STUDY OF ACRYLAMIDE FORMATION IN COFFEE DURING ROASTING

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ABSTRACT

Coffee is a popular beverage in society. Acrylamide is an undesirable substance that will metabolically activated to carcinogens.Acrylamide formation is found to occur during the browning process, which is called Maillard reaction. This is the reaction between sugars and asparagines at temperature above 120°C. The roasting temperature of coffee is at least 200°C, meaning that coffee surely contain acrylamide but the level of formation is different for different roasting conditions. In this research, study of the quantity acrylamide upon the local coffee powder, commercial coffee powder and different roasting parameters coffee have been done. The highest concentration of acrylamide is in coffee which was roasted at 220°C for 5 min. Besides that, the relationship between the carbohydrates and acrylamide also have been done but no simple linear regression relationship was found.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Coffee is the common name for the analyte in this project. It has a lot of scientific name but only *Coffea Arabica* and *Coffea Robusta Linden* are frequently used. It is the most important foreign exchange supplier for many agricultural oriented countries, the secondly most important raw material within the international trade, and a main contributor for tax yield. Coffee occupies one percent of total world trade that is over $2 billion US dollar per year. (Missouri Botanical Garden, 2006) Today the "coffee break" has become a regular morning ritual in our daily life.

It has at least a thousand years history. But the discovery of coffee is unknown. The first coffee plants probably grew in Kaffa, a place in southwestern Ethiopia and have provided coffee its name. Nowadays, Brazil is the biggest leading coffee-producing country and followed by Colombia, Mexico, Indonesia, Vietnam, Ethiopia, Uganda, and Guatemala. The leading consumer is The United States, Germany, Japan, France, Italy, Scandinavia, and Spain. (Danbury, Connecticut Mario Pei and James Adams Eichner, 2002)

Coffea Arabica and *Coffea Robusta* are the two main species of the coffee plant. The former is the older. *Robusta*, which contains about twice as much caffeine, has bitter taste than *Arabica* and can be cultivated in environments where *Arabica* will not thrive. While *Arabica* is more susceptible to disease, it is considered to have better taste than *Robusta*. Compared to *Arabica*, the price of *Robusta* is much lower; therefore it is much more popular among industrial clients.

Coffee is made from beans of the coffee tree. After plucking from the trees, coffee beans need to go through four steps to become coffee powder. Those are clean, dry, roast and ground. A lot of processing and human labour is required before coffee beans can be processed into roasted coffee. All coffee is roasted before being consumed. Roasting is an important step that has a great degree of influence on the taste of the final product.

The processing of coffee totally refers to the agricultural and industrial processes needed to deliver whole roasted coffee beans to their consumers. Some preparations are typically necessary to make this into a drink. The particular steps needed vary with the type of coffee desired, and with the raw material being worked with. Actually, coffee must be ground to varying roughness depending on the brewing method. After being brewed, it may be came out in a different ways such as only coffee powder, with or without sugar, with or without milk or cream, hot or cold, and others.

Coffee beans from different places, or coffee varietals, usually have distinctive characteristics such as flavour, caffeine content, and acidity. These are dependent on the local environment where the coffee plants are grown, their method of process, and the genetic subspecies.

Coffee is one of the most heavily researched commodities in the world today. It has been published in thousands of literature studies with a wide range of topics. Its potential benefits and hazards have been, and continue to be, widely studied and discussed. The main analytical technique used in this project to analysis the composition of coffee powder is High Performance Liquid Chromatography (HPLC) and UV-Visible Spectrophotometer. Coffee is a complex mixture of chemicals. Basically, it consists of carbohydrates, lipids, amino acids, vitamins, minerals, alkaloids and phenolic compounds. In green coffee the carbohydrates and amino acids are the main components that contribute to the formation of the typical aroma during roasting. (Linus Pauling Institute, 2006)

1.2 Problem Statement

1.2.1 Health Problem

Coffee is enjoyed as a drink by millions of people world-wide. However, some people say that stop drinking coffee is an important step towards healthy life. Some reports in the media suggest coffee drinking is unsafe and some scientific papers even claim that coffee increases the risk for getting cancer. (The pan-European Coffee Science Information Centre, 2006) Acrylamide, an undesirable substance that will metabolically activate to carcinogens will be formed during coffee roasting process. Study upon its quantity formation by using different parameter is utmost important towards our health. (Michael Murkovic, Karin Derler, 2006)

1.2.2 Correlation Between Carbohydrates and Acrylamide

Carbohydrates are one of the main components that contribute to the formation of the typical aroma during roasting. Sucrose is the main carbohydrate occurring in green coffee. (Michael Murkovic, Karin Derler,2006). The reducing sugars will reacts with Asparagine during Maillard reaction that will form flavour, colour and acrylamide as the intermediate product. (Vural Gökmen, Hamide Z. Senyuva, 2006)

