EFFECT OF SELENIUM PRE-TREATMENT ON ACRYLAMIDE FORMATION, PHYSICOCHEMICAL AND SENSORIAL PROPERTIES OF ROASTED COFFEE VIA CONVENTIONAL AND SUPERHEATED STEAM

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By

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

3-APA	3-aminopropanamide
AOAC	Association of Official Agricultural Chemists
BI	browning index
$BMDL_{10}$	benchmark dose level
BW	body weight
CGA	chlorogenic acid
CON	Conventional
CUPRAC	cupric reducing antioxidant capacity
DPPH	2,2-diphenyl-1-picrylhydrazyl
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
EU	European commission regulation
FA	furfuryl alcohol
FC	Folin–Ciocalteu
FDA	U.S. Food and Drug Administration
FRAP	ferric reduction antioxidant power
GC-FID	gas chromatography-flame ionization detector
GSH-Px	glutathione peroxidase
HAT	hydrogen atom transfer
HMF	3,4-dideoxyosone and 5-hydroxymethylfurfural
IARC	International Agency for Research on Cancer
ICO	International Coffee Organization
ICP-MS	inductively coupled plasma-mass spectrometry
MRP	Maillard reaction products
RDA	recommended dietary allowance
SCAA	Specialty Coffee Association of America
Se	Selenium
SeCys	Selenocysteine
SeMet	Selenomethionine
SET	single electron transfer
SHS	superheated steam

SPE	solid phase extraction
TPTZ	2,4,6-tripyridyl-s-triazine
UL	tolerable upper intake levels

LIST OF APPENDICES

KESAN PRARAWATAN SELENIUM KE ATAS PEMBENTUKAN AKRALIMIDA, SIFAT FIZIKOKIMIA DAN SENSORI KOPI YANG DIPANGGANG KONVENSIONAL DAN PEMANASAN STIM LAMPAU

ABSTRAK

Selenium adalah mikronutrien penting yang mempunyai aktiviti antioksidan yang signifikan yang boleh mengurangkan pembentukan akrilamida semasa pemanggangan pada suhu tinggi. Kajian ini bertujuan untuk menyiasat potensi selenium organik sebagai bahan antioksida tambahan dalam pengurangan pembentukan akrilamida semasa pemanggangan kopi menggunakan kaedah panggangan konvensional dan pemanasan stim lampau, mengkaji pengambilan selenium oleh biji kopi hijau, dan menilai aktiviti antioksidan, sifat fizikokimia, dan sifat sensori biji kopi yang diperkaya dengan selenium (Se-coffee). Pertama, kesan suhu panggangan (220-240 °C) terhadap pembentukan akrilamida dinilai terhadap kaedah panggangan yang berbeza (konvensional dan pemanasan stim lampau) keatas kopi Arabica dan Robusta. Panggangan pemanasan stim lampau telah mengurangkan pembentukan akrilamida dengan signifikan ($p<0.05$) pada semua suhu (220-240 °C) sebanyak 14% hingga 37% berbanding dengan panggangan konvensional. (Pembentukan akrilamida adalah terendah pada suhu 240 °C pada 306.2-429.4 μg/kg untuk panggangan pemanasan stim lampau dan pada 359.2-632.4 μg/kg untuk panggangan konvensional). Kemudian, kesan prarawatan selenium terhadap pengekalan selenium, aktiviti antioksidan, dan pembentukan akrilamida semasa pemanggangan kopi pada suhu 240 °C dinilai untuk kedua-dua kaedah panggangan. Prarawatan selenium dilakukan dengan menghancur, merendam semalaman dalam kepekatan selenium yang berbeza (200 dan 400 μg/L) dan mengeringkan biji kopi hijau (Se-coffee) sebelum pemanggangan kopi. Kesan prarawatan selenium dibandingkan dengan kawalan positif (prarawatan tanpa selenium) dan kawalan negatif (tanpa prarawatan). Pengambilan selenium oleh biji kopi hijau masing-masing adalah pada ~ 71 dan 77% daripada larutan selenium yang berkepekatan 200 dan 400 μg/L. Untuk kepekatan selenium yang tinggi, kehilangan selenium yang ketara diperhatikan semasa pengeringan (~53%) dan yang lebih rendah semasa pemanggangan (~30%) yang menghasilkan sampel Se-coffee Arabica dan Robusta dengan kandungan selenium akhir sebanyak 1012.0 dan 855.78 μg/kg, dan menyumbang hingga 24.7 dan 20.8% daripada saranan pengambilan harian (RDA) selenium untuk secawan kopi. Pembentukan akrilamida dalam sampel Se-coffee berkurang dengan signifikan (108.9-165.3 μg/kg) berbandingkan dengan kawalan positif dan negatif sebanyak 73.9% dan 52.8%. Prarawatan selenium telah meningkatkan aktiviti antioksidan sampel Se-coffee yang telah dipanggang walaupun prarawatan merendam biji kopi mengurangkan aktiviti antioksidan kopi hijau. Pengurangan akrilamida pada kopi yang telah dipanggang sangat berkorelasi dengan perubahan kapasiti antioksidan setelah pemaggangan (ΔFRAP, 0.858; ΔDPPH, 0.836). Gabungan prarawatan selenium dengan panggangan pemanasan stim lampau tidak mengurangkan pembentukan akrilamida secara signifikan kerana pengurangan akrilamida oleh panggangan pemanasan stim lampau hanya dilihat pada biji kopi tanpa prarawatan (kawalan negatif) sebanyak 32.4% selari dengan peningkatan aktiviti antioksidannya. Pada peringkat akhir, analisis komposisi, pH, warna, dan sensori keatas sampel Se-coffee yang dipanggang telah dibandingkan dengan kawalan negatif. Sampel Se-coffee menunjukkan peningkatan kandungan mineral (abu) dengan komposisi yang berlainan, warna yang setanding dan sifat sensori yang lebih unggul berbanding dengan sampel kawalan. Penemuan dari kajian ini menunjukkan bahawa sifat antioksidan selenium organik dapat menghalang

pembentukan akrilamida semasa pemanggangan kopi serta dapat memberikan nilai tambah selenium dalam minuman kopi, meningkatkan aktiviti antioksidan dan meningkatkan sifat deria kopi yang dipanggang.

