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Review

Some biochemical compounds in coffee beans and methods developed for their analysis

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In this paper, the literature review related to the chemical characteristics, composition and the various physiological and psychological effects of caffeine, chlorogenic acids in biological system and their relation with coffee quality have been presented. In addition, the contents of these biologically active compounds in coffee beans, the different chemical and physical methods used to analyze these compounds in coffee beans were reviewed.

Key words: Coffee, biochemical compounds of coffee, analysis, high pressure liquid chromatogdraphy (HPLC), spectroscopic techniques.

INTRODUCTION

The impacts of some biochemical compounds of coffee beans in biological system are currently a subject of intense research activity. Compounds like chlorogenic acid and its derivatives (Hydroxycinnamic acids) are useful for preventing different chronic degenerative disease. On the other hand, caffeine affects the physiology and psychology of human being. In addition, these compounds have a significant contribution for quality of coffee beans since they form aroma and flavor of coffee beverages. Hence the aim of this article is to review the chemical characteristics, composition and various applications of some biochemical compounds (hydoxycinnamic acids, caffeine) of coffee beans and the different chemical, physical techniques developed to analyze these compounds in coffee and others medicinal plants.

Chemical structure and composition of caffeine

Caffeine (1, 3, 7-trimetylxanthine), is one of the main alkaloid found in various kinds of foods and drinks that we consume in daily life (Mumin et al., 2006; Singh and Sahu, 2006; Najafi et al., 2003). It is naturally found in leaves, seeds or fruits of 63 plant species (Mumin et al., 2006). The most common sources of caffeine are coffee, coca beans, cola nuts and tea leaves (Mumin et al., 2006). The chemical formula for caffeine is $C_8H_{10}N_4O_2$ and chemical structure is shown in Figure 1. Pure caffeine occurs as odorless, white powder. It has molecular weight of 194.19 g, melting point of 236°C, sublimation point of 178°C and pH values in the range of 6 to 9 (Mumin et al., 2006; Clarke and Macare, 1985).

The caffeine contents of green coffee vary widely with difference in species. Even within species, there is a very wide range of values of caffeine (Silvarolla et al., 2004; Ky et al., 2001). The most common coffee species are Robusta and Arabica. Robusta coffee in general have a higher caffeine content with an overall mean value of 2.2%, while that of Arabica is about 1.2% with a range of 0.6 to 1.9% (Belay, 2010; Belay et al., 2008; Clarke and Macare, 1985; Franca et al., 2005). Intermediate value have also been reported for commercially less important species such as Liberica with mean value 1.35 and Arabusta about 1.72%, respectively (Clarke and Macarae, 1985). The coffee paracoffea genus in Africa and Asia are available with very low caffeine contents of about 0.2% (Clarke andMacarae, 1985).

The effects of environmental and agricultural factors are less important than genetic variation in controlling the

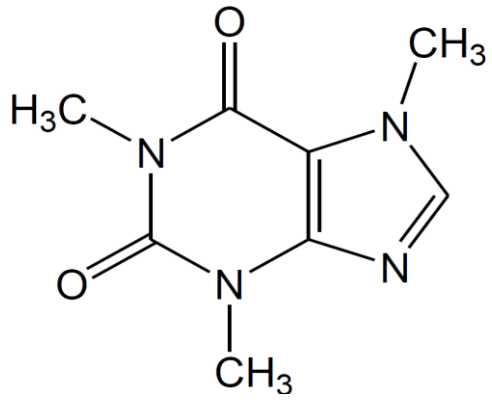

Figure 1. Chemical structure of caffeine.

caffeine contents of green beans and it is also reported that fertilizer in particular potassium, phosphate, magnesium and calcium do not have a significant effect on caffeine (Clarke and Macarae, 1985). Similarly, roasting at some extent did not affect the content of caffeine, other than causing a slight relative increase due to the loss of other compounds (Farah et al., 2006; Clarke and Macarae, 1985).

