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Research Article

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Comparative Study of Mineral Elements and Caffeine in Imported Coffee Varieties Affected by the Degree of Roasting by HPLC Analysis

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ABSTRACT

Factors influencing coffee consumption in the importing countries vary widely. The popularity and worldwide appeal of coffee, which stems from its unique flavor, make it currently one of the most desirable and frequently consumed beverages. Coffee beans found on the market are produced from two different species of Coffea genus: Coffea arabica and Coffea canephora syn. Coffea robusta. The objective of current study was to determine the content of caffeine in Green coffee beans of two varieties (Coffea Arabica Santus from Brazil and Coffea Canephora :Rubusta) from Vietnam as well as coffee beans roasted in three roasting degrees (light, medium and dark) . The coffee varieties analyzed in this study (Vietnam, Brazil) were obtained from importer coffee in Iran. Green beans of each coffee variety were ground with coffee mill and then roasted. Three different roasting degrees (coffee roasting at 250 ^{o}C for different roasting times - 8, 15 and 20 min called: light, medium and dark roasted coffee samples. Caffeine in coffee samples was extracted with water and magnesium oxide. After filtering the caffeine content in extracts was determined with HPLC and separation was performed on RP-C18 column by isocratic elution with UV detection in 272 nm. The caffeine concentrations were comparable with other reported where it is observed that the concentration depends principally on the genus or variety of coffee and the caffeine extraction method from coffee beans. Caffeine concentration in Arabica roasted coffee beans between 1.24 and 2.54% (w/w) and for Robusta was between 2.08-3.37 %. The results revealed that the content of caffeine content of different coffees vary depending on the coffee variety and are affected by the roasting. Light roasted Brazilian Arabica coffee contained the highest overall content of caffeine in all coffees, which exhibited a decrease with intensified roasting.

Key Words: Coffee Variety; Roasted coffee; HPLC analysis; Coffea Arabica; Coffea robusta

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INTRODUCTION

The coffee tree belongs to the Rubiaceae family, genus Coffea. Although more than 80 coffee species have been identified worldwide [1], only two are economically important. Coffea arabica, also known as Arabica coffee, is responsible for approximately 70% of the global coffee market, and Coffea canephora or Robusta coffee (commercial name of one of the main C. canephora cultivars) accounts for the rest [2-6]. Arabica and Robusta coffees are different in many ways, including their ideal growing climates, physical aspects, chemical composition, and characteristics of the brew made with the ground roasted seeds. Coffee may be steam-treated before roasting to make coffee less irritating to the stomach [7].

This type of coffee is sometimes referred to as stomach-friendly. The reduced stomach irritation has been attributed to the reduction of chlorogenic acids' content during steam treatment [8]. Carbohydrates are major constituents of coffee and may account for more than 50% of the dry weight. Lipids are major components of coffee, and their total content varies considerably between C. Arabica and C. Canephora species. The lipid fraction of coffee is composed mainly of triacylglycerols (approximately 75%), free fatty acids (1%), sterols (2.2% unesterified and 3.2% esterified with fatty acids), and tocopherols (0.05%), which are typically found in edible vegetable oils. This fraction also contains diterpenes of the kaurene family in proportions of up to 20% of the total lipid fraction [9-11] . The seed composition dramatically changes during roasting as a consequence of pyrolysis, caramelization, and Maillard reactions. Some compounds are destroyed and others are formed, including bioactive compounds and substances of high and medium volatility, which are important for the aroma and flavor of the brew. The final composition of roasted coffee varies according to the raw material, roasting degree, and other roasting variables such as roaster type and the time, temperature, and air-flow speed in the roasting chamber.