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ABSTRACT

Selenium is an essential micronutrient with significant antioxidant activity promising in mitigating the formation of acrylamide during high-temperature roasting. The study aimed to investigate the potential of organic selenium as an antioxidant additive in mitigating acrylamide formation during coffee roasting under conventional and superheated steam roasting, to study the selenium uptake by green coffee beans, and to evaluate the antioxidant activities, physicochemical properties, and sensory properties of the coffee beans enriched with selenium (Se-coffee). Firstly, the effects of roasting temperatures (220-240 °C) on acrylamide formation were evaluated under different roasting methods (conventional (CON) and superheated steam (SHS)) in Arabica and Robusta coffee beans. The superheated steam roasting significantly reduced (p<0.05) acrylamide levels at all temperatures (220-240 °C) by 14% to 37% compared to the conventional roasting. The lowest formation of acrylamide was at 240 °C at 306.2-429.4 μg/kg for superheated steam roasting and 359.2-632.4 μg/kg for conventional roasting. Subsequently, the effect of selenium pre-treatment on selenium retention, antioxidant activity, and acrylamide formation during coffee roasting at 240 °C was evaluated for both roasting methods. The selenium pre-treatment was conducted by crushing, overnight soaking in different selenium concentrations (200 and 400 μg/L), and drying the green coffee beans (Se-coffee) prior to coffee roasting. The effects of selenium pre-treatment were compared with the positive (pre-treated without selenium) and negative (untreated) controls. The selenium uptake by green coffee beans was at ~71 and 77% from 200

and 400 μg/L selenium solutions, respectively. Although significant loses of selenium was observed during drying (~53%) and lesser during roasting (~30%), the final selenium content in the roasted Se-coffee beans was exceptionally high at 101.2 and 85.6 μg/100 g for Arabica and Robusta coffee, respectively when pre-treated with high selenium concentration. This contributed up to 24.7 and 20.8% of the recommended daily allowance (RDA) of selenium in a cup of coffee. The acrylamide formation was significantly inhibited in Se-coffee (108.9–165.3 μg/kg) compared to the positive and negative controls by 73.9% and 52.8%, respectively. Selenium pre-treatment significantly increased antioxidant activity of the roasted Se-coffee beans after roasting although soaking pre-treatment significantly reduced antioxidant activity in the green beans. Acrylamide reduction in the roasted coffee beans strongly correlated with the change in antioxidant capacities after roasting (ΔFRAP, 0.858; ΔDPPH, 0.836). The combination of selenium pre-treatment with superheated steam roasting did not significantly reduce the acrylamide formation further as the reduction of acrylamide by superheated steam roasting was only observed in the untreated coffee beans (negative control) by 32.4% parallel to the increase in its antioxidant activity. In the final stage, proximate analysis, pH, colour, and sensory properties of the Se-coffee were conducted in comparison to the negative control. The roasted Se-coffee showed increased mineral (ash) content and modified composition with comparable colour and superior sensory properties to the control sample. The findings from this study suggest that the antioxidant properties of the organic selenium suppressed acrylamide formation during coffee roasting as well as provided selenium supplementation to a coffee beverage, increased the antioxidant activity, and improved the sensory properties of roasted coffee.

CHAPTER 1

INTRODUCTION

1.1 Background

Coffee is consumed widely around the world, mainly due to its refreshing and stimulating effects. Arabica (*Coffea arabica*) and Robusta (*Coffea canephora*) are two coffee species most widely cultivated with the former known for better organoleptic characteristics, while Robusta coffee possesses higher antioxidant activity but less favorable flavour. The potential benefits of coffee consumption have been well studied to associate with its rich phytochemicals and antioxidant properties originating from the green beans and complex bioactive compounds formed during coffee roasting (Hu et al., 2019). The main bioactive compounds in the coffee brew are chlorogenic acids, caffeine, pentacyclic diterpenes (cafestol and kahweol), trigonelline, and melanoidins which have been associated with reduced incidences of developing neurodegenerative diseases, several types of cancer, cardiovascular diseases, and type 2 diabetes (Gökcen & Şanlier, 2019; Higashi, 2019). Antioxidative properties of coffee brews are attributed to phenolic compounds predominantly from melanoidins, chlorogenic acids, and caffeine (Acidri et al., 2020; Liang & Kitts, 2014a; Liu & Kitts, 2011). The contribution of coffee to the daily intake of dietary antioxidants for many people is more than other food sources such as fruit, vegetables, and herbs (Lee et al., 2019; Torres & Farah, 2017).

Roasting of coffee beans at high temperatures involves a series of reactions including caramelization, Maillard reaction, Strecker degradation, and pyrolytic reaction responsible for the development of desirable organoleptic characteristics and antioxidant capacity of coffee (Chindapan et al., 2019; Opitz et al., 2014). Maillard reaction products (MRP) formed during roasting are important flavour and colour components in roasted coffee, chiefly Melanoidins, strong antioxidants that account for about 29% of coffee brew dry matter (Liu & Kitts, 2011). However, the Maillard reaction also contributes to the formation of undesired toxic components that may counteract the health benefits of coffee, such as acrylamide. Acrylamide is classified as carcinogenic compounds for human health (Group 2A) due to neural, reproductive, and genetic toxicities (IARC., 1994). Group 2A is defined as the compound that probably causes cancer according to the International Agency for research on cancer. Roasted coffee contributes to significant levels of dietary exposure of acrylamide, leading to the recent establishment of the benchmark level of acrylamide in roasted ground coffee at 400 μg/kg by the European Commission (Commission Regulation 2017/2158, 2017).

