SCREENING PARAMETERS ON THE BIOLOGICAL ACTIVE COMPOUNDS OF COFFEE BEAN MIXED WITH *NIGELLA SATIVA*

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BACHELOR OF CHEMICAL ENGINEERING

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Thesis submitted in partial fulfilment of the requirements
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Dedicated to my beloved family members and friends.

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ABSTRACT

The addition of *Nigella sativa* to coffee offers consumers to gain health benefits besides reducing the components which are bad to health. This research aimed to investigate the effect of significant parameters of coffee bean mixed with *Nigella sativa* in terms of biological compounds. The parameters studied included temperature, ratio of coffee to *Nigella sativa* and mixing time. The biological compounds included caffeine, total phenolic content and antioxidant activity. Design Expert was used to screen the parameters of the above factors. UV-VIS spectrophotometer was mainly used to measure the total phenolic content and antioxidant activity. From the screening process, three parameters which were temperature, ratio of *Nigella sativa* to coffee and mixing time contributed a significant effect to the caffeine content analysis for both black and white coffee with the P value<0.05. Interaction was found between temperature and ratio of *Nigella sativa* to coffee. As for total phenolic content, all three parameters(temperature, ratio of *Nigella sativa* to coffee and mixing time) contributed significant effects to the overall results for black coffee with P value< 0.05. Significant interactions between temperature and ratio of *Nigella sativa* to black coffee and between ratio of *Nigella sativa* to black coffee and mixing time. On the other hand, only ratio of *Nigella sativa* to coffee and mixing time resulted to significant contribution to the total phenolic content of white coffee. For antioxidant activity, only two parameters contributed significant effects for the results for both coffee. For black coffee, they were temperature and mixing time with P value<0.05. However, interaction was found between temperature and ratio of *Nigella sativa* to coffee. Whereas, ratio of *Nigella sativa* to coffee and mixing time significantly affected the antioxidant activity of white coffee with P value<0.05. The results showed that *Nigella sativa* worked better with black coffee than white coffee. All parameters were significant to black coffee, only two parameters played significant roles to white coffee. The parameters were ratio of *Nigella sativa* to coffee and mixing time. As a conclusion, the additional of *Nigella sativa* to the coffee had improved the quality of coffee by reducing the caffeine content and increasing the total phenolic content and antioxidant activity of coffee.

ABSTRAK

Penambahan *Nigella sativa* dalam kopi menawarkan pengguna banyak manfaat kesihatan selain megurangkan komponen kopi yang tidak baik. Kajian ini bertujuan untuk mengkaji kesan parameter terhadap penambahan *Nigella sativa* dalam kopi dari segi sebatian biologi. Parameter yang dikaji ialah suhu, nisbah *Nigella sativa* untuk kopi dan masa pencampuran. Sebatian biologi termasuk kandungan kafein, jumlah kandungan fenolik dan aktiviti antioksidan. Design Expert telah digunakan untuk menyaring faktor-faktor di atas. UV-VIS spektrofotometer terutamanya digunakan untuk mengukur jumlah kandungan fenolik dan aktiviti antioksidan. Tiga parameter termasuk suhu, nisbah *Nigella sativa* untuk kopi dan masa pencampuran menyumbang kesan yang besar kepada analisis kandungan kafein untuk kedua-dua kopi hitam dan putih dengan nilai $P < 0.05$. Bagi jumlah kandungan fenolik, ketiga-tiga parameter (suhu, nisbah *Nigella sativa* untuk kopi dan masa pencampuran) menyumbang kesan yang penting kepada keputusan keseluruhan untuk kopi hitam dengan nilai $P < 0.05$. Interaksi didapati berlaku antara suhu dengan nisbah *Nigella sativa* untuk kopi hitam dan nisbah *Nigella sativa* untuk kopi hitam dengan masa pencampuran. Bagi kopi putih, hanya nisbah *Nigella sativa* untuk kopi dan masa pencampuran menyumbangkan kesan penting kepada keputusan keseluruhan. Untuk aktiviti antioksidan, hanya dua parameter menyumbang kesan yang penting bagi keputusan untuk kedua-dua kopi. Bagi kopi hitam, suhu dan masa pencampuran ialah antaranya dua parameter yang menunjukan nilai $P < 0.05$. Pada masa yang sama, interaksi didapati berlaku antara suhu dengan nisbah *Nigella sativa* untuk kopi. Sementara itu, nisbah *Nigella sativa* untuk kopi dan masa pencampuran adalah parameter penting bagi aktiviti antioksidan kopi putih dengan nilai $P < 0.05$. Hasil kajian menunjukkan bahawa *Nigella sativa* bekerja lebih baik dengan kopi hitam daripada kopi putih. Ketiga-tiga parameter menyumbangkan kesan penting kepada analisis kopi hitam tetapi hanya dua parameter memainkan peranan yang lebih penting untuk kopi putih. Mereka adalah nisbah *Nigella sativa* untuk kopi dan masa pencampuran. Kesimpulannya, penambahan *Nigella sativa* dalam kopi telah meningkatkan kualiti kopi dengan mengurangkan kafein kopi di samping meningkatkan jumlah kandungan fenolik dan aktiviti antioksidan.

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LIST OF ABBREVIATIONS

oz	ounce
fl.oz	fluid ounce
PD	Parkinson Disease
TPC	Total Phenolic Content
DPPH	1,1- Diphenyl-2-picrylhydrazyl
UV-VIS Spectrophotometry	Ultraviolet-visible Spectrophotometry
MeOH	Methanol
GC	Gas Chromatographic
HPLC	High Performance Liquid Chromatography
GAE	Gallic acid equivalent
TQ	Thymoquinine
DIM	Dithymoquinone

CHAPTER 2

INTRODUCTION

2.1 Background of the Study

“Coffee is proof that mornings were created for a reason”, this is a quote from a coffee addict. According to "Global coffee consumption will increase by a third to 200 million bags"(2016), this year which is 2016, the production of coffee is about 140 million bags and it is estimated to increase by a third to 200 million bags by 2030. Today, coffee is enjoyed and consumed by people from all over the world. There are lots of sayings towards coffee consumption. Some say coffee can bring a lot of benefits. There is no doubt that coffee can bring us advantages. One of the reason is that coffee is antioxidant rich beverage. Yashin et al. (2013) showed that coffee contain 150 to 300 mg/g total antioxidant content which is higher than tea but lower than cocoa. Antioxidant activity is the biggest contributor to the benefits of coffee. Among the components contribute to antioxidant activity in coffee are chlorogenic, ferulic, caffeic, and n-coumaric acids. Even caffeine and trigonelline are considered as antioxidants.

