Effects of postharvest processing on the bioactive compounds in Arabica coffee

Author:
Punya-In, Sutthida

Publication Date:
2014

DOI:
https://doi.org/10.26190/unsworks/17059

EFFECTS OF POSTHARVEST PROCESSING ON THE BIOACTIVE COMPOUNDS IN ARABICA COFFEE

A thesis submitted as a fulfillment for the degree of Doctor of Philosophy

Sutthida Punya-in

Food Science and Technology

School of Chemical Engineering

Faculty of Engineering

The University of New South Wales

October 2014

Abstract

Coffee is known as the most popular beverage after water. Arabica coffee accounts for 70% of the overall world coffee production.

Coffee postharvest operations resulting in the production of green coffee, storage and roasting are important processing steps. Comparison of coffee postharvest treatments, storage and roasting temperatures were done to maximise yield of beneficial compounds. The study used the mechanically demucilaged coffee beans. The soaked and non-soaked parchment coffee samples were dried at 40 ^{o}C (20% RH), 50 ^{o}C (15% RH), 60 ^{o}C (15% RH) and 70 ^{o}C (10% RH). The soaked and non-soaked parchment coffee samples, dried at 40 ^{o}C (20 %RH), were selected to study effects of storage conditions (15 ^{o}C, 60% RH and 30 ^{o}C, 30% RH). After that, the samples were roasted and extracted. Moisture content, %weight loss and colour were measured to assure consistency of roasting levels of coffee samples.

The analysis by HPLC showed highest concentration of chlorogenic acid, caffeine, trigonelline, α-tocopherol in the coffee beans which were non-soaked and stored at 15 ^{o}C and 60% RH for 6 month and roasted at light level (180 ^{o}C, 3 min). Positive correlation was found between total phenolic compounds content and the anti-oxidant activities (FRAP and ORAC). Moreover, the highest contents of cafestol and kahweol were found in green coffee. The soaked and dark roasted coffee samples received the highest score of consumer acceptance.

Keywords: Arabica coffee, mechanical demucilaging, postharvest treatments, storage, roasting, chlorogenic acid, caffeine, trigonelline, α-tocopherol, cafestol, kahweol and antioxidant activity.

Table of contents

List of tables

List of figures

Acknowledgements

First of all, I would like to express my deeply gratitude to my supervisors Dr George Srzednicki and Dr John Craske for the very useful comments remarks and engagement through the learning process of this PhD research.

Furthermore, I would like to extend my thankfulness to the following people:

The owner and staff from Zentveld's coffee plantation, Newrybar, NSW for generous supply of demucilaged parchment coffee throughout the project.

Rajamangala University of Technology Lanna, Thailand for financial support in the form of my scholarship.

Professor Naresh Kumar from School of Chemistry, Faculty of Science, UNSW for his advice and guidance about ^{1}H NMR and other chromatograms. I would like to thank Dr Izabela Konczak from CSIRO for her advice and guidance about anti-oxidant activities. I would also like to thank Lewis Adler from Bioanalytical Mass Spectrometry Facility (BMSF) for his advice and guidance about HPLC methods.

I would like to express my appreciation to all the academic, technical and administrative staff, students and friends from Food Science and Technology program, School of Chemical Engineering, UNSW for all their help.

Special thanks for all my Thai friends and my other good friends for their help, their jokes and their unconditional friendship. I believe that laugh and smile boost pure energy.

Finally, I would like to express my love and grateful to my beloved parents, my sister, my brother and my little niece for their love and enormous support throughout my PhD life.

Nomenclature

*a**	Redness/greenness value
AAPH	2,2'-Azobis 2-methylpropionamidine dihydrochloride
ACN	Acetonitrile
ANOVA	Analysis of Variance
AOAC	Official Methods of Analysis Chemists
AUC	Area under the curve
*b**	Yellow/blueness
CFE	Caffeine
CFT	Cafestol
CGA	Chlorogenic acid
d.b.	Dry basis
DMRT	Duncan's multiple rage test
DR	Dark Roasting
DW	Dry weight
e	Equilibrium value
eq	Equivalent
exp	Experimental value
FC	Folin-Ciocalteu
Fe3+-TPTZ	(2,4,6-Tri(2-pyridyl)-s-triazine)
FRAP	Ferric reduction antioxidant power
GC	Green Coffee
^{o}h	Hue angle

HPLC	High Performance Liquid Chromatography
^{1}H NMR	Proton Nuclear Magnetic Resonance
KWL	Kahweol
*L**	Lightness
LR	Light Roasting
MC	Moisture Content
M_{db}	Moisture content (%dry basis)
M_e	Equilibrium moisture content (dry basis)
MeOH	Methanol
mM	milimolar
mmol	milimole
M_o	Initial moisture content (dry basis)
mol	Mole
MR	Medium Roasting
MR	Moisture ratio (see equation 2.1)
ORAC	Oxygen radical absorbing capacity
PBS	Phosphate buffered saline
r	Correlation coefficient
r^2	Coefficient of determination
RH	Relative Humidity
rpm	Revolution per minute
SD	Standard deviation
TE	Trolox equivalent
TGL	Trigonelline
TPC	Total Phenolic Compounds

Trolox	6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
Vit E	α-Tocopherol
w.b.	Wet basis
μM	micromole

1. Introduction

In recent years there has been an increase in research dealing with a healthy diet. Beverages are a considerable part of daily a diet. They include alcoholic and non-alcoholic beverages such as coffee, tea, juice and carbonated drinks. Among all beverages, coffee is the most popular after water.

Coffee contains chlorogenic acids, caffeine, trigonelline, cafestol, kahweol and α-tocopherol (Vitamin E).

Coffee postharvest operations including pulping, demucilaging, drying of parchment coffee, storage and roasting all have an effect on the compounds and the quality of coffee.

It has been demonstrated that mechanical demucilage uses significantly less water compared to other coffee processing methods and has lower negative impact on the environment.

Environmentally friendly processing and maximizing coffee quantity are becoming important concerns of the coffee industry.

Goal of this study

To devise appropriate postharvest treatments for drying of parchment coffee, storage and roasting degree, in order to preserve selected compounds and their antioxidant effects as well as the prevalent sensory characteristics of Australian coffee.

Sub-objectives

- To devise appropriate postharvest treatment, air-drying conditions and roasting degrees to enhance product quality, with emphasis given to preserving selected compounds and their anti-oxidant properties.
- To observe the effect of storage of parchment coffee beans on the selected compounds and anti-oxidant activities.
- To evaluate consumer acceptability of Arabica coffee subjected to different of postharvest and processing treatments.

2. Literature Review

2.1 Coffee

2.1.1 Historical background

Coffee was discovered in Yemen and the northeast region of Ethiopia. The Arab world was the first to cultivate coffee. The earliest credible evidence of coffee drinking appears in Yemen, southern Arabia, in the mid-fifteen century (Weinberg and Bealer, 2001).

The original coffee industry started in Ethiopia. By 1200 A.D. coffee beverage had spread along the Red Sea to Aden, Mecca, and Cairo. By 1300, Persians were also familiar with coffee, and by 1500 it had spread to Turkey. Shortly thereafter, coffee was being sold in Venice across the Mediterranean Sea. It is now a popular beverage worldwide. Coffee produced from roasted beans and its plants belongs to the genus *Coffea* (Sivetz and Desrosier, 1979).

2.1.2 Types of coffee

Coffea arabica (Arabica coffee) and *Coffea canephora* (Robusta coffee) are important species for trading. Two other species which are grown on a minor scale are *Coffea liberica*, (Liberica coffee) and *Coffea excelsa* (Excelsa coffee) (International Coffee Organisation, 2009; Wintgens, 2009)

The regions of the world where the different coffee species are grown are shown in Table 2.1.

Table 2.1 Worldwide distribution of cultivated coffee species.

Species	Regions	Area
C. arabica	Africa	Covers the highlands of the continent, Madagascar and the West Coast
	Asia	Toward the higher sea levels across the mainland from Arabia to the Philippines, Yemen, India, Papua New Guinea, Mauritius, Reunion, New Caledonia and Vietnam
	Americas	In the highlands of the American countries, mid sea level of South America, the high plateaux of the Caribbean Islands and Hawaii.
C. canephora	Africa	The lowlands of west and central Africa and mid-altitude zone in the East.
	Asia	Low and mid sea level regions India, Indonesia, Philippines, Malaysia, Thailand and China
	Americas	Moist and tropical zone in the North East of Brazil, Ecuador, Guyana and Mexico.
C. liberica	Asia	Low sea level zones Mainly in Malaysia, however also in Indonesia, the Philippines, Vietnam and Thailand.
	Africa	The west coast of central Africa and Liberia.
	Americas	Guyana and Surinam.
C. excelsa	Asia	Largely in Vietnam, however also in Indonesia and the Philippines.
	Africa	Central and west Africa, Chad, south Sudan, Madagascar, Mauritania and others.
	Americas	Mainly Puerto Rico.

Source: (Wintgens, 2009)

Coffea arabica – Arabica coffee

Linnaeus first described *Coffea arabica* in 1753. The best known varieties are 'Typica' and 'Bourbon'. From these two cultivars many other cultivars have been bred. A typical Arabica plant is a large bush with dark-green oval leaves. It is genetically different from other coffee species. The fruits are elliptical and ripen in 7 to 9 months; they usually contain two flat seeds (the coffee beans). Arabica coffee is susceptible to attacks by pests and disease and this is seen as a major challenge to plant breeding programs (International Coffee Organisation, 2009).

Coffea canephora – Robusta coffee

Robusta is the name of a widely cultivated variety of *C. canephora*. The height of Robusta shrub or small tree is approximately 10 metres, however it has a shallow root system. The fruits are mature within 11 months. The seeds are oval and smaller than those of *C. arabica.* (International Coffee Organisation, 2009).

The differences between Arabica and Robusta coffee are shown in Table 2.2.

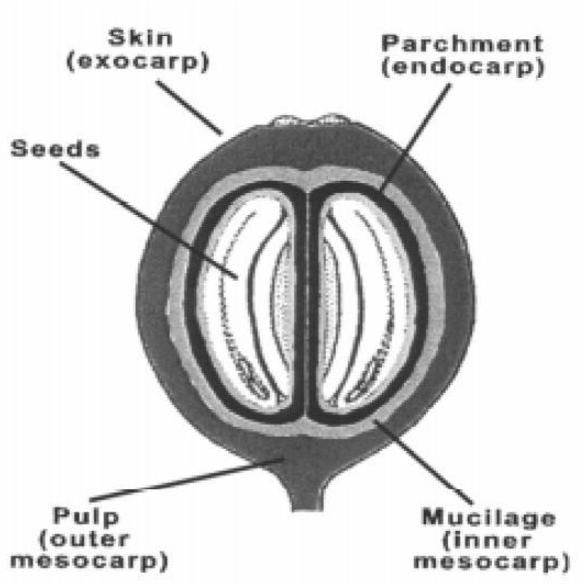
Table 2.2 The differences between *C. arabica* and *C. canephora*.

	Arabica	Robusta
Date species described	1753	1895
Chromosomes (2n)	44	22
Time from flower to ripe cherry	9 months	10-11 months
Flowering	After rain	Irregular
Ripe cherries	Fall	Stay on the tree
Yield (kg beans/ha)	1500-3000	2300-4000
Root system	Deep	Shallow
Optimum temperature (yearly average)	15-24 °C	24-30 °C
Optimal rainfall	1500-2000 mm	2000-3000 mm
Optimum altitude	1000-2000 m	0-700 m
Hemileia vastatrix (coffee rust)	Susceptible	Resistant
Ceratobasidium noxium (Koleroga)	Susceptible	Tolerant
Nematodes	Susceptible	Resistant
Tracheomycosis	Resistant	Susceptible
Coffee berry disease	Susceptible	Resistant
Caffeine content of beans	0.8-1.4%	1.7-4.0%
Shape of bean	Flat	Oval
Typical brew characteristics	Acidity	Bitterness, Full
Body	Average 1.2%	Average 2.0%

Source: (International Coffee Organisation, 2009)

2.1.3 Biology and ecology of coffee

Coffee belongs to the Rubiaceace family, which has over 6,000 species with some 500 genera. Most are tropical trees and shrubs that occur as undergrowth in forests. All species of coffee have woody tissues, ranging from small shrubs to large trees that are over 10 metres high. The leaves can be yellowish, dark green, bronze or tinged with purple (International Coffee Organisation, 2009). The first fruit of coffee is produced 3 years after seed germination. The coffee bean is formed inside the coffee cherry. The coffee cherries contain pulp which separated two the parchment enclose by a layer of mucilage (Wintgens, 2009). A cross-section of a coffee cherry is shown in Figure 2.1.



Source : Avallone *et al.* (2000)

Figure 2.1 Cross section of a coffee cherry

The multilayered structure of a coffee cherry is composed of a red or yellow exocarp (skin) when ripe, a gelatinous-pectic mesocarp (pulp),

which is 0.5-2 mm thick, rich in sugars and water, which is glued over the endocarp (parchment), enclosing each seed. The seeds (coffee beans), usually two per cherry, can vary in size, shape and density according to the growing conditions and genotype (Illy and Viani, 1995).

2.1.4 Physical properties of coffee

The density of coffee at various processing stages is shown in Table 2.3.

Table 2.3 Coffee densities at various processing stages

Coffee	Bulk Densities (kg/m^3)
Ripe cherry	800.85
Pulping and fermentation (50% moisture)	800.85
Dry green bean in parchment or hull (13% moisture)	400.42
Dry, hulled, polished green bean	704.74

Source: Sivetz and Desrosier (1979)

2. 1.5 Production and marketing

The agribusiness of coffee is popular worldwide. Coffee is produced to a large extent in the southern hemisphere and consumed in the northern hemisphere. Coffee production is always directed to support the palate of the consumers, either in the field, the great cities, in developed countries or in developing countries (Zylbersztajn *et al.*, 2008).

World coffee production is about 70% Arabica (*Coffea arabica*) and 30% Robusta (*C. canephora*). According to Vossen (2001), coffee production continues to show large annual fluctuations, but has generally increased by about 14% over the past 15 years: from 5.2 million tonnes per year in average over the years 1980-84, to 5.9 million over the period 1995-1999.

These data show that coffee is of great economic importance to developing countries where it is produced, with Arabica coffee gaining a wider share of the market than Robusta.

2.2 Harvesting

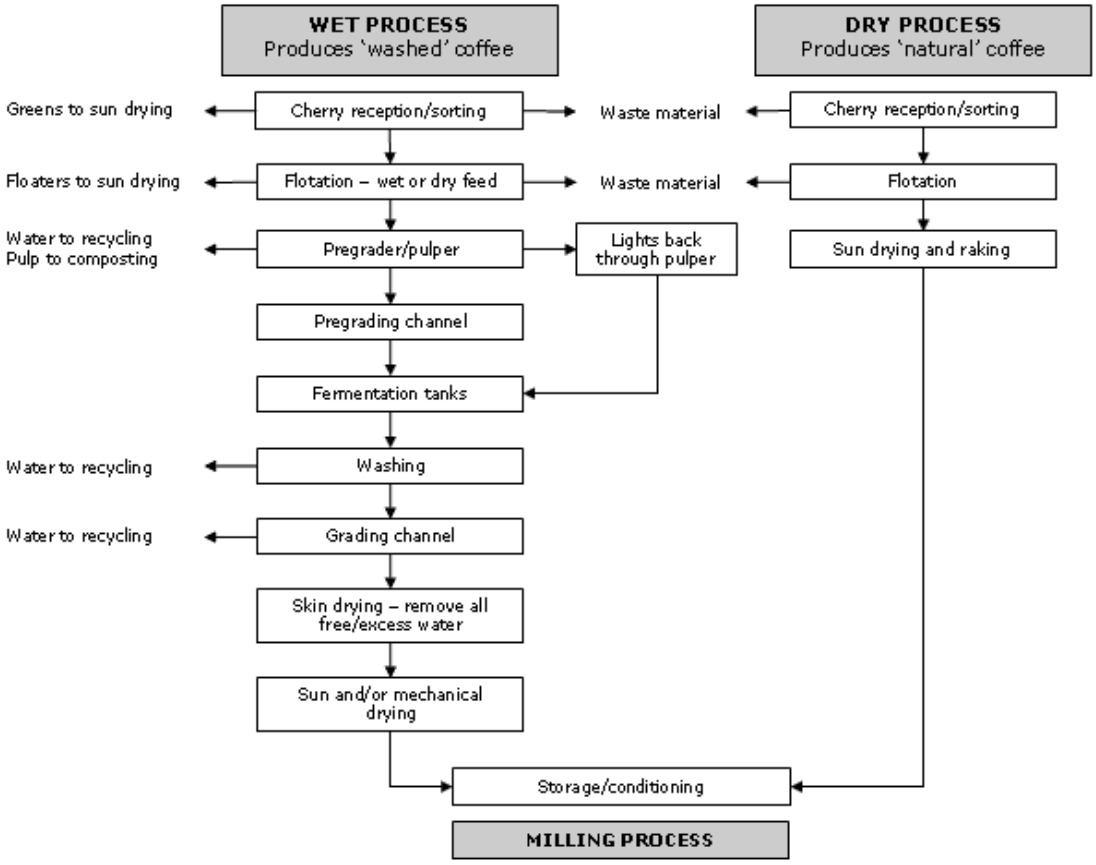
In most coffee growing countries, there is one major harvest a year. Harvesting is done in one of two ways, either by stripping or by picking.

Strip picking involves the entire crop being harvested at one time. This can either be done by machine or by hand. In either case, all of the cherries are stripped off from the coffee shrub at one time (National Coffee Association of USA, 2012)

With the picking or finger picking method, only ripe cherries are hand-picked and collected in baskets. In coffee regions with a well-defined season, all the cherries can be harvested at peak maturity. This method is generally used to harvest finer Arabica beans (Illy and Viani, 1995; National Coffee Association of USA, 2012).

2.3 Coffee processing methods

There are two main processing methods used to prepare green coffee. Firstly, the dry processing method is commonly used for Robusta coffee processing. This drying method is used in Brazil, Ethiopia, Haiti, Indonesia and Paraguay (Silva *et al.*, 2008). The whole fruits were dried immediately after harvest (Vincent, 1987). Secondly, the wet processing method, which is mostly used for preparing Arabica coffee (Vincent, 1987). The wet method is used in Columbia, Central America and Hawaii (Silva *et al.*, 2008). It includes fermentaion or other equipment to eliminate the mucilage (inner mesocarp) in the preparation process. The Overall scheme of coffee processing is shown in Figure 2.2.



Source: (Vincent, 1987)

Figure 2.2 Overview of green coffee processing

2.3.1 Dry processing method

The dry processing method is the simple method, which is very convenient and does not require a variety of machines. The fresh coffee cherries are immediately spread out in a thin layer (only 3-4 cm thick) on clean dry ground, tray, solid or concrete surface, then exposed to the sun or mechanical dryer. The moisture content at the end of drying process must be around 12% to prevent undesired fermentation and mould formation, which can cause off-flavours in the brewed coffee (Illy and Viani, 1995). Once the cherries are dry, all the layers are removed in one step by a hulling machine, then stored as raw coffee green beans (without parchment). The dry method is generally used for Robusta coffee (Vincent, 1987; Arya and Rao, 2001).

2.3.2 Wet processing method

The other method is known as wet processing. In this method, fresh coffee cherries are mechanically depulped, which means squeezing the fresh coffee cherries to remove the exocarp and the skin. At this stage, the fresh beans still retain the mucilage (inner mesocarp), which is rich in pectin and sugars. There are various means of wet processing depending on the production regions (Vincent, 1987; Illy and Viani, 1995). For example, in Mexico, classical green coffee processing uses microbial removal without using any waters while in Kenya, the coffee beans are fermented in water to remove all of the mucilage (Vincent, 1987). The pulping machine is shown in Figure 2.3.

(Author's own picture)

Figure 2.3 The pulping machine

2.3.2.1 Fermentation

Fermentation in coffee wet processing method is the stage of removing mucilage (inner mesocarp) which obstructs the heat transfer in coffee parchment drying stage. The fermentation can be carried out either without water or under water (Vincent, 1987; Avallone *et al.*, 2001).

The fermentation without water (semi-dry) processing is operated by spreading the depulped beans in a thin layer on the patio to allow further aerobic hydrolyse of the mucilage and also drying parchment in the sun until the moisture content reached 12% (Vincent, 1987; Vilela *et al.*, 2010).

The fermentation under water (wet) is carried out by placing the depulped beans into a series of concrete tanks to wash parchment under clean water, ideally for 24-48 h. The hydrolysis of mucilage is the purpose of fermentation. However, the reaction is sped up by different micro-organisms, such as *Saccharomyces,* which also have pectinolytic effects. The fermentation last until the mucilage is completely removed from the parchment. Then, the washed parchment coffee is ready for further drying (Vincent, 1987; Silva *et al.*, 2000).

Nevertheless, the fermentation can create undesirable volatile compounds by fungi and bacteria. This may be caused by carelessness handling of coffee cherries during the postharvest stage (Toci *et al.*, 2008).

2.3.2.2 Mechanical demucilaging

Mechanical demucilging is the process, in which the mucilage is removed from the parchment by applying shearing forces on the beans using a rotating cylinder within a cylindrical chamber. The machine produces parchment coffee with approximately 95% mucilage removed (Oliveros and Gunasekaran, 1995; Marsh and Chapman, 2006). This processing requires 0.6 litres of water per 1 kg of dry parchment coffee, while the conventional wet processing consumes at least 5 litres of water per 11 kg of dry parchment coffee (Roa *et al.*, 1997). The mechanical demucilaging process significantly reduces consumption of water in coffee processing.

All four coffee processes as mentioned above are presented in Figures 2.4-2.7.

(Author's own picture)

Figure 2.4 Dry processing of coffee

Source: (Brennan, 2012)

Figure 2.5 Semi-dry processing of coffee

(Author's own picture)

Figure 2.6 Wet processing of coffee

(Author's own picture)

Figure 2.7 Mechanical demucilaging process

The demucilaged coffee beans are shown in Fig 2.8

(Author's own picture)

Figure 2.8 Demucilaged coffee beans

2.3.3 Drying parchment coffee

2.3.3.1 Mechanism of drying

The drying mechanism can be explained as follows: the hot air is fanned over food. Water vapour diffuses through a boundary layer of the air surrounding the food and the moisture is removed by the moving air (Figure 2.9). The difference of water vapour pressure between inner moisture of the food and the drying air causes a driving force to remove the water. The difference of water vapour pressure between inner moisture of the food and the drying air leads to a driving force to remove water. The boundary film of the air surrounding the food affects the heat transfer and water vapour removed. A higher drying temperature, air velocity and low relative humidity should be lead to achieve a faster drying rate (Singh and Heldman, 2001; Fellows, 2002).

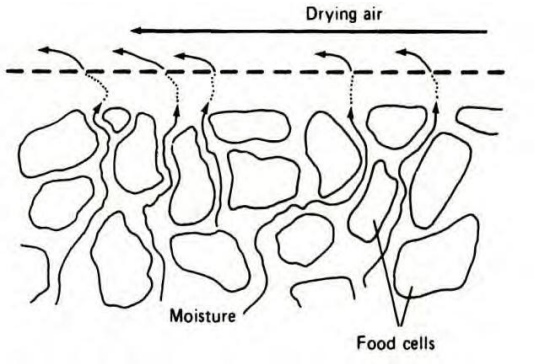


Figure 2.9 Transit of moisture during drying
---------- Boundary layer

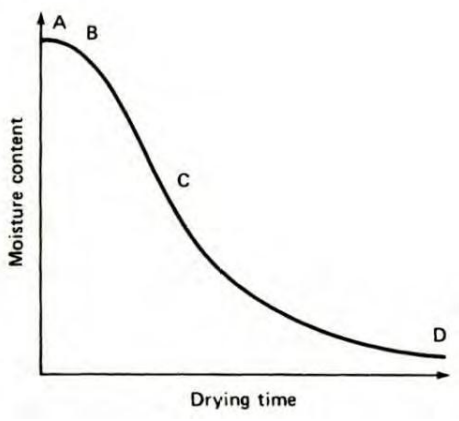
Source: (Fellows, 2002)

Rate of drying can be divided into three periods as shown in Figure 2.10.