Acrylamide forms in coffee during roasting via reaction of the carbonyl group mainly from degradation products of sugars and polysaccharides with an amino group of asparagine in Maillard reaction. Mitigation of acrylamide during coffee roasting should target for limiting the source of carbonyl and asparagine content of the coffee beans. Several studies have demonstrated the ability of antioxidants to inhibit the formation of acrylamide in several food matrixes by limiting the source of carbonyl pool, reacting with key Maillard reaction intermediates (e.g., 3-aminopropionamide), and with the acrylamide itself (Jin et al., 2013). However, phenolic antioxidant naturally found in green coffee containing carbonyl groups such as chlorogenic acid has been linked to the formation of acrylamide from Maillard reaction intermediates (Jin et al., 2013; Kocadagli et al., 2012). The antioxidants with various different functional groups such as α,β,γ,δ-diunsaturated carbonyl group, α-dicarbonyl groups and decarboxylated schiff base can induce the formation of reactive carbonyl pool at high temperatures and low moisture conditions (Jin et al., 2013). Moreover, most of the antioxidant additives successfully applied in different food system to reduce the acrylamide formation (Ciesarová et al., 2008; Fu, 2016; Kamarudin et al., 2018; Mekawi et al., 2019; Zhang et al., 2007) are not stable in high-temperature of roasting coffee (>200 °C). Alternatively, organic selenium, such as L(+)-selenomethionine, has high melting properties above 250 °C and remains stable at high temperatures (Kápolna et al., 2007). Therefore, it can be utilized as an antioxidant additive and supplemented to green beans for reducing the formation of acrylamide during coffee roasting.

Selenium is essential to human health with a recommended daily allowance (RDA) value of 55 μg/day and a tolerable upper intake of 400 μg/day for adults (Institute of Medicine, 2000). Selenium plays a role in several major metabolic pathways such as immune functions, antioxidants defense systems, and thyroid hormone metabolism (Kieliszek & Błazejak, 2016). Simultaneously, the deficiency of selenium is associated with cardiovascular and inflammatory diseases, cancer, cirrhosis, diabetes, asthma, and other free radical related problems such as premature aging. Recently, there has been intense interest in selenium supplementation due to its role in protecting the immune system, improve cardiovascular functions, and protection against cancer (Kieliszek & Błazejak, 2016; Kuršvietienė et al., 2020). Coffee contains a trace amount of selenium in the organic form, which is not significant for selenium supplementation (Messaoudi et al., 2018). The organic and inorganic selenium in particular selenomethionine have shown in vitro antioxidant activity in different food systems (Sentkowska & Pyrzyńska, 2019). Therefore, the supplementation of coffee with selenomethionine is expected to increase the antioxidant activity of coffee and is promising to reduce acrylamide formation during roasting.

1.2 The Problem Statements

Coffee is among the foods that contain highest acrylamide level (EFSA, 2015). The dietary exposure of acrylamide from coffee has been summarized by Arisseto & Vicente, (2015) in 21 countries worldwide. They reported that the daily exposures of acrylamide from coffee were 0.003–0.171 μg/kg BW/day and 0.059–0.456 μg/kg BW/day for average and high consumers, respectively. The higher level of acrylamide exposure from coffee alone has exceeded the Benchmark Dose Lower Confidence Limit ($BMDL_{10}$) of 0.43 μg/kg BW/day established from neurotoxicity and tumor incidences in animal studies by the European Food Safety Authority (EFSA, 2015). Due to its toxicity, the acrylamide in coffee has been regulated to be at 400 μg/kg of roasted coffee by European commission regulation which will ensure the safe limit is not exceeded from heavy and regular coffee consumption (Europen Commission, 2017).

The mitigation strategies of acrylamide in coffee have taken multiple approaches from the modification of raw material to remove potential precursors (e.g., steam and enzyme pre-treatments of green beans) and modification of the roasting process (e.g., vacuum and steam roasting) to the addition of external additives (e.g., adding amino acid) (Anese et al., 2014; Guenther et al., 2007; Lynglev & Schoesler, 2016; Narita & Inouye, 2014). However, the impact on the sensorial and physicochemical properties of coffee are among the limitations of the intervention studies. Superheated steam roasting demonstrated improvement in sensorial quality, increase antioxidant capacity, and reduce lipid oxidation of roasted coffee beans due to the generation of oxygen absent environment at higher temperature (Chindapan et al., 2019; Devahastin & Mujumdar, 2014; Maki &

Haruyama, 1997). Nevertheless, no information is available on the effect of superheated steam roasting on the fate of acrylamide in coffee.

Along with the formation of acrylamide, the reduction of antioxidant activity in roasted coffee has reported as a result of roasting (Budryn et al., 2015; Sacchetti et al., 2009). The supplementation of coffee beans with selenium is expected to increase the antioxidant activity of roasted coffee beans due to its stability at high temperature <250 °C. However, there is a concern of the selenium toxicity exceeding the recommended dietary allowances (RDA) when the coffee is enriched with selenium via soaking. Moreover, selenium uptake during soaking by the green beans can be affected by several factors such as soaking time and selenium concentration. It has been shown that roasting of coffee beans enriched with selenium resulted in volatile selenium species which affects the sensory properties of the final product of roasted coffee. This work aimed to study the effects of superheated steam and selenium pre-treatment on mitigation of acrylamide, the selenium uptake and selenium content in the roasted coffee beans, the antioxidant activity and physicochemical and sensory properties of coffee enriched with selenium (Se-coffee).

1.3 Research Objectives

The general objective of this study was to evaluate the potential of organic selenium in mitigating acrylamide formation during coffee roasting. The specific objectives of this study were:

(i) to investigate the effect of conventional (CON) and superheated steam (SHS) roasting on acrylamide formation at different temperatures of roasting;

(ii) to compare different concentration of selenium pre-treatment on green coffee beans (as a strategy of adding selenium to the coffee) on selenium uptake during soaking and retention,

(iii) to evaluate antioxidant capacity and acrylamide formation in selenium pre-treated coffee (Se-coffee) roasted via conventional and superheated steam roasting; and

(iv) to evaluate the physicochemical and sensory properties of roasted coffee pre-treated with selenium (Se-coffee).

