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Full Length Research Paper

Characterization and determination of chlorogenic acids (CGA) in coffee beans by UV-Vis spectroscopy

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In this research characterization and method for determination of CGA in green and roasted coffee beans have been reported by UV-Vis spectroscopy. The optical transition properties and solvent effects of CGA were measured in different polar-solvents. After characterization of the electron transition of the standard solutions, a method was developed for UV-Vis spectrophotometer determination of CGA in coffee beans. The method was resolved (deconvoluted), the overlapped spectra of CGA and caffeine using dichloromethane and a direct determination of CGA from the residue of the extraction, at peak height. The validations of the methods are limit of detection, limit of quantification, linearity, recovery study and precision calculated as coefficients of variation. The obtained results were in excellent agreement with those obtained with HPLC method, verified by the student t-test at 95% confidence level.

Key words: Coffee, CGA, UV-Vis spectroscopy, extraction, optical transition properties, solvent effects.

INTRODUCTION

Chlorogenic acids (CGA) are the main phenolic compounds in coffee, being an ester of trans-cinnamic acids, such as caffeic acid, ferulic and p-coumaric acids with (-) quinic acid (Clifford 2000; Clarke and Macarae, 1985). They are believe to have an antioxidant properties which are suggested to play an important role in protecting food, cells and any organ from oxidative degenerative(Cole, 1984; Moreira et al., 2005). Report indicate diet rich in CGA compounds play a great role in preventing various diseases associated with oxidative stress such as cancer, cardiovascular, aging and neurodegenerative disease (Manch et al., 2004; Fujioka et al., 2008). On the other hand CGA contributes a great role in the formation of pigments, taste and flavor of coffee beans, which determines the quality and acceptance of the beverages. Previous research reports have indicated the relation between the composition of the CGA and quality of coffee beans. According to Farah et al. (2006) the level of CGA has an inverse association with coffee quality with higher contents observed in lower quality coffee sample. Therefore establishing a rapid and cheap analytical method for determination of CGA in coffee beans has interests because of the wide use of this compound contributes.

Several methods have been developed for determination of chlorogenic acid and its derivatives in coffee beans and other plants. The most widely used methods are HPLC (Pedrosa et al., 2000; Ky et al., 1997, 2001; Negishi et al., 2004; Mozetic and Trebse, 2004; Farah et al., 2006a,b,c), capillary electrophoretic (Jiang et al., 2004; Polnsek et al., 2006), Miceller electrokinetic chromatography (Guan et al., 2006; Risso et al.,, 2007). Although the developed methods have been powerful for quantification the content of CGA and its derivatives, however; they have been criticized as being tedious, time consuming and others are limited to large sample volume extraction procedures and needs long time reaction. In addition most of these instruments are very expensive and not found in many laboratories. On the other hand the UV-Vis spectrophotometer method has been simple, fast and inexpensive for determination of CGA in coffee beans; but, a direct determination in coffee beans is impossible, because the spectral overlap with caffeine.

Therefore the objective of this research is developing a simple method for measuring the CGA in coffee beans of Ethiopia using UV-Vis spectroscopy which is available in most laboratories. The methods include characterizing the standard solutions in different polar-solvents and based

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Table 1. Shows the percentage of CGA for different coffee Arabica beans calculated by UV-Vis spectroscopy and HPLC.

Type of coffee samples	CGA in coffee beans by UV-Vis spectroscopy (w/w %)	HPLC
Sample -1	(6.06 ± 0.17)	5.8
Sample -2	(6.05 ± 0.33)	5.6
Sample-3	(6.19 ± 0.23)	6.1
Sample-4	(6.15 ± 0.25)	6.0
Sample-5	(6.25 ± 0.32)	6.1

on these developed methods for measuring CGA in coffee beans have been developed. The method was resolved (deconvoluted), the overlapped spectra of CGA and caffeine using dichloromethane and a direct determination of CGA from the residue of the extraction, at peak height. The developed method was validating in terms of limit of detection, limit quantification, linearity, recovery study and precision calculated as coefficient of variation moreover the obtained results were in excellent agreement with those obtained with HPLC method, verified by the student t-test at 95 % confidence level.

Table 2. Shows the percentage of CGA calculated for coffee roasted by light, medium and dark type.