However, Silletta & Marchioli (2008) said that coffee has either beneficial or harmful effect on health due to the presence of trigonelline and caffeine. Furthermore, among so many dietary products which contain caffeine, coffee contains the highest caffeine content in which the average caffeine content of brewed coffee is from 95mg to 200mg per 8 oz of coffee (Mayo Clinic, n.d.). Large amount of coffee consumption especially caffeine component in coffee will result in various chronic diseases such as cardiovascular disease, high blood pressure, high cholesterol level and others. Bonita et al. (2007) proved that caffeine in the coffee can increase the blood pressure and sympathetic nerve activity of non-habitual drinkers. However, for habitual drinkers, due

to mental stress problem caused by caffeine, they went through a lack of blood pressure increase although there is an increase in nerve activity.

The problem faced nowadays is the demand of coffee in this world is increasing. Coffee brings benefit but harms too. In this healthy lifestyle times, people start to seek for the way to consume coffee in the healthiest way. Decaffeinated coffee was produced before, however, study shows that it is not as good as we expected. This is mainly due to the solvent and coffee beans used in the decaffeinated process which will bring us adverse effects (Rafetto et al., n.d.).

Herbal coffee is one of the coffee products which receives well recognition in the current market as nowadays people keen in pursuing healthy lifestyle. The addition of herbs to coffee offers consumers to gain health benefits besides reducing the components which are bad to health. *Nigella sativa* herb plant which belongs to the botanical family *Ranunculaceae* can bring numerous great benefits. One of the benefits brought by *Nigella sativa* seed is anticancer. This is proved by a research of treating the human volunteer with whole seeds powder at doses of 1g twice per day for a duration of 4 weeks. From the results, the ratio of T-Lymphocytes helper T-cell to T-suppressor cell increased by 72% besides enhancing the T killer cell function and number (El-Tahir & Bakeet, 2006).

Thus, this research is done to study the effect of mixing *Nigella sativa* seeds with coffee by considering different parameters such as temperature, ratio of *Nigella sativa* to coffee and mixing time in order to find out the most significant parameter which bring significant effects to the nutritional value of the herbal coffee besides reducing the caffeine content of coffee.

2.2 Motivation

Nowadays, the demands of coffee are getting higher. Coffee lovers like to chill around the coffee shop or indulge their coffee cravings before they start their daily work routine or after a long working day. Coffee is rich in antioxidants, moderate consumption actually brings us many health benefits. However, due to the existence of

caffeine in coffee, an individual will get addicted to coffee uncontrollably. Harmful health effects will be resulted due to high consumption of coffee. According to European Food Safety Authority (2015), a daily consumption of 400mg of caffeine is considered safe for adults. However, for developed teens which are between 13 to 18 years old, consumption of caffeine should not be more than 100mg. As for pregnant woman, consumption of 200mg or less caffeine will cause a little risk to fetus. One cup of brewed coffee (8oz.) can contain 90mg to 200mg of caffeine. As for instant coffee, the caffeine content is in the range of 27mg to 173mg (Mayo Clinic, n.d.). People who are addicted to coffee can consume up to 5.6 cups of coffee per day. The caffeine level consumed definitely exceeds the safe limit of caffeine consumption (Leviton et al., 1994). The presence of decaffeinated coffee actually give a reason for coffee lovers to drink coffee without feeling guilty. However, problem associated with decaffeinated coffee arises too. People start to seek for the best and healthiest way to consume coffee. Herbal coffee is believed to receive more recognitions in the future especially when people are in the direction to pursuit healthy lifestyle. Febrianto & Rizki (2015) did a study on the addition of cardamom to the coffee. The study showed that this herbal coffee is potential to be developed as one of the coffee diversification products. *Nigella sativa*, a kind of herb plant, consists of numerous benefits. The seed itself contains more than 100 nutrients. The adding of *Nigella sativa* powder to coffee under the influence of different parameters is believed to increase the nutritional values of the coffee besides reducing the bad components of the coffee. Therefore, investigating the significant parameters which can produce coffee mixed with *Nigella sativa* powder with low level of caffeine and additional values without affecting the aroma of coffee has become the motivation for this research.

2.3 Problem Statement

Coffee is the most popular drink in this world besides water. People drink coffee for various reasons. A lot of them drink coffee for the sake of keep them awaken during working. Some seek for the best taste from various types of coffee. Compared to alcohol, coffee is always assumed as healthy alternative in any gatherings. For example, in Taiwan, instant coffee is ready available in a lots of places such as petrol station, mini market or convenient store. Similarly in Malaysia. People’s demand towards coffee is

getting higher and higher (Hsu & Hung, 2005). While coffee is moving towards leading the worldwide beverage, people encountered a lots of problems of drinking coffee, especially for those who start to get addicted to coffee. This is because coffee contains quite a high amount of caffeine. Caffeine overdose which is over 400mg per day will cause intoxication. This will result to anxiety, restlessness, psychomotor agitation and insomnia (Reissig et al., 2009). After realizing this problem, a lots of researches and developments start to produce coffee with low caffeine content which is known as decaffeinated coffee. However, according to Meikle (2005), drinking decaffeinated coffee may bring harms to our health. This is proved by a survey of 187 coffee drinkers on decaffeinated coffee. By referring to the results of survey, the coffee drinkers mentioned that decaffeinated coffee could raise "bad" cholesterol which can lead to arteries diseases. Besides, a 18% rise in fat in the blood was found through the study. Bonita et al. (2007) mentioned decaffeinated coffee will raise the blood pressure and increase the nerve activity for non-habitual drinkers. For a coffee drinker, the caffeine habit could induce temporary mental disorder. These included restlessness, excitement, nervousness, gastrointestinal upset and irregular heartbeat. All these are the signs of caffeine intoxication. However, there will be a lots of problems arise if the coffee drinkers stop drinking coffee immediately. This is known as coffee withdrawal symptoms (Parry, 2013). Therefore, there is a need that coffee is consumed in a healthier way in which the components which bring benefit should be retained or increased. At the same time, the components which are detrimental to health should be reduced.

2.4 Objectives

The following is the objective of this research:

- To investigate the effect of the significant parameters of coffee bean mixed with *Nigella sativa* in terms of biological active compounds.

2.5 Scopes of Study

The following are the scopes of this research:

- To investigate the effect of parameters such as temperature, ratio of *Nigella sativa* to coffee and mixing time on the biological active compounds (caffeine, antioxidants and total phenolic content) of black and white coffee..
- To screen the significant parameters of the above factors on biological active compounds of black and white coffee.

CHAPTER 3

LITERATURE REVIEW

3.1 Introduction

This chapter provides an overview of previous researches on knowledge sharing.

3.2 Coffee

Coffee culture is very well-known since 1950s-1960s in America and this culture is brought by Italian-American immigrants. Pay (2009) stated that the world largest coffee importer is European Union which contributing around 4 million tonnes in year 2008. In Malaysia itself, the coffee culture starts under the influential of British. After that, this culture develop from a small coffee stall and "kopitiam" until the retail shops such as Starbucks, Coffee Bean and so on in the local market today (Nurbaya & Rahman, 2010). Coffee has a big influence in our daily life. Bonita et al. (2007) mentioned that there are total 52% of United State citizens who consume coffee for more than 10 years. Besides, European community consume an average of 5.1 kg/year of coffee. In Malaysia itself, the yearly coffee consumption of Malaysian is 1.3 kg per capita. Leviton et al. (1994) concluded from a survey that people who used to consume coffee can consume up to 5.6 cups per day.