1.4 Thesis Outline

The attempt to mitigate the acrylamide levels in roasted coffee beans by selenium pre-treatment, and to make a new dietary source of selenium by exploiting one of the most consumed beverages in the world was presented in this thesis. To further expand, the acrylamide formation was investigated and compared in coffee beans roasted via conventional and superheated steam roasting at different roasting temperature. The effects of selenium pre-treatment were evaluated on coffee compositions and sensory properties of coffee beans. The main body of this dissertation comprises a general introduction and background, literature reviews, methods, results and discussion, the overall conclusion, and recommendations for future studies.

CHAPTER 1 comprises a general introduction on the background and rationale for this entire research, in which the reason for mitigating the acrylamide levels in roasted coffee and using the antioxidant as a potential strategy, were presented. Moreover, the objectives of this research were also presented in the current chapter.

CHAPTER 2 outlines the general literature review on the coffee, health benefits of coffee, coffee species, coffee treatment, and roasting technologies. As well as the knowledge of the acrylamide characteristics, carcinogenicity, formation, and reduction strategies. Furthermore, the general information on selenium characteristics, benefits, and dietary intake levels.

CHAPTER 3 clarifies the methods and approaches that were carried out for a roasting operation, selenium pre-treatment of coffee beans, acrylamide determination in roasted coffee, selenium quantification in green, antioxidant activity, proximate analysis, colour and browning index, acidity, quality, and sensory evaluation.

CHAPTER 4 demonstrates the result and discussion in detail as a comparison of acrylamide formation in different species of coffee roasted via conventional and superheated steam at different temperatures. Moreover, the effect of selenium pre-treatment on acrylamide formation, selenium content, the antioxidant activity, colour and browning index, acidity, and the composition of roasted coffee beans.

CHAPTER 5 consists of the overall conclusion on the entire research and several recommendations for future study on selenium pre-treatment, and acrylamide formation in coffee.

CHAPTER 2

LITERATURE REVIEW

2.1 Coffee

Coffee beverages that consumed by millions of people worldwide resulted from roasted beans of a plant belonging to the botanical genus *Coffea* in the family of flowering trees known as *Rubiaceae*. The discovery of coffee plants began in Africa and later expanded to countries throughout the world. Currently, coffee is a popular beverage and consumed widely around the world (Samoggia et al., 2019). Coffee has a significant impact on the economy in coffee-exporting countries. As an important commodity throughout the world, it has been considered as the most traded commodity, second after petrol (Ponte, 2002). According to the International Coffee Organization (ICO), coffee trading is getting larger year by year (ICO, 2020). Figure 2.1 shows the world coffee consumption 2014 -2020 with a 0.7-3.3 % increment annual.

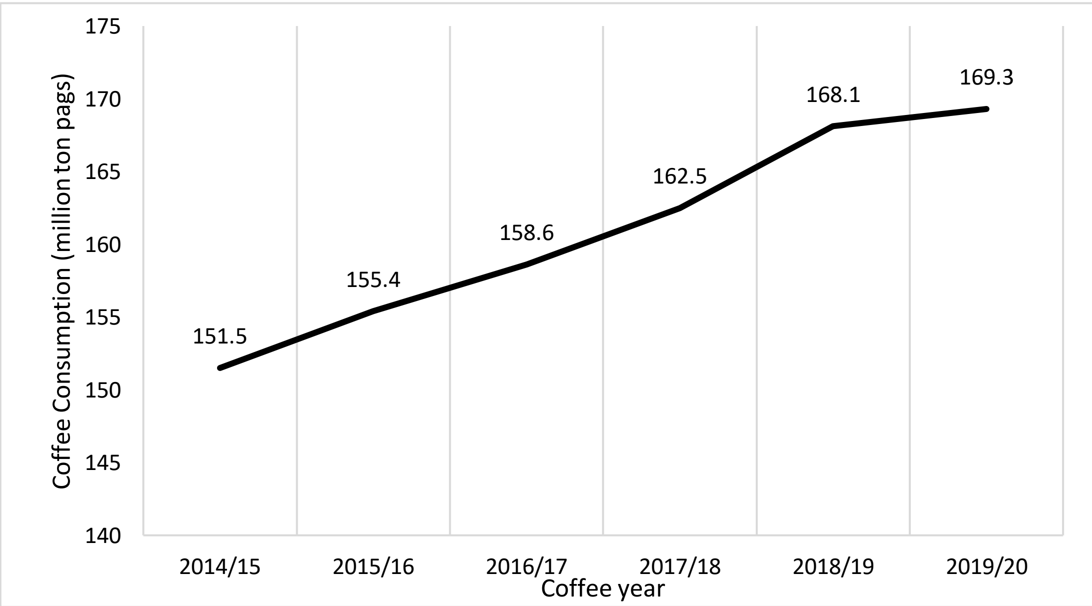

Figure 2.1: World coffee consumption 2014-2020 (ICO, 2020).

2.2 Coffee and Human Health

Among the popular drinks in the world, coffee has been demonstrated as one of the most beverage could affect human health (Higdon & Frei, 2006). Additionally, the reason for high coffee consumption is due to the presence of complex compounds composed of several chemicals responsible for several bioactivities and some compounds which have refreshing, stimulating effect and other beneficial health effects (Gokcen & Sanlİer, 2017). The health benefits of coffee are mostly due to the antioxidant compounds found in the coffee beans such as chlorogenic acid, caffeine, and melanoidins, and others (Liu & Kitts, 2011). A few of the significant bioactivities documented are antioxidant activity, anticarcinogenic activity, and antimutagenic activity (George et al., 2008). Coffee contributes to the daily intake of dietary antioxidants more than fruit, vegetables, green tea, black tea, and herbal infusions. In addition to that, it contains the highest total bio phenols content between the most consumed beverages in the world (Samoggia et al., 2019).