1. The short initial settling down period as the water around the food surface heats up. This stage is called initial rate period.
2. Water moves from the inside region of the food at the same rate as it evaporates from the surface. During this step, called the constant rate period,

water continues to evaporate until the moisture content has reached critical value.

3. The longest period of the drying operation is the falling rate period. At this stage the drying rate is slowly decreasing until the equilibrium moisture content is reached. Food can be damaged during this period because of the rise of the surface temperature (Fellows, 2002).



Source: (Fellows, 2002)

Figure 2.10 Drying curve.

A-B, initial rate period; B-C constant rate period;

C-D, falling rate period.

2.3.3.2 Drying models

Several drying models were developed over years to explain the drying behavior of food. They are useful in predicting the drying times for each drying condition. The Page's model has been used to characterise the drying rate of a thin layer of coffee at drying air temperature ranging from 40 to 80 ^{o}C as shown in equation (2.1) (Page, 1949).

$$MR = (M_{db}-M_e)/(M_o-M_e) \quad (2.1)$$

MR	Moisture ratio
M_{db}	Moisture content of coffee, decimal (dry basis)
M_e	Equilibrium moisture content (dry basis)
M_o	Initial moisture content (dry basis)

Subscripts

e	equilibrium value
eq	equivalent
exp	experimental value
o	initial value

Drying operation is the most important step in the coffee postharvest process. During this stage, moisture content is reduced from about 60% w.b. (in fresh parchment coffee beans) to 10-12% w.b. to stabilise and preserve the coffee beans from micro organisms and insect infestation and to allow for long storage time. Other benefits of dry parchment are its suitability for transportation and roasting (Amir *et al.*, 1991; Silva *et al.*, 2000; Corrêa *et al.*, 2006; Cirovelásquez *et al.*, 2010). Drying is the condition of heat and mass transfer which occur simultaneously. Moisture evaporats from the outer surface of the beans due to high partial vapour pressure (Cirovelásquez *et al.*, 2010).

2.3.4 Storage

Coffee in a producing country can be stored in the form of dried cherry, dry parchment coffee, and cured green coffee. Storage circumstances need not be exactly the same. Coffee beans can be stored in bulk. Green coffee is generally stored in jute bags in a cool room. It is important to keep coffee at low RH in order to prevent fungal spoilage. The most dangerous spoilage insect is *Aracecerus fascicalatus*, but *Lasioderma serricorne, Tribolium castaneum* and *Carpophilus sp.* can also cause spoilage (Vincent, 1987).

The moisture content in green beans has a significant effect on their shelf-life factor. High moisture content favours fungi and or bacteria which damage the coffee beans. The optimum moisture content for storage and transport is less than 12 % (w.b.) (Palacios-Cabrera *et al.*, 2004).

2.4. Roasting

The complicated part of roasting coffee beans is in applying the heat quickly and uniformly. During roasting coffee beans are heated at 200-240 ^{o}C. The level of roasting is associated with the type of green coffee bean and its position in the market. There is various degrees of roasting namely light, medium and dark, depending on the temperature and time in each process. This stage leads to physical, chemical and sensorial transformation (Sivetz and Desrosier, 1979; Clarke, 1987; Illy and Viani, 1995; Bita and Preda, 2005; Alessandrini *et al.*, 2008).

The transformation process involves a physical change a shift in the external colour from greenish to light brown to almost black, and also some change in the oil covers of the surface of the dark roasted beans. Furthermore, the high temperature

increases the volume, dehydration and weight loss of coffee beans (Clarke, 1987; Bita and Preda, 2005; Alessandrini *et al.*, 2008).

In addition, at 180-200 ^{o}C the endosperm was interrupted, beans cracked and bluish smoke appeared (Belitz, 1988; Bicho, 2011)

The colour of beans subjected to different degrees of roasting is illustrated in the Figure 2.11. The percentage of weight loss is shown in Table 2.4.

Figure 2.11 Colour of beans subjected to different degrees of roasting

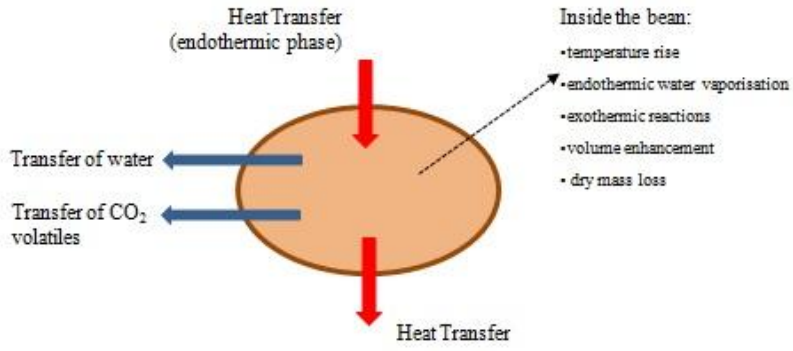
Table 2.4 Percentage weight loss for different degrees of roasting

Degrees of roasting	Percentage weight loss
Light	1-5
Medium	5-8
Dark	8-12
Very dark	> 12

Source: (Clarke, 1987)

In terms of chemical and sensorial change, the roasting process leads to the degradation of proteins, amino acids, and the reduction of sugar and water, which are the cause the Maillard's reaction. Moreover, the decrease in content of some compounds

such as trigonelline and chlorogenic acid occur. Chemical changes also have an effect on sensorial change, for example, acids present in green coffee amount to about 11% by weight, but decrease to about 6% by weight in roasted coffee beans. The aroma and flavour of coffee are also developed during the roasting process (Ginz *et al.*, 2000; Castillo *et al.*, 2002; Franca *et al.*, 2009). Figure 2.12 shows the mechanism of triggered reaction in coffee beans.



Source: (Eggers and Pietsch, 2001)

Figure 2.12 Heat transfer and its effect on a coffee bean during roasting

Fluidised bed roasting is accomplished by a high velocity hot gas blowing from the bottom of the roasting machine towards the beans. The beans are floating simultaneously. There are several advantages of a fluidised bed roaster. Firstly, there is a lower loss of small coffee particles (broken or small beans) resulting in a higher yield of roasted coffee beans. Secondly, there is a lower maintenance cost and cleaning is easy. Lastly, there is good control of process parameters and homogenisation of the product since there are no excessive temperature peaks (Clarke, 1987; Eggers and Pietsch, 2001).

2. 5 Coffee compounds and human health

Substantial research has been carried on the health promoting properties of coffee. Due to its popularity, epidemiological studies and also *in vito* and *in vivo* studies have been carried out to demonstrate the potential health benefits of coffee.

2.5.1 Coffee consumption and cancer

There are a variety of compounds in coffee, which include anticancer agents such as anti-oxidants and diterpenes (Fujioka and Shibamoto, 2008; George *et al.*, 2008; Butt and Sultan, 2011). Table 2.5 presents a summary of the studies.

Table 2.5 Coffee consumption and cancer

Cancer type	Research Group	Relationship with coffee
Breast cancer	(Kotsopoulos *et al.*, 2007)	64% reduction in breast cancer risk for daily drinker.
	(Oba *et al.*, 2006)	Daily coffee drinkers reduced risk of breast cancer
Liver cancer (Hepatocellular carcinoma)	(Larsson *et al.*, 2008)	Consuming 2 cups of coffee per day Reduces risk of liver cancer by 43%
Non- melanoma skin cancer	(Abel *et al.*, 2007)	10.8% lower prevalence; Drinking less than 6 cups/days reduces risk by 36%
Pancreatic cancer	(Luo *et al.*, 2008)	Reduced risk
	(Larsson *et al.*, 2008)	Lowers risk of the onset of cancer
Renal cell cancer	(Lee *et al.*, 2007)	Coffee consumption positively associated with risk reduction

Adapted from (Butt and Sultan, 2011)

2.5.2 Coffee consumption and diabetes mellitus

There is evidence that that a number of coffee components can affect hypoglycemic potential and this can improve glucose and insulin metabolisms. Table 2.6 shows coffee components that play a role in diabetes mellitus (van Dam, 2003; Greenberg *et al.*, 2006; Rodrigues *et al.*, 2007; Shearer *et al.*, 2007; van Dijk *et al.*, 2009; Zhang *et al.*, 2009)

Table 2.6 Benefit of the components of coffee related to diabetes mellitus

Functional Component in Coffee	Health Benefits
Caffeine	Lower risk of diabetes mellitus
	Reduces glucose storage
Chlorogenic acid	Improves glucose metabolisms
	Reduces insulin responses
	Antioxidant effects
	Inhibiting glucose-6-phosphates
	Improves mineral distribution
Trigonelline	Improves glucose metabolism

Source: adapted from (Butt and Sultan, 2011)

2.5.3 Coffee consumption and other health benefits

Epidemiological and *in vivo* studies have demonstrated that coffee/caffeine and tea may reduce the risk of Parkinson's disease. Neither gender nor smoking are considered factors (Ascherio *et al.*, 2004; Chade *et al.*, 2006).

According to recent studies on coffee consumption there is a reduction in the risk of Alzheimer's disease as a result of an increase of caffeine intake (Lindsay *et al.*, 2002; Arendash *et al.*, 2006). Moreover, it has been shown that the trigonelline in coffee leads to the memory improvement (Tohda *et al.*, 2005).

One of the benefits associated with coffee consumption included the improvement in the overall antioxidant capacity of the body and lesser oxidative stress, inflammation and carcinogenesis as a consequence of the anti-oxidants present in coffee (Butt *et al.*, 2008).

2.6. Selected compounds

2.6.1 Chlorogenic acid (CGA)

Chlorogenic acids are known as the main phenolic compound in coffee. They are esters of *trans*-cinnamic acid. The CGAs are common secondary metabolites in plants, frequently associated with plant protection against insect or microbial attack. They are present in relatively large quantities in coffee beans, in the form of potassium salts (Viani, 1985; Illy and Viani, 1995; Perrone *et al.*, 2008a; Jaiswal and Kuhnert, 2010). The structure of chlorogenic acid is presented in Figure 2.13.

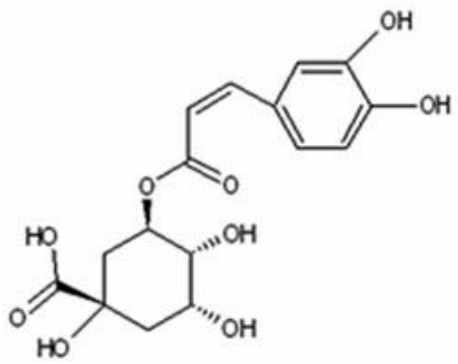


Figure 2.13 Chlorogenic acid structure

Various studies show that the CGA content in coffee ranges from 15 to 325 mg per cup of brewed coffee (Viani, 1985). In addition, the CGA content of coffee may be influenced by the kind of coffee beans used. Most commercial coffees are blended from both Arabica and Robusta beans. Arabica beans contain less CGA than Robusta beans, the CGA accounts for 5-8% and 7-10% of dry matter basis respectively (Ky *et al.*, 1997; Fujioka and Shibamoto, 2008).

According to Fujioka and Shibamoto (2008) and Perrone *et al.*(2008b) chlorogenic acid is a major contributor to the flavour and aroma of roasted coffee. The major and minor chlorogenic acids and chlorogenic acid lactones of green and roasted samples of Brazilian coffee have been investigated using LC-MS and synthetic standards. The authors suggest that they contribute significantly to the flavour, cup quality and the nutraceutical value and the biological properties of coffee (Fujioka and Shibamoto, 2008; Perrone *et al.*, 2008a)(Fujioka and Shibamoto, 2008; Perrone *et al.*, 2008a)(Fujioka and Shibamoto, 2008; Perrone *et al.*, 2008a)(Fujioka and Shibamoto, 2008; Perrone *et al.*, 2008a)(Fujioka and Shibamoto, 2008; Perrone *et al.*, 2008a)(Fujioka and Shibamoto, 2008; Perrone *et al.*, 2008a)

2.6.2 Caffeine (CFE)

A physiologically active component in coffee is caffeine. Its structure was determined by Runge in 1820 as 1,3,7- trimethylaxanthine. It is composed of needle-shaped crystals, melting at 236 ^{o}C. Coffee beans contain between 0.8 and 2.8% caffeine, depending on species and origin. Caffeine contributes 10 to 30% to the bitterness of coffee beverage. Caffeine is thermally quite stable. The roasting process brings about insignificant losses of caffeine. Caffeine plays a major part in relation to the physiological properties of coffee and also in determining the overall character of coffee

beverage. It may make a small contribution to its strength and body (Illy and Viani, 1995; Heilmann, 2001; Ramalakshmi *et al.*, 2011).

Caffeine is also present in coca-cola, chocolate and tea. Sensitive persons suffer from sleeplessness, nervousness, intestinal discomfort, heart stimulation and other effects after drinking one or two cups of coffee. People that habitually drink coffee require a larger dose for stimulation. This is because they develop a tolerance to the drink. Meanwhile, a non-coffee drinker will get a "stimulating reaction" from a single cup of coffee. Individual tolerances to dosages without obvious symptoms vary with age, sex, physical condition, environment, and other factors (Sivetz and Desrosier, 1979; Viani, 1985; Alonso-Salces *et al.*, 2009). The structure of caffeine is presented in Figure 2.14.

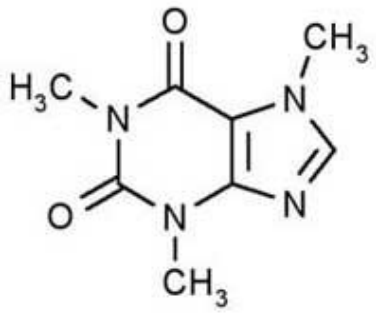


Figure 2.14 Caffeine structure

2.6.3 Trigonelline (TGL)

Trigonelline is an alkaloid belonging to the group of pyridine betaines posing as a quaternary amino group. Several health promoting properties of trignelline such as hypoglycemic, hypocholesterolemic, antitumor, antimigraine, and antiseptic effects have been reported. It is commonly reported as the second most abundant alkaloid in raw coffee beans. Demethylation of trigonelline during coffee roasting generates nicotinic acid, a water - soluble vitamin B also know as niacin. Moreover, trigonelline appears to have anti-invasive activity against cancer cells and may regenerate dendrites and axons, in addition to memory improvement in animal models. Trigonelline is partially degraded during roasting, There are losses of 60% occurring at 180 ^{o}C and 85% at 230 ^{o}C (Trugo and Macrae, 1984; Illy and Viani, 1995; Perrone *et al.*, 2008b; Sánchez-Hernández *et al.*, 2010) The structure of trigonelline is shown in Figure 2.15.

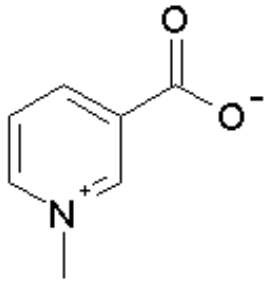


Figure 2.15 Trigonelline structure

2.6.4 Tocopherols

Tocopherols are a group of four lipid soluble amphipatic molecules (α, β, γ and δ) (Gilliland *et al.*, 2006). Vitamin E is only biosynthesised by plants as a consequently of this group of compounds being present at high concentration in edible plant oils (Hammond, 2003) in Alves *et al.* (2009a).

Vitamin E plays a role as a scavenger of free radicals, anti cancer and helps controlling cardiovascular diseases agents (Kamal-Eldin *et al.*, 2000).

The presence α-tocopherol was clearly established in coffee oil for the first time by Folstar *et al.* (1977) in Speer and Kölling-Speer (2001). α- Tocopherol is known among tocopherol compounds for its biological activity (Hammond, 2003) in Alves *et al.* (2009b). Two major tocopherols (α and β) were found in both unroasted and roasted Arabica and Robusta coffee. Tocopherol is also used in some studies as a standard to detect adulteration of commercial coffee (Jham *et al.*, 2007). The structure of α-tocopherol is shown in Figure 2.16

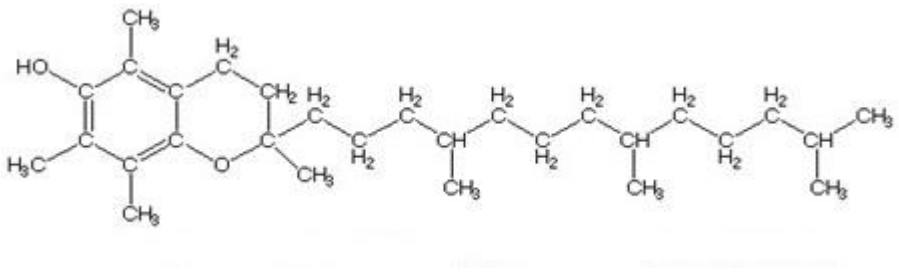


Figure 2.16 Structure of α-tocopherol

2.6.5 Cafestol (CFT) and kahweol (KWL)

Coffee oil contains a relatively high level of unsaponifiable matter rich in two diterpenes specific to coffee, namely kahweol and cafestol both of which are related to aglycones which are the product of hydrolysis of glycosides (Viani, 1985). They are present as fatty esters, mainly of palmitic and linoleic acids. The contents of kahweol and cafestol in coffee oil differ in various coffee products. These two components are sensitive to acids, heat and light. Arabica coffee contains more cafestol and kahweol than Robusta coffee. Cafestol raises serum cholesterol more significantly than kahweol does. A mixture of cafestol (60 mg/day) and kahweol (51 mg/day) increases serum cholesterol only slightly more in people who consume pure cafestol (64 mg/day). Results with pure kahweol are not available due to difficulties with purification and stability of this diterpene. Both compounds are extracted by hot water and are retained by a paper filter. This explains why Scandinavian boiled coffee, Turkish coffee and French press (cafetiere) coffee contain relatively high levels of cafestol and kahweol (6-12 mg/cup). In contrast, filtered percolated coffee and instant coffee contain low levels of cafestol and kahweol (0.2-0.6 mg/cup). Although diterpene concentrations are relatively high in espresso coffee, the small serving size makes it an intermediate source of cafestol and kahweol (4 mg/cup). It has also been claimed that both diterpenes have anti-carcinogenic effects. The structures of cafestol and kahweol are presented in Figure 2.17.

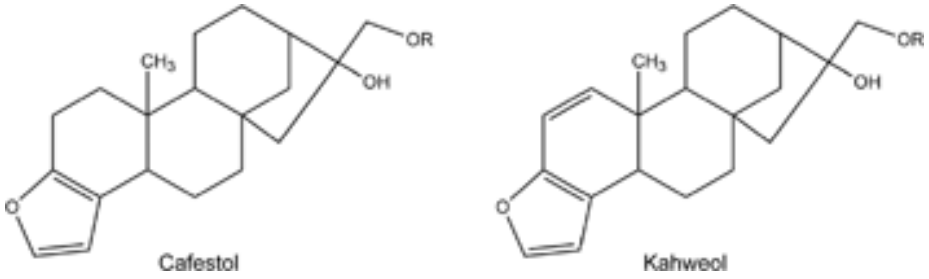


Figure 2.17 Cafestol and kahweol structure

These bioactive compounds are highly important to human health and thus this study attempts to determine what happens to the bioactive compounds during postharvest, storage and processing of Arabica coffee.

2.7 Estimation of total phenolic compounds (TPC)

Phenolic compounds are found in many foods. Popular foods that are high in phenolics include coffee and tea, chocolate, fruits and derived products, spices, and some whole grains. Hydroxycinnamic acids, flavonoids, anthocyanins and tannins represent the major classes of phenolics, which collectively account for approximately 40% of the organic carbon in the biosphere (Waterhouse, 2002; Ainsworth and Gillespie, 2007).

Folin-Ciocalteu (FC) colorimetry is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. Singleton *et al.* (1965) and (1999) adapted this method to wine analysis and have written two major reviews on its use. The products from metal oxide reduction have a blue colour that exhibits a broad light absorbtion with a maximium at 765 nm. The intensity of light absorption at that wavelength is proportional to the concentration of phenols. The FC method has been adopted as the official procedure for the total phenolic level in wine.Office International de la Vigne et du Vin (OIV), the only international body that certifies specific

procedures for wine analysis, accepts the FC method as the standard procedure for total phenolic analysis (Waterhouse, 2002).

2.8 Ferric reducing antioxidant power (FRAP)

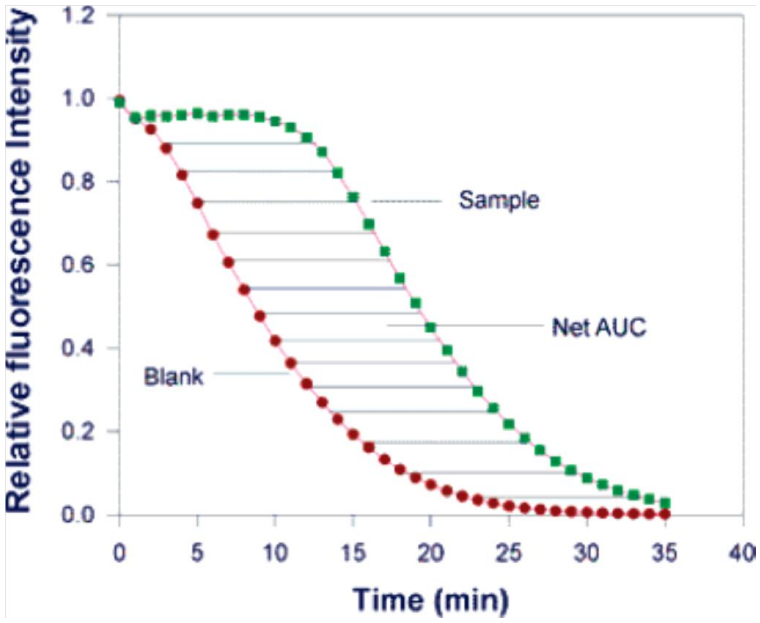
Antioxidant capacity of coffee is significantly influenced by the roasting process. Torrefacto coffee is produced by the addition of sugar at the end of the roasting process. The purpose of the work described by López-Galilea *et al.* (2006) is to characterise commercial torrefacto roasted coffee and to try to explain the prospective antioxidant capacity of coffee chemical compounds by means of multivariate statistical techniques.

In the FRAP method the yellow Fe3+-TPTZ (2,4,6-Tri(2-pyridyl)-s-triazine) complex is reduced to the blue Fe2+-TPTZ complex by electron-donating substances under acidic conditions. Any electron donation substances with a lower redox potential than Fe3+/Fe2+-TPTZ will produce a reaction and lead to the formation of the blue complex. The FRAP measures the ability of antioxidants to reduce the Fe3+-TPTZ complex. Reduction is quantified by change in absorption at 593 nm. (Mermelstel, 2010; Djordjevic *et al.*, 2011).