Coffee type	Light (%)	Medium (%)	Dark (%)
Sample-A	4.09	4.06	3.01
Sample-B	5.07	3.83	3.63
Sample-C	5.34	4.77	3.52

MATERIALS AND METHODS

Chemicals and samples

Dichloromethane and CGA were purchased from (Aldrich-Sigma, Germany), distilled water (AAU, Ethiopia), and Arabica coffee beans (washed at the center) were provided by Ethiopia Coffee Quality Inspection Center. The coffee samples were collected from south west of Ethiopia with out considering their varieties. The contents of CGA measured for green and roasted coffee beans are presented in Tables 1 and 2 respectively.

Instrumentation

For electronic absorption measurement of standard solutions and coffee samples different laboratory apparatus, a 1 cm quartz cuvette and double UV-Vis-NIR spectrometer, Perkin Elmer Lambda 19 (Perkin Elmer, D-7770 Ueberlingen, Germany) with wave length regions of 170-3200 nm were used. The instrument was operated by 4.3 UVCSS soft ware. Scanning speed 240 nm per min and with slit width 2 nm was used. Data acquisition performed by computer interfaced with spectrometer. The calibration of the instrument is performed by dichromate solution.

Standard solution preparation

For the standard solutions preparation a commercially bought CGA were dissolved in polar-solvents (ethanol, methanol, acetonitrile and water). The solutions were uniformly dissolved using magnetic stirrer, and absorbance measured immediately after stirring. Moreover to avoid from light interaction, it was stirred in dark room to avoid from light interaction. From UV-Vis absorption spectra, the optical transition properties (molar decadic absorption coefficient, oscillator strength, integrated absorption cross-section and transitional dipole moment) were calculated. For integrating the absorption coefficient and molar decadic absorption coefficient in the frequency regions origin6.1 software was used.

Coffee sample preparation for UV-Vis spectroscopy

Green and roasted (light, medium and dark) coffee beans were ground and screened through 250 µm sieve to get a uniform texture. An accurately weigh amount of sieved coffee (approximately 6 mg) was dissolved in 25 ml of distilled water. The solutions were stirred for one hour using magnetic stirrer and heated gently to increase the solubility of caffeine in water solution. In addition solution was filtered by glass filter to get rid of the suspended particles from the solutions.

Liquid-liquid extraction and absorption measurement procedures

In coffee sample, the spectra chlorogenic acids and caffeine make interference in the wavelength regions of 200 - 500 nm. To resolve (deconvolute) the two overlapped spectra; caffeine was extracted from water solution by dichloromethane. For caffeine extraction previously developed procedures by Belay et al. (2008) were used. After caffeine was extracted from the solution, it is the chlorogenic acids remain as residue in coffee beans. From the residual solution, concentration of chlorogenic acid was measured in the wavelength region of 200 - 500 nm against the corresponding blank (distilled water) by Beer-Lambert's Law at $\lambda_{max} = 324\ nm$. All glass wares, cuvette were thoroughly cleaned, rinsed with distilled water and dried before use.

HPLC analysis

For HPLC analysis, chlorogenic acids were extracted and purified from green coffee beans by following the method developed by (Ky et al., 1997). A chromatography consists of Hewlett packard with Quarternary pump, auto sampler, Shimadz SPD 10A UV-Vis detector, C18 pre-column and 250x4.6 mm phenomenex Luna 18(2) column with 5 micro meters pour size. The CGA were analyzed ac-