3.2.1 Types of coffee beans

Heckman et al. (2010) highlighted in the research that coffee contains the highest amount of caffeine compared to other beverages such as tea and coca-cola.

Among so many types of coffee, espresso contains the highest amount of caffeine which is 40mg caffeine per 1 ounce of coffee. Obviously, Espresso can be said as the foundation of all the other types of coffee and it has much more higher caffeine content than others. Casal et al. (2000) mentioned that *Coffea Arabica* and *Coffea Canephora* (Robusta) are the most important coffee bean in coffee industry. In year 2007, exports of *Coffea Robusta* contributed US$3.32 billion while exports of *Coffea Arabica* accounted US$9.38 billion (Pay, 2009). 75% of the world coffee production is *Coffea Arabica* while 24% is the *Coffea Robusta*. This data contributes to the determination of types of coffee to be used in this research. *Coffea Robusta* is larger in size and flat in shape compared to *Coffea Arabica* which is smaller in size and oval in shape. Robusta coffee bean contains lower level of chlorogenic acid but higher caffeine content when compared to Arabica coffee bean. Usually unroasted coffee seems to have higher protein, acid, sugar and caffeine compared to roasted coffee. However, unroasted coffee lacks of taste of roasted coffee due to Mallard or other chemical reaction during roasting (Hečimović et al., 2011).

Figure 3.2.1: Coffee Arabica and Coffee Robusta

(Costanzo, n.d.)

3.2.2 Decaffeinated coffee

Decaffeinated coffee is the coffee which contains trace amount of caffeine. Meikle (2005) conducted a survey regarding the cardiac health of 187 decaffeinated coffee drinker. The results of the survey showed that decaffeinated coffee can raise bad

cholesterol level. Rafetto et al. (n.d.) mentioned that decaffeinated process can be divided into few types which includes by using solvent, water, or supercritical carbon dioxide. The method of using methyl chloride is the most popular method among so many methods. This is because this method can retain the most flavor of coffee compared to other methods. However, methyl chloride is found to be carcinogenic and it is labeled as human carcinogen by the National Cancer Institute. As mentioned in Food and Drug Administration guidelines, a decaffeinated coffee should remove at least 97% of caffeine. In reality, this rule is not being followed and resulted in various caffeine contents in different decaffeinated coffee. Coffee drinkers always have the impression that decaffeinated coffee contains low amount of caffeine and this will cause them do not limit themselves in consuming coffee. Decaffeinated coffee will still contribute to the additive effect when the coffee is being consumed throughout the whole day. This will bring harmful effects to the one who is sensitive to the caffeine. Apart from that, decaffeinated coffee is made from *Coffea Robusta* which is believed to retain the more coffee flavor when comparing with other coffee beans. The main problem is *Coffea Robusta* contains higher level of caffeine thus it is more acidic. This will bring adverse effect to the individual who suffered from acid reflux, ulcers or other health problems. Decaffeinated coffee will greatly influence the pH system of the body. For instance, bone density will be decreased due to the acidic activity. As mentioned above, existence studies show that decaffeinated coffee can raise the bad cholesterol level. This is also mentioned be Rafetto et al. (n.d.) in the studies. This actually prove that decaffeinated coffee removes the caffeine in the coffee but other compounds in coffee will still contribute to certain detrimental effects. Therefore, although many coffee drinkers who start to look for healthy diet will start to switch from regular coffee to decaffeinated coffee, however, this does not really solve the problem due to the existence of so many doubts towards decaffeinated coffee.

3.2.3 Herbal coffee

Nowadays, coffee is no longer available in its purest form. Herbs are added to coffee to moderate its caffeine content as well as increase the nutritional values. It is believed herbal coffee can provide more antioxidant to the consumers based on the types of herbs used. Febrianto & Rizki (2015) did a study on the addition of cardamom

to the coffee. Cardamom (*Amonum cardomum*) is a commonly recognized herbs and spices for food flavoring. The study showed that this herbal coffee is potential to be developed as one of the coffee diversification products as it helps to reduce the caffeine content. Teeccino Herbal Coffee is one of the marketed herbal coffee products with low caffeine content.

3.2.4 Advantages of Coffee Consumption

Coffee contains of a lot of biologically active compounds which has far reaching effects for human body. Sochan (2016) mentioned coffee actually brings a lots of medical benefits because of its ingredients such as caffeine, chlorogenic acids, caffeol, polyphenols, phytoestrogens and diterpenes. According to "Caffeine Safe Limits: Determine Your Safe Daily Dose" (2016), for a healthy adult, 300mg to 400 mg (equally to 4 cups of coffee) of caffeine can be consumed without any adverse effect whereas for developing teens, they should not consume more than 100 mg daily. As for pregnant mother, it is advisable to consume 200 mg or less a day. Sochan (2015) stated that coffee consumption can reduce the risk of getting coronary disease and the new data suggests that 2 cups of coffee per day can protect against heart failure. For moderate coffee drinker (less than 300ml/day), the risk of getting coronary death can be decreased by 31% when comparing to non-coffee drinker. However, if one consumes the coffee in an amount which is more than 300ml/day, the risk of getting coronary death is directly proportional to the amount of coffee consumed (Bonita et al., 2007). Besides, coffee consumption can cut down the risk of stroke by as much as 25%. Data presented by European Meeting on Hypertension 2012 actually found that daily consumption of 1 to 3 cups of coffee may protect against ischemic stroke in general population. Ross et al. (2000) stated in the survey of the risk of getting Parkinson Disease(PD) due to amount of coffee intake and total dietary caffeine intake. From the results of finding, the higher amount of coffee and caffeine intake, the lower the risk of getting Parkinson Disease. Besides, coffee consumption may lower the risk of getting Type 2 Diabetes disease. This is because one cup of 8 fl.oz of coffee contains 7mg of Magnesium. The Magnesium plays a role as cofactor to lower the risk of the disease (Preedy, 2012).

3.2.5 Disadvantages of coffee consumption

However, with benefits come the risks, coffee consumption will bring certain negative effects, especially when one consume more than 4 cups of coffee per day. Mayo Clinic Research (2016) found that there is a 21% increase in all-cause mortality for men who drinks more than four 8 fl.oz. cups of coffee. Some people is over sensitive to caffeine molecules, this can cause allergic-like reaction happen in the body. Heavy coffee drinker may suffer from insomnia, headache, and even indigestion. According to Ostrow (2014), six genetic variants were found to be associated with coffee drinking based on Journal of Molecular Psychiatry. Besides, coffee consumption might cause the rise in blood pressure, especially to those who already suffered from hypertension. Mayo Clinic Research(2016) showed that as a result of consuming 160 mg caffeine, all the participants experience a marked rise in blood pressure. In addition, Dr. Lucio Mos found that after consuming the amount of caffeine equivalent to 4 cups of coffee, young adults had 4 times risks to be diagnosed with mild hypertension. This will lead to heart attack at the end. This actually shows that coffee consumption in a moderate amount is good in preventing coronary disease however, when in heavy consumption, the risks of getting heart diseases is increasing.