2.9 Oxygen radical absorbance capacity (ORAC)

ORAC is the standard method for investigating the antioxidant capacity of a substance (Cao *et al.*, 1993). This assay depends on the inhibition of the peroxyl-radical-induced oxidation initiated by thermal decomposition of azo-compounds such as 2,2'-Azobis 2-methylpropionamidine dihydrochloride (AAPH) and consequently, reflects the classical radical chain breaking antioxidant activity by H atom transfer (Glazer, 1990; Ou *et al.*, 2001)

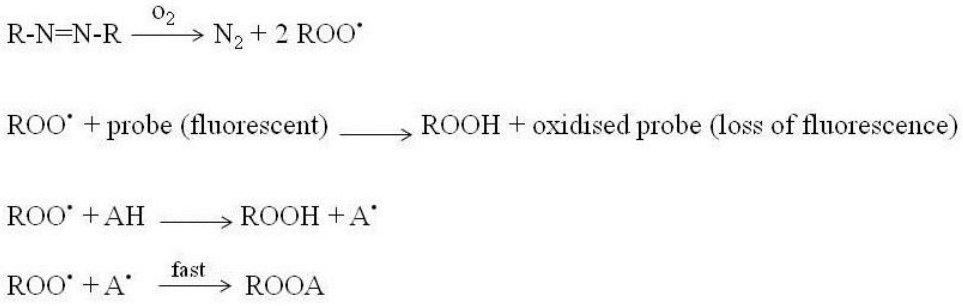
ORAC is expressed as the net area under the curve (AUC) as presented in Figure 2.18.



Source: (Prior *et al.*, 2005)

Figure 2.18 ORAC antioxidant activity of tested sample

The peroxyl radical reacts with a fluorescent probe to form a non fluorescent product. The decreased rate and the quantity of product formed over time is explained by a reaction shown in Figure 2.19.



Source (Prior *et al.*, 2005)

Figure 2.19 The reaction of a peroxyl radical

3. Materials and methods

3.1 Chemical reagents and equipment

The list of chemicals and reagents used and their suppliers is shown in Table 3.1. All chemical were of analytical and HPLC grade as required. In addition, the list of equipment used in the experiments is presented in Table 3.2.

Table 3.1 Chemicals and reagents used in the experiments

Chemicals	Suppliers
2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ)	Sigma Chemical Co., St. Louis, MO, USA
6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox)	Aldrich Chem. Co., Milwaukee, USA
Acetonitrile	Honeywell International, Burdick & Jackson, Morristown, NJ, USA
Cafestol	Chromadex, Irvine, CA (USA)
Caffeine	Sigma-Aldrich, Sydney Australia
Chlorogenic acid (3-Caffeoylquinic acid)	MP Biomedicals Australasia Pty Limited
Ethyl Acetate	Ajax Chemicals Pty. Ltd., Sydney, Australia
Folin-Ciocalteu reagent	Sigma-Aldrich, Sydney Australia
Gallic acid	Ajax Chemicals Pty. Ltd., Sydney, Australia
Glacial acetic acid	Ajax Chemicals Pty. Ltd., Sydney, Australia
Hydrochloric acid	Ajax Chemical Pty. Ltd., Sydney, Australia
Iron (III) chloride hexahydrate	Ajax Fine Chem, Sydney, Australia
Isopropanol,	Ajax Chemical Pty. Ltd., Sydney, Australia
Kahweol	Enzo Life Science inc., Farmingdale, NY (USA)
Methanol (Analytical)	Ajax Fine Chem, Sydney, Australia
Methanol (HPLC)	Ajax Fine Chem, Sydney, Australia
n-hexane (Analytical)	Ajax Fine Chem, Sydney, Australia
n-hexane (HPLC)	Honeywell International, Burdick & Jackson, Morristown

Table 3.1 Chemicals and reagents used in the experiments (continued)

Chemicals	Suppliers
Potassium Ferrocyanide	Ajax Fine Chem, Sydney, Australia
Sodium carbnate	Ajax Chemical Pty. Ltd., Sydney, Australia
tert-Butyl ether	Sigma, Aldrich, USA
Trifluoro acetic acid	Sigma-Aldrich, USA
Trigonelline	Sigma-Aldrich, Fluka, USA
Zinc acetate	Ajax Fine Chem, Sydney, Australia
α-tocopherol	Sigma Aldrich, USA

Table 3.2 Equipment used in the experiments

Equipment	Suppliers
Burr Grinder GVX2	KRUPS, China
Colour meter, Minolta Data Processor Dp-301	Minolta, Japan
MilliQ water system, 0.22 μm.	Academic MilliQ, France
Precision Coffee Roaster® Model 40203	Korea
Pulper/Demucilager, Model: UCBE 1500,	PENAGOS HERMANOS, Ltd.
Rotary Evaporator	FSE Scientific sales and service, Sydney, Australia
Shimadsu HPLC system DGU-20A5 Degasser LC-20AD liquid Chromatograph SIL-20A HT Auto sampler RF-10AXL Florescence detector SPD-M20A Diode Array UV-Detector CTO-20A Column oven	Shimadzu, Coporation, Kyoto, Japan
Soxhlet evaporation bath	LABEC, Sydney, Australia
Vacuum oven Townson	Townson and Mercer Ltd, Croydon, England
Water bath	Grant instrument (Cambridge) Ltd, Barrington, Cambridge, England

3.2 Raw materials

Mechanically demucilaged C. *arabica* (Arabica) was supplied from Zentveld's coffee plantation, Newrybar, New South Wales. All coffee cherries were harvested at the ripe stage, which was usual for hand harvesting. The samples were kept frozen at -20^{o}C until drying.

3.3 Pre-treatments

The coffee beans were divided into two batches, each of them being subjected to a pre-treatment namely soaking or non-soaking. For the soaking treatment, the weight ratio between water and mechanically demucilaged beans was kept at 3:1 in order to further remove mucilage. The beans were left overnight (approximately 10 hours) at ambient temperature. After the soaking treatment, the beans were drained and rinsed with water for 5 minutes until the beans were no longer slippery, then strained. In the case of the non-soaked treatment, the mucilaginous parchment beans were washed manually and the water was changed three times until the beans became less slippery.

3.4 Drying in a cabinet dryer

A laboratory scale cabinet dryer (Figure 3.1) was used in the experiments. The system included a fan, a heating section, a steam injection system and a drying chamber. The drying chamber walls were made from heavy gauge aluminium, filled with 5 cm thick fibre glass insulation. The drying chamber (50 cm wide, 30 cm deep and 70 cm long) was made from 2.5 cm thick plywood, painted both sides with water proof paint and an aluminium panel fitted on top. Air was circulated through the dryer by an axial

flow fan driven by a 1.5 kW three phase motor. The air speed was controlled using a MSC 3000-B Solid state speed controller (installed by W.E. & S.M. Bromley P/L). Heat was supplied by three 6 kW electric heaters and controlled by a Eurotherm 2404 controller (Eurotherm Intl. Pty Ltd, Sydney). The humidity was adjusted by steam injection, which vented into the drying chamber through a valve operated by an electrical actuator (model EFM 10). The relative humidity was controlled by a Eurotherm 2404 controller with an input from RTD temperature sensor and DC signal from a humidity sensor (Siemens/Landis and Gyr Model QFM 66) located in the duct before the drying chamber. A perforated tray was placed parallel to the air flow and supported with four rods, which passed through the bottom of the drying chamber to a Mettler Toledo PB 3002-s balance connected to a computer. In order to make a shelf stable product, the pre-treated samples needed to be dried until their moisture content reached 13% dry basis (db.).

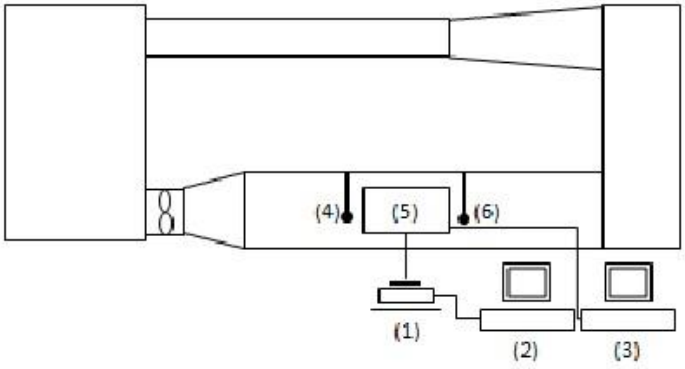


Figure 3.1 Diagram of lab scale cabinet dryer

adapted from Kashaninejad et al. (2007)

(1) Balance
(2) Computer for weight collecting
(3) Computer for recording of drying conditions
(4) RH and temperature probes

3.4.1 Moisture content determination

The initial and final moisture contents of the coffee beans were determined by the AOAC method (Dick, 1990) using a vacuum oven at 70 ^{o}C at 15 kPa for 48 hours.

3.4.2 Drying model fitting

After the data on initial moisture content (% dry basis) and weight of sample during drying were gathered, the calculated data were fitted into Page's model (see equation 2.1 chapter 2 topic 2.3.3.2).

3. 5 Roasting, grinding and colour measurement

3.5.1 Roasting

The dried coffee beans were manually dehulled. After that, deparched beans were stored at -20 ^{o}C prior to roasting. Un soaked green coffee beans were used as control. The roasting treatments were divided, light roasted, medium roasted and dark roasted. The coffee beans were roasted in batches of 100 g using a fluidised bed roaster (Precision Coffee Roaster® Model 40203, Korea). In addition, the three thermocouples were inserted into the roaster in order to collect the profile of roasting temperature over time.

3.5.2 Grinding

Green coffee beans were frozen with liquid nitrogen before grinding. The roasted coffee beans were ground at ambient temperature. All samples were ground by Burr Grinder GVX2, KRUPS, China. The granule size of all samples was 1 mm.

3.5.3 Colour

Colour measurements were accomplished on ground coffee samples using Minolta Data Processor Dp-301.9. *L**, *a** and *b** values were use to profile the degree of the roasting coffee.

Hue angle (Minolta, n.d.)

Hue angle equation is shown in Equation (3.1)

$$^{o}h = \tan^{-1}(b^*/a^*) \qquad (3.1)$$

*a**, *b** = Chromaticity coordinates in the *L**, *a**, *b** colour space.

3.6 Determination of selected compounds

3.6.1 Extraction

3.6.1.1 Hydrophilic compounds

The roasting, grinding and extraction processes described below were performed in triplicate for each sample. An amount of 5 g of ground coffee samples and MiliQ water at a ratio of 1:10 were refluxed for 15 minutes, and then filtered in a Büchner porcelain tunnel through no.1 filter paper. After the first filtration, the residues were washed with 50 mL of MiliQ water and re-filtrated. Furthermore, the mixture of residues was extracted as above for 2 more times. Then, all the filtered coffee was made to a volume of 50 mL. Finally, filtrate was kept in 50 mL centrifuge tubes wrapped with aluminium foil and stored at -20^{o}C in the darkness until analysis. The extracted samples were used within 2 weeks time.

3.6.1.2 Lipophilic compounds

3.6.1.2.1 Oil extraction for α-tocopherol quantification

About 5 g of each sample was extracted for 8 hours in a Soxhlet apparatus using n-hexane as the extraction solvent.

At the end of the extraction process, the flask containing the solvent and lipid is removed. The solution is removed in the rotary evaporator.

3.6.1.2.2 Cafestol and kahweol extraction

The method was adapted from Dias *et al.* (2010). About 2 g of each coffee sample were saponified with a 10 mL solution of 2.5 M potassium hydroxide in ethanol at 80 ^{o}C for 1 h. The saponification was followed by addition of 2 mL of purified water (MilliQ). In addition, extraction with methyl *tert*-butyl ether and clean up with MilQ water were carried out until the extract was clear. The solvent was removed in a rotary evaporator at 70 ^{o}C. In order to determine the compounds in sample, the extracts were re suspended in the mobile phase, then filtered by 0.45 μm Millipore filter and injected in to the HPLC.

The identity of CFT and KWL was confirmed by NMR. (see Appendix figure 7.7 and 7.8 respectively. CFT and KWL were purified by following steps. The coffee was extracted with methyl *tert*-butyl ether three times and all 5 fractions collected. Then, the fractions were separated using thin layer chromatography (ethyl acetate: methanol, 19:1). Three major bands were collected. The silica of each band was washed three times with ethyl acetate: methanol (1:1), the solvent of each extract was evaporated under vacuum tree fractions were collected. The identity of CFT and KWL was confirmed by ^{1}H NMR.

3.6.2 Quantification of selected compounds

3.6.2.1 Chlorogenic acid

This method was adapted from Fujioka and Shibamoto (2008). The coffee sample extracted as described in 3.6.1.1 were treated with Carrez I (21.9 g of zinc acetate, 3 g of glacial acetic acid in water). Then, the extracts were treated with Carrez II (10.6 g of potassium ferrocyanide in water). Subsequently, the solution was made up to 100 mL with water. This treatment was meant to remove polymeric components. Each extracted sample (6 ml) was mixed with 0.2 mL of Carrez I and II, and 1.6 ml of methanol, then vortex-mixed in a centrifuge tube and followed by allowed to stand for 10 min. The precipitate was separated by centrifuging at 5000 rpm for 10 min. The solution was decanted and filtered with a arodisc syringe filter with 0.2 µm membrane. Then, the filtrate was transferred to the HPLC vials.

Quantitative analyses of CGA were performed using a Shimadzu DGU-20A5 degasser, LC-20AD pump, RF-10A XL Fluorescence detector model HPLC with a GEMINI 3 µm C 18 110A Column (150x3.00 mm 3 micron) and guard cartridge system. Mobile phase A was 0.5% trifluoroacetic acid (TFA) in purified water and mobile phase B was 95% acetonitrile and 0.5% of mobile phase A. The gradient mode was initially set at A/B ratio of 100/0 from 0 to 1 min, and then linearly increased to 63/37 over 37 min, finally increased to 0/100 at 52 to 80 min. The detector was set at 325 nm for CGA. The flow rate was 0.3 mL/min. The detector was set to 325 nm. Samples were injected with an auto sampler using the full loop option (5 μL). Concentrations of CGA were calculated using the regression equation of their concentration and peak area. Linear standard curve (r^2>0.99) of concentration vs peak area was generated using duplicate injection of CGA (6.25 μg/mL, 12.5

μg/mL, 25 μg/mL, 50 μg/mL, 70 μg/mL, and 100 μg/mL). CGA peak was identified by retention time of known standards. Concentrate of 6.25 mM, 12.5 mM, 25 mM, 50 mM, 70 mM, and 100 mM were used. Measurements were done in duplicate. The chromatogram of CGA peak is shown in Figure 7.1

3.6.2.2 Caffeine and trigonelline

The filtrates from 3.5.2.1 were transferred into the HPLC vials.

The method was adapted from (Perrone *et al.*, 2008b). Quantitative analyses of CFE and TGL were performed using a Shimadzu DGU-20A5 degasser, LC-20AD pump, RF-10A XL fluorescence detector model HPLC with a GEMINI 3 μm C 18 110A Column (150x3.00 mm 3 micron) and guard cartridge system maintained at 40 ^{o}C constantly. The mobile phase consisted of 0.3% aqueous formic acid (solution A) and methanol (solution B), the flow rate was 0.4 mL/min. The column was equilibrated with 25% solution B. Then, solution B increased to 60% until the end of the run. The maximum absorption wavelength for caffeine (273 nm) and trigonelline (138 nm) were used, from the spectrum of each compound in the diode array detector. Samples were injected with an auto sampler using the full loop option (5μL) Linear standard curve (r^2>0.99) of concentration vs peak area was generated using duplicate injection of CFE and TGL (6.25 μg/mL, 12.5 μg/mL, 25 μg/mL, 50 μg/mL, 70 μg/mL, and 100 μg/mL). CFE and TGL peaks were identified by retention time of known standards. Measurements were done in duplicate. The chromatogram of CFE and TGL peaks are shown in Figure 7.2, 7.3 respectively.

3.6.2.3 α-Tocopherol

The method was adapted from Jham *et al.* (2007). Quantitative analyses of α-tocopherol was performed using a Shimadzu DGU-20A5 degasser, LC-20AD pump, RF-10A XL Fluorescence detector model HPLC with a Luna 5µ, NH_2 (250x4.60 mm) column and guard cartridge system. The isocractic elution (1 mL/min) with hexane: 2-propanol (99.5:0.5 v/v, made freshly daily) was pumped at 1 mL/min. Samples were injected with an auto sampler using the full loop option (20 μL). The fluorescence detector was set with the wavelength of 290 nm and emission wavelength of 330 nm. Linear standard curve (r^2>0.99) of concentration vs peak area was generated using duplicate injection of α-tocopherol (6.25 μg/mL, 12.5 μg/mL, 25 μg/mL, 50 μg/mL, 70 μg/mL, and 100 μg/mL). α-Tocopherol peak was identified by retention time of known standards. Measurements were done in duplicate. The Chromatogram of α-Tocopherol peak is shown in Figure 7.4.

3.6.2.4 Cafestol and Kahweol

The method was adapted from Dias *et al.* (2010). Quantitative analyses of CFT and KWL were performed using a Shimadzu DGU-20A5 degasser, LC-20AD pump, RF-10A XL Fluorescence detector model HPLC with a GEMINI 3 µm C 18 110A Column (150x3.00 mm 3 micron) and guard cartridge system. The isocractic elution (0.4 mL/min) with ACN/water mixture (55/45%; v/v) was applied. The maximum absorption wavelength for CFT (230 nm) and KWL (290 nm) were used, from the spectrum of each compound in the diode array detector.

Samples were injected by auto sampler using the full loop option (5μL). Linear standard curves (r^2>0.99) of concentration vs peak area were generated using duplicate injection of CFT and KWL (6.25 μg/mL, 12.5 μg/mL, 25 μg/mL, 50 μg/mL, 70 μg/mL, and 100 μg/mL). CFE and TGL peaks were identified by retention time of known standards. Measurements were done in duplicate. The chromatogram of CFT and KWL peaks shown in Figure 7.5, 7.6 respectively.

3.7 Antioxidant activities determination

The coffee samples were investigated for the content of bioactive compounds and their activity. The analyses included total phenolic compounds, ferric reducing antioxidant power and oxygen radical absorbance capacity.

3.7.1 Extraction for antioxidant activities

An amount of 5 g of samples and water at a ratio of 1:10 w/v was refluxed for 15 minutes. The extract was vacuum filtered through no.1 filter paper. The filtered coffee was made to 50 mL.

3.7.2 Determination of total phenolic compounds

Total phenolic compounds were determined calorimetrically using Folin-Ciocalteu reagent. The coffee extracts from 3.3.1 (2 mL) were diluted with distilled water 300 fold. Then, they were mixed with 1/10 water-diluted Folin-Ciocalteu reagent (10 mL). After 1-8 minutes (timing was not critical), 7.5% (w/v) Na_2CO_3 aqueous solution (8 mL) was added and the volume was made to 20 mL. After 2 hours at room temperature, the absorbance of the solutions was measured at 765 nm by using UV-1601 Shimadzu UV-visible spectrophotometer (Shimadzu Scientific Instruments, Ocenic Pty. Ltd., NSW, Australia). Gallic acid was used as a standard. The concentrations used to make the standard curve were 0.02, 0.04, 0.06, 0.08 and 0.10 mg/mL.

3.7.3 Determination of FRAP

The total antioxidant potential of the coffee samples was determined using a modification of the ferric reducing ability of plasma (FRAP) assay of Benzie and Strain (1996). FRAP reagent was prepared from 300 mmol/L acetate buffer, pH 3.6, 20 mmol/L ferric chloride and 10 mmol/L 2,4,6-tripyridyl-s-triazine made up in 40 mmol/L hydrochloric acid. All three solutions were mixed together in the ratio 100:10:10 (v:v:v). The FRAP assay was performed after preheating to 37 ^{o}C. To 100 µl of 600 times diluted samples, were added 2900 µL of reagent and then incubated at 37 ^{o}C for 2 h. Absorbance at 593 nm was measured relative to a reagent blank incubated at 37 ^{o}C. Coffee samples were diluted 1:600 prior to assay. Antioxidant potential of samples was determined against a standard curve of Trolox. The results were expressed in µM TE/mg samples. After 2 hours at room temperature, the absorbance of the solutions was

measured by using UV-1601 Shimadzu UV-visible spectrophotometer (Shimadzu Scientific Instruments, Ocenic Pty. Ltd., NSW, Australia).

3.7.4 Determination of ORAC

ORAC assays were conducted by preparation of 120 nM of fluorescein and 360 mM 2,2'-azobis(2-methylpropionamidine) dihydrocholride (AAPH). These solutions were freshly prepared daily in 75 mM, pH 7.0 phosphate buffer saline (PBS). The assay was performed in a microplate reader (POLARstar Omega BMG labtech, Germany). The extract from 3.5.1.1 was diluted 1000 times with PBS to ensure that it was in the range of the standard (6.25-75 µM Trolox). The determination started with, 20 µL PBS added in all 96 wells of black flat bottom plate. Then, 20 µL of diluted sample were added to the black flat bottom plate and a plate was inserted into the micro plate reader. A further 20 µl of AAPH solution and 160 µL of fluorescence were added automatically to the well to indicate the measurement. The intensity of fluorescence was recorded at a wavelength of 495 nm and an emission wavelength of 515 nm every 0.1 second until it reached zero. The area under the curve was integrated. The antioxidant activity was interpreted as µM TE/g DW.

3.8 Sensory evaluation

Sensory evaluations were conducted twice with semi-trained panelists selected among Food Science students from the School of Chemical Engineering, UNSW to illustrate consumer preference for coffee produced at different roasting temperature and subjected to different pre-treatments (soaked and non soaked parchment coffee). More than 30 panelists took part in the experiments (each time). A discrimination test (triangle test) and an affective test by using a hedonic scale ranging from 1 (extremely dislike) to 9 (extremely like) for evaluation of aroma, taste, after taste, colour and overall liking were concidered. The first evaluation related to light, medium and dark roasting degree, which were compared using parchment coffee beans which were soaked and dryed at 40 ^{o}C without storage (0 month).The second evaluation involved soaked and non-soaked parchment coffee that had been roasted at dark roasting degree.

3.9 Statistical analysis

The results of linearity were assessed by coefficient of determination (r^2) and uncertainties of slopes and intercepts from linear equations.

For all results, the data were evaluated using the analysis of variance (ANOVA) and Duncan's Multiple Range Test at 5% level of significance.

4. Results and Discussion

4.1 Drying parchment coffee in a cabinet dryer

The initial moisture content MC of soaked parchment coffee beans was higher than in non-soaked coffee (see Table 4.1). Some mucilage was remaining on the surface of the non-soaked beans.

The purpose of soaked and non-soaked treatments was to compare the drying time. This reason is related to the concerns about energy consumption and cost.

Table 4.1 Initial moisture content of fresh parchment coffee.

Pre-treatment	Moisture Content (%wb) (Average±S.D.)
Non-soaked	52.16 ± 0.61
Soaked	53.61 ± 0.21

Four drying treatments of parchment coffee were performed in the cabinet dryer. The drying curves of parchment coffee under four drying conditions are presented in Figures 4.1, 4.2, 4.3 and 4.4 respectively. The drying performances of the pretreated samples differed significantly. The soaked parchment coffee had a faster drying rate than the non-soaked coffee parchment.

These four conditions were selected to trial the optimum drying condition.