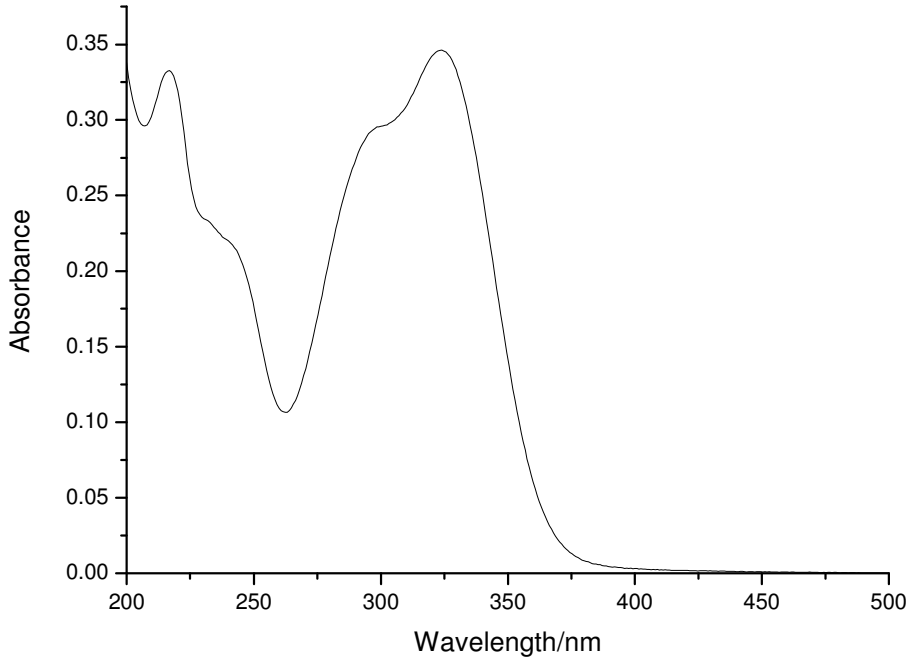

Figure 1. Shows the UV-Vis absorption spectra of pure CGA dissolved in water.

cording to the elution programme described by (Ky et al., 1997). The analyzed results are shown in Table 1 for comparison with UV-Vis absorption results.

RESULTS AND DISCUSSION

UV-Vis absorption spectra of CGA in distilled water

Figure 1 shows the UV-Vis absorption spectra of CGA measured in distilled water in the wavelength regions of 200 - 500 nm at room temperature. In these regions CGA has two maximum points; the first maximum being at 217 nm with shoulder at 240 nm and the second peak was at 324 nm with shoulder at 296 nm and the minimum point was at 262 nm. The peak point at 324 nm was the highest peak corresponding to the HOMO→LUMO transition presents mainly a $\pi\pi^*$ character with electron density localization on the benzene ring and carbon chain Cornard et al. (2008) and this large absorbance seem to be promising to improve the sensitivity for CGA determination in coffee beans. The other peak and shoulders are also presents the same $\pi\pi^*$ transitions.

Validation of the method

The UV-Vis absorption was validated in terms of limit of detection (LOD), limit of quantification (LOQ), linearity, and standard deviation (SD). The calibration graphs correlating the absorption intensity with the corresponding concentration was constructed for CGA at the highest peak for concentration range of (3.02 - 11.00) × 10^{-8} mol cm^{-3}. The Calibration equation is (Y = -0.00921 + 0.19252X, R = 0.99, S.D = 4.45%, N = 7, p < 0.0001) where Y, represents the peak height at $\lambda_{max}=324nm$ and X concentration in mg L^{-1}. The limit of detection (LOD) and quantification (LOQ) decide the sensitivity of the method and calculated from the peak-to-noise ratios. In the present study the LOD and LOQ values of CGA were 16 and 54 mg L^{-1}, respectively, and these values were similar with the result reported by Urakova et al. (2008) using column liquid chromatographic method.

Solvent effects of CGA

Figure 2 shows the overlapped spectra of CGA in different polar solvents (methanol, ethanol and acetonitrile) in the wavelength regions of 200 - 500 nm, generally a blue shift (hypsochromic) were observed as the polarity of the solvents are increased. The blue shift can be interpreted, in terms of a general dipole-dipole interaction between the solvents and CGA in the ground state. The solute solvent interaction in the ground sate greater than excited and leads to a blue shift of the spectrum. Moreover a reasonable linearity was observed between maximum absorbance versus the dielectric constant of the solvent except acetonitrile which has lack of hydrogen bonding. The deviation of maximum absorbance may be explained that a shift not only affected by dielectric constant of the solvent but also by hydrogen bonding.