Physiological and psychological effects of caffeine

The effects of caffeine on human being depend on concentrations. Consuming high concentration of this compound causes various physiological and psychological effects such as relaxation of bronchial muscle, stimulation of the central nervous system, gastric acid secretion and diuresis (Zhang et al., 2005; Minamisawa, 2004; Yukawa, 2004; Bolton and Null, 1981). The increases in concentration of caffeine *in vivo* are also a key mark for various disorder including heart disease, kidney malfunction and asthma. Moreover, our sleeping habit, performance, and concentration are modified by caffeine (Zhang et al., 2005; Najafi et al., 2003; Singh and Sahu, 2000; Bolton and Null, 1981). Caffeine has a tendency of rapidly and completely absorbed from gastrointestinal tract within a short period of time and distributed in the body (Kervigan and Lindsey, 2005; Clarke and Macarae, 1985); however, it is not removed from the circulation until metabolized initially into paraxanthine, thephylline and thyobromine then into derivative of uric acid and diaminourcil, which is eventually removed from the circulation. So the plasma half life of caffeine in man, that is, the time required for its level to be diminished by 50% as a result of biotransformation and excretion is 5 to 6 h (Kervigan and Lindsey, 2005; Clarke and Macarae, 1985).

When the peak of plasma level of caffeine concentration is 15 to 30 M, effects like, mild anxiety, respiratory stimulation, cardiovascular effects, diuresis and increase in gastric secretion would be observed. When the levels are in between 150 to 200 M, a symptom of acute toxicity may appear. These include severe restlessness, excitement, muscular tension, twitching and cardiovascular disturbance such as tachycardia (Barone and Roberts, 1996; Clarke and Macarae, 1985). The International Olympic Committee classified caffeine as a drug of abuse when it is present in human urine with concentration higher than 12 µg/ml (Aragao et al., 2005; L-Martinez et al., 2003).

On the other hand, besides the physiological and psychological effects of caffeine, the chemical analyses of caffeine in coffee beans have been used as an additional tool for evaluating coffee quality. It has been reported that higher caffeine contents is associated with less quality samples compared to other Arabic samples (Farah et al., 2006, 2005b). Due to the adverse effects mentioned previously, there is an increased demand for de-caffeination of coffee. About 10% of the world production of green coffee and 20% of the coffee import to some European countries have gone for decaffeination in coffee industry (Clarke and Macarae, 1985). Decaffeination of coffee by chemicals in industry is expensive and it also changes the taste and aroma of coffee and results in reduction of cup quality of coffee. Recently, Nara Institute of Science and Technology in Japan created transgenic plant with 70% reduced caffeine contents. The researchers design to repress a gene, which activated one of the three enzymes, involved in caffeine biosynthesis (Ogita et al., 2003). However, the genetic engineering process takes longer time and is not cost effective. Considering the problems associated with the process of decaffeination, researchers have started searching for naturally decaffeinated coffee beans. Accordingly, Brazilian researchers after screening 300 Ethiopian coffee trees discovered three naturally decaffeinated varieties, which they named AC1, AC2 and AC3. Analysis of these varieties showed they contain 0.07% caffeine compared to the caffeine found in natural coffee beans (Sivarolla et al., 2004). It is hoped that this finding provides an alternative to the artificial decaffeinated coffee already on the market. Thus, it is important to develop a simple method of analyzing caffeine in coffee beans in order to identify the origin of decaffeinated coffee beans obtained in Ethiopia.

Methods developed for analysis of caffeine

Due to aforementioned facts, many chemical and physical methods have been developed for the determination of caffeine in coffee and other beverages. The most widely used methods for the determination of caffeine in beverages are UV-Vis spectrophotometer and partial least square (L-Martinez, 2003; Oztem et al.,

