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The first report on stability of green coffee extract and studies of stability pattern of chemical constituents during storage for three years

Kothathi Puttaiah, Holalu Rajanna Harshan, Mysore Shivanna Nitin, Sujay Kumar, Yedugani Lingam*

Natural & Essential Oils Pvt. Ltd., # 74/2B, Manadavadi Road, Srirampura, Mysore-570008, (INDIA)

E-mail: lingam@nesso.com

ABSTRACT

The current study aims to establish the stability studies of green coffee extract during storage for three years. The stability of chemical constituents in green coffee extract like Total Chlorogenic acids and Caffeine were studied at room temperature for three years. In this study, we have also studied the loss on drying pattern (LOD) of green coffee extract for three years. Results indicated that there are no major changes in chemical constituents after three years stability studies at room temperature.

KEYWORDS

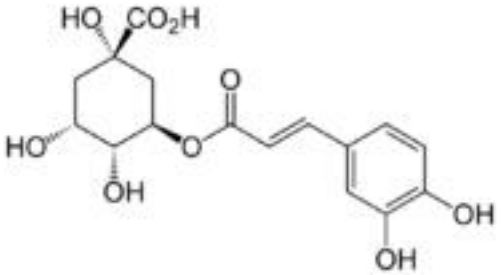
Chlorogenic acids;
Stability;
Green coffee extract;
Oxidation.

INTRODUCTION

Coffee is one of the world's most popular beverages[1]. It is also the most important consumed and traded food commodity worldwide and ranks second, after crude oil, among all commodities.

Most coffee beverage consumed around the world is produced from the species Coffea *arabica* (Arabica) and Coffea *canephora* (Robusta). The former one is considered to be superior due to its sensory properties and, therefore, gets higher prices in the international market. Coffee extracts have extremely strong antioxidant properties against lipid oxidation, higher than many other food products or common antioxidants[2] For several years, the antioxidant effect of coffee, mainly roasted, was the subject of numerous studies on extending the shelf life of food products, in particular those containing significant amounts of fat, susceptible to oxidative damage[3-6]

Chlorogenic acid[7] is a naturally occurring phenolic compound found in all higher plants. This component, being the ester of caffeic acid with quinic acid, is an important biosynthetic intermediate and plays an important role in the plant's response to stress. Potential uses of chlorogenic acid are suggested in pharmaceuticals, foodstuffs, feed additives, and cosmetics due to its recently discovered biomedical activity. This finding caused new interest in chlorogenic acid properties, its isomers, and its natural occurrence. It has been found that as many as nine compounds (chlorogenic acid derivatives and its reaction product with water). These chlorogenic extracted from green coffee beans have extremely strong antioxidant properties against lipid oxidation, higher than many other food products or common antioxidants[8,9] This applies



Chlorogenic Acid (1)

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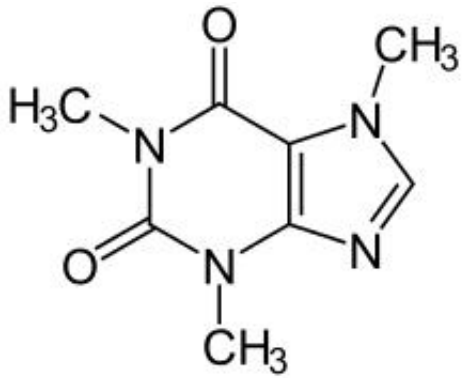

Caffeine (2)

to both roasted and green coffee; however, the latter contains even ten times higher concentration of polyphenols.

Till date there are no reports on stability of Green Coffee extract at room temperature for three years. In the present study the major and minor chemical constituents have been investigated and established the stability data of three years.

Earlier, there are several reports on green coffee like Stability of hydroxycinnamic acids and caffeine[10] from green coffee extracts after heating in food model systems. The mechanisms of these transformations and their significance for the human diet remain largely unknown.

The main objective of the present study was to assess whether degradation of Green coffee extract chemical constituents especially the Chlorogenic acid (1) and Caffeine (2) during storage for three years at room temperature.

EXPERIMENTAL

Materials and methods

Solvents and organic modifiers used in the HPLC mobile phases were Acetonitrile HPLC grade purchased from SIGMA ALDRICH and Formic acid AR Grade (assay ≥ 98%) purchased from RANKEM. The HPLC grade water used in analysis was purchased from RANKEM. The reference standards Chlorogenic acid standard ≥ 95% and Caffeine Standard ≥ 99% were purchased from SIGMA ALDRICH.