Coffee contains bioactive compounds that can improve overall health and provide beneficial effects against several pathologies such as metabolic disorders (type 2 diabetes), neurological diseases (Parkinson's and Alzheimer's diseases), liver dysfunctions (cirrhosis), and psychoactive responses (alertness, mood change) (Ciaramelli et al., 2018). In 2017, Harvard Medical School (HMS) published data that explains the consumption of three to four cups of coffee per day for adults people can lead to a longer lifespan, 8% to 15% reduction in the risk of death, and reduce risk of some disease such as cardiovascular disease (including heart attack, heart failure, and stroke), type 2 diabetes, Parkinson's disease, uterine and liver cancer, cirrhosis, and gout (Robert et al., 2017). However, the over-consumption of coffee can lead to other problems for human health such as anxiety, insomnia, nervousness, a rise in blood pressure, and lung and gastric cancers (Samoggia et al., 2019).

2.3 Green Coffee Beans

The overall organic and chemical contents of different green coffee species are influenced by soil composition, soil fertilization, plantation weather, and altitude, and final cultivation and drying methods used. Coffee trees are usually to grow mainly in temperature and humid climates like tropical and subtropical regions of central and South America, Africa, and Southeast Asia. Recently, Brazil is by far the largest grower and exporter of green coffee beans in the world – followed by Indonesia, Ethiopia, Philippines, Mexico, Vietnam, and India – exporting nearly 22.3 million tons of green coffee beans per year (ICO, 2020).

The wild varieties of coffee trees have been discovered to reach over 100 species under the genus *Coffea* are catalogued (Davis, 2001). However, Coffea Arabica (Arabica coffee), Coffea Canephora (Robusta coffee), and Coffea Liberica (Liberica coffee) are only used for the commercially cultivated (Couto et al., 2018). According to the United State Department of Agriculture (USDA) around 57% and 42% of the coffee produced worldwide from Arabica and Robusta, respectively (USDA, 2019); Other species with not much commercial value like Coffea Liberica and Coffea Excelsa represent only 1% (Rubayaza & Meurens, 2005). Robusta is known to be more resistant to tree diseases and exhibits less ecological requirements in terms of humidity, temperature, and altitude of the plantation than Arabica (Mander & Liu, 2010). Contrary, Arabica coffee is superior to Robusta in the terms of quality, price, and consumer acceptance; such superiority is due to the differences in major chemical compositions of their green beans. Arabica coffee contains lower content of caffeine and free amino acids, but higher contents of sugars, lipids, and organic acids (Chindapan et al., 2019). That is why Arabica coffee is generally sweeter and exhibits more acidity and fruity flavour.

However, the origin and variety of coffee beans are related to the aroma profile of roasted coffee. Aldehydes, Acetaldehyde, and Propanal are responsible for a fruity taste in Arabica coffee, while the Pyrazines give the earthy odor (Wang, 2012). In comparison, Robusta beans carry stronger roasty and “sulphury” note due to the presence of a greater amount of selenium and sulfur-containing compounds (Meija et al., 2003; Sanz et al., 2002). Thus, blends Arabica is often added for the aroma effect while Robusta is used for enhancing the body, earthy and phenolic notes of the coffee blend (Parliment & Stahl, 1995). Besides contributing to balanced flavour profiles, Robusta coffee is often blended with Arabica for cost reduction purposes. Robusta beans are lower in cost since the crops are hardier to grow (more resistant to infestation) and easier to harvest (grown in regions of low elevation) than the Arabica counterpart.

Coffee cherries are harvested when they become bright-red, glossy, and firm, either by selective hand-picking or non-selective stripping of whole branches' mechanical harvesting. After harvesting, the coffee fruits are separated from the pulp, which is carried out by dry or wet processing (Ghosh & Venkatachalapathy, 2014). The whole cherries are dried under the sun in the open air, followed by the separation of the hull (dried pulp and parchment) mechanically to yield the green beans. The drying process should be applied to about 12% moisture content and storing below 26 °C under a dry environment (50-75% RH) to maintain the bean quality and to prevent the mold's growth (Ghosh & Venkatachalapathy, 2014). Under optimal conditions of storage, green coffee may be stored with a shelf life for more than 3 years (Bucheli et al., 1998). In factory operations, after drying beans, the bags of green coffee beans are opened by hand or machine, dumped into a hopper, and screened to remove debris. The green beans are then weighed and transferred by belt or pneumatic conveyor to storage hoppers. From the storage hoppers, then the green beans are conveyed to the roaster.

2.4 Roasted Coffee Beans

Green coffee beans provide neither the characteristic aroma nor flavour of brewed coffee until they are roasted. Moreover, the roasting process increases the value of coffee beans, by 100-300% of the raw material (Yeretzian et al., 2002). As a time-temperature-dependent process, roasting is the main thermal treatment affecting the physicochemical and organoleptic properties of roasted coffee beans. Roasting of coffee beans typically takes place at 200-250°C for different times depending on the desired characteristics of the final product. This process is responsible for the development of the characteristics to obtain a good quality cup of coffee with specific organoleptic properties (flavour, aroma, and colour). However, events that take place during roasting are complex, destroying some compounds initially present in green beans and the formation of volatile compounds that are important contributors to the characteristic of coffee’s aroma. The chemical compositions of green and roasted coffee are shown in Table 2.1.

Table 2.1: General chemical composition of green and roasted coffee beans (Barker, 2004; Wang, 2012).