1.2.3 Solution for the Problems

In order to get the precise composition data from coffee powder, High Performance Liquid Chromatography (HPLC) and UV-Visible Spectrophotometer will be used in this project. It allows separations and measurements to be made in a matter of minutes. (Skoog *et al*, 2004)

1.3 Objectives

The objectives of this project are:

1.2.1. To determine the effect of roasting onto the main component inside coffee powder.

1.2.2. To analysis the composition of different coffee powder.

1.4 Scopes of Project

Based on the objectives, the scopes of this project are:

1.3.1 To determine the total concentration of carbohydrates (sucrose and glucose), asparagine and acrylamide at different time and different temperature.

1.3.2. Comparing the composition of self roasted coffee powder, local coffee powder and commercial instant coffee powder.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Before studying the concentrations inside a substance, getting information needed, knowing the purpose and the type of analyzed sample are necessary. The initial step to know the needs to study the concentrations of the sample is very important. A sample may contain more than 10 million compounds but perhaps a few elements are of interest. After the required measurement is defined, several factors will decide the analytical methods that will be used. The factors include the analyst's skills and training in different techniques and instruments; the facilities, equipment and instruments available; the sensitivity and precision demanded; the cost and budget allowed; the period for analysis given and the time to get the results. (Dean, 2002)

A lot of studies have done upon coffee. Although caffeine has received the most attention from scientists, it is vastly complex, capable of producing thousands of chemicals that can be perceived, depending on the origin, roast, and age of the coffee. These various chemicals can enhance each other, cancel each other out, operate independently of one another, or combine to create new perceptions. Great strides have been made in instrumentation, but the simple, less expensive, and replicable results are obtained by analysis coffee powder using analytical equipment. (Coffee Analyst, 2006)

2.2 Coffee Roasting Process

The coffee roasting process consists essentially of cleaning, roasting, cooling, grinding, and packaging operations. We will see the brown coffee bean come from a red cherry fruit, through a washing tank and fermentation. Then it will be dried and readied for grading and shipping. With little room for mechanization, the coffee industry is steadily growing even in today's modern world.

Processing of coffee is the method converting the raw fruit of the coffee plant (cherries) into the commodity green coffee. The cherry has the fruit or pulp removed leaving the seed or bean which is then dried. While all green coffee is processed the method that is used varies and can have a significant effect on the flavor of roasted and brewed coffee.

Coffee processing is divided into two types. These are wet process and dry process. Most of the world's green coffee has gone through some sort of wet processing including most of the premium coffee.

Figure 2.1: Traditional methods of roasting coffee using by industry.

Figure 2.1 shows the traditional methods of roasting coffee in industry, especially in small industry. An old large-capacity coffee roaster made from cast iron. It is wood fired.

Figure 2.2: The ways of drying coffee after washing

Figure 2.2 shows that the ways of drying coffee after washing the coffee and pucking down from trees. At this step, some sands, stones or even iron will most probably mix inside the coffee and sell out to the manufacturer. That is the reason why coffee is full of impurities. (Sai Kee Coffee (M) Sdn bhd, May 2006)

Figure 2.3: Unroasted coffee

Figure 2.4: Roasted coffee

Figure 2.3 and figure 2.4 show the difference of coffee beans physically. The unroasted coffee is brown in colour but the roasted coffee is dark in colour and oily. When roasted, the green coffee bean expands to nearly double its original size, changing in color and density. As the bean absorbs heat, the color shifts to yellow and then to a light "cinnamon" brown then to a dark and oily color. During roasting oils appear on the surface of the bean. The roast will continue to darken until it is removed from the heat source. (Wikipedia Foundation, 2006)

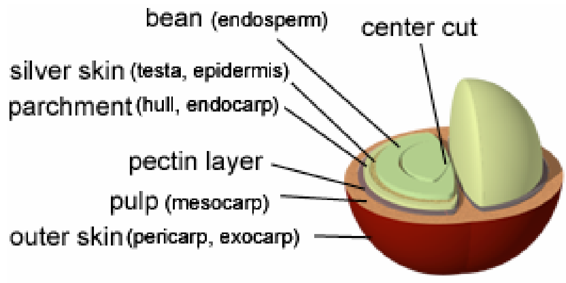


Figure 2.5: Coffee beans structure

Coffee beans are divided into a few layers. The typical structure of the coffee beans is shown in figure 2.5. The coffee beans are covered by its shell. To get the bean inside, the outset shell and a few layers that surrounded it, are needed to be removed.