Instruments

We have used two HPLC systems for our research work; conventional binary pump HPLC system 1525 made by Waters equipped with an UV-VIS detector 2489, and a shimadzhu HPLC system (model: LC-2010CHT) equipped with an UV-VIS detector and an auto injection with a 20 µl loop, consisted of a computer controlled system with VP 3.20 software, Analytical Balance. Make Essay model #GR202.(5 digit balance and having resolution 0.01mg.)

HPLC Analysis

Chromatographic conditions

The chromatographic separation was performed on a C18(ODS-3, 250mm x 4.6mm,5µ) reversed-phase column. Column temperature 25± 2°C, Wave length of detection 330 nm for total chlorogenic acids and 275 for caffeine, Injection volume 20 µl, Run Time 35 min.

Mobile phase

A: 0.1 % Formic Acid in 25 % Acetonitrile, B: 0.1 % Formic Acid in 10 % Acetonitrile.

Gradient flow program

The flow-rate of the mobile phase was 1.5 ml/min. The gradient elations of 100% mobile phase B at initial. During the first 20 min mobile phase B decreased from 100% to 0%, isocratic flow of mobile phase A for next 10min, gradient flow program mobile phase B increased from 0% to 100% for 2 min, and finally 100% mobile phase B for 3 min before the next injection.

Peak areas were integrated and final concentrations were calculated in comparison with a known standard response.

Determination of Total Chlorogenic acids, 5-CQA and caffeine by HPLC

(a) Preparation of mobile phase-A

Mix well 750ml of Double distilled water and 0.75 ml of formic acid (assay ≥ 98%), add 250ml of HPLC grade Acetonitrile and mixed well. Filter through 0.45µ membrane filter paper and degassed by sonication OR required quantity has to be prepared by same ratio.

(b) Preparation of mobile phase-B

Mix well 900ml of double distilled water and 0.9ml of formic acid (assay ≥ 98%), add 100ml of HPLC grade Acetonitrile and mix well. Filter through 0.45µ membrane filter and degassed by sonication OR required quantity has to be prepared by same ratio.

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(c) Preparation of diluent

HPLC Grade water.

(d) Standard solution preparation-1

Weigh accurately 0.025 to 0.03g of Total chlorogenic acid working standard and transfer into 50ml volumetric flask dissolved in 35 ml of diluent and sonicate for 2-3 min. Dilute up to mark with diluent.

(e) Standard solution preparation-2

Weigh accurately 0.01 to 0.025g of 5-CQA Reference standard and transfer into 50ml volumetric flask dissolved in 35 ml of diluent and sonicate for 2-3 min. Dilute up to mark with diluent.

(f) Standard solution preparation-3

Weigh accurately about 0.01 g of Caffeine working standard and transfer into 50 ml volumetric flask dissolve in 35 ml of diluent and sonicate for 2-3 min. Dilute up to mark with diluent.

(j) Test solution preparation

Weigh accurately about 0.02 to 0.04 g of sample and transfer in to 50 ml volumetric flask dissolved in 35 ml of diluent and sonicate for 2-3min. Dilute up to mark with diluent. If the test solution is hazy filter the solution through 0.45µ nylon syringe filter.

(k) Quantification of areas for standardization

The quantification of catechins and caffeine were performed by quantifying the areas of standardization, where [Sample] g.mL–1 = Area standard × [default g.mL–1] / sample area. The results obtained in g.mL–1 were expressed in%.

RESULTS AND DISCUSSION

We followed the standard GMP protocol for stability studies of green coffee extract and tested samples at regular intervals like initial, 3rd, 6th, 9th, 12th, 18th, 24th and 36th months. The storage conditions are as per GMP norms and both samples were in dark place at room temperature. We have analyzed both batches GCCA-60-1201001 and GCE-

TABLE 1 : Stability data of Batch No:GCCA-60-1201001

S.No	Parameters	Initial	3rd	6th Month	9th	12th	18th Month	24th Month	36th Month
1	Loss on drying at 105°C	3.50%	3.60%	3.80%	3.90%	3.80%	4.00%	4.10%	4.30%
2	Total chlorogenic acids By HPLC	61.50%	62.10%	61.80%	61.60%	62.00%	61.50%	60.60%	60.80%
3	Caffeine by HPLC	1.80%	1.90%	1.80%	1.80%	1.90%	1.90%	1.90%	1.80%