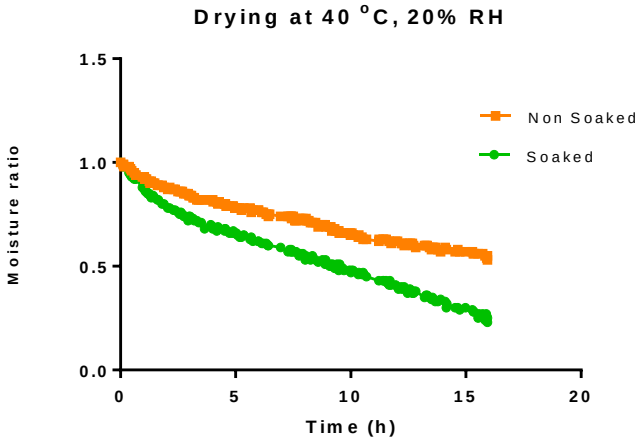


Figure 4.1 Effects of pre-treatments on parchment coffee: drying curves at 40 ^{o}C, 20% RH

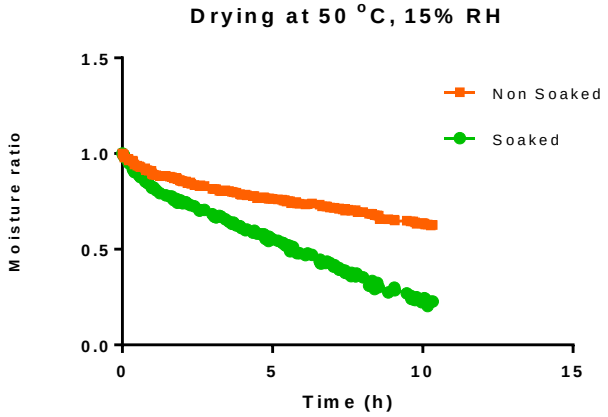


Figure 4.2 Effects of pre-treatments on parchment coffee: drying curves at 50 ^{o}C, 15% RH

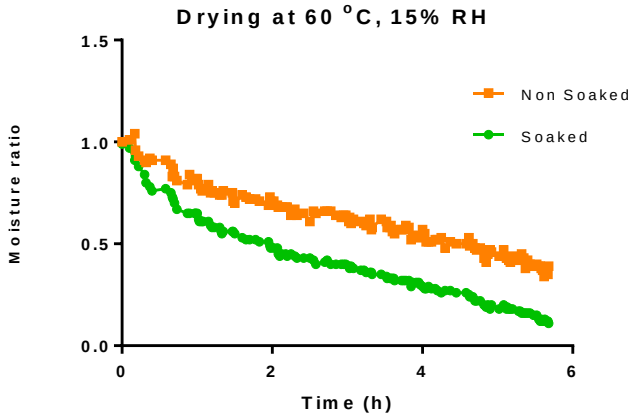


Figure 4.3 Effects of pre treatments on parchment coffee: drying curves at 60 ^{o}C, 15% RH

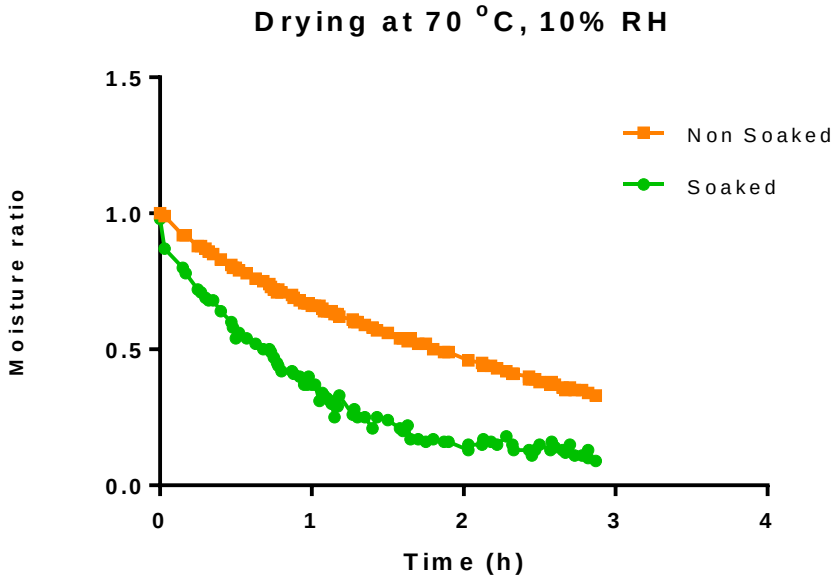


Figure 4.4 Effects of pre treatments on parchment coffee: drying curves at 70 ^{o}C, 10% RH

Total drying time was reduced from 16 h at 20% RH (40 ^{o}C) to 10 h at 15% RH (50 ^{o}C) then to 6 h at 15% RH (60 ^{o}C) and finally to 3 h at 10% RH (70 ^{o}C).

Although the drying rates were higher for soaked samples, the same drying time was used for samples subjected to both pre-treatments.

The soaked parchment showed faster drying rate because of the presence of mucilage which contains a significant amount of sugar. The latter partially covers the parchment. This phenomenon causes the lower drying rate of the non-soaked parchment.

The appearance of non-soaked and soaked parchment coffees is illustrated in Figure 4.5.

Figure 4.5 Effects of pre-treatments on the appearance of dried parchment coffee.

4.2 Storage and roasting

4.2.1 Moisture content and colour of samples

MC of green coffee prior to storage and of the roasted samples is shown in Table 4.2.

Table 4.2 Average moisture content (% wb) of raw fresh vs roasted samples

Pre-treatment	Drying Temperature	Roasting Degree	Moisture Content (%wb)		
Soaked	40 °C	Control*	12.12^{k}	±	0.43
	50 °C	Control	12.32^{k}	±	0.72
	60 °C	Control	11.77^{j}	±	0.17
	70 °C	Control	11.76^{j}	±	0.83
Non Soaked	40 °C	Control	12.76^{l}	±	0.59
	50 °C	Control	12.18^{k}	±	0.78
	60 °C	Control	11.69^{j}	±	0.46
	70 °C	Control	12.36^{k}	±	0.79
Soaked	40 °C	Light	5.14^{f}	±	0.42
	50 °C	Light	6.61^{i}	±	0.35
	60 °C	Light	5.72^{g}	±	0.97
	70 °C	Light	5.75^{g}	±	0.51
Non Soaked	40 °C	Light	5.35^{f}	±	0.53
	50 °C	Light	5.79^{g}	±	0.4
	60 °C	Light	6.21^{h}	±	0.21
	70 °C	Light	5.20^{f}	±	0.6
Soaked	40 °C	Medium	2.28^{d}	±	0.22
	50 °C	Medium	2.51d^{e}	±	0.56
	60 °C	Medium	2.67^{e}	±	0.47
	70 °C	Medium	2.73^{e}	±	0.79
Non Soaked	40 °C	Medium	2.29^{d}	±	0.41
	50 °C	Medium	2.75^{e}	±	0.27
	60 °C	Medium	2.24^{d}	±	0.03
	70 °C	Medium	1.88^{c}	±	0.47
Soaked	40 °C	Dark	1.13^{a}	±	0.28
	50 °C	Dark	1.51^{ab}	±	0.06
	60 °C	Dark	1.74^{c}	±	0.27
	70 °C	Dark	1.25^{a}	±	0.23
Non Soaked	40 °C	Dark	1.41^{ab}	±	0.16
	50 °C	Dark	1.20^{a}	±	0.41
	60 °C	Dark	1.41^{ab}	±	0.73
	70 °C	Dark	1.34^{a}	±	0.5

All results are expressed as mean ± SD
Values followed by different letters (a-l) differ significantly at a $p<0.05$ level.
* Green coffee

The effects of roasting on weight loss and colour of coffee samples after roasting are shown in Table 4.3

Table 4.3 Average values of %weight loss and colour of raw fresh vs roasted samples

Pre treatment	Drying Temperature	Roasting Degree	% weight loss	Lightness (L^*)	Hue angle (^oh)
Soaked	40 ^oC	Control*	-	$51.84^{q} \pm 0.74$	$78.58^{k} \pm 0.50$
	50 ^oC	Control	-	$59.34^{t} \pm 2.00$	$75.78^{j} \pm 0.18$
	60 ^oC	Control	-	$59.38^{t} \pm 0.40$	$80.96^{l} \pm 0.74$
	70 ^oC	Control	-	$56.92^{rs} \pm 0.90$	$80.30^{l} \pm 0.32$
Non Soaked	40 ^oC	Control	-	$57.99^{s} \pm 0.17$	$81.16^{l} \pm 0.34$
	50 ^oC	Control	-	$56.07^{r} \pm 0.53$	$85.15^{m} \pm 0.81$
	60 ^oC	Control	-	$56.41^{r} \pm 0.40$	$80.26^{l} \pm 0.25$
	70 ^oC	Control	-	$46.84^{p} \pm 0.85$	$84.99^{m} \pm 0.72$
Soaked	40 ^oC	Light	$3.47^{b} \pm 0.53$	$34.20^{l} \pm 0.92$	$68.45^{i} \pm 0.35$
	50 ^oC	Light	$3.70^{bc} \pm 0.43$	$34.06^{l} \pm 0.88$	$64.67^{g} \pm 0.43$
	60 ^oC	Light	$4.46^{de} \pm 0.45$	$29.84^{k} \pm 0.13$	$67.21^{h} \pm 0.37$
	70 ^oC	Light	$4.83^{e} \pm 0.86$	$26.31^{i} \pm 0.23$	$67.52^{h} \pm 0.54$
Non Soaked	40 ^oC	Light	$2.58^{a} \pm 0.79$	$39.09^{m} \pm 0.45$	$63.15^{f} \pm 0.53$
	50 ^oC	Light	$3.63^{bc} \pm 0.81$	$42.14^{n} \pm 0.34$	$61.94^{e} \pm 0.49$
	60 ^oC	Light	$4.14^{cd} \pm 0.27$	$39.65^{m} \pm 0.47$	$67.23^{h} \pm 0.42$
	70 ^oC	Light	$4.46^{de} \pm 0.43$	$44.43^{o} \pm 1.17$	$68.94^{i} \pm 0.39$
Soaked	40 ^oC	Medium	$5.33^{f} \pm 0.15$	$21.04^{cd} \pm 0.04$	$60.65^{abc} \pm 0.28$
	50 ^oC	Medium	$5.71^{fg} \pm 0.76$	$24.44^{gh} \pm 0.47$	$60.26^{ab} \pm 0.56$
	60 ^oC	Medium	$5.99^{gh} \pm 0.22$	$24.81^{h} \pm 0.53$	$60.56^{abc} \pm 0.49$
	70 ^oC	Medium	$6.43^{hi} \pm 0.57$	$27.62^{j} \pm 0.75$	$61.02^{bcde} \pm 1.12$
Non Soaked	40 ^oC	Medium	$6.72^{i} \pm 0.45$	$23.20^{f} \pm 0.84$	$60.92^{bcd} \pm 0.39$
	50 ^oC	Medium	$6.33^{hi} \pm 0.03$	$23.58^{fg} \pm 0.15$	$60.72^{abc} \pm 0.44$
	60 ^oC	Medium	$7.33^{j} \pm 0.48$	$23.90^{fgh} \pm 0.67$	$65.18^{g} \pm 0.57$
	70 ^oC	Medium	$8.04^{k} \pm 0.91$	$23.52^{fg} \pm 0.41$	$60.08^{ab} \pm 0.21$
Soaked	40 ^oC	Dark	$10.82^{lm} \pm 0.36$	$21.09^{cd} \pm 0.16$	$61.70^{de} \pm 0.88$
	50 ^oC	Dark	$11.59^{n} \pm 0.5$	$20.01^{abc} \pm 0.57$	$61.09^{bcde} \pm 0.34$
	60 ^oC	Dark	$11.75^{n} \pm 0.67$	$20.71^{bcd} \pm 0.43$	$61.31^{cde} \pm 0.53$
	70 ^oC	Dark	$12.33^{o} \pm 0.38$	$21.90^{de} \pm 0.66$	$60.67^{abc} \pm 0.58$
Non Soaked	40 ^oC	Dark	$10.51^{l} \pm 0.37$	$21.91^{de} \pm 0.65$	$60.34^{abc} \pm 0.65$
	50 ^oC	Dark	$11.28^{mn} \pm 0.85$	$22.91^{ef} \pm 0.22$	$60.46^{abc} \pm 0.17$
	60 ^oC	Dark	$11.62^{n} \pm 0.4$	$19.74^{ab} \pm 0.42$	$59.89^{a} \pm 0.43$
	70 ^oC	Dark	$12.85^{p} \pm 0.52$	$18.91^{a} \pm 0.24$	$61.09^{bcde} \pm 0.44$

All results are expressed as mean ± SD.

Values in the same column followed by different letters (a-t) differ significantly at $p<0.05$ level

* Green coffee

4.2.2 Storage conditions

After drying parchment coffee beans, the effects of two storage temperatures namely 30 ^{o}C (30% RH) and 15 ^{o}C (65% RH) and times (6 and 12 months) on dried parchment coffee were investigated. Samples of parchment coffee dried at 40 ^{o}C were chosen for the storage.

Theis samples dried at 40 ^{o}C were chosen for storage because this temperature was the most suitable for drying of coffee parchment. It is the slow drying process that leads to the perfect bean after hulling.

Comparison of the MC of parchment coffee stored under 2 different storage conditions for up to 12 months is shown in Figure 4.6. MC in all samples slightly decreased over the first 6 months and continued decreasing over the following 6 months. It appears that storage at 15 ^{o}C leads to lower losses MC than storage at 30 ^{o}C.

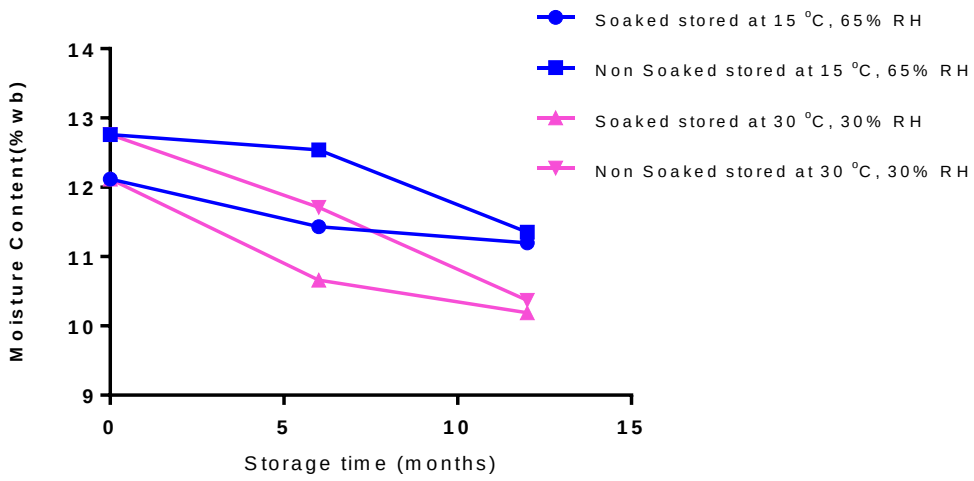


Figure 4.6 Moisture content of green coffee stored for 12 months under different conditions.

The criterion for selecting the storage conditions was the ability to reduce the risk of occurrence of microbiological and biochemical processes causing deterioration of the beans.

4.2.3 Roasting temperature and time

Raw samples after storage for 6 and 12 months were roasted with constant heat input at different temperatures as shown in Figure 4.6. The different roasting levels were confirmed by colour measurement. Table 4.4 shows MC (%w.b.) of samples subjected to different pre-treatments and roasting temperatures.

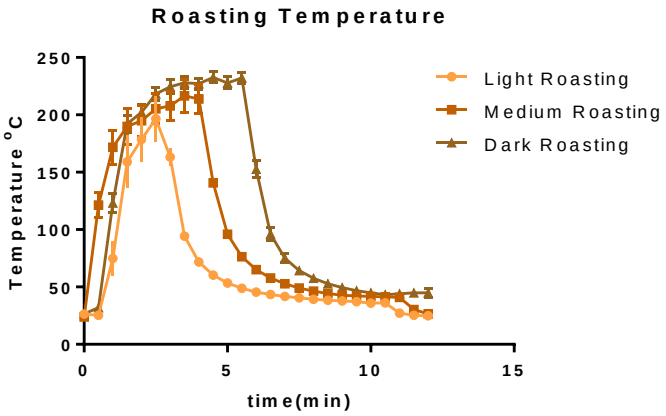


Figure 4.7 Temperature and time profiles during coffee roasting

The results in Table 4.4 show MC after storage prior and after roasting. The weight loss and colour of samples after storage before and after roasting are shown in Table 4.5.

Table 4.4 Average values of moisture content (% wb) of raw samples after storage under different conditions for 6 and 12 months before and after roasting

Storage time(months)	Storage Condition	Pre treatment	Roasting Degree	Moisture Content (%wb)
6	15 °C	Soaked	Control*	11.43^{kl} ± 0.84
			Light	5.36^{g} ± 0.04
			Medium	2.49^{d} ± 0.83
			Dark	1.48^{ab} ± 0.22
		Non Soaked	Control	12.54^{m} ± 0.38
			Light	4.22^{e} ± 0.45
			Medium	2.39^{cd} ± 0.36
			Dark	1.53^{ab} ± 0.64
	30 °C	Soaked	Control	10.66^{j} ± 0.51
			Light	4.44^{ef} ± 0.26
			Medium	2.29^{cd} ± 0.21
			Dark	1.44^{ab} ± 0.72
		Non Soaked	Control	11.71^{l} ± 0.34
			Light	4.28^{e} ± 0.19
			Medium	2.47^{cd} ± 0.41
			Dark	1.28^{ab} ± 0.44
12	15 °C	Soaked	Control	11.20^{k} ± 0.35
			Light	5.87^{h} ± 0.68
			Medium	2.58^{d} ± 0.59
			Dark	1.43^{ab} ± 0.43
		Non Soaked	Control	11.35^{k} ± 0.44
			Light	4.78^{ef} ± 0.54
			Medium	2.52^{d} ± 0.97
			Dark	1.18^{a} ± 0.52
	30 °C	Soaked	Control	10.19^{i} ± 0.48
			Light	4.50^{ef} ± 0.43
			Medium	2.22^{cd} ± 0.24
			Dark	1.64^{b} ± 0.47
		Non Soaked	Control	10.37^{ij} ± 0.32
			Light	4.30^{e} ± 0.35
			Medium	2.10^{c} ± 0.38
			Dark	1.26^{ab} ± 0.42

All results are expressed as mean ± SD
Values followed by different letter (a-l) differ significantly at a $p<0.05$ level.
* Green coffee

Table 4.5 Average values of % weight loss and colour of samples after storage under different conditions for 6 and 12 months before and after roasting.

Storage time (months)	Storage condition	Pre treatment	Roasting degree	% weight loss	Lightness (L*)	Hue angle (^{o}h)
6	15 ^{o}C	Soaked	Control*	-	84.14^{j} ± 1.65	78.58^{k} ± 0.52
			Light	3.57^{a} ± 0.587	68.05^{i} ± 0.63	75.78^{j} ± 0.43
			Medium	5.41^{b} ± 2.722	60.35^{e} ± 1.04	80.96^{l} ± 0.09
			Dark	11.43^{d} ± 0.106	59.40^{cd} ± 0.03	80.30^{l} ± 0.51
		Non Soaked	Control	-	86.29^{l} ± 0.66	68.45^{i} ± 0.43
			Light	3.50^{a} ± 0.474	63.26^{g} ± 0.49	64.67^{g} ± 0.42
			Medium	7.74^{c} ± 0.834	60.92^{e} ± 0.66	67.21^{h} ± 0.67
			Dark	11.90^{d} ± 0.092	59.24^{c} ± 0.35	67.52^{h} ± 0.74
	30 ^{o}C	Soaked	Control	-	59.11^{c} ± 0.68	60.65^{abc} ± 0.22
			Light	3.42^{a} ± 0.361	62.31^{f} ± 0.3	60.26^{ab} ± 0.7
			Medium	7.38^{c} ± 0.148	60.81^{e} ± 0.64	60.56^{abc} ± 0.52
			Dark	12.5^{1d} ± 0.46	59.15^{c} ± 0.11	61.02^{bcde} ± 0.4
		Non Soaked	Control	-	86.45^{l} ± 0.92	61.70^{de} ± 0.61
			Light	3.47a ± 0.085	60.12^{de} ± 0.82	61.09^{bcde} ± 0.33
			Medium	8.12c ± 0.297	60.72^{e} ± 0.52	61.31^{cde} ± 0.67
			Dark	12.55d ± 0.424	60.70^{e} ± 0.29	60.67^{abc} ± 0.55
12	15 ^{o}C	Soaked	Control	-	68.04q ± 0.82	84.16j ± 0.45
			Light	4.10ab ± 0.247	43.24ij ± 1.36	60.55e ± 0.44
			Medium	7.27c ± 0.233	21.17cd ± 0.53	60.42e ± 0.26
			Dark	11.75d ± 0.106	21.14cd ± 0.23	60.33e ± 0.34
		Non Soaked	Control	-	57.25l ± 0.59	86.02kl ± 0.56
			Light	3.32a ± 0.304	42.32hi ± 0.66	60.59e ± 0.57
			Medium	7.63c ± 0.311	23.95ef ± 0.34	53.45a ± 0.45
			Dark	12.41d ± 0.156	19.78b ± 0.31	62.08f ± 0.32
	30 ^{o}C	Soaked	Control	-	62.87n ± 0.86	85.35k ± 0.43
			Light	3.65a ± 0.276	41.79h ± 0.73	64.31h ± 0.13
			Medium	7.69c ± 0.276	23.24e ± 0.94	54.48b ± 0.58
			Dark	12.09d ± 0.325	19.57b ± 0.39	64.26h ± 0.63
		Non Soaked	Control	-	57.87l ± 0.38	83.50j ± 0.52
			Light	4.02ab ± 0.651	42.69hij ± 0.54	62.40fg ± 0.57
			Medium	7.31c ± 0.021	23.59ef ± 0.71	55.20b ± 0.68
			Dark	12.64d ± 0.205	20.18bcd ± 0.58	62.86fg ± 0.54

All results are expressed as mean ± SD.
Values in the same column followed by different letter (a-q) differ significantly at a $p<0.05$ level.
* Green coffee

Table 4.4 shows that the moisture contents decreased considerably from green coffee (11-12 %wb) to light roasting (5-6 %wb) then to medium roasting (1.8-2.8 %wb) and finally to dark roasting (1-1.8 %wb).

Table 4.5 indicates that the roasting temperatures have a significant effect on % weight loss after roasting. It amounts to 3-5% after light roasting, to 5-8% after medium roasting and to 10-13% after dark roasting. As for the effects of roasting degree on

colour, the control (green coffee) presents the highest *L value, which is between 46-60 and hue angle from 75-80. This explains that green coffee has the brightest colour (see Figure 4.8).

Figure 4.8 Colour of ground coffee subjected to different roasting temperatures.

Heating time affects the physical change (weight, volume, texture and colour). The *L** colour and H^o parameter was use as a criterion for roasted coffee colour classification namely light, medium and dark (Jokanović *et al*., 2012; Bicho 2012).

A similar study was performed by Clarks (1987) and Alessandrini *et al.* (2008) who observed percentage of weight of weight loss with the degrees of roasting. The comparison between those results could be in the same range of % weight loss. Furthermore, Alessandrini *et al*. (2008) represented the coffee colour in terms of L* and ^{o}h. Their results were in similar range as those of the present study.

4.3 Extraction technique

Comparison of extraction efficiency between coffee percolator and reflux boiling with respect to TPC values expressed in mg GAE/g DW are shown in Figures 4.9 and 4.10.

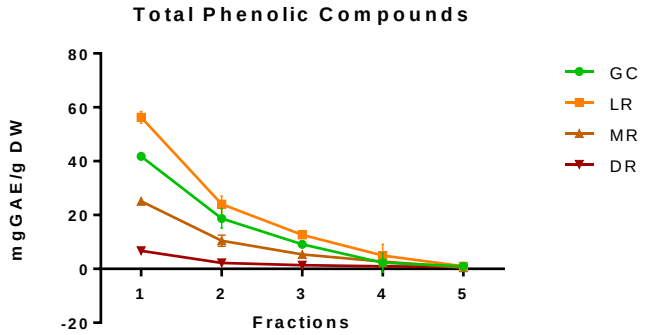


Figure 4.9 The fractions of non-soaked roasted coffee sample extracted using coffee percolator

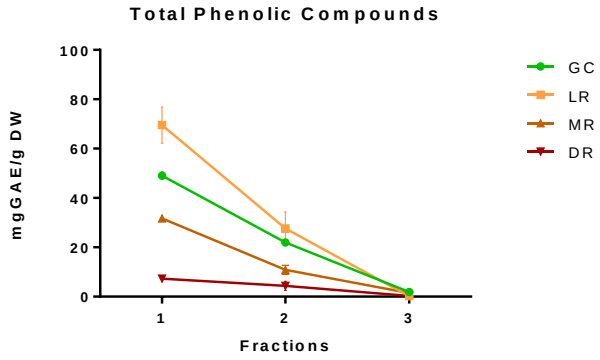


Figure 4.10 The fractions of non-soaked roasted coffee sample extracted using reflux boiling and Büchner funnel methods.

