chicory; 0.14-0.17; 0.67-1.00 for soybean and 0.00-0.05; 0.07-0.08; 0.80-0.82; 0.88-0.94 for wheat. It is observed that there are zones of retention factor that are specific to each sample and that can make the difference between coffee samples and possible adulterants.

CONCLUSIONS

This work led to the development a new cost-effective and fast method for the evaluation of the adulteration of coffee proving that the HPTLC with statistics analysis could be used to detect the adulterants in coffee.

The developed method can be used for quality control both in the food industry and the product sales market.

The development of the method taking into account other common like husks, barley and sticks adulterants is advised in order to enlarge the method for all adulterants. Also, future research may concentrate on detection more than one adulterant as the coffee may be generally counterfeit with.

EXPERIMENTAL SECTION

Materials and chemicals

All reagents and solvents were purchased from Merck (Darmstadt, Germany) and were of analytical purity. The coffee, soybeans, whole wheat and chicory were purchased from a local store.

Samples preparation

First, the soybeans and wheat were roasted to a very dark coffee-like color. Then, all materials were grounded and 1g of each was mixed with 10ml of ethanol 80%. The samples were macerated for 14 days, filtered and kept in the refrigerator until they were analyzed.

Spectrophotometric analysis

The absorption spectra of each sample were recorded in the wavelength range between 190 and 900 nm using a T80+ double beam spectrophotometer (PG Instruments Limited). The samples were diluted 400 times before the UV-Vis spectrophotometric analysis.

Thin-Layer Chromatographic analysis

The chromatographic analysis was carried out on HPTLC glass plates, with silica gel $60F_{254}$, 10x10 cm, using different solvent mixtures as mobile phases. The elution of the plates was carried out at room temperature in a chromatographic chamber (Camag) presaturated with the mobile phase for 30 minutes. Aliquotes of 10 µL of each coffee sample, 5 µL of the chicory sample and 20 µL each of the soy and wheat samples were applied to the chromatographic plate using a semi-automatic applicator (Linomat 5, Camag) as bands of 8 mm, at a distance of 15 mm from the base of the plate and 13 mm from the left edge, with a flow rate of 40 nL/s. After elution the plate was heated to 110 ^{o}C for 20 minutes and then was immersed for 2 seconds in the solution of ethanolic solution of vanillin and sulfuric acid (10%). Visualization of the compounds was performed in visible light and in UV light at 254 nm and at 366 nm using the Reprostar 3 (Camag) visualization device.

Statistical analysis

In order to reveal characteristic zones of retention that differentiate coffee samples from adulterants, the Factor Analysis method with Varimax rotation algorithm was applied on data matrix from digitized images of the chromatographic plate using the Statistics 11.0 software package (StatSoftinc., USA).

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