Chemical composition	Green coffee (%)	Roasted Coffee (%)
Soluble carbohydrates	9	10
Water	12	2
Non-volatile acids	7	7
Caffeine	1	1
Protein	12	13
Ash	3	4
Oil	11	13
Trigonelline	1	1
Cellulose (Non-Hydrolyzable)	18	17
Cellulose (Hydrolyzable)	13	14
Starches and pectins	13	14

Briefly, when the temperature of roasting reaches 100 °C, the moisture content reduced from 8-12% in green coffee beans to about 5% in roasted coffee (Wang, 2012) The smell of the bean’s changes from an herb-like green bean aroma to bread-like, the colour turns from green to yellowish, and the structure changes from strength and toughness to crumblier and more brittle. However, the colour darkened slightly when the internal temperature of the bean reaches 100 °C, due to the vaporization of water. At 160-170 °C, the beans getting darker in colour, and with roasting continues Maillard and pyrolytic reactions start to take place, resulting in gradually darkening of the beans (Hernández et al., 2007). Finally, after roasting, the fresh-roasted coffee beans are quickly cooled to stop roasting (Yeretzian et al., 2002).

However, the conventional roasting coffee consists of a single operation, the raw green coffee is placed in the roaster and subjected to the heat (the time and temperature setting depending on the degree of roasting required) without intermission until the roasting process is completed (Heilig, 1994). Figure 2.2 shows the schematic diagram of conventional roaster (convection type).

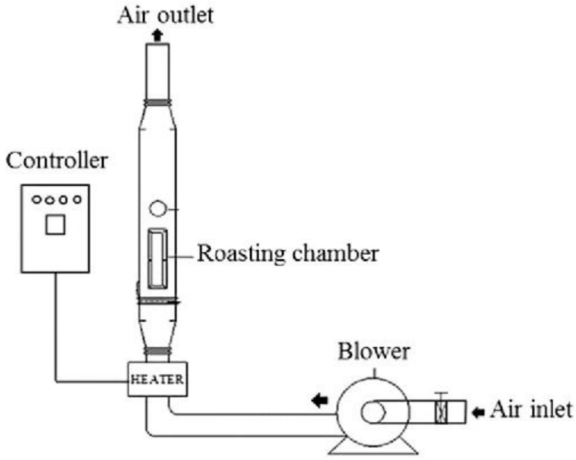

Figure 2. 2: The schematic diagram of oven coffee roaster (convection type).

Regardless of the type of roaster, roasting coffee occurs at high temperatures above 200 °C (Gloess et al., 2014). For laboratory roasting, 220-230 °C for 24 min was evaluated as optimal conditions for the acceptable sensory properties for the coffee beverage (Bagdonaite et al., 2008). However, these values can vary greatly based on several factors such as the degree of roasting required (light, medium or dark), type of roaster used, and the variety, age, moisture content of the coffee beans, and other factors, such as the metal drum type and thickness, and the way of heat transfer from the heat source to the beans.

The final quality of roasted coffee is influenced by the design of the roasters and time-temperature profiles used. Although heat transfers during roasting can involve conduction by using hot metal surfaces, convection using hot air as the heating medium, and radiation using infrared roasters, convection by far is the most important mode of heat transfer that determines the rate and uniformity of roasting

(Bagdonaite et al., 2008). Coffees roasted in the fluidized-bed roaster that is almost exclusively based on convective heating can result in low density and high yield coffee. On the other hand, coffees roasted in drum roaster that involves mainly conductive heat transfer have less soluble solids, more degradation of chlorogenic acids, more burnt flavour, and higher loss of volatiles than the fluidized bed roasters (Nagaraju et al., 1997).

Conduction method in this system only heat from the externally heated open metal drum is used to roast the beans through direct contact and the drum should rotate to prevent scorching beans, while the convection method uses heated air to heat the beans and “float it in the air to reduce burning (Poss, 2007). The roaster should be equipped with a fan to carry away the evaporated moisture.

2.4.1 Superheated Steam Roasting

Over the past 100 years, superheated steam has been proposed as an alternative method for roasting and drying, attracting serious attention in the last 20 years (Devahastin & Mujumdar, 2014). Superheated steam has a good advantage and useful for the food industry because it is providing high production rates, better pollution control, safe, absence of oxygen reducing food oxidation, and requiring low energy consumption. Additionally, superheated steam technology provides high qualities of roasted products with low shrinkage and high porosity (Li et al., 1999; Moreira, 2001).

Recently, many researchers have been focused on and succeeded in the application of superheated steam in the coffee roasting as an alternative method. The basic principle of the application of the superheated steam as the heating saturated steam above the boiling temperature of water more than 100 °C that is a colourless, dry and transparent gas to carries away the evaporated moisture (Devahastin & Mujumdar, 2014). In a typical set-up, saturated steam from a steam generator is heated up in a heater and becomes superheated steam, which is then introduced into a drying chamber wherein a product to be dried is placed (Yodkaew et al., 2017). However, the products roasted with superheated steam was not oxidized at the same level of hot air, because the air in the system is replaced with superheated steam and thus, the coffee can be heated or dried in an oxygen-free environment (Cooper et al., 2007). Figure 2.3 shows the superheated steam roaster which consists of a stainless steel cylindrical roasting chamber with the diameter of 20 cm and height of 140 cm, a 1.5-kW backward-curved blower, a 12-kW electric heater, and a 20-kW electric steam boiler capable of generating 30 kg/hr of saturated steam at one bar (Chindapan et al., 2019).

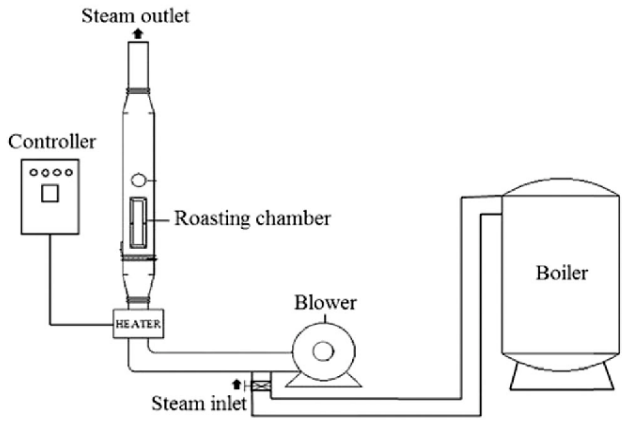

Figure 2.3: Schematic diagram of the superheated steam roasting process (Yodkaew et al., 2017).