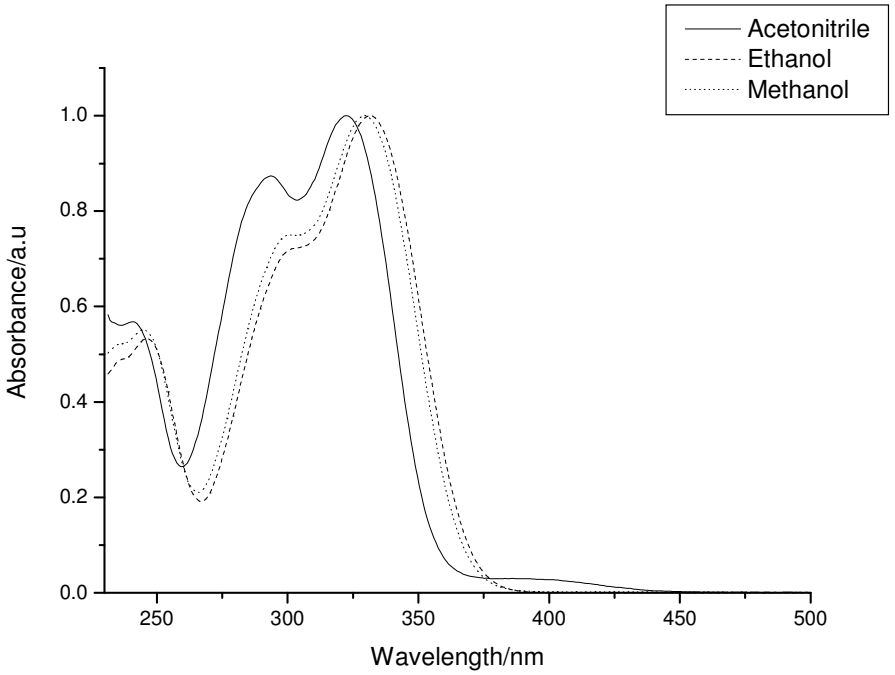

Figure 2. Shows the overlapped spectra of CGA in different polar solvents (Acetonitrile, Ethanol, and Methanol).

Optical transition properties of CGA

From UV-Vis absorption spectra the optical transitional properties of CGA were calculated in solvents to compare the strength of transition. The optical transition properties of the molecules are important for characterize an electron transition and to interpret the absorption spectra. The molar decadic absorption coefficient measuring the intensity of optical absorption at a given wavelength was calculated using Beer-Lambert's law equation Liptay (1969) and integrated absorption coefficient is the sum of absorption coefficient for all frequencies. It is independent of line function which may be varying due to pressure, temperature, interaction of solute and solvent interaction (Milonni and Eberly, 1988; Michale, 1999). The integrated absorption coefficient in the certain frequency regions can be expressed by (Milonni and Eberly, 1988),

$$a_t = \int a_\lambda d\nu \quad (1)$$

Using the equation (1) the integrated absorption cross-section was expressed by the following equation (Milonni and Eberly, 1988).

$$\sigma_t = \frac{1}{N}\int a_\lambda d\nu \quad (2)$$

Where a_t is the integrated absorption coefficient, a_λ is absorption coefficient at given wavelength v, frequency σ_t, the integrated absorption cross-section and N, number density of the molecules.

The other important parameter which providing the relative strength of electron transition is the oscillator strength. It is one of the fundamental quantities in analytical spectroscopy. In practice, it determines the sensitivity of a given atomic resonance line and needs to be accurately known if one to relate the magnitude of the absorption signal to its concentration. Oscillator strength related to molar decadic absorption coefficient by the following equation (Georgakopoulous et al., 2004)

$$f = 4.32\times10^{-9}\int \varepsilon d\nu \quad (3)$$

The transition dipole moment is a vector that depends on both ground state and excited state and couple the transition to the electric field of light. The transition dipole moment related to the molar decadic absorption coefficient by the following equation (Liptay, 1969; Michale, 1999).

$$\frac{1}{3}S\left|\mu_{fi}\right|^2 = \int \frac{\varepsilon(\nu)}{\nu} d\nu \quad (4)$$

Where; S = 2.9352 × 10^{60} C^{-2} mol^{-1}, μ_{fi} transition dipole moment and ε molar decadic absorption coefficient.

Using the above equations the optical transition properties of CGA calculated in different polar solvents are

Table 3. The optical transition properties of chlorogenic acid in different polar solvents.