The moisture content of roasted coffee (1.5%–5%) is much lower than that of green coffee, and varies depending on the roasting degree [12-14]. A portion of the coffee protein is degraded, and free amino acids and peptides are consumed by Strecker reactions. Some of the amino acids react with reducing sugars to form (via Maillard reaction) low-molecular-weight compounds and melanoidins that incorporate into their structures other components, such as chlorogenic acids, galactomannans, and arabinogalactan-proteins [15]. Melanoidin polymers, which exhibit variable composition and molecular mass, are responsible for the brown color of roasted coffee and approximately 25% of its dry matter [16]. Different studies suggest that melanoidins are partially responsible for the antioxidant, antibacterial, and metal-chelating properties of coffee beverages and therefore may be considered bioactive compounds [17-20]. However, their physiologic relevance in humans is unknown. Because of thermal instability, chlorogenic acids undergo many changes during roasting, namely, isomerization, epimerization, lactonization, degradation to low-molecularweight compounds (including phenols and cathecols), and incorporation into melanoidins, contributing to color and flavor development. Their degradation follows firstorder Arrhenius-compliant kinetics; however, distinct models should be used for C. Arabica and C. canephora samples [21]. Depending on the roasting degree, the total chlorogenic acids content is reduced to less than 1% of the original content. Chlorogenic acid contents in commercial roasted coffees may vary from 0.5–6 g/100 g, dry weight, depending on the type of processing, blend, roasting degree, roasting method, and analytical conditions [22-23]. Fast high-temperature roasting (230◦C) reduces the loss of these compounds [24-26]. Caffeine is not significantly altered during coffee roasting, but small losses may occur due to sublimation. However, an increase in caffeine content may be observed due to the loss of other compounds. Roasting degrades trigonelline, producing a variety of compounds including nicotinic acid (3%) and volatile compounds such as pyrrols (3%), pyridines (46%), pyrazines, and methyl nicotinate [27 -28]. Nicotinic acid, also called niacin, vitamin B3, or vitamin PP, is formed via trigonelline demethylation [29]. The lipid fraction including triacylglycerols and sterols is relatively heat stable. Although diterpenes are more sensitive to heat, reasonable amounts (0.2–0.9 g/100 g dry weight) may still be found in roasted coffee, especially in C. Arabica. Tocopherol content also decreases during roasting. Depending on the degree of roasting, -tocopherol, -tocopherol, and total tocopherols may be reduced 79%–100%, 84%–100%, and 83%–99%, respectively [30]. The objective of current study was to determine the content of caffeine in Green coffee beans of two varieties (Coffea Arabica Santus from Brazil and Coffea Canephora :Rubusta) from Vietnam as well as coffee beans roasted in three roasting degrees (light, medium and dark) . The coffee varieties analyzed in this study (Vietnam, Brazil) were obtained from importer coffee in Iran.

EXPERIMENTAL SECTION

Chemicals and preparing samples

Methanol and water (HPLC grade), and High grade Magnesium oxide was supplied from Merck (Germany). Pure Caffeine powder (1, 3, 7-trimethylxanthine; 1, 3, 7-trimethyl-1H-purine-2, 6(3H, 7H)-dione; methyltheobromine; C8H10N4O2) were purchased from Sigma. During the experience just we used pure analytical grades and only distilled water was applied for our research. In this paper, caffeine contents in Green coffee beans of two varieties (Coffea Arabica Santus from Brazil and Coffea Canephora (Rubusta) from Vietnam in different degrees of roasting were studied. The coffee varieties analyzed in this study (Vietnam, Brazil) were obtained from importer coffee in Iran. Green beans of each coffee variety were ground with coffee mill and then roasted in three roasting degrees (Pacorini coffee roaster, Italy). All coffee varieties were processed in triplicate for each temperature regime of lab-scale roasting used to mimic industrial processing of coffee. Each batch consisted of 500 mg of green coffee beans.

Roasted beans were packed in airtight plastic bags. Caffeine in coffee samples was extracted with water and magnesium oxide. After filtering the caffeine content in extracts was determined with HPLC and separation was performed on RP-C18 column by isocratic elution with UV detection in 272 nm.

Roasting of coffee beans

Randomly selected coffee bean samples were placed into a pan and then roasted using a muffle roaster. Each time the pan was positioned in the same place of the muffle roaster in an effort to ensure uniform roasting conditions. After roasting, the beans were cooled immediately using an electric fan. Three different roasting degrees (coffee roasting at 250^{o}C for different roasting times - 8, 15 and 20 min called: light, medium and dark roasted coffee samples.

The aqueous extraction was carried out with water at 90˚C which allows a rapid and easy extraction. After the extraction a cloudy solution was obtained because of polysaccharides, colloidal proteins and others colloidal compounds that are contained in the coffee beans. The preliminaries assays were carried out injecting caffeine standard solutions of 5, 10, 15, 20 and 40 mg/L by using a C18 minicolumn. According to the best result was obtained by Maria E. Salinas-Vargas and Maria P. Canizares-Macias in Mexico in 2014, a mixture composed of 25% methanol in water (v/v) with well-shaped peaks was used. Amounts of methanol higher than 25% did not improve the caffeine elution.

HPLC mobile phase

240 mL methanol (1:4) was reached to 1 liter with water and after mixing, was filtered through a 0.45μm membrane filter.

Caffeine standard solutions

200 mg of caffeine was mixed with warm water in order to dissolve caffeine, and then was cooled in room temperature. After that, was reached to 1 liter with water. The solution was stored in refrigerator in 4˚C.