Superheated steam has been applied for roasting of many products including rice, sesame seeds, cacao beans, peanuts, and coffee beans. All of the studies reported the quality improvement in terms of taste, flavour, colour, texture, and retention of antioxidant compounds (Chindapan et al., 2019; Idrus et al., 2012; Ling et al., 2018; Yodkaew et al., 2017; Zzaman et al., 2017). The lack of oxygen is the most important characteristic of the superheated steam roasting. The air in the system is replaced by superheated steam and, thus, the samples can be heated under an oxygen void environment, which provides products that are not oxidized (Chindapan et al., 2019; Devahastin & Mujumdar, 2014; Zzaman et al., 2017). Furthermore, roasting coffee under superheated steam has been noted to decrease the unsaturated aldehydes content, which is generated during roasting by lipid oxidation, due to the lack of oxygen (Maki & Haruyama, 1997). It has also reported that the coffee bean roasted under superheated steam had lower pH and higher sugar content (Chindapan et al., 2019).

2.5 Roasting and Sensory Properties of Coffee

Green coffee beans during roasting imparts physical and chemical changes to the coffee beans when subjected to high temperatures >200 °C. Physical changes are mainly reflected in the dramatic changes in the shape, water content, density, color, and internal structure of the beans (Schenker et al., 2000). The chemical changes are marked with Maillard reaction and caramelization reaction to produce pleasant or unpleasant substances, which can directly decide the quality of the beverage (Baggenstoss et al., 2008; Liu et al., 2019; Steen et al., 2017). During roasting the structure of coffee beans starts to change at 50 °C and thereafter protein denaturation and water evaporation increase. Above 100 °C the beans undergo browning, due to thermal decomposition and organic compounds pyrolysis. Gaseous substances (mainly water vapor, carbon dioxide, and carbon monoxide) are released and the bean volume increases at about 150 °C. At 180–200 °C, with the disruption of the endosperm, bean cracking occurs, caramelization develops, bluish smoke appears, and aroma develops (Belitz & Grosch, 1988).

In the past few decades, in addition to direct use of instrumentation combined with expert assessment to find flavor components, represented by gas chromatography–olfactometry-mass spectrometry (GC-O-MS) (Zou et al., 2018), instrumental detection combined with multivariate analysis has also been tried for the excavation of flavor substance and quality control of roasted coffee beans (Sittipod et al., 2019). For example, proton transfer reaction-mass spectrometry was employed in the discrimination of coffee beans of different roasting degree (Romano et al., 2014), and near infrared spectroscopy was exploited as an analytical tool for on-line monitoring of acidity during coffee roasting (Worku et al., 2016). However, beverage quality can be usually determined by sensory analysis in which a panel of trained, specialized "cuppers" evaluates coffee quality using either a table with scoring values (scoring method) or a sensory lexicon (descriptive method) (Worku et al., 2016). The most widely adopted evaluation standard is the "Coffee Cupping Protocol of Specialty Coffee Association of America" (SCAA), which includes ten sensory indicators: aroma, flavor, aftertaste, acidity, body, overall, clean up, uniformity, sweetness, and balance.

The aroma indicates smell of the coffee when infused with hot water, while the flavour represents coffee's principal character (SCAA, 2015). The aftertaste is defined as the length of positive flavour qualities emanating from the back of the palate and remaining after the coffee is swallowed. The body indicates the tactile feeling of the liquid in the mouth, the fragrance indicates the smell of the ground coffee when still dry; Acidity is often described as "brightness" when favorable or “sour” when unfavorable. Uniformity refers to the consistency of flavour of the different cups of the sample tasted. Sweetness refers to a pleasing fullness of flavour as well as any obvious sweetness and its perception is the result of the presence of certain carbohydrates. The balance showed how all the various aspects of flavour, aftertaste, acidity, and body of the sample work together and complement or contrast to each other.

2.6 Heat-induced Coffee Contaminants

High temperature of roasting >200 °C of coffee beans results in series of chemical reactions such as Maillard reaction, Strecker degradation, pyrolytic reactions, and caramelization which change the composition of roasted coffee beans. Formation of undesirable compounds in coffee as a result of roasting has been reported including acrylamide, furfuryl alcohol (FA), furan, and 5-hydroxymethylfurfural (HMF) (Albouchi et al., 2018; Alves et al., 2010; Bertuzzi et al., 2020; Granby et al., 2004; Tareke et al., 2002; Zzaman et al., 2017). In particular, the level of acrylamide reported in roasted coffee is exceptionally high which could pose health risk due to their known toxicity toward human health (IARC 1994).

During roasting, the acrylamide formation in coffee beans starts when the roasting temperature >120 °C and reach the maximum levels when the temperature 175-177 °C, then start to decrease with the heat increasing (Bagdonaite et al., 2008; Bertuzzi et al., 2020; Pastoriza et al., 2012). Bagdonaite et al. (2008) have reported that the acrylamide formed at the first minutes of the roasting process of coffee with the highest amount 500 μg/kg and 3800 μg/kg for Arabica and Robusta coffee, respectively; then start to decrease to reach 374 μg/kg and 708 μg/kg in coffee

Arabica and Robusta, respectively. The acrylamide levels are expected to reduce during roasting at high temperatures (>177 °C) due to partial thermal degradation and chemical interaction of acrylamide with melanoidins in coffee beans.

2.7 Acrylamide Characteristics and Uses

Acrylamide (2-propenamide) is an organic compound with a chemical structure C_3H_5NO. This solid monomer is a white colourless crystal that is soluble in water and polar solvents such as, acetone, methanol, dimethyl ether, and ethanol. Acrylamide has a molecular mass of 71.08 Da and a boiling point of 84.5 °C at 25 mmHg (Kusnin et al., 2015). Figure 2.4 shows the chemical structure of acrylamide.