2.2.1 Wet Process

Firstly, the green coffee is immersed in water. During the immersion, bad or unripe fruit will float and the good ripe fruit will sink. To remove the skin of the cherry and some of the pulp, pressing the fruit by machine in water through a screen. However, the bean will still have a significant amount of the pulp clinging to it that needs to be taken off.

The remainder of the pulp is removed by breaking down the cellulose in the ferment and wash method of wet processing by fermenting the beans with microbes for several days and then washing them with large amounts of water. Nowadays, wet processing fermentation is not used to separate the bean from the remainder of the pulp but it is scrubbed off by a machine.

The left after removing the pulp is two additional layers, the silver skin and the parchment. The beans must be dried to a water content of about 10% before they are stable. Coffee beans can be dried under the sun or by machine. Most of the industries dry the coffee beans under the sun to 12-13% moisture and use machine to bring down to the moisture to 10%. Most of the coffee is dried on large raised tables where the coffee is turned by hand. There are good points for using this way, which are allowing air to circulate better around the beans to increase the drying rate but this methods will consume a significant labor cost. Finally, the parchment is removed from the bean and what remains is green coffee. (Wikipedia Foundation, 2006)

2.2.2 Dry Process

An unwashed or natural coffee and the oldest method of processing coffee is dry process. To dry the cherries, they are placed under the sun on tables or in thin layers on patios about ten days to two weeks to make sure the entire cherries are completely dry. However, they need to be raked regularly to prevent mildew while they dry. Pulp and parchment will be removed once they are dry. (Minister coffee (M) Sdn Bhd, 2006)

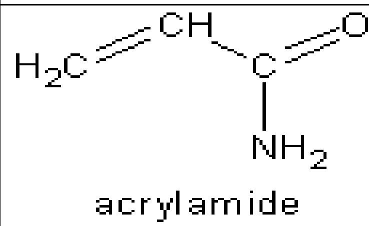
2.3 Coffee Quality

The most important among the criteria used for assessing coffee quality is based on sensory analysis and is referred to as cup quality. The presence of defective coffee beans is quite relevant to coffee quality. However, the majority of data on coffee properties are restricted to supposedly good quality beans. Therefore, the present study is aimed at an evaluation of physical and chemical attributes of coffees of different qualities, both green and roasted. (Swiss Society of Food Science and Technology, 2004)

2.4 Acrylamide

Acrylamide (acrylic amide) is a synthetic monomer. Table 2.1 summaries the properties of acrylamide.

Table 2.1: Properties of acrylamide

Systematic name	2-Propenamide
Structure	
Chemical formula	C_3H_5NO
Molar mass	71.08 g/mol
Density	1.13 g/cm^3
Solubility in water	204 g/100 ml (25 °C)
Physical properties	1. White odorless crystalline solid. 2. Soluble in water, ethanol, ether and chloroform.
Chemical properties	1. Acrylamide monomer is a potent neurotoxin. It is incompatible with acids, bases, oxidizing agents, iron and iron salts. It decomposes non- thermally to form dimethylamine and thermal decomposition produces carbon monoxide, carbon dioxide and oxides of nitrogen.
Applications	1. In industries, mainly use as a precursor in the production of several polymers, such as polyacrylamide are used in water and wastewater treatment processes, pulp and paper processing, electrophoretic separations, mining mineral processing and the manufacture of permanent press fabrics. Some acrylamide is used in the manufacture of dyes and the manufacture of other monomers.(Wikipedia Foundation, 2006)
Sources	1. Acrylamide in fried or baked goods is produced by the reaction between asparagine and sugars (sucrose, glucose, etc.) or reactive carbonyls. Acrylamide is not created by cooking by

	normal boiling, and nearly all uncooked foods do not contain any detectable amounts. Browning or over-cooking of foods should be avoided. (Dominique Taeymans *et al*, 2004) 2. Acrylamide in olives and prune juice comes through another process. It has been suggested that environmental pathways, such as the breakdown of the herbicide glyphosate (Roundup). 3. Smoking is also a major acrylamide producer.
Effects	1. Occupational or experimental exposure to acrylamide produces neurotoxicity characterized by ataxia, weight loss, and nerve damage. 2. Acrylamide has also been implicated as a potential mutagen or is metabolically activated to carcinogens and reproductive toxicant. (Barber *et al*, 2001) 3. Evidence proves that exposure to large doses can cause damage to the male reproductive glands. 4. Exposure to pure acrylamide directly by inhalation, skin absorption, or eye contact irritates the exposed mucous membranes, such as the nose can also cause sweating, urinary incontinence, nausea, myalgia, speech disorders, numbness, paresthesia, and weakened legs and hands.