Considering results shown in Figure 4.9 and 4.10, it appears that extraction using boiling under reflux and Büchner funnel was more efficient than that using a coffee percolator. Ground coffee samples were extracted by continually cycling brew using gravity. Meanwhile, the coffee extractions using boiling under reflux and Büchner funnel, which are continuously boiling. In addition, the coffee extracted through the filter via the force of vacuum system. This given more compounds was present in the

refluxed and Büchner funnel under vacuum system than the extraction using coffee percolator.

The advantage of the reflux method is that the solvent always boils at a certain temperature and the boiling process is cycling continuously. Furthermore, the coffee is extracted through the filter using vacuum which improves the extraction efficiency. This leads to more compounds being released from the samples. More compounds were present in the refluxed samples that was filtered through Büchner funnel under vacuum than in those extracted using a coffee percolator.

The effects of pre-treatment on extraction efficiency with respect to TPC values are shown in Figures 4.11 and 4.12

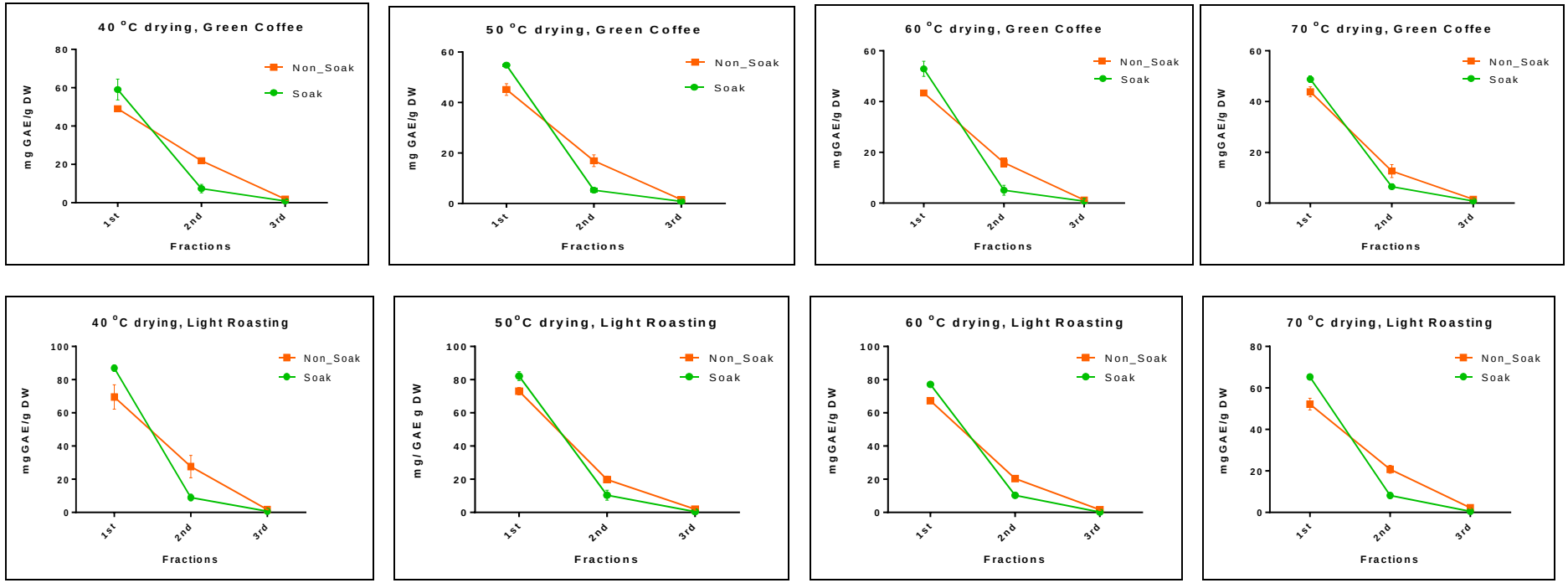


Figure 4.11 Effects of pre-treatments and drying temperature on total phenolic compounds in raw and in light roasted coffee.

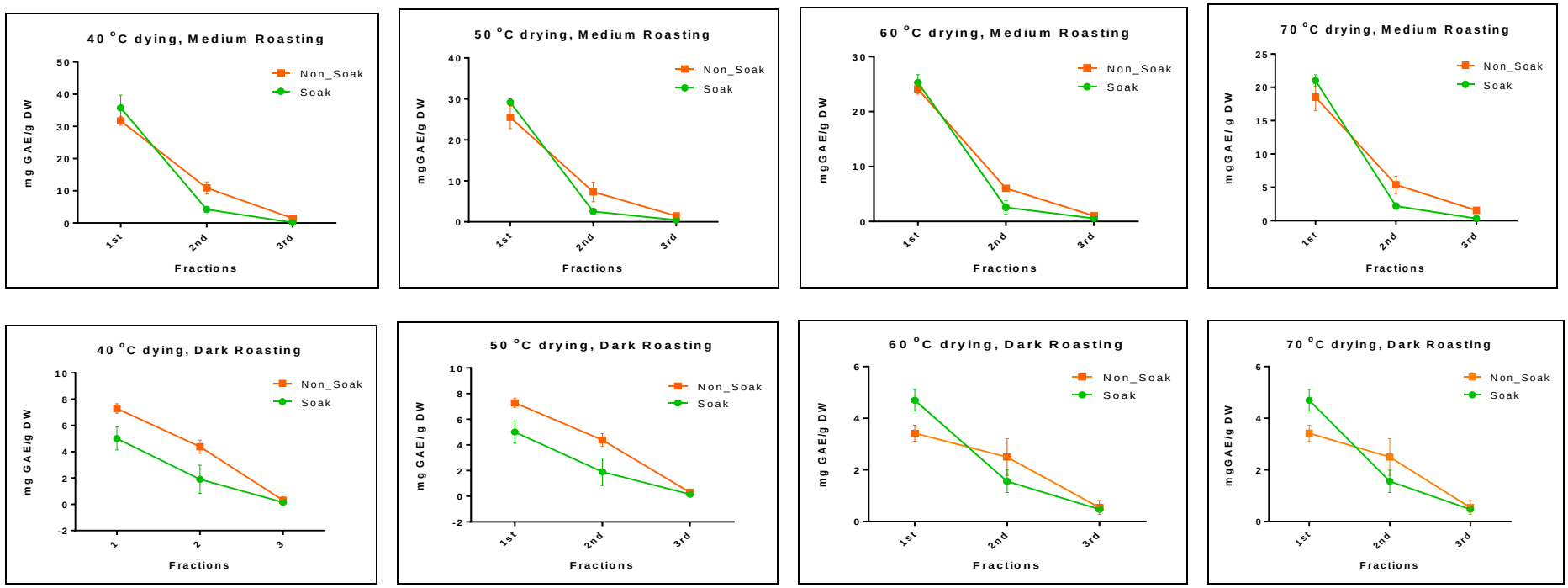


Figure 4.12 Effects of pre-treatments and drying temperature on total phenolic compounds in medium and in dark roasted coffee.

Figure 4.11 and 4.12 show that the soaked coffee beans produced extracts with higher TPC values than non soaked coffee beans after drying and roasting

The effects of storage condition, storage time and roasting degree on TPC are shown in Figure 4.13 and 4.14

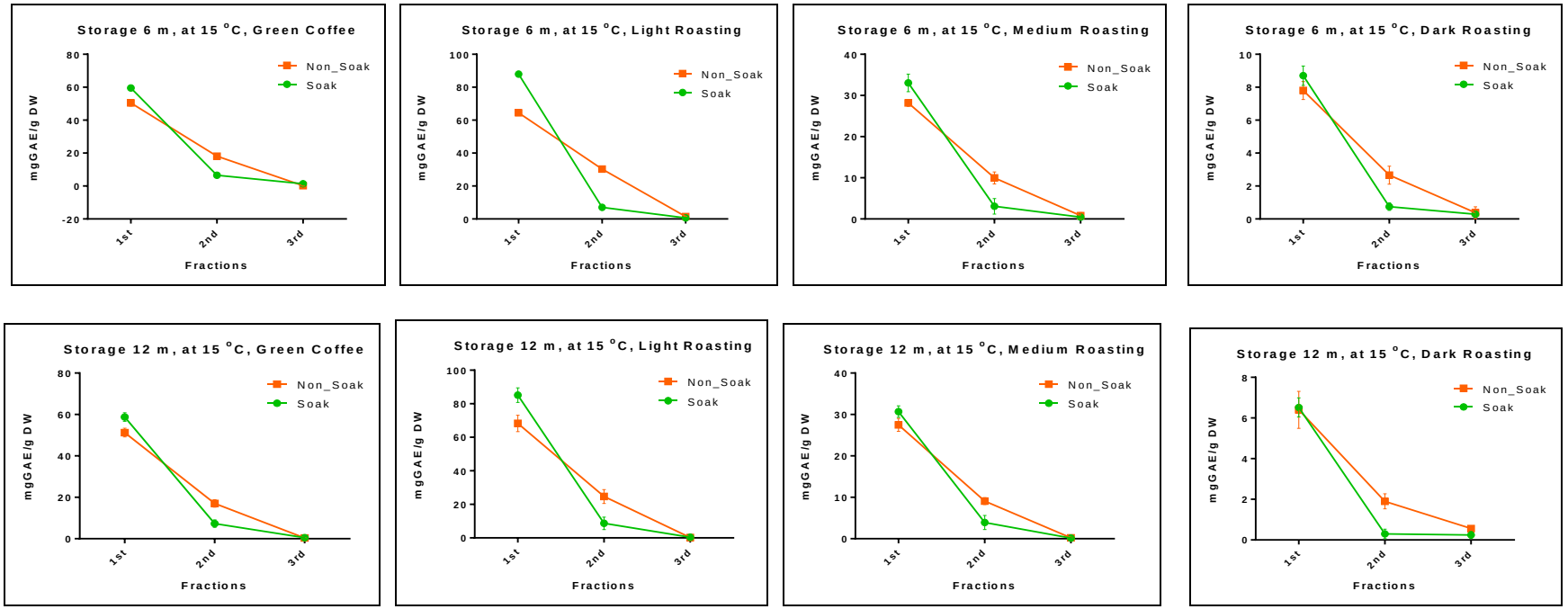


Figure 4.13 Effects of pre-treatments and storage temperature on total phenolic compounds in raw and in roasted coffee.

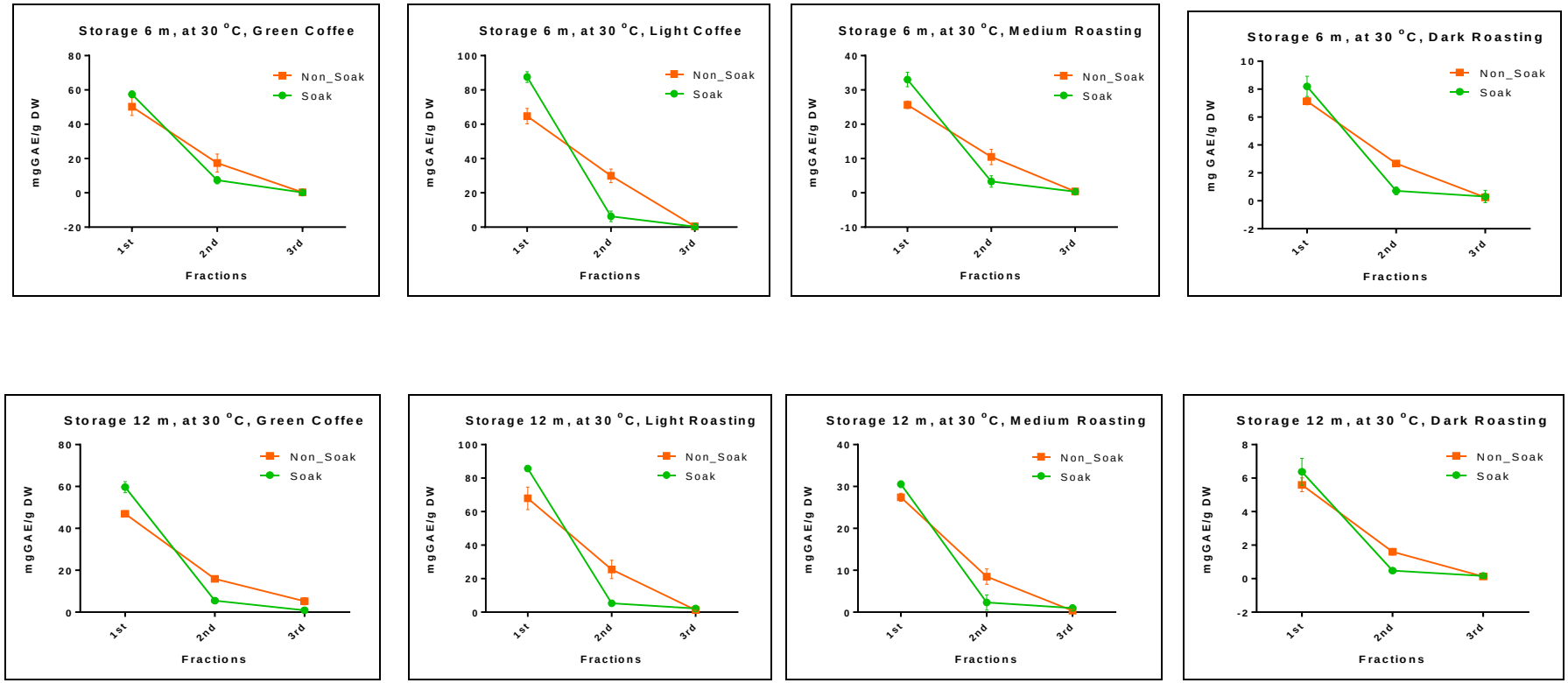


Figure 4.14 Effects of pre-treatments and storage temperature on total phenolic compounds in medium and dark roasted coffee.

These diagrams show that the soaked samples have a better extracted efficacy than the non-soaked ones. It appears in all diagrams that show that all total phenolic compounds were present in the second fraction.

4.4 Effects of pre-treatments, drying, storage conditions and roasting degree on selected coffee compounds

In order to maximise the CGA, CFE and TGL content in coffee beans, it was important to study their content in samples subjected to various treatments.

4.4.1 Effects of different pre treatments, drying conditions and roasting degree on CGA, CFE and TGL.

Table 4.6 shows the effects of pre-treatments, drying condition and roasting degree on CGA, CFE and TGL content in four coffee samples.

Table 4.6 Chlorogenic acid (CGA), caffeine (CFE) and trigonelline (TGL) content in coffee samples subjected to different pre-treatments, drying conditions and roasting degree in fresh coffee.

Pre treatment	Drying temperature	Roasting degree	CGA mg/g DW			CFE mg/g DW			TGL mg/g DW		
Soaked	40 °C	Control*	3.62^{l}	±	0.31	1.62^{f}	±	0.06	1.99^{e}	±	0.27
	50 °C	Control	2.81^{jk}	±	0.08	1.34^{bc}	±	0.1	2.50^{f}	±	0.18
	60 °C	Control	2.43^{gh}	±	0.17	1.37^{bc}	±	0.04	1.81^{e}	±	0.16
	70 °C	Control	2.29^{fg}	±	0.12	1.19^{b}	±	0.05	1.56^{d}	±	0.18
Non-soaked	40 °C	Control	3.74^{l}	±	0.17	1.93^{g}	±	0.06	2.82^{g}	±	0.14
	50 °C	Control	2.99^{j}	±	0.2	1.61^{ef}	±	0.05	2.48^{f}	±	0.17
	60 °C	Control	2.76^{ij}	±	0.09	1.44^{cdef}	±	0.03	1.83^{e}	±	0.17
	70 °C	Control	2.40^{gh}	±	0.11	1.39^{bcde}	±	0.03	1.22^{c}	±	0.1
Soaked	40 °C	Light	5.30^{p}	±	0.09	3.64^{k}	±	0.19	4.59^{k}	±	0.16
	50 °C	Light	4.36^{n}	±	0.12	3.25^{j}	±	0.08	4.34^{jk}	±	0.1
	60 °C	Light	4.12^{m}	±	0.05	2.75^{i}	±	0.1	4.29^{j}	±	0.07
	70 °C	Light	3.36^{k}	±	0.21	2.23^{h}	±	0.15	4.15^{j}	±	0.06
Non-soaked	40 °C	Light	5.76^{q}	±	0.05	3.86^{l}	±	0.12	5.37^{m}	±	0.21
	50 °C	Light	4.77^{o}	±	0.22	3.53^{k}	±	0.08	5.11^{l}	±	0.21
	60 °C	Light	4.28^{mn}	±	0.15	3.16^{j}	±	0.18	4.37^{jk}	±	0.24
	70 °C	Light	4.25^{mn}	±	0.14	2.84^{i}	±	0.07	4.39^{jk}	±	0.21
Soaked	40 °C	Medium	2.43^{gh}	±	0.06	6.71^{q}	±	0.08	3.77^{i}	±	0.07
	50 °C	Medium	2.16^{f}	±	0.25	6.49^{p}	±	0.17	3.42^{h}	±	0.13
	60 °C	Medium	1.30^{d}	±	0.14	6.31^{op}	±	0.08	3.35^{h}	±	0.28
	70 °C	Medium	1.20^{cd}	±	0.1	5.76^{m}	±	0.09	3.23^{h}	±	0.1
Non-soaked	40 °C	Medium	2.67^{i}	±	0.14	6.81^{q}	±	0.07	3.86^{i}	±	0.07
	50 °C	Medium	2.60^{hi}	±	0.22	6.20^{no}	±	0.12	3.46^{h}	±	0.14
	60 °C	Medium	1.62^{e}	±	0.09	6.35^{op}	±	0.11	3.28^{h}	±	0.13
	70 °C	Medium	1.61^{e}	±	0.05	6.02^{n}	±	0.24	3.21^{h}	±	0.08
Soaked	40 °C	Dark	1.12^{cd}	±	0.03	1.64^{f}	±	0.25	0.82^{b}	±	0.17
	50 °C	Dark	0.83^{ab}	±	0.09	1.65^{f}	±	0.23	0.66^{b}	±	0.12
	60 °C	Dark	0.85^{ab}	±	0.11	1.36^{bc}	±	0.1	0.78^{b}	±	0.1
	70 °C	Dark	0.63^{a}	±	0.11	1.23^{bc}	±	0.09	0.38^{a}	±	0.07
Non-soaked	40 °C	Dark	1.13^{cd}	±	0.04	2.06^{gh}	±	0.29	0.84^{b}	±	0.07
	50 °C	Dark	0.96^{bc}	±	0.02	1.60^{def}	±	0.16	0.76^{b}	±	0.1
	60 °C	Dark	0.78^{ab}	±	0.09	1.37^{bcd}	±	0.03	0.77^{b}	±	0.03
	70 °C	Dark	0.65^{a}	±	0.03	0.90^{a}	±	0.09	0.71^{b}	±	0.09

All results are expressed as mean ± SD.
Values in the same column followed by different letter (a-n) differ significantly at $p<0.05$ level.
* Green coffee

4.4.2 Effects of pre treatments, roasting degree, storage conditions and time on CGA, CFE and TGL

Table 4.7 shows the effects of pre-treatments, storage condition and time and roasting degree on CGA, CFE and TGL contents in coffee samples.

Table 4.7 Chlorogenic acid (CGA), caffeine (CFE) and trigonelline (TGL) content in coffee samples subjected to storage conditions and time as well as different roasting degrees.

Storage time (months)	Storage condition	Pre treatment	Roasting degree	CGA mg/g DW	CFE mg/g DW	TGL mg/g DW
6	15 °C	Soaked	Control*	2.79f^{g} ± 0.14	1.38^{a} ± 0.05	1.27^{bc} ± 0.13
			Light	5.36^{j} ± 0.07	3.24^{c} ± 0.07	5.73^{m} ± 0.11
			Medium	1.45^{d} ± 0.16	5.37^{fg} ± 0.23	3.51^{f} ± 0.11
			Dark	0.84^{bc} ± 0.11	1.32^{a} ± 0.09	0.89^{a} ± 0.08
		Non-soaked	Control	2.92^{g} ± 0.09	1.53^{a} ± 0.1	1.71d^{e} ± 0.16
			Light	5.49^{j} ± 0.1	3.59^{cd} ± 0.23	5.90^{n} ± 0.06
			Medium	1.53^{d} ± 0.06	5.39^{fg} ± 0.35	4.56^{ij} ± 0.16
			Dark	0.90^{c} ± 0.17	1.43^{a} ± 0.05	1.25^{bc} ± 0.06
	30°C	Soaked	Control	2.41^{e} ± 0.17	1.43^{a} ± 0.04	1.34^{bc} ± 0.15
			Light	4.86^{i} ± 0.8	3.49^{cd} ± 0.19	5.26^{l} ± 0.11
			Medium	1.43^{d} ± 0.06	5.12^{f} ± 0.16	3.62^{fg} ± 0.22
			Dark	0.83^{bc} ± 0.1	1.51^{a} ± 0.27	0.85^{a} ± 0.06
		Non-soaked	Control	2.82^{fg} ± 0.15	1.49^{a} ± 0.04	1.81^{e} ± 0.15
			Light	5.35^{j} ± 0.12	3.61^{d} ± 0.22	5.67^{mn} ± 0.24
			Medium	1.56^{d} ± 0.06	5.50^{g} ± 0.3	4.19^{h} ± 0.2
			Dark	0.66^{abc} ± 0.1	1.38^{a} ± 0.16	1.22^{b} ± 0.05
12	15°C	Soaked	Control	2.64^{efg} ± 0.26	1.32^{a} ± 0.06	1.51^{cd} ± 0.15
			Light	4.25^{h} ± 0.13	3.53^{cd} ± 0.28	5.37^{l} ± 0.2
			Medium	1.36^{d} ± 0.07	5.49^{g} ± 0.2	3.81^{g} ± 0.11
			Dark	0.51^{ab} ± 0.07	1.34^{a} ± 0.12	0.85^{a} ± 0.09
		Non-soaked	Control	2.66^{efg} ± 0.2	1.43^{a} ± 0.06	1.76^{de} ± 0.19
			Light	4.23^{h} ± 0.09	3.52^{cd} ± 0.36	5.45^{lm} ± 0.17
			Medium	1.40^{d} ± 0.15	5.55^{g} ± 0.26	4.35^{hi} ± 0.22
			Dark	0.74b^{c} ± 0.09	1.46^{a} ± 0.09	1.24^{bc} ± 0.09
	30°C	Soaked	Control	2.52^{ef} ± 0.23	1.35^{a} ± 0.03	1.34^{bc} ± 0.14
			Light	4.24^{h} ± 0.12	3.29^{cd} ± 0.33	4.74^{jk} ± 0.08
			Medium	1.36^{d} ± 0.07	4.64^{e} ± 0.14	3.53^{f} ± 0.25
			Dark	0.38^{a} ± 0.11	1.25^{a} ± 0.14	0.92^{a} ± 0.09
		Non-soaked	Control	2.52^{ef} ± 0.25	1.39^{a} ± 0.03	1.66^{de} ± 0.07
			Light	4.37^{h} ± 0.1	2.78^{b} ± 0.22	4.94^{k} ± 0.14
			Medium	1.30^{d} ± 0.13	5.11^{f} ± 0.17	3.68^{fg} ± 0.21
			Dark	0.57^{abc} ± 0.12	1.51^{a} ± 0.24	1.32^{bc} ± 0.04

All results are expressed as mean ± SD.
Values in the same column followed by different letters (a-n) differ significantly at $p<0.05$ level.
* Green coffee

Coffee beans dried at 40 ^{o}C, non-soaked and lightly roasted were found to have the highest content of CGA and TGL. Moreover, the CFE content appears to be positive correlated with the CGA content in samples subjected to higher drying temperature.