3.3 Caffeine content

There are a few ways to extract caffeine from coffee. According to Nyamien et al. (2015), the solvent which consist of water, ethanol, and methanol in a ratio of 2:1:1 can extract the highest amount of caffeine compared to other solvents. In addition, the caffeine content extracted increases when the solid and liquid ratio increase from 1:100 to 3:100 but decreases until ratio 6:100. However, from Pradeep (2015), solvent chloroform extracts a much higher caffeine when compared to other solvents.

In purification of caffeine crude, there are two ways in purifying the caffeine. The two ways included vacuum sublimation and atmospheric sublimation. Sublimation point of caffeine is 178°C. Therefore, at 178°C, caffeine will turn from solid to gas and when the gas rises up, it will condense. White solid of caffeine can be collected. Other than sublimation, recrystallization is another way in purifying caffeine. Recrystallization

happens by dissolving the crude caffeine in hot solvent such as acetone. The solution is then cooled in ice bath and the crystal will form. Vacuum filtration is then used to collect the final purified product. (“Extraction of Caffeine from Tea Techniques & Principles: Week One”, n.d)

Caffeine content can be determined by measuring the weight of caffeine extracted or measured by using equipment. Instead of using UV-VIS spectrophotometer to determine the amount of caffeine, they used High Performance Liquid Chromatography to determine the caffeine content and purity. This is because it is non-destructive and sensitive. Similarly, as mentioned in “Extraction of Caffeine from Tea” (2016), dichloromethane is used to extract caffeine because caffeine is more soluble in dichloromethane(140mg/ml) than water (22mg/ml).

Liew et al. (2001) mentioned in the research about various ways to measure the caffeine contents. These ways include spectrophotometric method, Gas Chromatographic(GC) method and High Performance Liquid Chromatographic(HPLC) method. From the results of the research, it shows that HPLC without defatting step is the most accurate method which achieve 98.6%. At the same time, spectrometry and HPLC with defatting step show a satisfactory accuracy too. Belay et al. (2008) mentioned that UV-VIS spectrometry cannot be used to determine the caffeine content in coffee bean directly due to the matrix effect of UV absorbing substances in the simple matrix. Therefore, it is suggested to extract the caffeine by using chloromethane before measuring the caffeine content using UV-VIS spectrometer.

3.4 *Nigella sativa*

Nigella sativa, a flowering plant, native to Southwest Asia with a height of 20-30 cm. It’s seed, which is also known as Black Cumin, has numerous great functions. This seed consists of over 100 nutrients. Due to the medical benefits, *Nigella sativa* seeds can be used as food preservatives or as a spice. People also consume *Nigella sativa* in different ways. Some mix the seed in food or mix with honey for consumption. (Shabana et al., 2013)

Figure 3.4: Nigella sativa and seeds

(Shabana et al., 2013)

3.4.1 Benefits of *Nigella sativa*

Nigella sativa contains few major components which contributes to the benefits. Thymoquinine(TQ) and dithymoquinone (DIM) have great potential in traditional medical properties. The presence of these two components are found to have resistant towards tumour cell in vitro (Swamy & Tan, 2000). Thymoquinine also plays an important role as cholesterol-lowering agent due to its high antioxidant activity (Ismail et al., 2010). Besides, *Nigella sativa* is found to have ability to reduce the toxicity of body because of the antioxidant activity. In addition, *Nigella sativa* possesses anti-histaminic properties. This is shown by the active ingredient in *Nigella sativa*, Nigellone which has certain impact towards inflammatory diseases caused by histamine. Besides, *Nigella sativa* has been reported to have various anti-microbial activities. For example, anti- fungal, anti-bacterial and so on (Sahebkar et al., 2016). As for disease Diabetes Mellitus, *Nigella sativa* plays an important role in improving the metabolic factors which are glycemic status and liquid profile, This is mainly due to its antioxidants characteristics and also the effect exerted by *Nigella sativa* to glucose absorption and insulin secretion (Heshmati & Namazi, 2015).

3.5 Total phenolic content and antioxidant activity

According to Naidu et al. (2008), Folin-Ciocalteu method was used to measure the total phenolic content of coffee samples. Gallic acid was used as standard. 765nm of wavelength was used to measure the absorbance of the samples. Castillo et al. (2002) mentioned in the research that three ways were being used to measure the antioxidant activity of coffee. The ways included gel filtration chromatography, UV-visible spectrophotometry, and capillary electrophoresis. For UV-VIS spectrophotometry, the sample was determined at 280nm and 420nm. It was found that the medium roasted coffee sample detected the highest value at absorbance 280nm whereas at 420nm, the highest value was detected by dark-roasted coffee sample. According to Castillo et al. (2002), wavelength 280nm was chosen is mainly because most of the components including caffeine, trigonelline and proteins absorb at this wavelength. On the other hand, 420nm was chosen is because the development of brown colour is to be monitored. Besides, for the gel filtration chromatography, the profiles of the coffee was also extracted at 280nm and 420nm. The results showed that with the increasing of roasting level up to medium, the overall strength of chromatogram is increasing too. According to Naidu et al. (2008), the method used for antioxidant activity measurement is through using 1,1-diphenyl-2-picrylhydrazyl (DPPH) to measure the free radical scavenging activity. The changes in absorbance were measured at 515nm.

3.6 Screening process

Experimental variables and interactions that have influence to the results can be determined through screening experiment and it can show one or more responses. In screening process, there are few common interaction models can be used such as full factorial or factional factorial designs. Throughout these process, the variable which has the largest influence on the final result will be determined. Most of the experimental values, interaction effects and factors are investigated through factorial design. A factorial design will consist of 2^k experiments. For instance, 3 factors will render $2^3=8$ experiments (Lundstedt et al., 1998). There are few benefits in carrying out the factorial design. The first benefit is when comparing with other alternative designs, factorial design requires less experimental subjects. The second benefit is when comparing with

manipulating one factor at a time, factorial design is able to investigate the interactions between factors at one time (Collins et al., 2009). As for whether to choose complete factorial design or fractional factorial design, there are few different perceptions. According to Collins et al. (2009), complete factorial design is not recommended as some combinations of levels of the factors will produce impractical and undesirable experiment conditions. On the other hand, fractional factorial design is more generally approached because it makes simultaneous investigation of multiple independent variables more flexible.

CHAPTER 4

METHODOLOGY

4.1 Introduction

This chapter clearly defines the research method used to conduct the research.