Acrylamide is metabolized to the expoxide, glycidamide (2,3-expoxypropanmide) as shown in figure 2.8, which has been shown to have neurotoxic potential. In light of this finding, it is possible that conversion to glycidamide is an important step in the mechanism of acrylamide neutrotoxicity. (Barber *et al*, 2001)

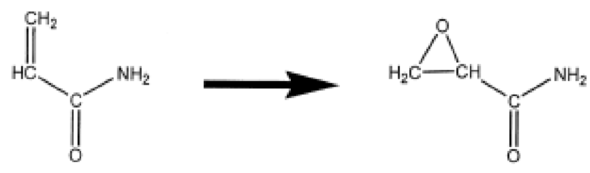


Figure 2.6: Structures of acrylamide and its epoxide metabolite, glycidamide

2.4.1 Acrylamide in Food

In 24 April 2002, the Swedish National Food Administration announces the presence of acrylamide predominantly in heat-treated carbohydrate-rich foods has sparked intensive investigations into acrylamide, consisting the occurrence, chemistry, agricultural practices, and toxicology, in order to get know about is there any potential risk to human health from the presence of this contaminant in the human diet. This review addresses the analytical and mechanistic aspects of the acrylamide issue and summarizes the progress made to date by the European food industries in these key areas. Importantly, it presents experimental results generated under laboratory model conditions, as well as under actual food processing conditions in high levels in fried, baked, grilled, toasted or microwaved carbohydrate-rich foods covering different food categories, such as potatoes, biscuits, cereals, and coffee. (Kit Granby, Sisse Fagt, 2004) Estimates for the proportion in the diet coming from the consumption of coffee range from twenty to forty percent, however roasted coffee also contains a range of anti-cancer compounds and antioxidants.

"The major contributing foods to total exposure for most countries were potato chips (16-30 per cent), potato crisps (6-46 per cent), coffee (13-39 per cent), pastry and sweet biscuits (10-20 per cent) and bread and rolls/toasts (10-30 per cent). Others foods items contributed less than 10 per cent of the total exposure," the Swedish National Food Administration committee reports. (Lindsey Partos, 07/03/2005)

Although trace amounts of acrylamide has been shown in 2002 that can be formed by boiling, significant formation generally requires a processing temperature of 120 °C or higher. Most acrylamide are accumulated during the final stages of baking, grilling or frying processes as the moisture content of the food falls and the surface temperature rises, with the exception of coffee where levels fall considerably at later stages of the roasting process. (Lindsey Partos, 31/03/2005)

Acrylamide is stable in the large majority of the affected foods, again with the exception of ground coffee for which levels can decline during storage over months. Since formation is dependent on the exact conditions of time and temperature used to cook or heat-process a food, there can be large variations between brands of the same product and between batches of the same brand. Large variations are also to be expected during cooking although this aspect has been less well documented.

Since acrylamide formation is closely linked to food composition, factors such as the presence of sugars and availability of free amino acids are also considered. Many new findings that contribute towards a better understanding of the formation and presence of acrylamide in foods are presented. Many national authorities across the world are assessing the dietary exposure of consumers to acrylamide, and scientific projects have commenced to gather new information about the toxicology of acrylamide. These are expected to provide new scientific knowledge that will help to clarify whether or not there is a risk to human health from the consumption of foods containing low amounts of acrylamide. (Dominique Taeymans *et al*, 2004)

2.4.2 Cancer Link

Some sources claim that people in the modern world consume daily on average around 25 micrograms of acrylamide, and that this accounts for a significant number of cases of cancer. There is as yet no consensus among the scientific and medical community as to the dangers of acrylamide in food; this topic is still being actively researched and investigated by government bodies in several different countries. But some research has revealed the connection between acrylamide intake via food and cancer.

Swedish Livsmedelsverket (National Food Administration) announced that acrylamide can be found in baked and fried starchy foods and concern was raised as it may be a carcinogen. On the other hand, the preceding research might not have been able to isolate the effects of acrylamide due to it being ubiquitous in western diets.

On 2005-08-26, California attorney general Bill Lockyer filed a lawsuit against top makers of french fries and potato chips to warn consumers of the potential risk of consuming acrylamide.

According to Dr.Carl Winter, a food toxicologist employed by UC Davis, acrlyamide is easy to avoid. Says he "If you want to avoid exposure to acrylamide, don't eat foods that have acrylamide in them."