Storage condition and time did not seem to affect the content of these three compounds. Significant difference appeared when samples subjected to different roasting conditions were compared. It appears that, the roasting temperature had a major effect on these three compounds. The results presented in Table 4.6, show that light roasted coffee beans contained the highest amount of CGA and TGL. In contrast, the medium roasted coffee beans contained the highest amount of CFE.

Results from similar studies, shows that there are lower amounts of CGA, CFE and TGL in defective coffee beans (Franca *et al.*, 2005). However, there are higher CGA contents in commercial coffee beans (Fujioka and Shibamoto, 2008). This is due to blending of Arabica and Robusta commercially produced coffee beans.

Consequently, in this study, the drying temperature of 40 ^{o}C was selected for all samples. A high concentration of selected compounds was found and evaluated with regard to the selection of appropriate processing method leading to adequate consumption of brewed coffee.

4.4.3 Effects of different pre-treatments, drying conditions and roasting degree on α-tocopherol (VitE)

The effects if pre-treatments, drying conditions and roasting degree on α-Tocopherol content are shown in Table 4.8

Table 4.8 α-Tocopherol content in coffee samples subjected to different pre-treatments, drying conditions and roasting degree.

Pre treatment	Drying temperature	Roasting degree	VitE µg/g DW		
Soaked	40 °C	Control*	44.05^{k}	±	0.33
	50 °C	Control	45.30^{l}	±	0.74
	60 °C	Control	42.77^{ij}	±	0.49
	70 °C	Control	41.93^{ij}	±	0.85
Non-soaked	40 °C	Control	45.4l^{1}	±	0.30
	50 °C	Control	43.00^{j}	±	1.43
	60 °C	Control	41.75^{i}	±	0.48
	70 °C	Control	40.30^{f}	±	0.40
Soaked	40 °C	Light	56.24^{r}	±	0.39
	50 °C	Light	55.30^{r}	±	0.62
	60 °C	Light	53.94^{q}	±	0.22
	70 °C	Light	53.97^{q}	±	0.17
Non-soaked	40 °C	Light	55.87^{r}	±	0.43
	50 °C	Light	55.82^{r}	±	0.72
	60 °C	Light	55.56^{r}	±	0.76
	70 °C	Light	54.10^{q}	±	0.78
Soaked	40 °C	Medium	49.97^{no}	±	0.32
	50 °C	Medium	49.12^{n}	±	0.59
	60 °C	Medium	47.42^{m}	±	0.76
	70 °C	Medium	45.20^{l}	±	0.78
Non-soaked	40 °C	Medium	51.49^{p}	±	0.69
	50 °C	Medium	50.85^{op}	±	0.42
	60 °C	Medium	49.94^{no}	±	0.58
	70 °C	Medium	49.63^{n}	±	0.52
Soaked	40 °C	Dark	39.88^{ef}	±	0.76
	50 °C	Dark	39.02^{cde}	±	0.69
	60 °C	Dark	38.02^{bc}	±	0.56
	70 °C	Dark	38.24^{cd}	±	0.28
Non-soaked	40 °C	Dark	39.16^{de}	±	0.54
	50 °C	Dark	38.70^{cd}	±	0.56
	60 °C	Dark	37.09^{b}	±	0.63
	70 °C	Dark	35.39^{a}	±	0.64

All results are expressed as mean ± SD.
Values in the same column followed by different letters (a-r) differ significantly at $p<0.05$ level.
* Green coffee

4.4.4 Effects of pre-treatments, roasting degree, storage conditions and time on α-tocopherol

Table 4.9 shows the effects of pre-treatments, storage condition and time and degree of roasting on α-Tocopherol content in coffee samples.

Table 4.9 α-Tocopherol content in coffee samples subjected to different storage conditions and time as well as different roasting degree.

Storage time (months)	Storage condition	Pre treatment	Roasting degree	VitE µg/g DW		
6	15 °C	Soaked	Control*	43.79[b]	±	0.52
			Light	56.84[de]	±	0.71
			Medium	52.27[c]	±	0.33
			Dark	39.49[a]	±	1.14
		Non-soaked	Control	43.94[b]	±	0.59
			Light	57.25[def]	±	0.38
			Medium	52.66[c]	±	0.64
			Dark	40.31[a]	±	0.40
	30 °C	Soaked	Control	44.19[b]	±	0.61
			Light	56.24[d]	±	0.52
			Medium	52.65[c]	±	1.18
			Dark	39.60[a]	±	1.20
		Non-soaked	Control	43.91[b]	±	0.42
			Light	56.75[de]	±	0.60
			Medium	51.87[c]	±	1.22
			Dark	40.45[a]	±	0.56
12	15 °C	Soaked	Control	43.86[b]	±	0.65
			Light	55.95[d]	±	0.41
			Medium	52.76[c]	±	0.58
			Dark	39.70[a]	±	0.92
		Non-soaked	Control	44.22[b]	±	0.88
			Light	58.46[f]	±	1.24
			Medium	52.53[c]	±	0.98
			Dark	39.57[a]	±	0.97
	30 °C	Soaked	Control	43.54[b]	±	0.39
			Light	56.16[d]	±	0.21
			Medium	51.33[c]	±	0.73
			Dark	39.97[a]	±	0.83
		Non-soaked	Control	43.95[b]	±	0.59
			Light	57.98[ef]	±	0.50
			Medium	52.41[c]	±	1.53
			Dark	40.04[a]	±	0.68

All results are expressed as mean ± SD. Values in the same column followed by different letters (a-f) differ significantly at $p<0.05$ level. * Green coffee

According to Table 4.8, the amount of α- tocopherol in Arabica coffee was similar for both pre treatments and the drying temperatures. However, the different roasting degrees play a major role to the content of α- tocopherol in Arabica coffee beans. As the results shows light roasting of coffee beans results in the highest amount of α- tocopherol in samples that have been soaked then dried at 40 ^{o}C. Furthermore, the coffee samples which are non-soaked and dried at 40 or 50 ^{o}C and also the coffee beans those were soaked and dried at 50 ^{o}C have similar results.

According to Table 4.9, the storage time and condition also affected the amount of α-tocopherol. The highest amount was found in the non-soaked samples which were stored at 15 ^{o}C for 12 months and were light roasted.

Similar amounts and trends were found in Columbian coffee, Santos non fermented Arabica stored for 1-2 year and espresso coffee (Folstar *et al.*, 1977; Alves *et al.*, 2009b; Alves *et al.*, 2010).

4.4.5 Effects of different pre-treatments, drying condition and roasting degree on cafestol and kahweol.

The effects of pre-treatments, drying condition and roasting degree on cafestol and kahweol content in coffee samples are shown in Table 4.10.

Table 4.10 Cafestol and kahweol content in coffee samples subjected to different pre-treatments, drying conditions and roasting degree in fresh coffee.

Pre treatment	Drying temperature	Roasting degree	CFT µg/g DW			KWL µg/g DW		
Soaked	40 °C	Control*	32.70^{mn}	±	4.19	27.83^{mn}	±	1.7
	50 °C	Control	31.03^{m}	±	3.52	27.17^{mn}	±	1.91
	60 °C	Control	26.60^{l}	±	3.18	25.53^{lm}	±	3.02
	70 °C	Control	28.13^{l}	±	1.27	19.33^{k}	±	0.57
Non-soaked	40 °C	Control	35.03^{n}	±	3.25	28.93^{n}	±	0.81
	50 °C	Control	34.53^{n}	±	3.73	27.93^{mn}	±	1.7
	60 °C	Control	26.37^{l}	±	4.67	24.07^{l}	±	5.21
	70 °C	Control	26.67^{l}	±	3.46	18.57^{jh}	±	3.16
Soaked	40 °C	Light	17.83^{bc}	±	1.51	16.93^{jh}	±	2.41
	50 °C	Light	17.20^{bc}	±	1.42	15.87^{ij}	±	0.45
	60 °C	Light	18.37^{bc}	±	0.8	13.67^{i}	±	2.33
	70 °C	Light	17.03^{bc}	±	0.47	9.43^{h}	±	2.00
Non-soaked	40 °C	Light	25.13^{de}	±	3.07	10.77^{h}	±	2.06
	50 °C	Light	25.30^{de}	±	2.59	9.97^{h}	±	1.85
	60 °C	Light	25.43^{de}	±	3.62	9.50^{h}	±	0.92
	70 °C	Light	24.03^{d}	±	2.01	8.70^{gh}	±	0.79
Soaked	40 °C	Medium	15.03^{b}	±	3.85	6.53^{fg}	±	0.72
	50 °C	Medium	15.53^{bc}	±	1.35	6.27^{efg}	±	0.47
	60 °C	Medium	17.67^{bc}	±	1.17	6.07^{defg}	±	1.21
	70 °C	Medium	14.97^{b}	±	1.86	5.93^{cdefg}	±	0.42
Non-soaked	40 °C	Medium	16.57^{bc}	±	2.15	6.14^{defg}	±	1.5
	50 °C	Medium	17.63^{bc}	±	1.11	6.07^{defg}	±	1.8
	60 °C	Medium	17.83^{bc}	±	1	5.90^{cdefg}	±	0.46
	70 °C	Medium	18.53^{bc}	±	1.29	5.70^{bcdefg}	±	0.6
Soaked	40 °C	Dark	8.03^{a}	±	0.38	3.76^{abcdef}	±	0.49
	50 °C	Dark	8.93^{a}	±	0.61	3.33^{abcde}	±	0.55
	60 °C	Dark	8.87^{a}	±	0.91	3.07^{abcde}	±	1.29
	70 °C	Dark	7.33^{a}	±	0.57	2.67^{ab}	±	0.5
Non-soaked	40 °C	Dark	7.73^{a}	±	1.01	3.07^{abcde}	±	0.7
	50 °C	Dark	8.57^{a}	±	1.08	2.97^{abcd}	±	0.47
	60 °C	Dark	7.97^{a}	±	1.07	2.80^{abc}	±	0.4
	70 °C	Dark	5.80^{a}	±	0.36	2.47^{a}	±	0.25

All results are expressed as mean ± SD.
Values in the same column followed by different letters (a-n) differ significantly at $p<0.05$ level.
* Green coffee

4.4.6 Effects of pre-treatments, storage conditions and time and roasting degree on cafestol and kahweol

The effects of pre-treatments, storage condition and time and roasting degree on cafestol and kahweol content in coffee samples are shown in Table 4.11.

Table 4.11 Cafestol and kahweol content in coffee samples subjected to storage conditions and time as well as different roasting degree.

Storage time (months)	Storage condition	Pre treatment	Roasting degree	CFT µg/g DW	KWL µg/g DW
6	15 °C	Soaked	Control*	45.30^{h} ± 2.76	33.63^{e} ± 2.63
			Light	30.73^{de} ± 4.74	16.07^{b} ± 1.82
			Medium	16.50^{b} ± 1.87	12.57^{b} ± 1.76
			Dark	7.13^{a} ± 1.85	3.37^{a} ± 0.87
		Non-soaked	Control	45.60^{h} ± 3.35	32.00^{e} ± 4.94
			Light	30.97^{de} ± 2.95	16.63^{b} ± 2.15
			Medium	14.87^{b} ± 2.24	14.10^{b} ± 1.47
			Dark	5.87^{a} ± 2.15	3.50^{a} ± 0.79
	30 °C	Soaked	Control	43.10^{gh} ± 3.32	25.73^{d} ± 2.97
			Light	24.67^{c} ± 3.23	14.63^{b} ± 1.15
			Medium	12.37^{b} ± 0.78	13.77^{b} ± 1.16
			Dark	6.63^{a} ± 1.67	3.70^{a} ± 0.98
		Non-soaked	Control	45.90^{h} ± 2.29	31.80^{e} ± 3.58
			Light	33.17^{def} ± 4.16	16.37^{b} ± 3.14
			Medium	16.20^{b} ± 2.17	12.73^{b} ± 1.94
			Dark	6.73^{a} ± 1.07	4.27^{a} ± 0.55
12	15 °C	Soaked	Control	37.70^{fg} ± 0.85	23.27^{cd} ± 1.32
			Light	27.57^{cd} ± 5.49	23.23^{cd} ± 1.1
			Medium	13.57^{b} ± 2.15	13.73^{b} ± 1.46
			Dark	5.83^{a} ± 1.78	3.07^{a} ± 0.74
		Non-soaked	Control	45.83^{h} ± 3.01	32.17^{e} ± 2.75
			Light	33.87^{ef} ± 7.45	22.57^{cd} ± 3.75
			Medium	17.53^{b} ± 0.7	14.33^{b} ± 1.82
			Dark	4.93^{a} ± 1.55	3.80^{a} ± 0.7
	30 °C	Soaked	Control	41.30^{gh} ± 1.41	24.67^{cd} ± 2.47
			Light	27.90^{cd} ± 5.4	21.17^{c} ± 2.08
			Medium	13.43^{b} ± 1.07	12.97^{b} ± 0.78
			Dark	5.50^{a} ± 2.52	3.97^{a} ± 0.72
		Non-soaked	Control	43.80^{h} ± 6.79	32.40^{e} ± 1.21
			Light	34.80^{ef} ± 6.41	25.07^{cd} ± 3.12
			Medium	14.47^{b} ± 1.31	15.30^{b} ± 2.07
			Dark	5.57^{a} ± 2.75	4.00^{a} ± 0.75

All results are expressed as mean ± SD.
Values in the same column followed by different letter (a-h) differ significantly at a $p<0.05$ level.
* Green coffee

Results in Table 4.10 (time 0) show that the highest level of CFT and KWL were recorded in green coffee. The lowest drying temperature for parchment coffee resulted in the highest value of both compounds. Levels of both compounds decrease as roasting degree become higher. For each roasting level pre-treatments have made little different to the content of CFT and KWL (Table 4.11). Storage conditions of parchment coffee also had little effect on these compounds.

A similar study on CFT and KWL in Arabica coffee was performed by Sridevi *et al*. (2011). Their results agreed with those of the present study.

4.5 The effects of pre treatment, drying conditions, storage conditions and roasting degree on antioxidant activities

4.5.1 Effects of different pre-treatments, drying conditions and roasting degree on total phenolic compounds

The effects of pre-treatments, drying conditions and roasting degree on TPC content are shown in Table 4.12

Table 4.12 Total phenolic compounds content in coffee samples subjected to different pre-treatments, drying conditions and roasting degree.

Pre treatment	Drying temperature	Roasting degree	mg GAE/g DW		
Soaked	40 °C	Control*	67.32^{n}	±	5.22
	50 °C	Control	60.94^{l}	±	0.78
	60 °C	Control	58.75^{kl}	±	0.33
	70 °C	Control	56.08^{j}	±	0.53
Non-soaked	40 °C	Control	72.86^{o}	±	0.61
	50 °C	Control	63.60^{m}	±	0.51
	60 °C	Control	60.55^{l}	±	0.83
	70 °C	Control	57.99^{jk}	±	0.64
Soaked	40 °C	Light	96.61^{rs}	±	1.51
	50 °C	Light	92.97^{q}	±	0.63
	60 °C	Light	87.65^{p}	±	1.40
	70 °C	Light	73.93^{o}	±	0.51
Non-soaked	40 °C	Light	98.81^{s}	±	0.82
	50 °C	Light	94.87^{qr}	±	0.44
	60 °C	Light	89.33^{p}	±	0.70
	70 °C	Light	75.25^{o}	±	1.58
Soaked	40 °C	Medium	40.13^{h}	±	3.77
	50 °C	Medium	32.18^{fg}	±	1.94
	60 °C	Medium	28.40^{e}	±	0.16
	70 °C	Medium	23.52^{d}	±	1.12
Non-soaked	40 °C	Medium	44.10^{i}	±	0.96
	50 °C	Medium	34.27^{g}	±	0.65
	60 °C	Medium	31.14^{f}	±	0.88
	70 °C	Medium	25.46^{d}	±	1.06
Soaked	40 °C	Dark	7.07^{ab}	±	1.59
	50 °C	Dark	7.77^{b}	±	0.21
	60 °C	Dark	6.73^{ab}	±	0.11
	70 °C	Dark	4.767^{a}	±	0.60
Non-soaked	40 °C	Dark	11.98^{c}	±	0.11
	50 °C	Dark	7.97^{b}	±	0.18
	60 °C	Dark	6.46^{ab}	±	0.21
	70 °C	Dark	5.16^{a}	±	0.12

All results are expressed as mean ± SD.
Values in the same column followed by different letters (a-h) differ significantly at $p<0.05$ level.
* Green coffee

4.5.2 Effects of pre treatments, storage conditions and time and roasting degree, on total phenolic compound content

Table 4.13 shows the effects of pre-treatments, storage condition and time and roasting degree on TPC content in coffee

Table 4.13 Total phenolic compounds (TPC) content in coffee samples subjected to different storage conditions and time as well as different roasting degree.

Storage time (months)	Storage condition	Pre-treatment	Roasting degree	mg GAE/g DW		
6	15 °C	Soaked	Control*	67.40^{hij}	±	1.85
			Light Roasting	95.59^{lm}	±	0.54
			Medium Roasting	36.57^{e}	±	0.55
			Dark Roasting	9.74^{bc}	±	0.54
		Non-soaked	Control	68.78^{j}	±	0.85
			Light Roasting	96.14^{m}	±	0.92
			Medium Roasting	38.98^{f}	±	0.89
			Dark Roasting	10.84^{c}	±	0.99
	30 °C	Soaked	Control	65.05^{g}	±	0.90
			Light Roasting	94.09^{kl}	±	0.50
			Medium Roasting	36.69^{e}	±	0.67
			Dark Roasting	9.22^{b}	±	0.88
		Non-soaked	Control	67.90^{ij}	±	0.41
			Light Roasting	95.09^{lm}	±	0.66
			Medium Roasting	36.53^{e}	±	1.08
			Dark Roasting	10.05^{bc}	±	0.32
12	15 °C	Soaked	Control	66.53^{hi}	±	0.86
			Light Roasting	94.20^{kl}	±	0.72
			Medium Roasting	34.76^{d}	±	0.42
			Dark Roasting	7.06^{a}	±	0.46
		Non-soaked	Control	68.66^{j}	±	0.59
			Light Roasting	95.25^{lm}	±	0.84
			Medium Roasting	36.81^{e}	±	1.46
			Dark Roasting	8.86^{b}	±	0.71
	30 °C	Soaked	Control	66.05^{gh}	±	0.84
			Light Roasting	93.19^{k}	±	0.72
			Medium Roasting	33.83^{d}	±	0.88
			Dark Roasting	7.03^{a}	±	0.70
		Non-soaked	Control	68.11^{j}	±	0.18
			Light Roasting	94.72^{lm}	±	0.92
			Medium Roasting	36.27^{e}	±	1.32
			Dark Roasting	7.33^{a}	±	0.31

All results are expressed as mean ± SD. Values in the same column followed by different letter (a-h) differ significantly at a $p<0.05$ level.*Green coffee

The results in Table 4.12 show the highest TPC value in light roasted coffee. Non-soaked coffee gave little higher TPC value. The levels of TPC content were lower in green coffee, medium and dark roasted samples respectively. TPC content is sensitive to the drying temperature. In most cases the optimal drying temperature was 40 ^{o}C. According to Table 4.13 storage temperature had a marginal effect on TPC content, however shorted storage time provided slightly better results.

4.5.3 Effects of different pre-treatments, drying conditions and roasting degree on FRAP

The effects of pre-treatments drying conditions and roasting degree on FRAP are shown in Table 4.14

Table 4.14 FRAP values in coffee samples subjected to different pre-treatments, drying conditions and roasting degree.

Pre-treatment	Drying temperature	Roasting degree	µMol Trolox/gDW		
Soaked	40 °C	Control*	641.81^{k}	±	39.86
	50 °C	Control	446.09^{e}	±	11.57
	60 °C	Control	382.49^{c}	±	19.17
	70 °C	Control	327.11^{a}	±	8.57
Non-soaked	40 °C	Control	1267.44^{s}	±	35.61
	50 °C	Control	845.04^{p}	±	6.73
	60 °C	Control	795.68^{o}	±	4.25
	70 °C	Control	504.00^{g}	±	10.57
Soaked	40 °C	Light	847.27^{p}	±	20.35
	50 °C	Light	739.88^{n}	±	18.36
	60 °C	Light	546.16^{h}	±	3.93
	70 °C	Light	418.11^{d}	±	17.52
Non-soaked	40 °C	Light	673.11^{m}	±	18.51
	50 °C	Light	556.32^{hi}	±	6.00
	60 °C	Light	447.49^{e}	±	18.41
	70 °C	Light	340.27^{a}	±	19.13
Soaked	40 °C	Medium	848.07^{p}	±	3.07
	50 °C	Medium	669.84l^{m}	±	7.15
	60 °C	Medium	598.49^{j}	±	4.40
	70 °C	Medium	492.51^{fg}	±	2.81
Non-soaked	40 °C	Medium	1092.76^{r}	±	11.45
	50 °C	Medium	908.74^{q}	±	3.81
	60 °C	Medium	808.30^{o}	±	1.95
	70 °C	Medium	645.79^{kl}	±	14.52
Soaked	40 °C	Dark	756.32^{n}	±	2.59
	50 °C	Dark	558.35^{hi}	±	5.86
	60 °C	Dark	472.14^{f}	±	11.67
	70 °C	Dark	344.42^{ab}	±	4.09
Non-soaked	40 °C	Dark	572.83^{i}	±	22.55
	50 °C	Dark	484.37^{fg}	±	2.49
	60 °C	Dark	424.78^{de}	±	6.91
	70 °C	Dark	366.02^{bc}	±	11.62

All results are expressed as mean ± SD. Values in the same column followed by different letters (a-h) differ significantly at $p<0.05$ level.*Green coffee.

4.5.4 Effects of pre-treatments and storage conditions and time and roasting degree on FRAP

The effects of pre-treatments storage condition and time and roasting degree on FRAP value are show in Table 4.15.

Table 4.15 FRAP value in coffee samples subjected to different pre-treatments storage conditions and time as well as different roasting degrees.