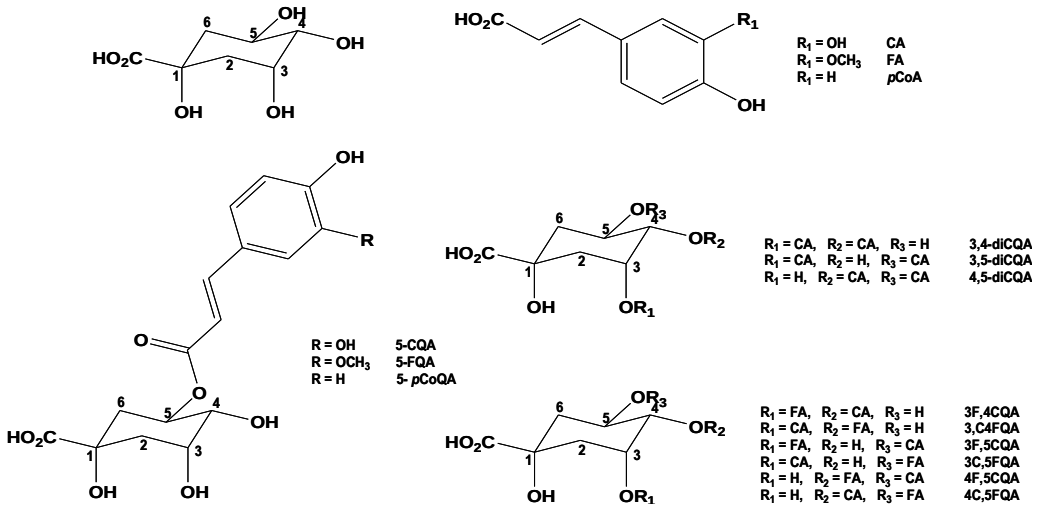

Figure 2. Chemical structure of chlorogenic acid and its derivative. (Source J. Plant Physiol., 18(1):23-26, 2006).

2003), UV-Vis spectrophotometer (Singh and Sahu, 2006; Belay et al., 2008; Belay, 2010), luminescscence (Wei et al., 2005; Masatoki and Hirokazu, 1989; Andino et al., 1987), derivative spectrophotometer (Alpdogan et al., 2002; Lau et al., 1992), HPLC (Mumin et al., 2006; Aragao et al., 2005; Minamisawa, 2004; Levent, 2002; Qi et al., 2002; Ortega-Burrales et al., 2002; Casal et al., 2000), Fourier transform infrared spectroscopy (Najafl et al., 2003; Paradkar and Irudayaraj, 2002; Bousain et al., 1999), near infrared reflectance (NIR Reflectance) spectrometry (Chen et al., 2006; Huck et al., 2005; Rodriquez-Saona et al., 2005), Raman spectroscopy (Edawards et al., 2005) and capillary electrophoresis (Zhang et al., 2005) are very commonly used techniques.

Spectrophotometer method is fast, simple, accurate, reproducible and inexpensive procedure as compared to other methods; however, it is not possible to determine caffeine directly in coffee beans by conventional UV-Vis absorption measurement due to the spectral overlap of UV absorbing substances in the sample (Belay, 2010; Belay et al., 2008; Zhang et al., 2005). The derivative spectrophotometry is relatively easy; but, it is not reliable for the small concentration of caffeine in samples. By HPLC methods, many caffeine contents were determined in various foods using different procedures since it provides the most reliable method. However, the use of expensive equipments and the demand for more operator attention prevents its applications in small industrial laboratories where only a few analyses are performed each day (Zhang et al., 2005; Alpdogan et al., 2002). Other methods such as Fourier transform infrared (FTIR), Raman and near infrared reflectance (NIR Reflectance) reflectance spectrometry are equally versatile and powerful analytical tools for analyzing contents of caffeine in coffee (Huck et al,. 2005; Rodriquez- Saona et al., 2005). Moreover, they do not require expensive chemicals; however, such instruments are not available in most laboratories and involve huge capital costs. Although, there are many reports on analysis of caffeine in coffee beans and other beverages by different techniques, however, qualitative and quantitative characterization of caffeine in coffee beans by a UV-Vis spectroscopy is not yet reported. The characterization and analysis include the determination of the optical transition properties of caffeine and its composition in coffee beans using Beer-Lambert's and integrated absorption techniques.

The other active compounds which have useful applications in biological systems and found in coffee beans by high amounts are chlorogenic acids. Thus, following, a review related to chlorogenic acids would be presented.