2.5 Maillard Reaction

The link of acrylamide in foods to the Maillard reaction and, in particular, to the amino acid especially asparagine has been a major step forward in explaining the first feasible chemical route of formation during the preparation and processing of food. Other probably minor pathways have also been proposed, including acrolein and acrylic acid. Maillard reaction take a important place in the thermal reaction in preparing foods. Amino group of a free or protein-bound amino acid and sugars contribute to this reaction as shown in figure 2.7. Variables such as the reaction conditions, for example heating time and temperature; phsico-chemical properties of the system likes water activity or pH, chemical composition and nature of reagents can affect the kinectic of the reaction and this lead to the formation of Maillard reaction products with different structure and chemical composition and consequently different properties.

Initial results on acrylamide content indicated carbohydrate-rich foods to generate relatively more acrylamide. Several researchers have established that the main pathway of formation of acrylamide in foods is linked to the Maillard reaction and in particular, the amino acid asparagines as shown in figure 2.8 (Imre Blank, 2005)

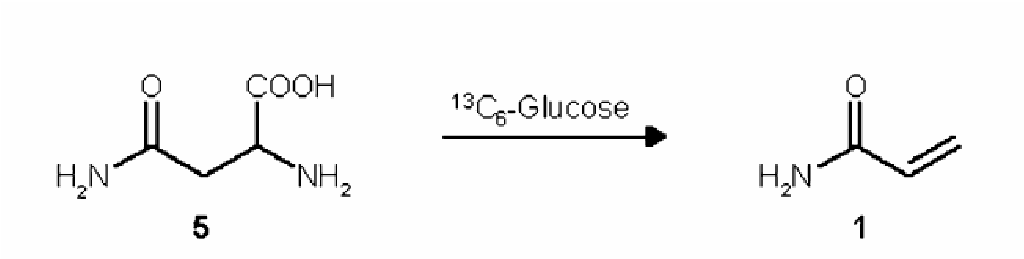


Figure 2.7: The formation of acrylamide (structure 1) from asparagines (structure 5)

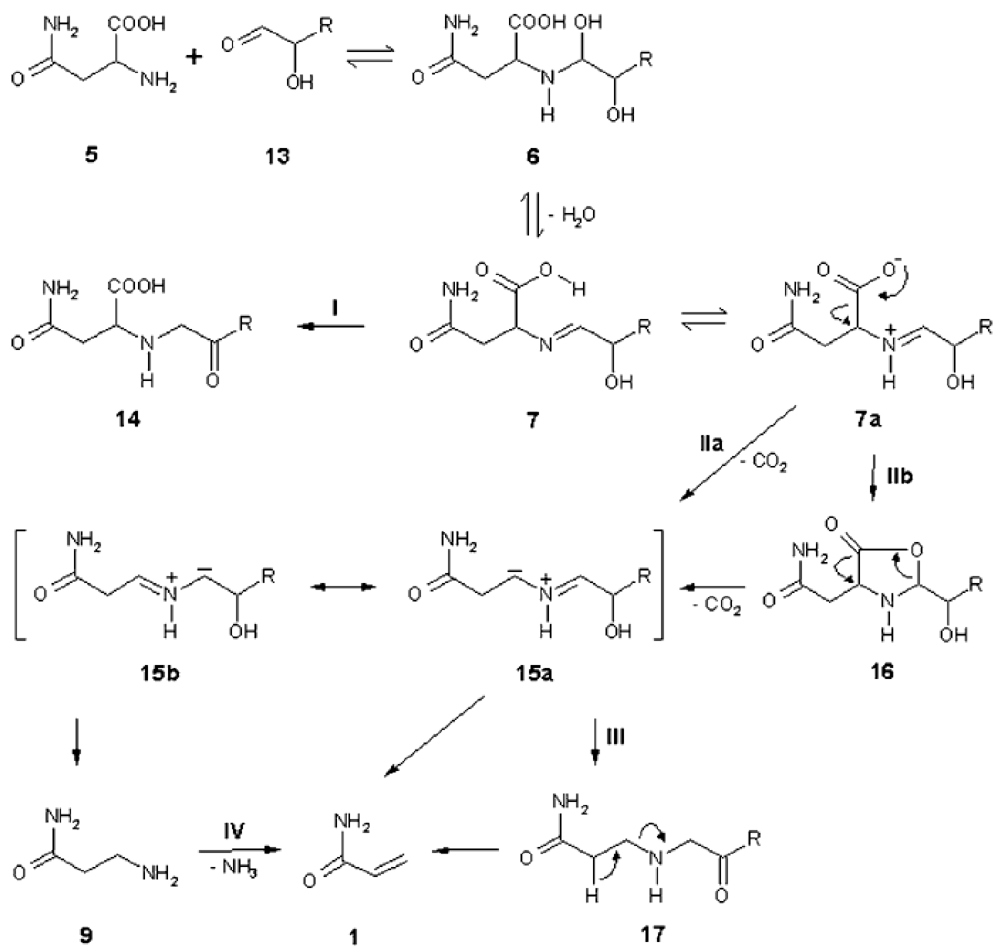


Figure 2.8: The process of acrylamide formation.