Storage time (months)	Storage condition	Pre-treatment	Roasting degree	uMol TE/gDW		
6	15 °C	Soaked	Control*	629.33^{bcd}	±	11.59
			Light Roasting	1075.33^{g}	±	57.57
			Medium Roasting	680.00^{def}	±	28.35
			Dark Roasting	538.00^{a}	±	17.35
		Non Soaked	Control	657.67^{cde}	±	14.22
			Light Roasting	1225.67^{h}	±	55.25
			Medium Roasting	669.33^{cdef}	±	58.00
			Dark Roasting	638.67^{cd}	±	33.62
	30 °C	Soaked	Control	632.33^{bcd}	±	12.66
			Light Roasting	1125.00^{g}	±	103.44
			Medium Roasting	635.00^{cd}	±	19.47
			Dark Roasting	547.33^{a}	±	46.93
		Non Soaked	Control	654.00^{cde}	±	13.75
			Light Roasting	1196.67^{h}	±	31.09
			Medium Roasting	728.67^{ef}	±	18.15
			Dark Roasting	607.33^{abcd}	±	19.66
12	15 °C	Soaked	Control	628.33^{bcd}	±	9.50
			Light Roasting	1093.67^{g}	±	41.04
			Medium Roasting	660.00^{cdef}	±	37.64
			Dark Roasting	555.33^{a}	±	34.79
		Non Soaked	Control	650.00^{cd}	±	4.36
			Light Roasting	1213.00^{h}	±	70.55
			Medium Roasting	733.00^{f}	±	17.35
			Dark Roasting	673.67^{cdef}	±	23.18
	30 °C	Soaked	Control	629.00^{bcd}	±	20.52
			Light Roasting	1088.33^{g}	±	72.23
			Medium Roasting	560.33^{ab}	±	31.90
			Dark Roasting	539.67^{a}	±	22.90
		Non Soaked	Control	659.33^{cdef}	±	20.31
			Light Roasting	1241.33^{h}	±	46.69
			Medium Roasting	667.33^{cdef}	±	25.77
			Dark Roasting	598.67^{abc}	±	27.02

All results are expressed as mean ± SD. Values in the same column followed by different letters (a-h) differ significantly at a $p<0.05$ level.
* Green Coffee

Results in Table 4.14 show the highest FRAP values in light roasted coffee. Non-soaked parchment coffee samples show slightly higher FRAP values. The levels of FRAP values were lower in green parchment coffee, medium and dark roasted respectively. FRAP value is sensitive to the drying temperature. In most cases the optimal drying temperature was 40 ^{o}C. According to Table 4.15, storage temperature had little effect on anti-oxidant activity expressed as TE content. However, a shorted storage time provided slightly better results.

4.5.5 Effects of pre-treatments, storage conditions and roasting degree on ORAC

Table 4.16 shows ORAC values in coffee sample subjected to different storage conditions and roasting degree.

Storage time (months)	Storage condition	Pre-treatment	Roasting degree	umol TE./gDW		
12	15 ^{o}C	Soaked	Control*	4209.46^{f}	±	70.16
			Light Roasting	9122.40^{i}	±	318.96
			Medium Roasting	3743.21^{cd}	±	183.15
			Dark Roasting	3528.30^{abc}	±	99.65
		Non-soaked	Control	4713.33^{g}	±	91.06
			Light Roasting	9392.33^{j}	±	49.08
			Medium Roasting	3852.00^{de}	±	44.71
			Dark Roasting	3669.67^{bcd}	±	173.58
	30 ^{o}C	Soaked	Control	4019.67^{ef}	±	36.75
			Light Roasting	8915.00^{h}	±	48.77
			Medium Roasting	3604.33^{bc}	±	57.59
			Dark Roasting	3392.33^{a}	±	48.64
		Non-soaked	Control	4072.67^{f}	±	43.29
			Light Roasting	9101.67^{hi}	±	72.39
			Medium Roasting	3715.33^{bcd}	±	41.24
			Dark Roasting	3502.00^{ab}	±	92.37

All results are expressed as mean ± SD. Values in the same column followed by different letter (a-h) differ significantly at a $p<0.05$ level.
* Green coffee

Results in Table 4.16 show the highest ORAC value in light roasted coffee. Non-soaked samples gave slightly higher ORAC value. The levels of ORAC values were lower in green coffee, medium and dark roasting respectively. ORAC value is also sensitive to the drying temperature. In most cases the optimal drying temperature was 40 ^{o}C.

4.6 Sensory evaluation

Out of total 81 panelists made up of staff and Food Science students of the School of Chemical Engineering, UNSW, 44 attended the first test and 37 attended the second test. According to Meilgaard *et a*l. (2006), the number of panelists used was adequate in order to get reliable sensory results (discrimination test and affective test).

4.6.1 Results of discrimination test

Discrimination test (triangle test) is based on the perceived difference between two different products. The probability of correct judgment in this test is 1/3 (Stone and Sidel, 1993). The results of the triangle test are shown in Table 4.17

Table 4.17 Results of discrimination test (triangle test)

Total number of panelists	Correct Results	Incorrect Results
44	40	4
37	25	12

Considering Table 4.17, it appears that 40 and 25 out of 44 and 37 panellists respectively were able to differentiate the coffee samples.

4.6.2 Results of affective test

This experiment used brewed coffee to determine the roasting degree preferred by the consumer. Subsequently, the results were leading to the selection of the degree of roasting for further experiments.

Results from the effective test are shown in Figure 4.15 and 4.16.

A total of 5 attributes (aroma, taste, after taste, colour, overall) were examined.

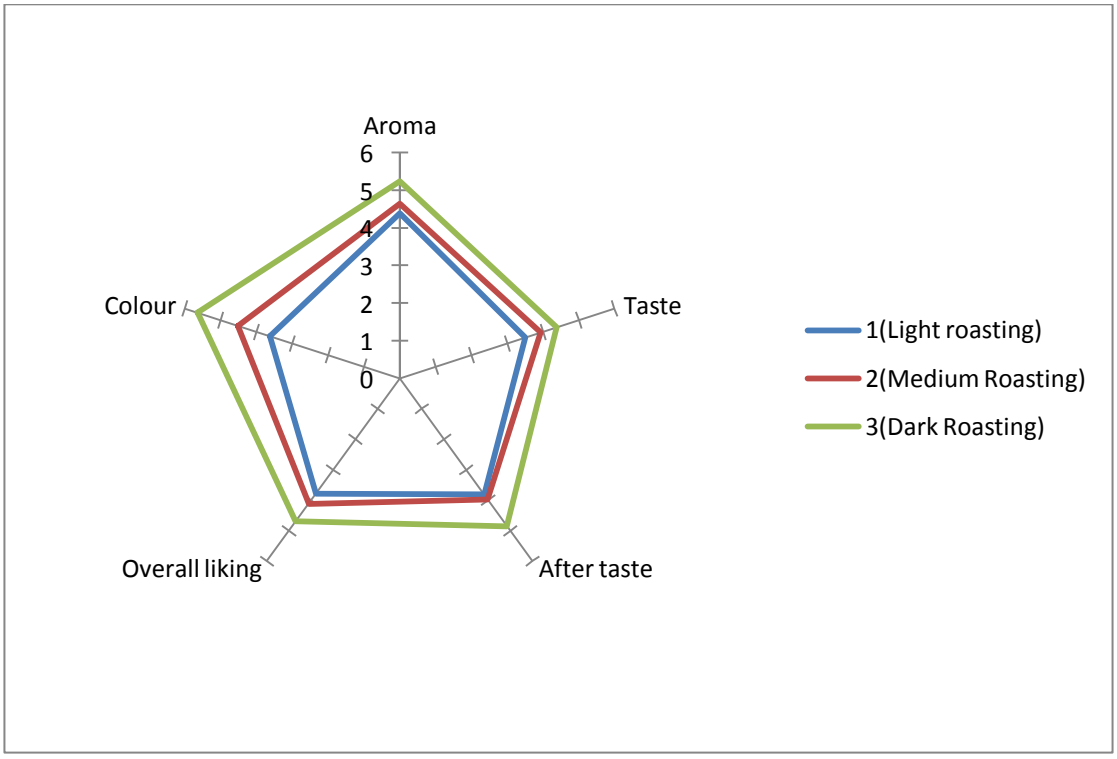


Figure 4.15 Compiled results from the affective test on effects different roasting degree of coffee beans.

Results show that dark roasting produced the strongest aroma. The after taste results for light and medium roasting were similar. Therefore, with regard to the after taste of light roasted and medium roasted coffee beans the results were similar. Hence, considering the score of three samples it stands out that the panelists preferred the dark roasted coffee. Out of the three samples dark roasting had the highest score.

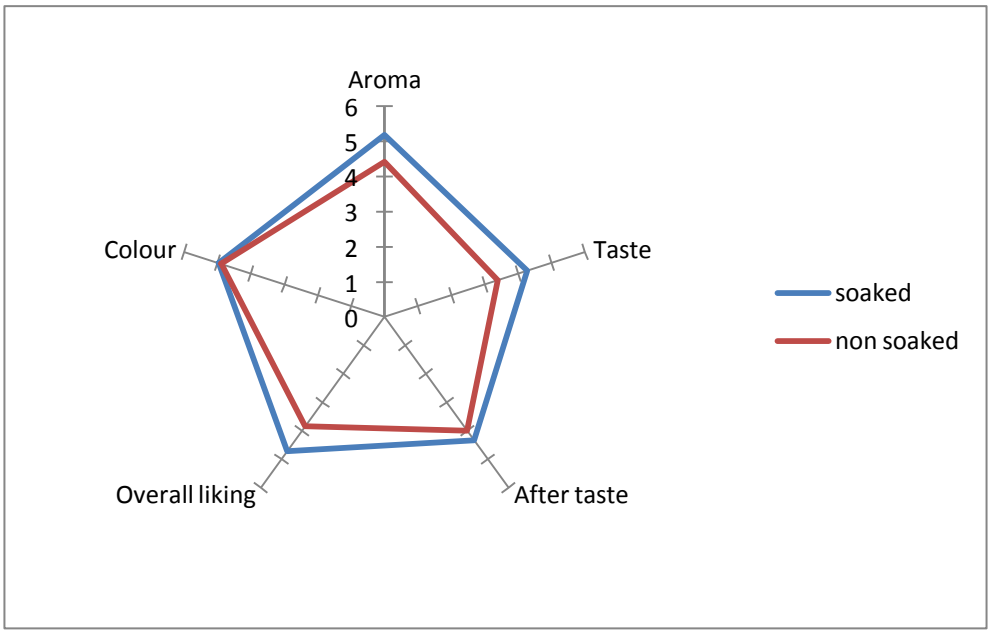


Figure 4.16 Compiled results from the affective test on effects of different pre-treatments on coffee beans using dark roasted coffee.

According to Figure 4.16 the soaked parchment coffee obtained the highest overall liking score. Both pre-treatments faired evenly in the after taste category. The soaked parchment coffee beans received the highest overall score.

ANOVA analysis was performed on all attributes to measure the preference between coffee samples. There was a significant difference between the level of roasting and pre-treatments. Therefore, the highest acceptance of panelists was for the soaked and dark roasted coffee.

Gonzalez-Rios *et al.* (2007) conducted aroma test on green coffee from different pre-treatments, namely mechanical mucilage removal (without soaking), fermentation in water, dry fermentation (using disc pulper) and dry fermentation (using vertical drum)

using GC. The results from this study show that coffee obtained after mechanical demucilaging (without soaking) received the lowest aroma score.

5. Conclusions and recommendations

Demucilaged soaked in water and non-soaked brewed coffee beans were used in this study in various drying conditions. Coffee samples were roasted at different temperatures. All processing was carried out in order to maximise product quality in terms of bioactive compounds (CGA, CFE, TGL, CFT, KWL and VitE) and antioxidant activities.

This study has found that, generally, the soaked demucilaged parchment coffee beans bring about a shorter drying time than the non-soaked demucilaged parchment coffee beans. This is probably due to some remaining mucilage has covering parchment coffee beans.

High drying temperature (70 ^{o}C for 3 h, 60 ^{o}C for 6 h and 50 ^{o}C for 8 h until moisture content reaches 11-12% w.b.) resulted in shorter drying time and the loss of CGA, CFE, TGL, CFT, KWL, VitE and anti-oxidants as compared to low temperature drying at 40 ^{o}C for 16 h.

Investigations into storage conditions showed that storage at 15 ^{o}C can preserve all selected compounds and antioxidants. This can be attributed mainly to lower temperature, which reduce the risk of compound degradation caused by heat.

One of the more significant findings from this study is that light roasting degree resulted in the highest level of all compounds and anti- oxidant activity. This may be due to the optimum temperature profile which increases and breaks down the bonds of

compounds from unroasted coffee. In contrast, this level of roasting did not degrade compounds in the way medium roasting and dark roasting did.

Finally, sensory evaluation has shown that consumers like dark roasted coffee beans that had been soaked in water (to get rid of all mucilage) before the drying process.

The following recommendations could be suggested:

- The nano-filtration for coffee extract could be applied at lab scale. The nano-filtration is likely to influence the process parameters such as recirculation flow rate, pressure and temperature, thus leading to appropriate flux and total solid rejection.
- The microencapsulation of green coffee extracts could be applied for their use as health products.
- The colour and roasting degree data logging software could be used in order to assure a constant quality of roasted coffee.

6. References

Abel, E. L., Hendrix, S. O., McNeeley, S. G., Johnson, K. C., Rosenberg, C. A., Mossavar-Rahmani, Y., Vitolins, M. and Kruger, M., 2007. Daily coffee consumption and prevalence of nonmelanoma skin cancer in Caucasian women. European Journal of Cancer Prevention, vol 16(5):pp 44-452.

Ainsworth, E. A. and Gillespie, K. M., 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nature Protocols, vol 2(4):pp 875-878.

Alessandrini, L., Romani, S., Pinnavaia, G. and Rosa, M. D., 2008. Near infrared spectroscopy: An analytical tool to predict coffee roasting degree. Analytica Chimica Acta, vol 625:pp 95–102.

Alonso-Salces, R. M., Serra, F., Reniero, F. and Berger, K. R. H., 2009. Botanical and Geographical Characterization of Green Coffee (*Coffea arabica* and *Coffea canephora*): Chemometric Evaluation of Phenolic and Methylxanthine Contents. Journal of Agricultural and Food Chemistry, vol 57:pp 4224–4235.

Alves, R. C., Casal, S. and Oliveira, M. B. P. P., 2009a. Determination of Vitamin E in Coffee Beans by HPLC Using a Micro-extraction Method. Food Science and Technology International, vol 15(57):pp 57-63.

Alves , R. C., Casal, S. and Oliveira, M. B. P. P., 2009b. Tocopherols in espresso coffee: Analytical method development and validation. Food Chemistry, vol 115:pp 1549–1555.

Alves, R. C., Casal, S. and Oliveira, M. B. P. P., 2010. Tocopherols in coffee brews : influence of coffee species, roast degree and brewing procedure. Journal of Food Composition and Analysis, vol 23:pp 802-808.

Amir, E. J., Grandegger, K., Esper, A., Sumarsono, M., Djaya, C. and Muhlbauer, W., 1991. Development of a Multi-purpose Solar Tunnel Dryer for Use in Humid Tropics. Renewable Energy, vol 1(2):pp 167 176.

Arendash, G. W., Schleif, W., Rezai-Zadeh, K., Jackson, E. K., Zacharia, L. C.,

Cracchiolo, J. R., Shippy, D. and Tan, J., 2006. Caffeine Protects Alzheimer's Mice Against Cognitive Impairment and Reduces Brain-Amyloid Production. Neuroscience, vol 142:pp 941-952.

Arya, M. and Rao, L. J. M., 2001. An Impression of Coffee Carbohydrates. Critical Reviews in Food Science and Nutrition, vol 47:pp 51-67.

Ascherio, A., Weisskopf, M. G., O'Reilly, E. J., McCullough, M. L., Calle, E. E., Rodriguez, C. and Thun, M. J., 2004. Coffee Consumption, Gender, and Parkinson's Disease Mortality in the Cancer Prevention Study II Cohort: The Modifying Effects of Estrogen. American Journal of Epidemiology, vol 160(10):pp 977-984.

Avallone, S., Guiraud, J.-P., Guyot, B., Olguin, E. and Brillouet, J.-M., 2000. Polysaccharide Constituents of Coffee-Bean Mucilage. Journal of Food Science, vol 65(8):pp 1308-1311.

Avallone, S., Guiraud, J.-P., Guyot, B., Olguin, E. and Brillouet, J.-M., 2001. Fate of Mucilage Cell Wall Polysaccharides during Coffee Fermentation. Journal of Agricultural and Food Chemistry, vol 49:pp 5556-5559.

Belitz, H. B. G., W. , 1988. Quimica de los alimentos. . Zaragoza: , Editorial Acribia S.A.

Benzie, I. F. F. and Strain, J. J., 1996. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of ''Antioxidant Power'': The FRAP Assay. Analytical Biochemistry, vol 239:pp 70-76.

Bicho, N. C. e. a., 2011. Identification of nutritional descriptors of roasting intensity in beverages of Arabica and Robusta coffee beans. International Journal of Food Sciences and Nutrition, vol 62(8):pp 865-871.

Bicho , N. C. L., A.,E. ; RAmalho,J., C.; and Lidon, F.,C.. 2012. Use of colour parameters for roasted coffee assessment. Food Science and Technology (Campinas), vol 32(3):pp 436-442.

Bita, M. G. and Preda, M., 2005. The Effect of Temperature and roasting degree on the total phenolic content of coffee brews. Scientific Study & Research, vol 6(2):pp 239-242.

Brennan, C., 2012. From Cherry to Cup: Operations Case Studies in the Coffee Industry. Wollongong's coffee cherry harvest. Accessed Date 29 June 2013, from http://www.hscbusinessresources.com.au/coffeecasestudies/?p=350.

Butt, M. S., Nazirb, A., Sultana, M. T. and Schroën, K., 2008. *Morus alba* L. nature's functional tonic. Trends in Food Science & Technology, vol 19:pp 505-512.

Butt, M. S. and Sultan, M. T., 2011. Coffee and its Consumption: Benefits and Risks. Critical Reviews in Food Science and Nutrition, vol 51:pp 363–373.

Cao, G., Alessio, H. M. and Cutler, R. G., 1993. Oxygen-radical Absorbance Capacity Assay for Antioxidants. Free Radical Biology and Medicine, vol 14:pp 303-311.

Castillo, M. D., Ames, J. M. and Gordon, M. H., 2002. Effect of Roasting on the Antioxidant Activity of Coffee Brews. Journal of Agricultural and Food Chemistry, vol 50:pp 3698-3703.

Chade, A. R., Kasten, M. and Tanner, C. M., 2006. Nongenetic causes of Parkinson's disease. Journal of Neural Transmission, vol 70:pp 147–151.

Cirovelásquez, H. J., Abudcano, L. C. and Pérezalegría, L. R., 2010. Numerical Simulation of Thin layer Coffee Drying by Control Volumes. Dyna, vol 77(163):pp 270-278.

Clarke, R. J., 1987. Roasting and Grinding. Coffee. Clarke, R. J. and Macrae, R. 2**:** 321.

Corrêa, P. C., Resende, O. and Ribeiro, D. M., 2006. Drying Characteristics and Kinetics of Coffee Berry. Revista Brasileira de Produtos Agroindustriais, vol 8(1):pp 1-10.

Dias, R. C. E., Campanha, F. G.-A., Vieira, L. G. E., Ferreira, L. P., Pot, D., Marraccini, P. and Benassi, M. D. T., 2010. Evaluation of Kahweol and Cafestol in Coffee Tissues and Roasted Coffee by a New High-Performance Liquid Chromatography Methodology. Journal of Agricultural and Food Chemistry, vol 58:pp 88-93.

Dick, R. H., 1990. Coffee and tea. Official Method of the Association of Official Analytical Chemists. Helrich, K. (ed),Virginia, USA, AOAC. 2.

Djordjevic, T. M., Šiler-Marinkovic, S. S. and Dimitrijevic-Brankovic, S. I., 2011. Antioxidant Activity and Total Phenolic Content in Some Cereals and Legumes. International Journal of Food Properties, vol 14:pp 175–184.

Eggers, R. and Pietsch, A., 2001. Technology I: Roasting. Coffee Recent Developments. Clarke, R. J. and Vitzthum, O. G. (ed), Blackwell Science.

Fellows, P. J., 2002. Food processing technology. . New York, CRC Press.

Folstar, P., van der Plas, H. C., Pilnik, W. and de Heus, J. G., 1977. Tocopherols in the Unsaponifiable Matter of Coffee Bean Oil. Journal of Agricultural and Food Chemistry, vol 25(2):pp 283-285.

Franca, A. S., Mendonca, J. C. F. and Oliveira, S. D., 2005. Composition of green and roasted coffees of different cup qualities. LWT-Food Science and Technology, vol 38:pp 709-715.

Franca, A. S., Oliveira, L. S., Oliveira, R. C. S., Agrest, P. C. M. and Augusti, R., 2009.

A preliminary evaluation of the effect of processing temperature on coffee roasting degree assessment. Journal of Food Engineering, vol 92:pp 345–352.

Fujioka, K. and Shibamoto, T., 2008. Chlorogenic acid and caffeine contents in various commercial brewed coffees. Food Chemistry, vol 106:pp 217–221.

George, S. E., Ramalakshmi, K. and Rao, L. J. M., 2008. A Perception on Health Benefits of Coffee. Critical Reviews in Food Science and Nutrition, vol 48: pp 464-486.

Gilliland, L. U., Magallanes-Lundback, M., Hemming, C., Supplee, A., Koornneef, M., Bentsink, L. and Penna, D. D., 2006. Genetic basis for natural variation in seed vitamin E levels in *Arabidopsis thaliana*. Proceedings of the National Academy of Sciences of the United States of America, vol 103(49):pp 18834-18841.

Ginz, M., Balzer, H. H., Bradbury, A. G. W. and Maier, H. G., 2000. Formation of aliphatic acids by carbohydrate degradation during roasting of coffee. European Food Research and Technology, vol 211:pp 404–410.

Glazer, A. N., 1990. Phycoerythrin fluorescence-based assay for reactive oxygen species. Methods in Enzymology, vol 186:pp 161–168.

Gonzalez-Rios, O., Suarez-Quiroza, M. L., Boulangerb, R., Barelb, M., Guyotb, B., Guiraudc, J. P. and Galindo, S. S., 2007. Impact of 'ecological' post-harvest processing on the volatile fraction of coffee beans: I. Green coffee. Journal of Food Composition and Analysis, vol 20:pp 289-296.

Greenberg, J. A., Boozer, C. N. and Geliebter, A., 2006. Coffee, diabetes, and weight control. The American Journal of Clinical Nutrition, vol 84:pp 682-693.

Hammond, E. W., 2003. Vegetable oils – composition and analysis. Encyclopedia of food sciences and nutrition. Caballero, B. and Trugo, L. C. F., P. M. (Eds.). United Kingdom, Elsevier Science Ltd.,.

Heilmann, W., 2001. Decaffeination of Coffee. Coffee recent developments. Clarke, R. J. and Vitzthum. England,
New York, Blackwell Science Ltd.

Illy, A. and Viani, R., 1995. Espresso Coffee: The Chemistry of Quality. London, Academic Press.

International Coffee Organisation, 2009. About Coffee. Botanical Aspects. Accessed Date 11 June 2010, from http://www.ico.org/botanical.asp#bot.

Jaiswal, R. and Kuhnert, N., 2010. Hierarchical scheme for liquid chromatography/multi-stage spectrometric identification of 3,4,5-triacyl chlorogenic acids in green Robusta coffee beans. Rapid Communications : In Mass Spectrometry, vol 24:pp 2283–2294.

Jham, G. N., Winkler, J. K., Berhow, M. A. and Vaughn, S. F., 2007. γ -Tocopherol as

a Marker of Brazilian Coffee (*Coffea arabica L.*) Adulteration by Corn. Journal of Agricultural and Food Chemistry, vol 55:pp 5995-5999.

Jokanović. M. R., Džinić, N. R., Cvetković, B. R., Gruzić, S. and Odžaković, B. 2012. Changes of Physical Properties of Coffee Beans During Roasting. Acta periodica technologica, vol 43:pp 21-31.

Kamal-Eldin, A., Görgen, S., Pettersson, J. and Lampib, A., 2000. Normal-phase high-performance liquid chromatography of tocopherols and tocotrienols. Comparison of different chromatographic columns. Journal of Chromatography A, vol 881:pp 217-117.

Kashaninejad, M., Mortazavi , A., Safekordi , A. and Tabil, L. G., 2007. Thin-layer drying characteristics and modeling of pistachio nuts. Journal of Food Engineering, vol 78:pp 98-108.

Kotsopoulos, J., Ghadirian, P., El-Sohemy, A., Lynch, H. T., Snyder, C., Daly, M., Domchek, S., Randall, S., Karlan, B., Zhang, P., Zhang, S., Sun, P. and Narod, S. A., 2007. The CYP1A2 genotype modifies the association between coffee consumption and breast cancer risk among BRCA1 mutation carriers. Cancer Epidemiology, Biomarkers and Prevention, vol 16(5):pp 912-916.