TABLE 2 : Stability data of Batch No: GCE-12-A060

S.No	Parameters	Initial	3rd	6th Month	9th	12th	18th Month	24th Month	36th Month
1	Loss on drying at 105°C	2.4%	2.6%	2.5%	2.6%	2.7%	2.9%	3.0%	3.2%
2	Total chlorogenic acids By HPLC	70.7%	70.4%	70.1%	70.1%	69.5%	68.9%	69.6%	70.1%
3	Caffeine by HPLC	1.5%	1.4%	1.5%	1.6%	1.5%	1.7%	1.4%	1.4%

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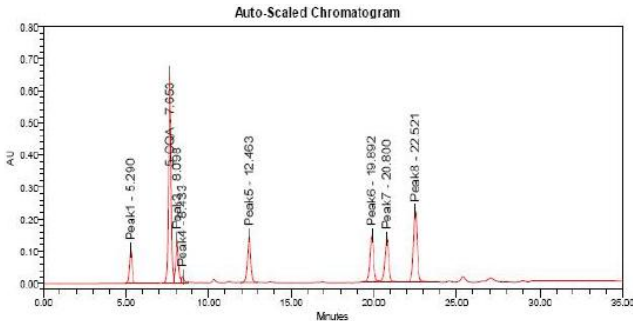

Figure 1 : HPLC Chromatgram GCCA-1201001(Initial)

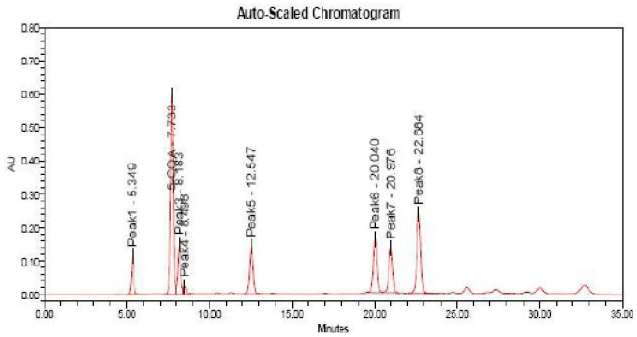

Figure 2 : HPLC Chromatgram GCE-1A060 (Initial)

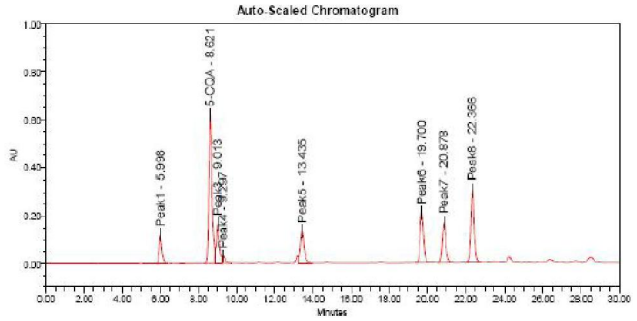

Figure 3 : HPLC Chromatgram GCCA-1201001(36th Month)

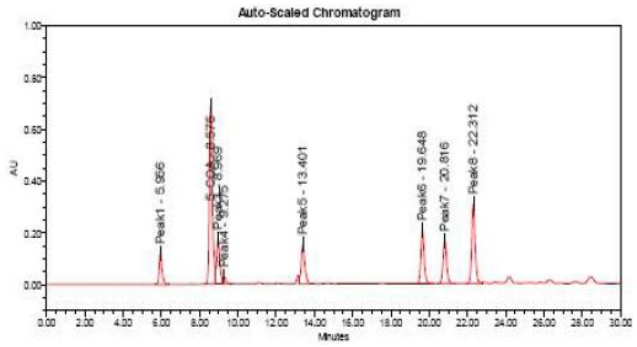

Figure 4 : HPLC Chromatgram GCE-1A060 (36th Month)

12-A060 for loss on drying (LOD), Total Chlorogenic Acid, Caffeine content.

Our internal description of green coffee extract is yellowish brown to pale brown colored powder. The both samples were complies with the description and it was very much complies with our customer specification. However, our experimental analysis proved that there was slight color change in both the samples. The color change in both samples may be due to oxidation of Chemical constituents present in the green coffee extract. In both batches (GCCA-1201001and GCE-1A060), the number peaks remain same after three years and there are no other extra peaks were observed after three years stability studies. It is very clear from our studies that the main chemical constituents of green coffee extract were remained stable at room temperature.

We have conducted the loss on drying test (LOD) at 105°C and value was increased in both batches. There is no major difference in the percentage of total chlorogenic acid and caffeine.

CONCLUSION

To our knowledge, this is the first report on green coffee extract stability studies for long term storage for three years at room temperature. These results are the beginning step for all interesting researchers to do many scientific studies on Yemeni coffee which is national crops in Yemen.

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