CHLOROGENIC ACIDS

Chemical structure and composition of chlorogenic acids

Chlorogenic acids (CGA) are the main phenolic compound found in green coffee beans and have been studied for more than a century (Clifford, 1979). They belong to hydroxycinnamic acids classes and chiefly consists of caffeic acid (3, 4-dihydroxycinnamic acid), ferulic acid (3-methoxy-4-hydroxycinnamic acid), p-coumaric (4-hydroxycinnamic acid), and sinapic acid (3,5-dimethoxy-4-hydroxycinnamic acid) (Zhu et al., 2006; Manach et al., 2004). The chemical structures of compounds are shown in Figure 2. Chlorogenic acids are

subdivided according to the nature and number of cinnamic substituents and their esterification position in the cyclohexane ring of quinic acid. The IUPAC number system for quinic acid (1L-1(OH), 3, 4, 5-terahydroxy-cyclohexane carboxyl acid) has axial hydroxyl group on carbon 1 and 3 and equatorial hydroxyl on carbon 4 and 5. Esters of this acid are usually formed on 5 but also on carbon 3, 4 and less commonly on carbon 1 (Clifford, 2000; Clifford, 1979). The main groups of CGA compounds found in green coffee beans are shown in Figure 2. These groups of compounds are, caffeoylquinic acids with 3 isomers (3-, 4-, and 5-CQA); dicaffeoylquinic acids (diCQA) with 3 isomers (3-,4-diCQA; 3,5-diCQA; 4,5-diCQA); feruloquinic acids (FQA), with 3 isomers (3-, 4-, and 5-FQA); p-coumaroylquinic acids (pCoQA) with 3 isomers (3-, 4-, and 5-pCoQA) and six mixed diesters of caffeoylferuloy-quinic acids (CFQA) (Clifford, 2003; Trugo and Macrae, 1984b; Clifford and Wight, 1976).

FQA represent 98% of CGA (Farah and Donangelo, 2006; Clarke and Macarae, 1985) and minor class, such as diferuloylquinic acids ((diFQA), di-p-coumaroylquinic acid (di-p-CoQA), dimethoxycinnamoylquinic acids and others, which together constitute less than 1% of the total CGA content as recently identified (Perrone et al., 2008). The major representative of hydroxycinnamic acid is caffelic acid, which occurs in food mainly as an ester with quinic acid called chlorogenic acids (Manach et al., 2004; Kono et al., 1995; Clifford, 1999).

The total CGA content of green coffee beans is varying according to genetic species, degree of maturation and less importantly agricultural practices, climate and soil (Farah et al., 2005b; Clifford, 1985). In general, the percentage of CGA for regular green coffee beans on the dry matter varies from 4 to 8.4% for Arabic and 7 to 14.4% for Canephora with some hybrids presenting intermediate levels (Farah et al., 2005a, 2005b; Trugo and Macrae, 1984b; Clifford and Wight, 1976). On the other hand, a low CGA content of 1.2% was reported in beans of coffea pseudozanquebariae, caffeine free species native of East Africa. Such low contents have been also observed in some other low caffeine species from Africa (Clifford, 1985).

Antioxidant properties of chlorogenic acids

Chlorogenic acids are the most abundant poly phenols in coffee which are responsible for substantial part of coffee antioxidants (Svilaas et al., 2004; Wen et al., 2004; Pellegrini et al., 2003; Richelle et al., 2001). Epidemiological study indicates that due to chlorogenic acids, its derivatives coffee shows tea catechin analog biological effect like antioxidation, antimutation, anticarcinogenic, antibiotic, antihypercholesterolemia, antihypertensive, metal chelation and inflammatory action (Svilaas et al., 2004; Wen et al., 2004; Yukawa, 2004). Moreover, there are also reports that hydroxycinnamic acid derivative have a promising role in protection from radiation and photooxidation (Zhu et al. 2006).

In addition to the aforementioned, commercially antioxidants are also manufactured from coffee that consists of 55% chlorogenic acids which regulates the blood sugar and perhaps help for the management of obesity (Abidoff, 1999). Several studies related to the effects of this compound on blood sugar regulation are also noted. Clinical evolution indicated the chlorogenic groups had lowered the increase in blood glucose level by 15 to 20% (Abidoff, 1999). Furthermore, the risks of type-2 diabetes also decreased with higher consumption. It is expected that the chlorogenic acids are the main active component to antidiabetic effect according to pharmacology studies (Salazar-martinez et al., 2003; Vandam and Feskens, 2002; Abidoff, 1999).