4.2 Materials and methods

Black coffee bean, white coffee bean(*Coffea Arabica*) and *Nigella sativa* seeds were purchased from local market; distilled water; sodium carbonate; Dichloromethane; ethanol; pure caffeine; 1,1- Diphenyl-2-picrylhydrazyl (DPPH); gallic acid; Folin Ciocalteu Reagent

4.2.1 Preparation of coffee, *Nigella sativa* seeds and mixed coffee and *Nigella sativa* seeds

The white and black coffee beans and *Nigella sativa* seeds were ground into powder and screened through 250 μm of sieve to get uniform texture (Belay et al., 2008). Approximately 20g of black coffee and different amounts of *Nigella sativa* seeds were weighed and mixed to be dissolved in 150ml of distilled water. The amount of *Nigella sativa* seeds used were in the range of 0.1g to 0.5g after considering the aroma of the coffee. The solution was stirred. Three parameters which included temperature, mixing time of *Nigella sativa* seeds with coffee and ratio of *Nigella sativa* seeds and coffee were studied. One-factor-at-a-time method (OFAT) was the method used in preliminary experiment.

For the temperature effect, samples(black coffee and white coffee) were collected at temperature 60 ℃, 70 ℃, 80 ℃, 90 ℃ and 100 ℃ with the mixing time fixed.

As for the effect of ratio of *Nigella sativa* powder to black and white coffee, 0.1g, 0.2g, 0.3g,0.4g and 0.5g of *Nigella sativa* powder were added to black and white coffee separately with the temperature and mixing time were fixed.

Lastly, for the effect of mixing time of *Nigella sativa* powder with black and white coffee, the mixing time of 1minute, 2minutes, 3minutes, 4minutes and 5 minutes were studied with the temperature and amount of *Nigella sativa* added were fixed.

4.3 Extraction and purification of caffeine

Caffeine was extracted from the sample by using liquid-liquid extraction. 30ml of dichloromethane was added to the 150ml of sample by instalment. The mixture was stirred and poured into separate funnel. The layer of dichloromethane was extracted and poured into a clean beaker. The dichloromethane was then sent to rotary evaporator to separate dichloromethane from the crude caffeine. The temperature of rotary evaporator was set at 40°C which is the boiling point of dichloromethane. The crude caffeine was purified through sublimation process. The crude caffeine obtained from the rotary evaporator was allocated in a preheated conical flask on a hotplate. The conical flask was then sealed. The conical flask with crude caffeine was heated directly until 178°C by using hot plate. The caffeine sublimed during melting. At the end, white colour caffeine was obtained (Hill & Barbaro, 2005).

4.4 Analysis

4.4.1 Determination of caffeine content

After extraction and purification done, the mass of weighing boat was taken before and after the caffeine was purified. Mass of purified caffeine was calculated and as in equation 1.

Mass of purified caffeine
= (Mass of weighing boat + purified caffeine) – Mass of weighing boat

(Equation 1)

4.4.2 Determination of antioxidant activity

The black, white coffee and coffee mixed with *Nigella sativa* went through antioxidant activity analysis. The sample collected at preliminary experiment were analysed using UV-VIS spectrophotometer.

4.4.2.1 Antioxidant activity analysis

A 6.1 X 10^{-5} M of 1,1- Diphenyl-2-picrylhydrazyl (DPPH) methanol solution was prepared immediately before use. 50µL of diluted sample (1:100) was added to DPPH solution in the cuvette. The sample will be incubated in darkness and at temperature of 25℃ for 30 minutes. After 30 minutes, the resultant absorbance will be recorded at 515nm by using UV-VIS spectrophotometer. The control antioxidant will be measured. (The absorbance of DPPH radical without antioxidants). The data will be recorded as % scavenging radical activity (Mariod et al., 2009).

4.4.3 Determination of total phenolic content

The black, white coffee and coffee mixed with *Nigella sativa* went through total phenolic content analysis. The sample collected at preliminary experiment were analysed using UV-VIS spectrophotometer.

4.4.3.1 Gallic acid standard calibration

Few concentrations of Gallic Acid equivalent (mg GAE/100g) with range from 20-100ppm were prepared. 1ml of standard gallic acid (20,40,60,80 and 100 ppm) was positioned into centrifuge tube. 5ml of distilled water and 0.5ml of Folin Ciocalteu's reagent was added and shaken. After 5 minutes, 1.5ml of 7.5% of sodium carbonate was added and the volume was made up to 10ml with water. The solution was allowed to

incubate for 2 hours at room temperature. After incubation, the absorbance were measured at 760nm wavelength by using UV-VIS spectrophotometer. Calibration curve will be plotted (Chen & Ho, 1997).

4.4.3.2 Phenolic content analysis

1ml of sample was positioned into centrifuge tube. 5ml of distilled water and 0.5ml of Folin Ciocalteu's reagent was added and shaken. After 5 minutes, 1.5ml of 7.5% of sodium carbonate was added and the volume was made up to 10ml with water. The solution was allowed to incubate for 2 hours at room temperature. After incubation, The absorbance were measured at 760nm wavelength by using UV-VIS spectrophotometer (Kaur et al., 2008; Mariod et al., 2009).

4.5 Screening process

Design Expert factorial design was performed to screen the three parameters and investigate the effects of these parameters in biological compounds of black and white coffee. Three parameters which included temperature, mixing time of coffee with *Nigella sativa* and ratio of coffee and *Nigella sativa* will be screened by using either complete factorial design or fractional factorial design. This method will be decided once the results of preliminary experiment are obtained. Eight formulations of sample for black and white coffee which combined testing three parameters simultaneously were determined. The experiment and analysis were carried out to determine the reading for caffeine content, antioxidant activity and total phenolic content respectively.

Table 3.5(a): Eight formulations of black coffee samples for screening process.

Select	Std	Run	Factor 1 A:Temperature °C	Factor 2 B:Ratio of N.S to coffee g	Factor 3 C:Mixing Time min	Response 1 Caffeine mg	Response 2 Total Phenolic Content microgram/ml	Response 3 Antioxidant % scavenging activity
	4	1	100.00	0.40	1.00	71.1333	50.1961	40.6218
	2	2	100.00	0.20	1.00	103.167	44.3987	44.9032
	5	3	80.00	0.20	4.00	66.5667	41.7367	42.3547
	6	4	100.00	0.20	4.00	85.5667	45.2661	45.5657
	3	5	80.00	0.40	1.00	62.8667	39.1317	40.6728
	1	6	80.00	0.20	1.00	80.9333	39.7759	39.2457
	8	7	100.00	0.40	4.00	61	57.0308	43.578
	7	8	80.00	0.40	4.00	48.9333	45.2661	43.3741

Table 3.5(b): Eight formulations of white coffee samples for screening process.