50mL of stored caffeine stock solution was poured in a 250mL flask with pipet and mixed with water to reach 250mL. Diluted standard solution was prepared daily

A Calibration plot with high accuracy was used.

Membrane filter units

Several 0.45μm membrane filters was used in order to filter the extracts.

High Performance Liquid Chromatography (HPLC)

Waters HPLC which was equipped with isocratic elution, Waters 2487 dual λ absorbance detector coupled with UV and an integration system, was used in 272nm.

Chromatography column for HPLC

A 125mm Column, filled with C18 by 5μm particles. Magnetic shaker with heater and water bath was applied. About 1g of each sample (green and different degrees of roasted) was weighted and transferred in a 250 mL flask. To each test portion 5mg magnesium oxide and 200mL water was added. The flasks were transferred to a 90˚C water bath and remained there for 20 min. after that they were cooled with tab water in order to reach to room temperature and then water was added to gain 250ml. and waited until the solid sediment.

System adjustment for HPLC analysis

a) Flow rate: 1.0 mL/min

b) Injection volume: 0.1 mL or 100μ

10 μL of standard solution was injected in HPLC. Then 10 μL of samples extracts were injected with regular distances.

Caffeine evaluation

The amount of caffeine was calculated with following equation in g/100g og coffee:

$$W_x = \frac{A_x \rho_c V \times 100}{A_c m_s} = \frac{A_x \rho_c \times 25}{A_c m_s}$$

Ac: peak area for caffeine standard solution
Ax: peak area for sample extract
Ms: sample mass in g
V: sample volume in L
Pc: caffeine concentration in standard solution

Statistical analysis

All measurements and analyses were carried out in triplicate. The results were analyzed statistically using the Statistica 7.0 program to determine the average value and standard error. Variance analysis, with a significance level of a = 0.05%, was performed to determine the differences in the phenolic content due to different extraction conditions, as well as to establish the differences in the content of these compounds among the coffee extracts. Correlation analysis was also run with the same statistical package.

RESULTS AND DISCUSSION

This study presents the content of caffeine in Green coffee beans of two varieties (Coffea Arabica Santus from Brazil and Coffea Canephora (Rubusta) from Vietnam as well as coffee beans roasted in three roasting degrees (light, medium and dark) were analyzed and studied. The quantitative determination of caffeine content was carried out using a calibration curve of standard (R^2 = 0.9841).

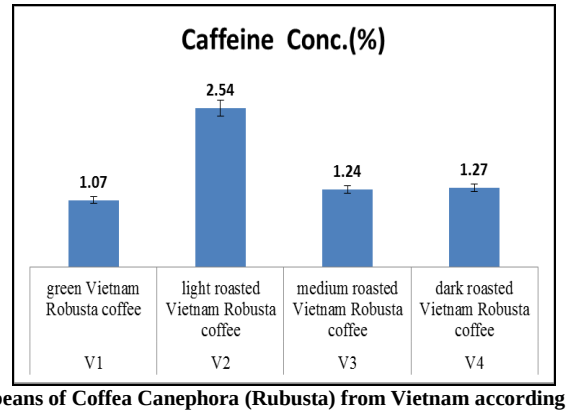

Figure 1: Caffeine content in coffee beans of Coffea Canephora (Rubusta) from Vietnam according to the roasting degrees: Green, light, medium and dark

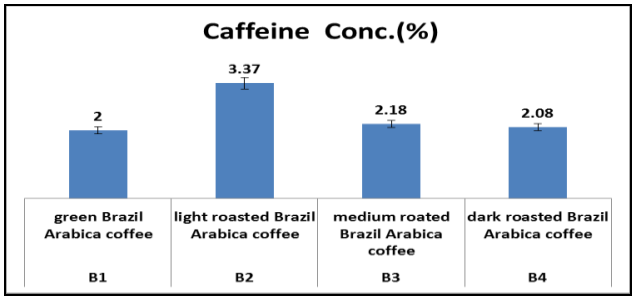

Figure 2: Caffeine content in coffee beans of Coffea Arabica Santus from Brazil according to the roasting degrees: Green, light, medium and dark

The dependency of extractable compounds on grinding time has probably the great direct importance to coffee drinkers that grind beans at home, it is also of interest to determine which of the many factors that change with

grinding are responsible for changes in extractable compound concentration. Therefore in the current study the dependence of the caffeine content of brewed coffee on two factors that increase with longer grinding times have examined: percolation time and the presence of grounds in the brewed coffee. While percolation time and the amount of grounds that pass the filter into the brewed coffee are not directly influenced by grinding (they are a function of several aspects of the brewing process, including the brewing device used), it is reasonable to expect that finer particles resulting from longer grinding will contribute to longer percolation times and a higher fraction of particles fine enough to pass the filter, as well as greater surface area for a given mass of coffee. By considering the effect of increased percolation time and also the spice of samples figures 1 and 2 respectively declare the same trend of caffeine content in both of coffee Varieties.