In figure 2.8, formation of acrylamide 1 from asparagines 5 in the presence of α-hydroxycarbonyls 13.R represents the rest of carbonyl moiety.

The Maillard reaction is of primary importance to the food manufacturer, since it is frequently responsible for the aromas and colours that develop during heating or storage of food products. Our ability to control the reaction is still very limited, although recent studies have indicated how it may be manipulated, particularly with regard to aroma development. The introduction of new processing and cooking techniques, such as microwave heaving, presents a constant challenge to the manufacturer to find ways of manipulating the reaction. The chemistry of the reaction and the factors affecting it are reviewed. (Jennifer M. Ames, 2003)

Reactive intermediates are formed by a variety of pathways and these can yield both volatile flavour components and brown melanoidins of higher molecular weight. The formation of these compounds is desirable in the heating (cooking) of many food products (meat, coffee, bread) but their occurrence during storage is undesirable and leads to a reduction in quality. The mechanism of the Maillard reaction will be explained and the most important intermediates and reaction products will be pointed out and their properties described. Reaction conditions for the Maillard reaction and methods for its inhibition will be discussed together with a description of methods currently available for the early identification of the Maillard reaction in foods. (Werner Baltes, 1982)

The high molecular weight compounds formed in the last stage of Maillard reaction are called melanoidins; they are widely distributed in foods and could exert different functional properties such as antioxidant, antimicrobial and metal-biding activity. In recent years, several studies have been mainly focused in the effect of melanoidins on the human diet and their possible nutritional, biological and health implications. Melanoidins could be responsible in part of the antimicrobial activity found in Maillard model systems derived from amino acid and sugar mixutures heated at different temperatures.

2.6 Chemical Changes During Roasting

During roasting, the most noticeable changes for the coffee bean occur from caramelization. The starches in the bean are changes to simple sugars due to the intense heat, where they begin to brown. This adds the recognizable color and also a slight sweetness to the coffee bean. The floral aromatics, fruit acids and caffeine weaken as the bean continues developing a dark color. Yet, as these characteristics reduce, the smoothness and other flavor compounds that we associate with our coffee are developed. People always misunderstand the darker roast coffee contain higher caffeine levels. According to the research, dark roasts such as the contents of French or espresso roasts less 15-20% caffeine than a lighter roast (mindburp).

Major changes occur in the carbohydrates during roasting. Sucrose is reduced fast during the roasting process, even in a lightly roasted coffee, only about 3-4% of its original content in the green bean remains. At a medium roast, some 1% may be found, whereas with very darkly roasted coffee it is completely lost. Other simple sugars present, in particular glucose and fructose and arabinose, are progressively destroyed. Polysaccharides are also greatly reduced. (University of Washington state, 1999)

Coffoel is one of the main flavor compounds developed from carmelization. It is coffee oil, formed generally around 200°C in the heating process and can be solute in water. In addition, it is a compound that has a tendency to absorb other flavors easily. Hence the reason coffee is carefully stored away from other intense flavors.

At the initial step, roasting remove all free moisture and proceeds further to force out bound moisture. This force of the moisture will expand the bean and produce crackling and snapping noises when it is leaving. The oils have not volatilized and colour is not dramatically change at this point. However, when the internal temperature reaches 400° F, the darkening of the bean will be seen and the oil will start to appear. The initial crackle along with a light brown color is a good indication of the coffoel formation. The crackles are also an indication of pyrolysis, which is the temperature (around 465°F/240°C) at which gasses are produced in the roasting coffee beans that combust, causing them to emit their own heat. This results in an increase in temperature within the roasting chamber. In this stage the color will start to darken drastically and an oil formation will begin to develop (Davids, 1976). Roasting is subdued at the point the bean attains the sought after color.

2.7 Carbohydrates

Carbohydrates constitute the major fraction of both green coffee and commercial (roast and soluble) products therefrom. Roasting is an important step in coffee production for the formation of various types of ‘flavour’ compounds. The formation of these compounds are significantly contributed by the conversion of carbohydrates. (A.Oosterveld, A.G.J. Voragen, H.A. Schols, 2003)

Carbohydrates, the largest group of organic molecules in nature, containing about 50 % of the earth’s biomass. The number of carbon inside carbohydrates is range from three to thousands. The chemistry of carbohydrates can be complex. Carbohydrates comprise many functional groups, which can be react either each other and with other reagents. Basically, they divided into four types, that are monosaccharides, disaccharides, oligosaccharides and polysaccharides according to the number of monosaccharide units linked in a molecule.