Select	Std	Run	Factor 1 A:Temperature °C	Factor 2 B:Ratio of N.S to coffee g	Factor 3 C:Mixing Time min	Response 1 Caffeine mg	Response 2 Total Phenolic Content mg/ml	Response 3 Antioxidant % scavenging activity
	4	1	100.00	0.40	1.00	82.2333	4.209	33.5959
	8	2	100.00	0.40	4.00	60.2	5.3445	36.7687
	2	3	100.00	0.20	1.00	101.133	3.3165	30.5581
	7	4	80.00	0.40	4.00	48.5	4.6039	35.2385
	5	5	80.00	0.20	4.00	62.2333	4.2686	32.1512
	1	6	80.00	0.20	1.00	83.8	3.5894	27.2727
	6	7	100.00	0.20	4.00	71.8	4.5364	37.1737
	3	8	80.00	0.40	1.00	82.4	3.6748	28.3078

CHAPTER 5

RESULTS AND DISCUSSION

5.1 Introduction

This chapter is about results gathered throughout the whole research. All the findings of this research will be discussed.

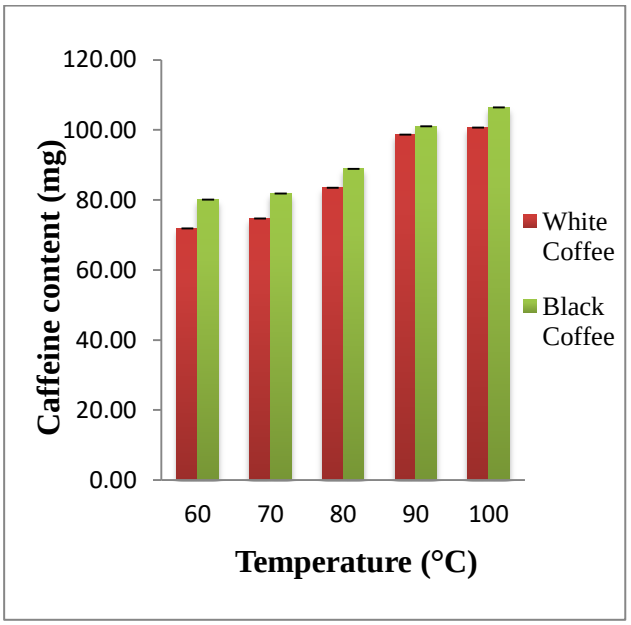
5.2 Caffeine content analysis

According to figure 5.2(a), the caffeine content increased when the temperature of water increased. At 60℃, the caffeine content of white and black coffee were 71.83mg and 80.07 mg respectively. At 100℃, the caffeine content of white and black coffee were 100.67mg and 106.43mg respectively, white coffee recorded an increase of 28.65% caffeine content while black coffee recorded an increase of 24.77% caffeine content. There was not much difference between the caffeine content of white and black coffee at different temperatures. Caffeine is quite stable during the roasting process, therefore, the caffeine content changes a little amount throughout the whole roasting process (Scribblers, 2011). This is due to the high thermastability of caffeine in both types of coffee beans. This explained why there was no significant difference in caffeine content of white coffee and black coffee (Ranken et al., 1997). Caffeine's solubility was mainly driven by the water temperature. At higher temperature, there will be more caffeine dissolve in water when comparing to lower temperature. This is because cold water extracted components in the coffee beans slower when compared to hot water (Brones, 2015).

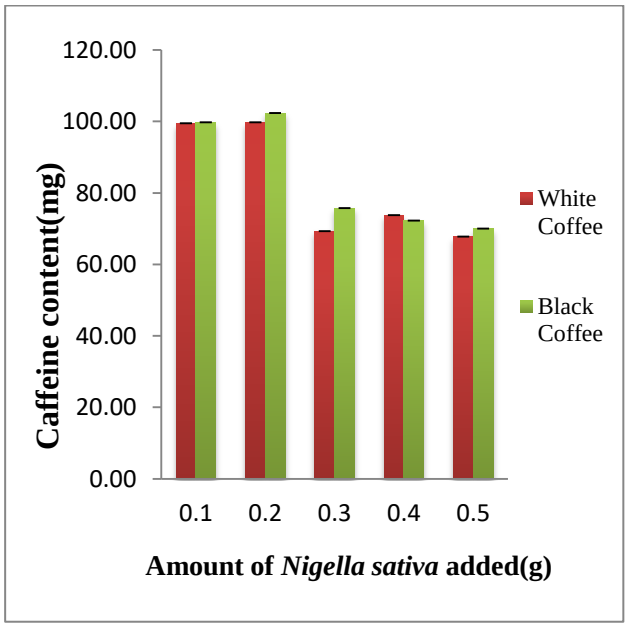
According to figure 5.2(b), when 0.1g to 0.5g of *Nigella sativa* powder was added to coffee under 100℃ and 2 minutes mixing time, the overall caffeine content was decreased. However, there was only slight difference in the caffeine level when 0.4g of *Nigella sativa* and 0.5g of *Nigella sativa* powder were added. When 0.5g of *Nigella sativa* added, the caffeine content were 67.77mg and 70.00mg for white and back coffee. When compared to coffee without adding *Nigella sativa*, there is a decrease of 32.68% and 34.23% of caffeine content for white and black coffee. This implied that the adding of *Nigella sativa* powder did contributed certain effect in reducing the caffeine content of coffee.

Besides, according to figure 5.2(c), the caffeine content of black coffee fluatuated between 1 minute to 3 minutes mixing time. After 3 minutes, the caffeine content started to increases. At 3 minutes mixing time, the caffeine level of black coffee was greatly reduced The caffeine content of black coffee at 3 minutes was 63.03mg. After that time, *Nigella sativa* could not contribute much effect to the overall results. However, for white coffee, the caffeine content fluatuated between 1 minute to 4 minutes and the lowest caffeine content was achieved at 4minutes mixing time which was 65.73mg. From figure 5.2(c), it could be seen that when mixing time of coffee increased to 5 minutes, the caffeine content increased again. This may be due to the longer the coffee was being brewed under high temperature, the more the caffeine was being extracted (Brones, 2015).