CONCLUSION

There are many differing views as to what constitutes 'quality'. But it can be said that the quality of a parcel of coffee comes from a combination of the botanical variety, topographical conditions, weather conditions, and the care taken during growing, harvesting, storage, export preparation and transport. Botanical variety and topographical conditions are constants and therefore dominate the basic or inherent character of a coffee. Weather conditions are variable and cannot be influenced, resulting in fluctuating quality from one season to another. Growing, harvesting, storage, export preparation and transport are variables that can be influenced. They involve intervention by human beings, whose motivation is a key factor in the determination of the end quality of a parcel of green coffee. Depending on their marketing priorities people's efforts will fluctuate between the highest possible level, regardless of the cost, and the bare minimum, in order to reduce costs and optimize revenues and margins. The caffeine concentrations were comparable with other techniques reported where it is observed that the concentration depends principally on the genus or variety of coffee and the caffeine extraction method from coffee beans. Caffeine concentration in Arabica roasted coffee beans between 1.24 and 2.54% (w/w) and for Robusta was between 2.08-3.37 %.

When the seed temperature reaches 130◦C, sucrose caramelizes, and the seeds begin to brown and swell. At temperatures higher than 160◦C, a series of exothermic and endothermic reactions take place; the seeds become light brown, their volume increases considerably, and aroma formation begins. The chemical reactions responsible for the aroma and flavor of roasted coffee are triggered at approximately 190◦C. During the Maillard and Strecker reactions, which involve carbohydrates (reducing sugar), proteins, and other classes of compounds, low- and high-molecular-weight compounds such as melanoidins are simultaneously degraded and produced. During this process the light brown seeds can become almost black [12, 31].

The loss of mass during roasting may be a useful parameter to evaluate roasting degree in small-scale production, but may be difficult to control in large-scale production. Visual inspection continues to be the most accepted method to determine the degree of roasting. In addition, roasting parameters such as the amount of coffee in the roaster, temperature, roasting time, and speed of hot air circulation (in the case of fluid and spouted bed roasters) used to reach a single roasting degree can vary considerably. The speed at which the seed reaches the desired color affects a number of physical–chemical and chemical parameters and therefore the flavor and bioactivity of the beverage. Thus, two samples of the same coffee roasted to the same degree may have distinct chemical compositions if roasted under different conditions. For example, coffees roasted at higher temperatures for a shorter time tend to exhibit higher acidity, more soluble solids, and a different volatile profile than those roasted for longer periods at lower temperatures [32].

The seed composition dramatically changes during roasting as a consequence of pyrolysis, caramelization, and Maillard reactions. Some compounds are destroyed and others are formed, including bioactive compounds and substances of high and medium volatility, which are important for the aroma and flavor of the brew. The final composition of roasted coffee varies according to the raw material, roasting degree, and other roasting variables such as roaster type and the time, temperature, and air-flow speed in the roasting chamber.

Caffeine is not significantly altered during coffee roasting, but small losses may occur due to sublimation. However, an increase in caffeine content may be observed due to the loss of other compounds. It is only during roasting that the complex aroma of coffee is formed by pyrolysis, Strecker degradation, and Maillard reaction. The variety and concentrations of volatile compounds in roasted coffee depend on the composition of nonvolatile compounds in the raw seeds and on roasting conditions. Therefore, factors such as genetics, soil, agricultural practices, climate, and degree of maturation influence the final composition of the volatile fraction of roasted coffee [32]. The roasting degree and roasting parameters affect the volatile composition of coffee. The effect of roasting degree is readily apparent because the aroma of a light roasted coffee differs considerably from that of a dark roasted coffee. The

formation of volatile compounds depends on the stability of their precursors and location within the seed. In addition, different volatile profiles have been observed in coffee samples roasted under different conditions to achieve the same roasting degree. Also, many other studies have reported that caffeine content increased during the roasting process, Belay in 2008 reported that caffeine ranging from 10.10 to 11.90 mg/g[33,34] , Farah et al., in 2006 reported caffeine contents from 9.60 to 12.60 mg/g [12] and from 20.44 to 25.15 mg/g, and it was higher in the French roast [12,23].

The results of current study revealed that the content of caffeine content of different coffees vary depending on the coffee variety and are affected by the roasting. Light roasted Brazilian Arabica coffee contained the highest overall content of caffeine in all coffees, which exhibited a decrease with intensified roasting.

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