Carbohydrates are storehouses of chemical energy. They known as saccharides occur naturally in plants and are one of the three principal classes of animal food. They commonly referred to as sugars and starches are polyhydroxy aldehydes and ketones or compounds that can be hydrolyzed to them. They are synthesized in green plants and algae by photosynthesis, a process that uses the energy from the sun to convert carbon dioxide and water into glucose and oxygen. This energy is released when glucose is metabolized. The oxidation of glucose is a multi step process that forms carbon dioxide, water and great deal of energy. (Janice Gorzynski Smith, 2006) The amounts of energy available from carbohydrates is about 17 kJ/g (4 kcal/g). Figure 2.9 shows chemical reaction of carbohydrates.

$$6H_2O + 6CO_2 \xrightarrow[chlorophyll]{hv} C_6H_{12}O_6 + 6O_2$$

Figure 2.9: Chemical reaction of carbohydrates

2.7.1 Glucose

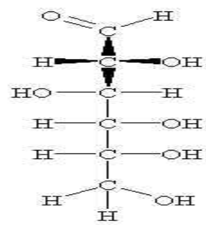


Figure 2.10: Structure glucose

Glucose ($C_6H_{12}O_6$), in the figure 2.10, is one of the most common Monosaccharide. Monosaccharides are carbohydrates that cannot be hydrolyzed to simpler carbohydrate units. It is an aldohexose found in the free state in plants and animal tissue, generally known as *dextrose* or *grape sugar* and is a component of the disaccharides sucrose. Among the common sugars, glucose is of intermediate sweetness. (Joel K.Swadesh, 2001)

Glucose is the key sugar of the body and is carried by the blood stream. The concentration of glucose in the blood is normally 80 - 100 mg per 100 ml of blood. Sometimes, it is called as *blood sugar* because it is the most abundant carbohydrate in the blood. It can be given to patient who cannot eat food by mouth intravenously as it does not need digestion. For those who find glucose in their urine are people having diabetes mellitus (sugar diabetes).

2.7.2 Sucrose

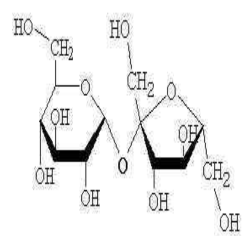


Figure 2.11: Sucrose chemical structure

Figure 2.11 shows the chemical structure of sucrose, $C_6H_{12}O_6$ and is the most important disaccharides in coffee. Disaccharides contain two monosaccharides joined together by a glycosidic linkage. Sucrose is commonly known as *table sugar*, is found in the free state throughout the plant kingdom. It consists of one glucose unit and one fructose unit as shown in figure 2.12.

Disaccharides are not used directly in the body but are first hydrolyzed to monosaccharides. It yields two monosaccharide molecules when hydrolyzed in the laboratory at elevated temperatures in the presence of hydrogen ions (acids) as catalyst. In biological systems, enzymes (biochemical catalyst) carry out the reaction. For each different hydrolysis of disaccharide, different enzyme is required. For sucrose, sucrase is needed. The structure of disaccharide is derived from two monosaccharide molecules by elimination of a water molecule between them. (Janice Gorzynski Smith, 2006)

$$\text{Sucrose} + \text{water} \xrightarrow{\textit{Sucrase}} \text{Glucose} + \text{Fructose}$$

Figure 2.12: Hydrolysis of sucrose

2.8 Amino Acids

Proteins are the third important class of foodstuffs. All proteins are polyamides formed by joining amino acids together. Naturally occurring amino acids have an amino acids group (NH_2) bonded to the α carbon of a carboxy group (COOH), and thus are called α-amino acids as showing in figure 2.13. The 20 amino acids that occur naturally in the proteins differ in the identity of the R group bonded to the α carbon. The R group is called the side chain of the amino acid.

$$\begin{array}{c} R \\ | \\ H_2N\text{-}C\text{-}COOH \\ | \\ H \end{array}$$

Figure 2.13: α-amino acids

All amino acids have common names. These names can be represented by either a one-letter or a three-letter abbreviation. Alanine is the amino acid with the highest concentration followed by asparagines in coffee. (Michael Murkovic, Karin Derler, 2006)

2.8.1 Asparagine

It is neutralized version of aspartic acid. Asparagine carries a hydrophilic acidic group with strong negative charge. Usually it is located on the outer surface of the protein, making it water-soluble. It binds to positively-charged molecules and ions, often used in enzymes to fix the metal ion. When located inside of the protein, aspartate and glutamate are usually paired with arginine and lysine. (Wikipedia Foundation, 2006)

CHAPTER 3

METHODOLOGY

3.1 Introduction

This research is divided into two main experiments. In these two experiments, two parameters will be studied, these are time and temperature. For the self-roasted coffee, 200°C and 220°C will be used to roasted coffee because the coffee will start roasted at 200°C while 220°C roasted coffee powder is the most popular among Malaysian. (Minister Coffee Powder Sdn Bhd, May 2006.)