(a)


120.00
100.00
80.00
60.00
40.00
20.00
0.00
Caffeine content (mg)
60
70
80
90
100
Temperature (°C)
White Coffee
Black Coffee

(b)


120.00
100.00
80.00
60.00
40.00
20.00
0.00
Caffeine content(mg)
0.1
0.2
0.3
0.4
0.5
Amount of Nigella sativa added(g)
White Coffee
Black Coffee

(c)

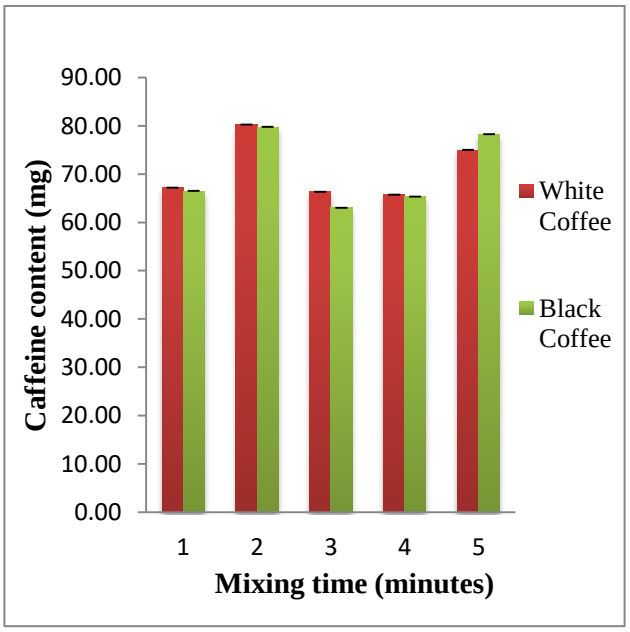


Figure 5.2: Graph of caffeine content of black and white coffee; (a) At different temperature with mixing time fixed at 2 minutes and without adding *Nigella sativa*; (b) Coffee mixed with different amount of *Nigella sativa* with temperature fixed at 100 ℃ and mixing time fixed at 2 minutes; (c) At different mixing time with temperature fixed at 100 ℃ and amount of *Nigella sativa* added fixed at 0.5g

5.3 Total phenolic analysis

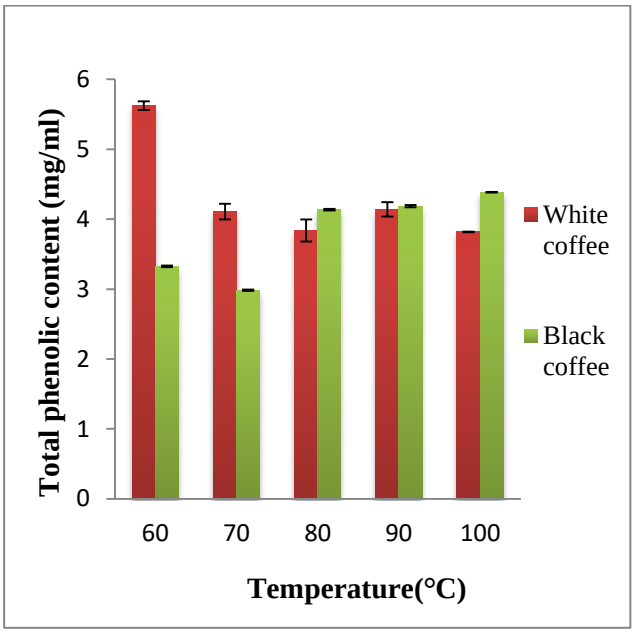
By referring to figure 5.3(a), when looking at the trend of total phenolic content of black and white coffee separately, for white coffee, the total phenolic content was highest at 60 ℃ but after that the concentration fluatuated between 3.8 mg/ml to 4.2mg/ml from 70 ℃ to 100 ℃. Temperature affected the solubility and volality of coffee solubles. At higher temperature, more coffee components were being extracted into water, meanwhile, the volality also increased and escaped to the air. This explained why the fluatuation of total phenolic content occurred from 70 ℃ to 100 ℃. As for black coffee, when temperature went up from 80 ℃ to 100 ℃, the total phenolic content of black coffee started to increase in a small amount. The highest total phenolic content was achieved at 100 ℃ with the value of 4.38 mg/ml. This may be due to at higher temperature, the water extracted the components of coffee faster when compared to lower temperatures (Harold, 2011). At lower temperature, the total phenolic content of

white coffee was higher than black coffee. According to Bita & Preda (2005), when the coffee beans were roasted in a higher temperature, the higher the possibility of coffee bean to be degraded. For instance, protein, chlorogenic acid and amino acids will be degraded under high roasting temperature. Therefore this had explained why at 60℃ and 70 ℃ white coffee had a higher total phenolic content when compared to black coffee due to the lower roasting temperature of white coffee bean.

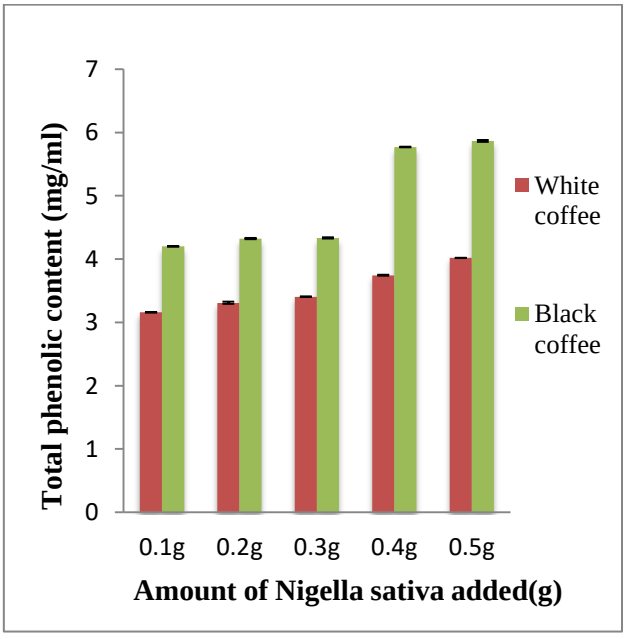
According to figure 5.3(b), when *Nigella sativa* powder was added to coffee, significant increased of total phenolic content showed by black coffee. At 100℃ and 2 minutes mixing time, when 0.5g of Nigella sativa was added, the total phenolic content achieved for black coffee was 5.87 mg/ml. There was a total of 25.38% of increase in total phenolic content. As for white coffee, less significant effect showed after adding *Nigella sativa* powder. There was only around 5% increasement in total phenolic content. The difference may be due to different roasting level of coffee bean. White coffee used light roasted coffee bean whereas black coffee used dark roasted coffee bean. The roasting level will cause the change of physical and chemical properties of the coffee bean. When the roasting time increased, the more changes will be experienced by the coffee bean (Wang & Advisor, n.d.). Therefore, there was a possible that *Nigella sativa* could bring much significant effect to black coffee than white coffee due to the unstability of the coffee bean.

Lastly, by refering to figure 5.3(c), 5 minutes mixing time was showed to be the time which produced the highest total phenolic content for both types of coffee. At 5 minutes mixing time when 0.5g of *Nigella sativa* was added under 100°C, white coffee achieved 4.47 mg/ml while black coffee achieved 6.07 mg/ml. The longer the mixing time, the more components were being extracted from *Nigella sativa* and coffee bean into the coffee. This result was contrasted with the caffeine content in which the optimum mixing time was 3 minutes, however, it was believed that at mixing time 5 minutes, the caffeine content increased. Caffeine as source of total phenolic content contributed to the high overall total phenolic content and this had led to high total phenolic content at 5 minutes of mixing time.