Chlorogenic acids have also physiological function in coffee plant. It contributes to control seed germination and cell growth through regulation of the level of indolacetic acids (Clifford, 1985). Moreover, it has been reported that due to antioxidants and antibiotic properties, hydroxycinnamoylqunic acids are involved in numerous biological plant functions such as pest and disease resistance (Matsuda et al., 2003; Takahama, 1998). The mono (Szalma et al., 2005) and dicaffeoylqunic acids (Cole, 1984) are involved in the insect resistance in different cultivated species.

Besides the antioxidant properties and physiological function, chlorogenic acids play a great role in the formation of pigments, taste and flavor of coffee beans, which determine the quality and acceptance of the beverages. They contribute to the final acidity of the beverages, as a result of maillard and strecker's reaction bitterness and the formation of lactones and other phenol derivatives responsible for flavor and aroma (Variyar et al., 2003; Trugo and Macrae, 1984a; Clifford and Wight, 1976). According to literature report, a total CGA level in coffee has an inverse association with coffee quality with higher content observed in lower quality samples. Farah et al. (2006) observed a strong association between the level of CQA and FQA and low cup quality. The lower CGA level of coffee Arabica in beverage quality when compared with coffee Canephora also appear to explain this situation. The large difference in CGA content of these species was considered one of the factors responsible for flavor difference between the two species (Ky et al., 2001; Trugo and Macrae, 1984b).

Methods developed for analysis of chlorogenic acids

Several methods have been developed for determination of chlorogenic acids and its derivatives in coffee beans and other plants. The most widely used methods are HPLC (Farah et al., 2006, 2005b; Negishi et al., 2004; Ky

et al., 2001; Pedrosa et al., 2000), capillary electrophoretic (Polnsek et al., 2006; Jiang et al., 2004), and Miceller electrokinetic chromatography (Risso et al., 2007; Guan et al., 2006). Although these methods are powerful for quantification of the content of chlorogenic acids and its derivatives, however, they have been criticized as being tedious, time consuming and some are limited to large sample volume extraction procedures and requiring long time for reaction. Moreover, most of these instruments are very expensive which are not available in moderate laboratories.

There are also some physical methods developed for qualitative and quantitative characterization of chlorogenic acids. UV-Vis and fluorescence spectroscopy are the methods used to study the suppression of N-nitrosating reaction by chlorogenic acids (Kono et al., 1995). Rates of oxidation of hydroxycinnamic by HRP/H2O2 or tyrosinase /O_2 (Moridani et al., 2001), the application of laser flash photolysis technique to investigate the antioxidant properties of CGA (Zhu et al., 2006) and the UV-Vis spectrophotometer for determination of CGA contents in fresh and processed potatoes (Dao and Friedman, 1992) and in coffee beans (Belay et al., 2009) were also reported.

Despite the characterization of the compound using the aforementioned techniques, it is worthwhile to mention that simple and systematic method for measuring the CGA in green and roasted coffee beans using UV-Vis spectroscopy was applied by Belay et al. (2009). Moreover, the investigation of the optical transition properties (molar decadic absorption coefficient, integrated absorption cross-section, oscillator strength and transition dipole moment) of CGA, the effects of solvents, and the self-association and hetero-association were not explored by aforementioned instrument in the past.

CONCLUSION

Biochemical compounds in coffee beans are useful applications in food and pharmaceutical industries. The natural health product company has developed antioxidants from chlorogenic acid of coffee that regulate blood sugar. Other research reports also show the risks of type -2 diabetes decreases with higher coffee consumption due to the main active components of coffee chlorogenic acids. Similarly, caffeine, one of the main constituents of coffee, has also a variety of pharmacological and cellular response in a wide spectrum of biological system. On the other hand, the biochemical compounds have a great contribution in characterizing the quality and thus determine the relative price as well as the usefulness of a given quantity of coffee. Therefore, obtaining information about the chemical compositions of these compounds and methods of the analysis of these compounds are necessary.

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