Solvent type	Integrated absorption cross-section in (cm molecule^{-1}) $\sigma=\frac{\int a d\nu}{N}$	Molar decadeic absorption coefficient in (m^2 mol $^{-1}$) $\varepsilon_{max}=\frac{A}{cl}$	Oscillator strength $f=4.32\times10^{-9}\int \varepsilon d\nu$	Transitional dipole moment in (C m) $\left(\frac{3}{s}\int\frac{\varepsilon d\nu}{\nu}\right)^{\frac{1}{2}}$
Ethanol	(216±2) ×10^{-15}	2003±28	0.56±0.01	(20±0.15)×10^{-30}
Methanol	(204±2)×10^{-15}	1866±20	0.53±0.01	(19±0.10)×10^{-30}
Water	(217±2) 10^{-15}	1882±30	0.56±0.01	(20±0.10)×10^{-30}
Acetonitrile	(177±2) 10^{-15}	1476±28	0.46±0.01	(18±0.09)×10^{-30}

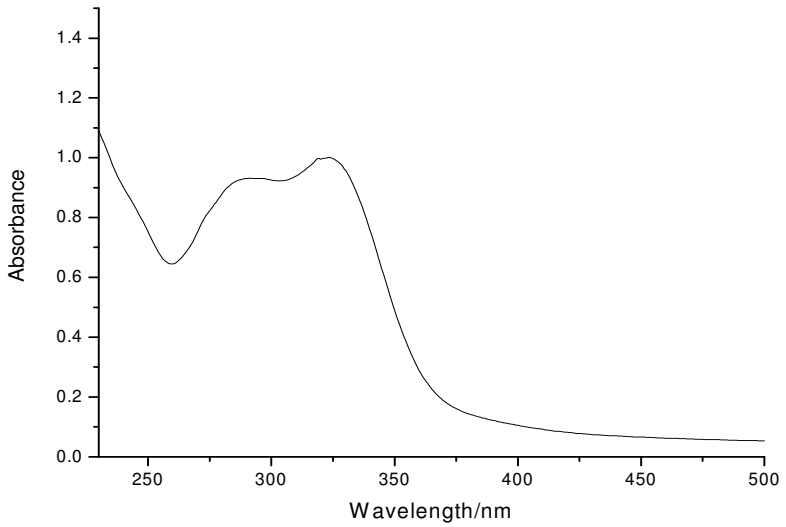

Figure 3. Show UV-Vis absorption spectra of coffee beans dissolved in water.

presented in Table 3. The molar decadic absorption coefficient of CGA calculated at λ_{max}=324, 322, 330 and 332 nm for water, acetonitrile, methanol and ethanol respectively and these results are agree with the one reported by Moridani et al. (2001). The corresponding integrated absorption cross-section, oscillator strength and transitional dipole moment were also calculated in the wave number regions of 25000-38,109 cm^{-1} are also mentioned in Table 3. The results show CGA has less transition probability in acetonitrile than other solvents at room temperature.

Determination the contents of chlorogenic acid in green and roasted coffee beans

By UV-Vis spectrometry a direct determination of CGA content in coffee beans are impossible. It was observed that, there is spectral interference from caffeine and CGA in the wavelength regions of 200 - 500 nm. Figure 3 shows the spectrum of coffee beans dissolved in water. Two peaks were observed in these wavelength regions, the first peak was observed in the region of 250 - 300 nm which belongs to the peak of caffeine and the other peak was observed in the regions of 300 - 350 nm and this peak corresponds to the peak of CGA. For quantitative determination of CGA in coffee beans using UV-Vis spectrometry the overlapped spectral band, should be resolved or deconvoluted. In this research the two overlapped spectra were deconvoluted by extracting caffeine from water solution using dichloromethane. After caffeine extracted from coffee solution the remaining residue is the CGA of coffee beans. Figure 4 shows the overlapped spectra of pure CGA dissolved in water and CGA after caffeine extracted from green coffee bean solution. The two spectra are exactly similar to each other both in peaks and shapes. The similarity in peak and shape of the two spectra show there is no overlap band from other components of coffee in these regions, and this shows the specificity of the method.