Ky, C. L., Noirot, M. and Hamon, S., 1997. Comparison of Five Purification Methods for Chlorogenic Acids in Green Coffee Beans (*Coffea* sp.). Journal of Agricultural and Food Chemistry, vol 45:pp 786-790.

Larsson, S. C., Männistö, S., Virtanen, M. J., Kontto, J., Albanes, D. and Virtamo, J., 2008. Coffee and Tea Consumption and Risk of Stroke Subtypes in Male Smokers. Journal of the America Heart Association, vol 39:pp 1681-1687.

Lee, K. J., Choi, J. H. and Jeong, H. G., 2007. Hepatoprotective and antioxidant effects of the coffee diterpenes kahweol and cafestol on carbon tetrachloride-induced liver damage in mice. Food and Chemical Toxicology, vol 45:pp 2118-2125.

Lindsay, J., Laurin, D., Verreault, R., Hébert, R., Helliwell, B., Hill, G. B. and McDowell, I., 2002. Risk Factors for Alzheimer's Disease: A Prospective Analysis from the Canadian Study of Health and Aging. American Journal of Epidemiology, vol 156(5):pp 445-453.

López-Galilea, I., Peña, M. P. d. and Cid, C., 2006. Correlating chemical compounds with antioxidant capacity in torrefacto roast coffees. . Proceedings of the 21st International Conference on Coffee Science. Montpellier, France. vol: pp.

Luo, J., Butelli, E., Hill, L., Parr, A., Niggeweg, R., Bailey, P., Weisshaar, B. and Martin, C., 2008. AtMYB12 regulates caffeoyl quinic acid and flavonol synthesis in tomato: expression in fruit results in very high levels of both types of polyphenol. The plant journal, vol 56:pp 316-326.

Marsh, A. and Chapman, K., 2006. Lao Coffee Industry: Evaluation of VINACAFÉ pulper/demucilager. Research Experimentation Centre Lao PDR.

Meilgaard, M. C., Civille, G. V. and Carr, B. T., 2006. Sensory evaluation techniques. CRC Press.

Mermelstel, N. H., 2010. Dealing with Antioxidant Assay Issues. Food Technology. Chicago. vol 64: pp 72-76.

National Coffee Association of USA, 2012. Ten Steps to Coffee. Accessed Date 11 June 2012, from http://www.ncausa.org/i4a/pages/index.cfm?pageid=69.

Oba, S., Shimizu, N., Nagata, C., Shimizu, H., Kametani, M., Takeyama, N., Ohnuma, T. and Matsushita, S., 2006. The relationship between the consumption of meat, fat, and coffee and the risk of colon cancer: A prospective study in Japan. Cancer Letter, vol 244:pp 260-267.

Oliveros, C. E. and Gunasekaran, S., 1995. Rheological Characterization of Coffee Mucilage. Journal of Food Process Engineering, vol 19(3):pp 331-342.

Ou, B., Hampsch-Woodill, M. and Prior, R. L., 2001. Development and Validation of an Improved Oxygen Radical Absorbance Capacity Assay Using Fluorescein as the Fluorescent Probe. Journal of Agricultural and Food Chemistry, vol 49: pp 4619-4626.

Page, G. E., 1949. Factors influencing the maximum rates of air drying shelled corn in thin layers. Purdue University. Master Thesis.

Palacios-Cabrera , H., Taniwaki , M. H., Menezes , H. C. and Iamanaka, B. T., 2004. The production of ochratoxin A by Aspergillus ochraceus in raw coffee at different equilibrium relative humidity and under alternating temperatures. Food Control, vol 15:pp 531-535.

Perrone, D., Farah , A., Donangelo , C. M., Paulis , T. and Martin, P. R., 2008a Comprehensive analysis of major and minor chlorogenic acids and lactones in economically relevant Brazilian coffee cultivars. Food Chemistry, vol 106 :pp 859-867.

Perrone, D., Donangelo, C. M. and Farah, A., 2008b. Fast simultaneous analysis of caffeine, trigonelline, nicotinic acid and sucrose in coffee by liquid chromatography–mass spectrometry. Food Chemistry, vol 110:pp 1030-1035.

Prior, R. L., Wu, X. and Schaich, K., 2005. Standardized Methods for the Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements. Journal of Agricultural and Food Chemistry, vol 53 :pp 4290-4302.

Ramalakshmi, K., Hithamani, G., Asha, K. R. and Rao, L. J. M., 2011. Separation and characterisation of chlorogenic acid-rich conserves from green coffee beans and their radical scavenging potential. International Journal of Food Science and Technology, vol 46:pp 109–115.

Roa, G., Oliveros, C. E., Alvarez, J., Sanz, J. R., Ramirez, C. A., Dávila, M. T., Alvarez, J. R., Zambrano, D. A., Puerta, G. I. and Rodriguez, N., 1997. Ecological Processing of Coffee at the Farm Level. Centro Nacional De Investigaciones De Café, Pedro Urobe Mejia, Proceedings of the Annual Conference of the Hawaii Coffee Association 25-26 July 1997, Kona (Hawaii).

Rodrigues, C. I., Marta, L., Maia, R., Miranda, M., Ribeirinho, M. and Ma´guas, C., 2007. Application of solid-phase extraction to brewed coffee caffeine and organic acid determination by UV/HPLC. Journal of Food Composition and Analysis, vol 20:pp 440–448.

Sánchez-Hernández, L., Puchalska, P., Garci´a-Ruiz, C., Crego, A. L. and Marina, M. L., 2010. Determination of Trigonelline in Seeds and Vegetable Oils by Capillary Electrophoresis as a Novel Marker for the Detection of Adulterations in Olive Oils. Journal of Agricultural and Food Chemistry, vol 58 :pp 7489–7496.

Shearer, J., Sellars, E. A., Graham, T. E. and Wasserman, D. H., 2007. Effects of chronic coffee consumption on glucose kinetics in the conscious rat. Canadian Journal of Physiology and Pharmacology, vol 85:pp 823-830.

Silva, C. F., Batista, L. R., Abreu, L. M., Dias, E. S. and Schwan, R. F., 2008. Succession of bacterial and fungal communities during natural coffee (*Coffeaarabica*) fermentation. Food Microbiology, vol 25:pp 951–957.

Silva, C. F., Schwan, R. F., Dias, E. S. and Wheals, A. E., 2000. Microbial diversity during maturation and natural processing of coffee cherries of Coffea arabica in Brazil. International Journal of Food Microbiology, vol 60:pp 251–260.

Singh, R. P. and Heldman, D. R., 2001. Introduction to Food Engineering. San Diego, Academic Press.

Singleton, V. L., Joseph, A. and Rossi, J. J., 1965. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. American Journal of Enology and Viticulture, vol 16(3):pp 144-158.

Singleton, V. L., Orthofer, R. and Lamuela-Raventós, R. M., 1999. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. Methods in Enzymology, vol 299:pp 152-178.

Sivetz, M. and Desrosier, N., 1979. Coffee Technology. Westport Connecticut, The AVI publishing company.

Speer, K. and Kölling-Speer, I., 2001. Lipids. Coffee Recent Developments. Clarke, R. and Vitzthum, O. G. Great Britain, Blackwell Science Ltd,.

Sridevi, V., Giridhar, P. and Ravishankar, G. A., 2011. Evaluation of Roasting and Brewing effect on Antinutritional Diterpenes-Cafestol and Kahweol in Coffee. Global Journal of Medical research, vol 11(5):pp 17-22.

Stone, H. and Sidel, J. L., 1993. Sensory Evaluation Practices. San Diego, Acedemic

Press Inc.

Toci, A. T., Benedetti, M. and Farah, A., 2008. New volatile compounds as Brazilian defective coffee seeds' markers. . 22nd International Conference on Coffee Science, ASIC 2008. Campinas, SP, Brazil, . vol: pp 263-269.

Tohda, C., Kuboyama, T. and Komatsu, K., 2005. Search for Natural Products relate d to Regeneration of the Neuronal Network. Neurosignals, vol 14:pp 34-45.

Trugo, L. C. and Macrae, R., 1984. A Study of the Effect of Roasting on the Chlorogenic Acid Composition of Coffee using HPLC. Food Chemistry, vol 15:pp 219-227.

van Dam, R. M., 2003. The epidemiology of lifestyle and risk for type 2 diabetes. European Journal of Epidemiology, vol 18:pp 1115-1125.

van Dijk, A. E., Olthof, M. R., Meeuse, J. C., Seebus, E., Heine, R. J. and van Dam, R. M., 2009. Acute Effects of Decaffeinated Coffee and the Major Coffee Components Chlorogenic Acid and Trigonelline on Glucose Tolerance. Diabetes Care, vol 32(6):pp 1023-1025.

Viani, R., 1985. Physiologically Active Subsatances in Coffee. Coffee. Clarke, R. J. and Macrae, R. England, New York, Elsevier Applied Science Publ. Ltd. 3..

Vilela, D. M., Pereira, G. V. M., Silva, C. F., Batista, L. R. and Schwan, R. F., 2010. Molecular ecology and polyphasic characterization of the microbiota associated with semi-dry processed coffee (*Coffea arabica L.*). Food Microbiology, vol 27:pp 1128-1135.

Vincent, J., 1987. Green Coffee Processing. Coffee : Technology. Clarke, R. J. and Macrae, R. London, New York, Elsevier Applied Science. 2**:** 1-33.

Vossen, H. V., 2001. Agronomy I: Coffee Breeding Practices. Coffee recent developments. Clark, R. J. and Vitzthum, O. G. The United Kingdom, The Blackwell Science Ltd. 184-201.

Waterhouse, A. L., 2002. Determination of Total Phenolics. Current Protocols in Food Analytical Chemistry,

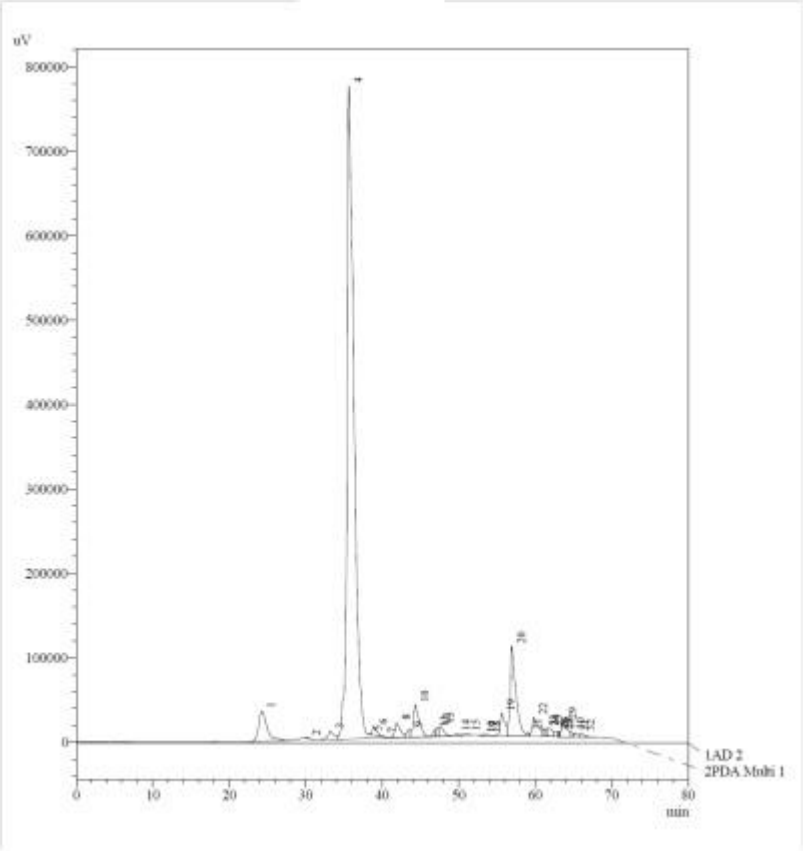
Weinberg, B. A. and Bealer, B. K., 2001. The World of Caffeine: The Science and Culture of the World's Most Popular Drug. New York, Routledge.

Wintgens, J., 2009. Coffee: Growing, Processing, Sustainable Production. Wintgens, J., Wiley.

Zhang, W. L., Lopez-Garcia, E., Li, T. Y., Hu, F. B. and van Dam, R. M., 2009. Coffee consumption and risk of cardiovascular events and all-cause mortality among women with type 2 diabetes. Diabetologia, vol 52:pp 810-817.

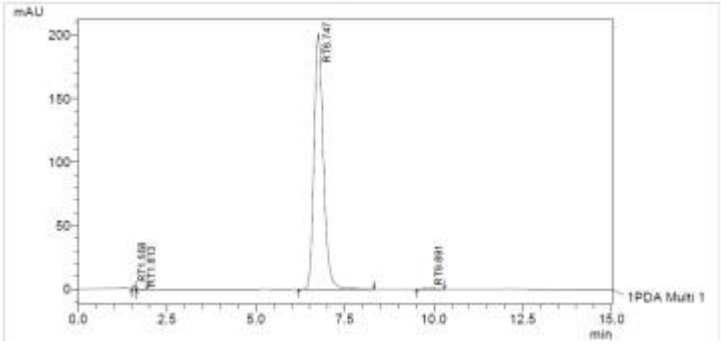
Zylbersztajn, D., Saes, M. S. M. and Cunha, C. F. D., 2008. Globalized Market and the Agro-Industrial System of the Coffee. International Conference on Coffee Science. Campinas, Brazil Association for Science and Information on Coffee (ASIC).

7. Appendices



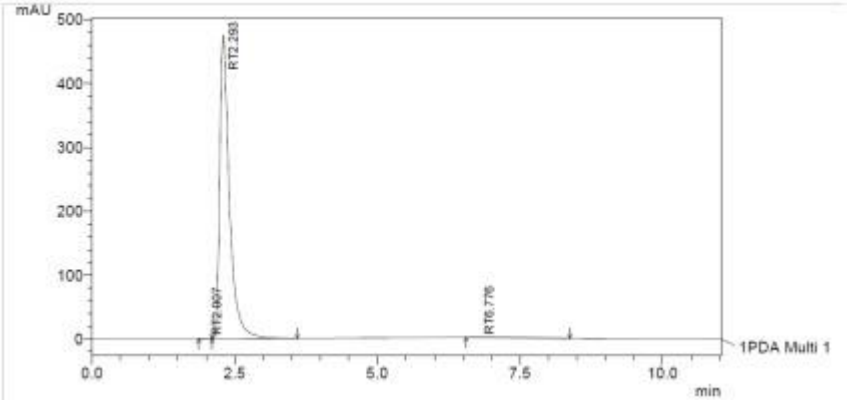
λ nm	Retention time (min)
325	35.617

Figure 7.1 Chromatogram of CGA (3-caffeoylquinic acid) obtained by HPLC



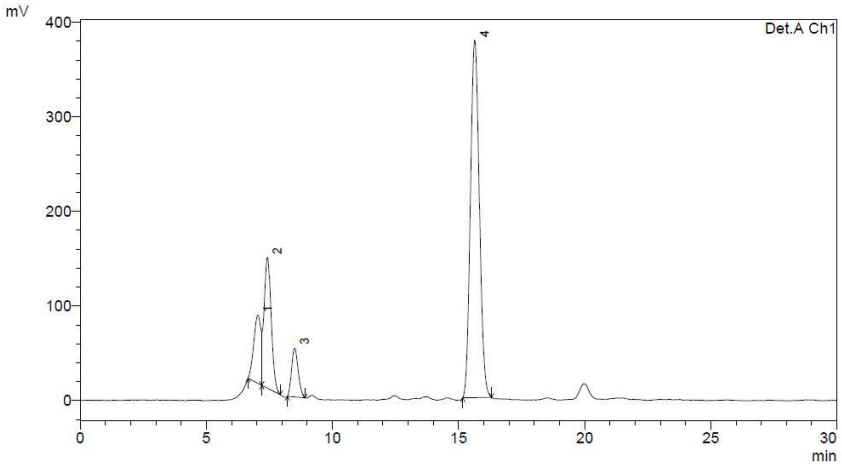
λ nm	Retention time (min)
273	6.747

Figure 7.2 Chromatogram of CFE obtained by HPLC



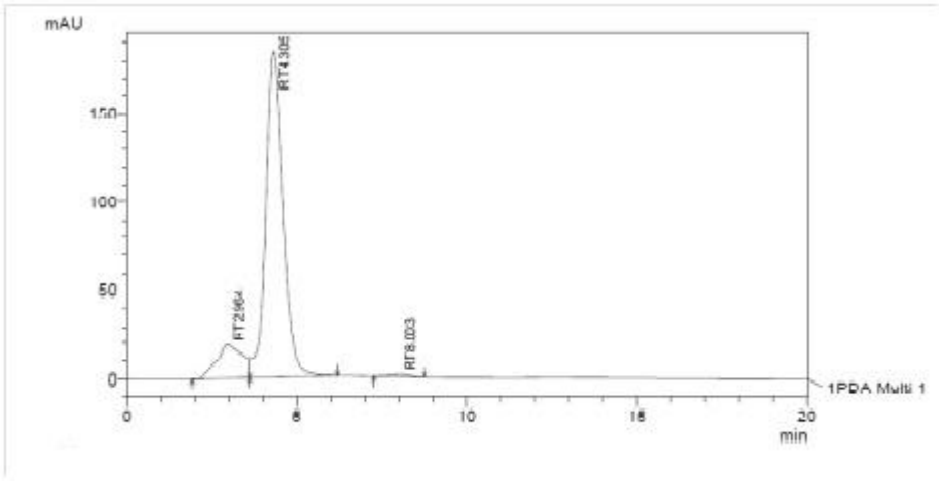
λ nm	Retention time (min)
138	2.293

Figure 7.3 Chromatogram of TGL obtained by HPLC



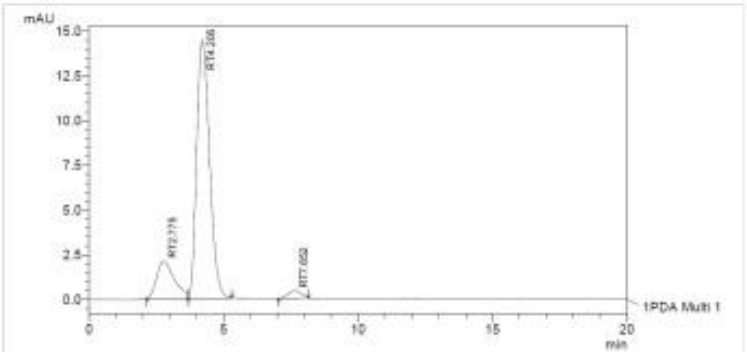
λ nm	Retention time (min)
290	15.22

Figure 7.4 Chromatogram of VitE obtained by HPLC



λ nm	Retention time (min)
230	4.306

Figure 7.5 Chromatogram of CFT obtained by HPLC



λ nm	Retention time (min)
290	4.206

Figure 7.6 Chromatogram of KWL obtained by HPLC

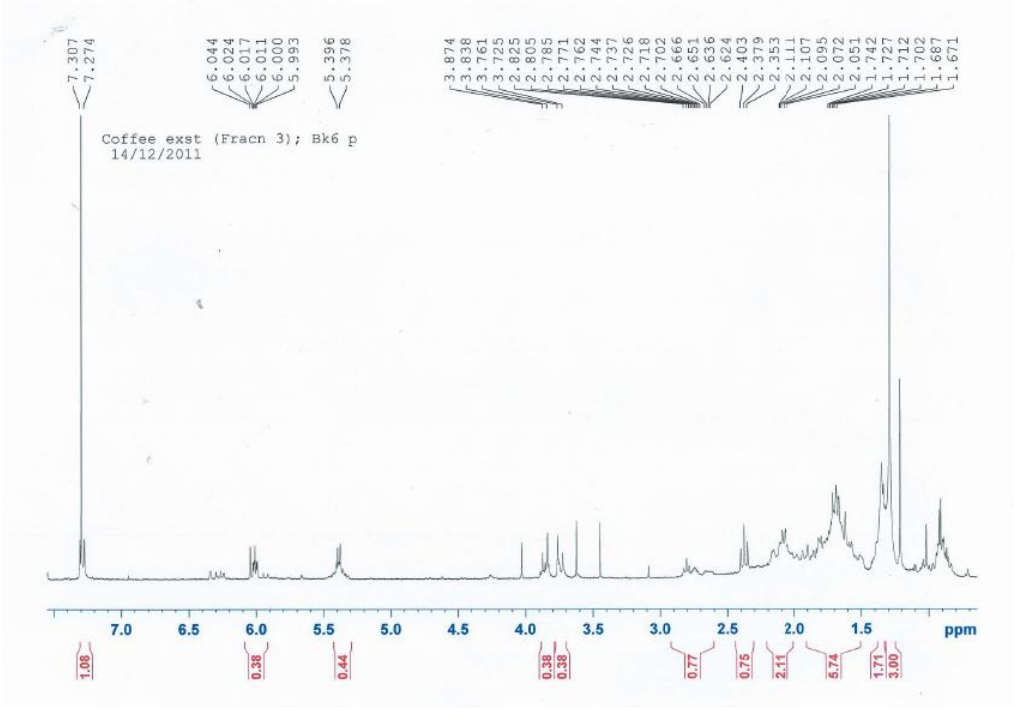


Figure 7.7 ^{1}H NMR spectra of CFT

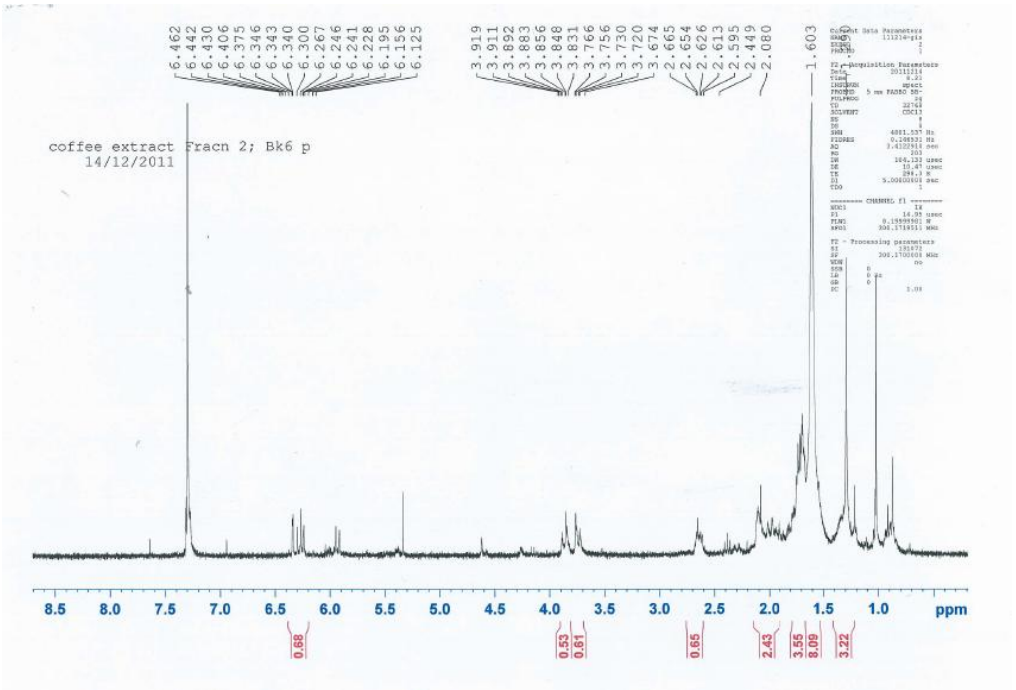


Figure 7.8 ^{1}H NMR spectra of KWL