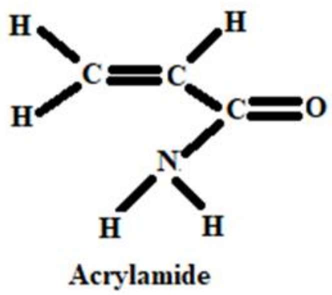

Figure 2.4: Chemical structure of acrylamide.

Industrially, acrylamide is used to make polyacrylamide, which is mainly used in treating effluent from water treatment plants and industrial processes. Besides, acrylamide monomers and polymers are used in the production of cosmetics, dyes and organic chemicals, contact lenses, toiletries, permanent-press fabrics, paper, and textile production, pulp, and paper production, ore processing, sugar refining, and as a chemical grouting agent and soil stabilizer for the construction of tunnels, sewers, wells and reservoirs (Chemical Safety facts, 2019). However, the acrylamide discovery in food was in April 2002 by Eden Tareke in

Sweden, the Eritrean scientist found the acrylamide compounds foods that contain reducing sugar and amino acids such as potato chips (potato crisps), French fries (chips), and the bread and coffee that had been heated higher than 120 °C (Tareke et al., 2002). A later study has been found acrylamide in coffee as one of the greatest foods contributes to acrylamide dietary intake (Mucci et al., 2005).

2.8 Carcinogenicity of Acrylamide

According to the International Agency for Research on Cancer (IARC), acrylamide compounds have been defined as a potential cause of a spectrum of toxic effects and it has been classified as a carcinogenic for human health (Group 2A) (IARC, 2014). Also, the benchmark levels of acrylamide have been established by the Environmental Protection Agency (EPA) and the U.S. Food and Drug Administration (FDA) in water and food. In water: less than 0.5 μg/L, in food: 12 μg of acrylamide per person per day are safe in terms of the nervous system (CSPI, 2003). Furthermore, in 2015, the European Food Safety Authority (EFSA) reported the levels of dietary exposure to acrylamide across age groups indicates a concern due to its carcinogenic effect (EFSA, 2015).

However, epidemiological studies have evaluated various cancer endpoints in association with the dietary acrylamide exposure of humans. Some epidemiological publications indicate the associations of acrylamide with cancer risk, such as lung and liver cancer (Conti et al., 2019). Additionally, Hogervorst et al., (2007) have been published that there is a positive association between acrylamide and ovarian cancer risk through effects on sex hormones. A positive association for endometrial cancer was observed in two prospective cohort studies (Hogervorst et al., 2007; Wilson et al., 2010). Also, a positive association has been observed in the cohort study between acrylamide consumption with multiple myeloma and follicular lymphoma in all men and never-smoking men (Bongers et al., 2012). Moreover, acrylamide may generate the metabolite glycidamide, which could be metabolized to more genotoxic and mutagenic forms and may damage the DNA (Hu et al., 2015). Theoretically, acrylamide is a hydrophilic compound and it can diffuse throughout all body tissues even lymphoid tissues, which may cause acrylamide carcinogenicity (Friedman, 2003).

2.9 Acrylamide Dietary Sources

In 2002, the Swedish national food Administration (NFA) announced that some food products contain significant levels of acrylamide (Tareke et al., 2002). These food products which contain a high level of acrylamide obtained from the plant sources are a naturally rich source of asparagine and carbohydrate (Pundir et al., 2019). High-temperature cooking, such as frying, roasting, or baking, is most likely to cause acrylamide formation. while boiling and steaming do not typically form acrylamide. Generally, acrylamide is found mainly in foods made from plants, such as potato products, grain products, or coffee. Additionally, acrylamide does not form, or forms at lower levels, in dairy, meat, and fish products. However, acrylamide is more likely to accumulate when cooking is done for longer periods or at higher temperatures (FDA, 2017). Due to the accumulating the toxic acrylamide in different food matrices, the U.S. Food and Drug Administration (FDA) and European Food Safety Authority (EFSA) and others were established the benchmark levels, which is the highest safe level for acrylamide consumptions. The benchmark levels of acrylamide have been determined by the Environmental Protection Agency (EPA) and the U.S. Food and Drug Administration (FDA) in water and food respectively for less than 0.5 µg/L and 12 µg/ person/ day are safe in terms of the nervous system (CSPI, 2003). Also, in 2015, the EFSA has established the Benchmark Dose Lower Confidence Limit "$BMDL_{10}$" as 0.43 µg/kg BW/day as the safe limit in term of neurotoxicity (EFSA, 2015). According to the (EFSA) acrylamide was found at the highest levels in coffee, solid coffee substitutes, and potato fried products. The mean and 95th percentile content of acrylamide in different processed food were ranges 27µg/kg to 1504µg/kg and 90µg/kg to 3976 µg/kg, respectively. For the dietary acrylamide exposures during age groups, the mean and 95th percentile was ranging 0.4 to 1.9 µg/kg BW per day and 0.6 to 3.4 µg/kg BW per day, respectively (EFSA, 2015).

2.9.1 Acrylamide Levels in Coffee

Coffee as a research object is important because of its high consumption in some countries and therefore possible hazardous influence on human health. According to the EFSA 2015, the mean and 95th percentile of acrylamide levels in coffee was 522µg/kg and 1054 µg/kg respectively (EFSA, 2015). Table 2.2 shows the mean of acrylamide in different types of coffee. Arabica coffee contains significant levels of acrylamide lower than Robusta coffee (Bagdonaite et al., 2008; Lantz et al., 2006; Soares et al., 2006; Surma et al., 2017). The difference may be contributed by higher amount of free amino acids (e.g. asparagine) reported in green Robusta coffee bean at 486±97 µg/g compared to green Arabica bean at 797±23 µg/g (et al., 2019 Bagdonaite et al., 2008; Baskar & Aiswarya, 2018; Murkovic & Derler, 2006).

2.9.2 Acrylamide Exposure from Coffee