The accuracy and matrix effects of the method were

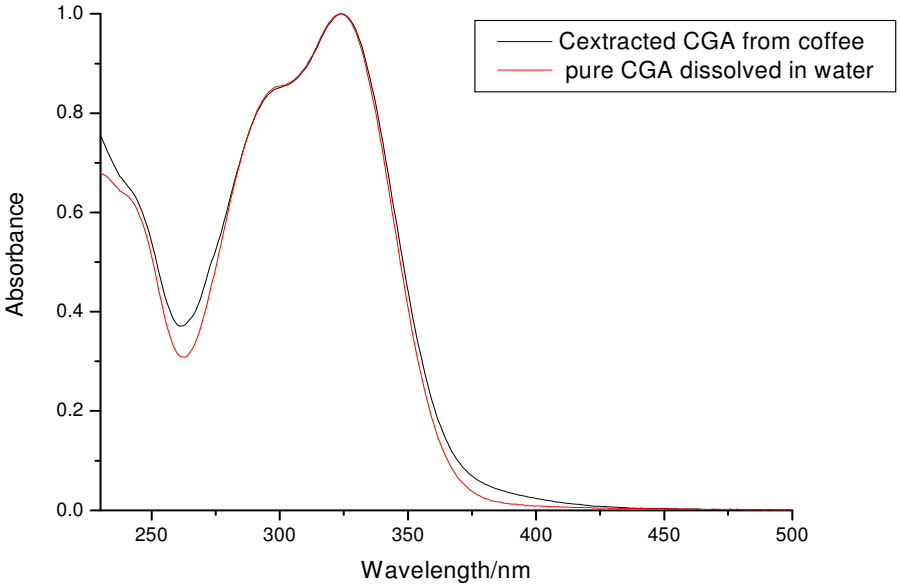

Figure 4. The overlapped spectra of pure CGA and CGA after caffeine extracted from coffee beans by dichloromethane. The dot shows pure CGA dissolved in distilled water and line shows CGA after caffeine extracted from coffee solution.

also evaluated using the recovery tests. The recovery of the method was performed by adding a known amount of pure 5-QCA concentration to the coffee solution. The percent of recovery obtained in different coffee varieties were 91 - 98.43%. From the result of recovery test the matrix effects in the sample studies were minor. Thus the method is valid statistically for determination of CGA in coffee beans. The recovery test obtained in this research is similar with the one reported by (Fujioka and Shibamoto, 2008) for 3-CGA, caffeic acid, ferulic in brewed coffee. The precision (repeatability) of the method was observed by measuring the absorbance of the solution (about 6 mg of coffee samples were dissolved in 25 ml of water) and a coefficient of variation 2.80 - 5.45% were obtained for different coffee varieties. From the results of coefficient variation the new methods are valid in terms of precision.

By this method, the percentage of CGA in various green coffee beans collected from south west of Ethiopia was reported. The percentage of chlorogenic acids determined by this method ranged from [(6.05± 0.33) - (6.25±0.23)%] shown in Table 1. A comparative determination of CGA in coffee beans in the same sample by HPLC was also carried out using method developed by (Ky et al., 1997). Results obtained by UV-Vis spectroscopy (new method) were found to be slightly higher as compared to that of HPLC method Table 1. This may be due to minor interference of water soluble compounds, other than CGA. The results obtained from both methods were in excellent agreement, evaluated by the student t-test at the 95% confidence level shows no significance difference ($p < 0.02$) between the results obtained by both methods. The contents of CGA reported by this method for green coffee beans are also with in the range of previously reported by HPLC techniques. The level of CGA in green coffee beans reported by (Farah et al., 2006b; Clifford and Wight, 1976; Trugo and Macarae, 1984b; Ky et al., 1997, 2001) were about 4 - 8.4% for Arabica coffee type. On the other hand the con-tent of CGA in various green coffee beans (21 species) from Cameroon and Congo ranged from 0.8 - 11.9% on dry matter basis as reported by Campa et al. (2005). In addition Perrone et al. (2008) recently report a similar result with a total CGA contents for coffee Arabica 6.3 and 5.5 g/100 g using LC-MS.

By similar method the contents of CGA were measured in coffee roasted at light, medium and dark temperature. There is a significant decline in CGA as the roasting temperature increases and the results shown in Table 2. For light and dark roasting the CGA present in green coffee beans were decomposed by 25 - 30% and 45 - 52% respectively, and these results are agree with one reported by (Fujioka and Shibamoto, 2008) for brewed coffee roasted at similar temperature.

Conclusion

The optical characterization and method for determination of CGA in coffee beans were developed by UV-Vis spectrometry. The newly investigated optical transition properties are useful for experimental and theoretical studies of CGA. On the other hand the developed method for analysis CGA in coffee beans is simple, fast and sensitive. Moreover chemicals and equipments necessary to carry out the analysis are available in most common labo-

ratories.

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