Carbohydrates and asparagines as the main precursors of acrylamide, will be analysis among these nine samples to find out the relationship of the acrylamide formation. While the acrylamide will be analysis to find out the composition of it in the coffee. These three experiments undergo the same common procedures as shown in figure 3.1.

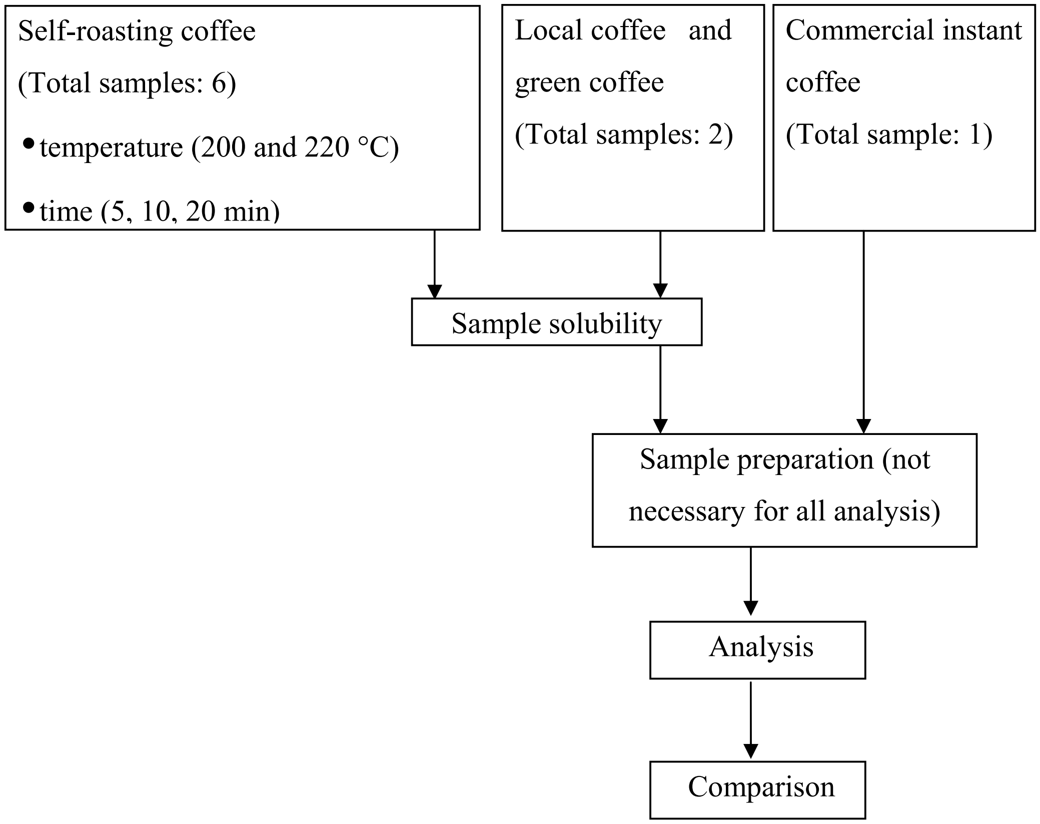


Figure 3.1: Overall analysis steps for coffee

First and foremost, green coffee beans will be divided into seven categories. Six of them are roasted in an oven for different temperature and time. The remain one will be green coffee, that is also known as unroasted coffee. After that, all of these seven samples will be ground by grinder for obtaining the fine powder.

The nature characteristic of coffee is insoluble with water. Thus, they need to be gone through some solubility steps prior to analysis. Different components will be used in different ways of solubility steps but with some similarity. The similarity of them are mixing with water and vortex, then centrifuge and lastly, been filtered. The commercial coffee no needs to do solubility process as it is instant coffee, can solute into water. It will be treated as the amount of chemicals and water that add into the solution as other insoluble coffee. Then, these nine samples will be prepared to do analysis. Mostly, the sample preparation is sample purification process and is to ensure there are free of solids inside the samples that will affect the analysis result. As usual, this step is done by filtration.