(a)


Total phenolic content (mg/ml)
0
1
2
3
4
5
6
60
70
80
90
100
Temperature(°C)
White coffee
Black coffee

(b)


Total phenolic content (mg/ml)
0
1
2
3
4
5
6
7
0.1g
0.2g
0.3g
0.4g
0.5g
Amount of Nigella sativa added(g)
White coffee
Black coffee

(c)

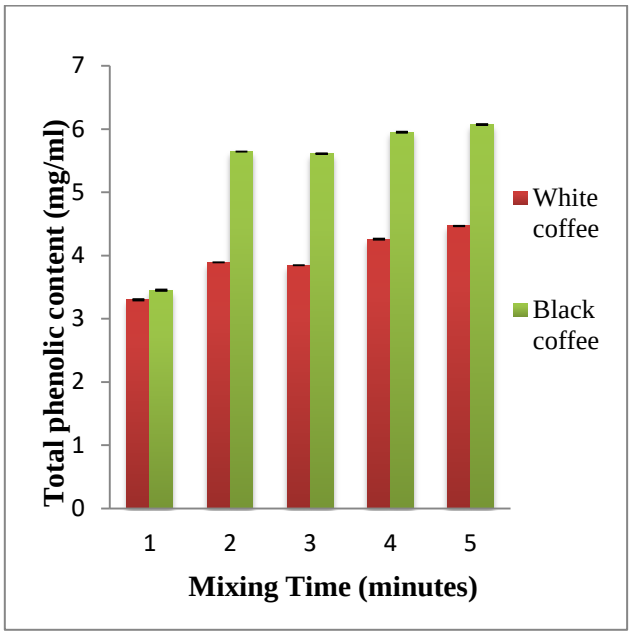


Figure 5.3: Graph of total phenolic content of black and white coffee; (a) At different temperature with mixing time fixed at 2 minutes and without adding *Nigella sativa*; (b) Coffee mixed with different amount of *Nigella sativa* with temperature fixed at 100 ℃ and mixing time fixed at 2 minutes; (c) At different mixing time with temperature fixed at 100 ℃ and amount of *Nigella sativa* added fixed at 0.5g

5.4 Antioxidant activity analysis

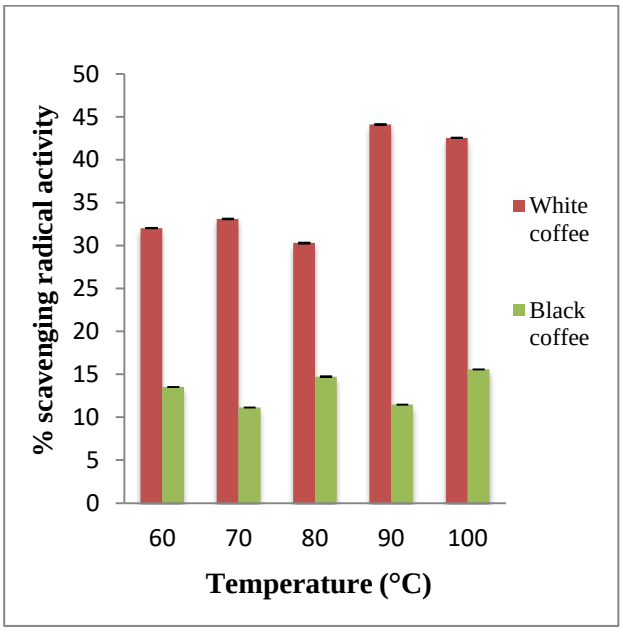
According to figure 5.4(a), the antioxidant activity of white coffee fluatuated from 60 ℃ to 100 ℃. The highest antioxidant activity was achieved at 90 ℃ with the % of scavenging radical activity of 44.12%. Similar condition happened to black coffee, fluatuation occurred between 60 ℃ to 100 ℃ and the highest antioxidnat activity was achieved at 100 ℃ with the % of scavenging radical activity of 15.57% followed by 80 ℃ with 14.73% of scavenging radical activity. Higher antioxidant activity was detected in white coffee which was light roasted coffee beans (Smrke et al., 2013). During roasting, antioxidant activity of melanoid and chlorogenic acid were affected a lots. Chlorogenic acid as the main source of antioxidant was degraded slowly during the roasting process. This explained why there was a gap between % of scavenging radical activity of black coffee and white coffee. From the results obtained, at temperature 90°C and 100°C, both black and white coffee showed high percentage of scavenging activity.

This may be due to hot water extracted the phenolic compound from the coffee bean in a faster rate when compared to lower temperature.

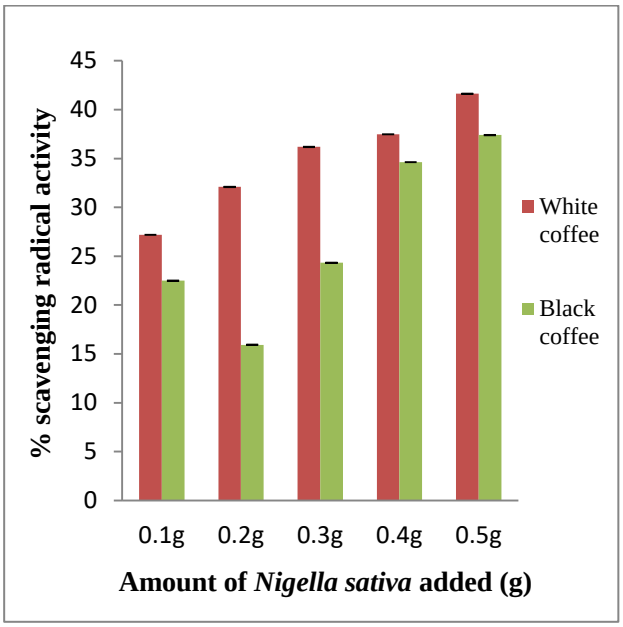
Next, by referring to figure 5.4(b), after *Nigella sativa* powder was added, there was a significant increase in antioxidant activity of black coffee especially when 0.4g and 0.5g of *Nigella sativa* powder was added. When 0.5g *Nigella sativa* was added to black coffee under temperature 100℃ and 2 minutes mixing time, the % of scavenging radical activity showed was 37.40%. When compared to 15.57% achieved when *Nigella sativa* was not added, there was an increase of 21.83%. However, when *Nigella sativa* was added to white coffee, there was not much increase in antioxidant activity. This may be due to the white coffee bean is more stable than black coffee bean resulted from different roasting level and this cause *Nigella sativa* easier to contribute effect to the components of the coffee (Wang & Advisor, n.d.).

According to figure 5.4(c), 5 minutes mixing time was showed to be the time which produced the highest antioxidant activity for both types of coffee. At 5 minutes mixing time when 0.5g of *Nigella sativa* was added under 100℃, white coffee achieved 42.32% while black coffee achieved 41.70%. This result was similar to total phenolic content where it was belived at 5 minutes, the caffeine extracted into coffee was higher and contributed to the overall high antioxidant activity.

(a)



(b)



(c)

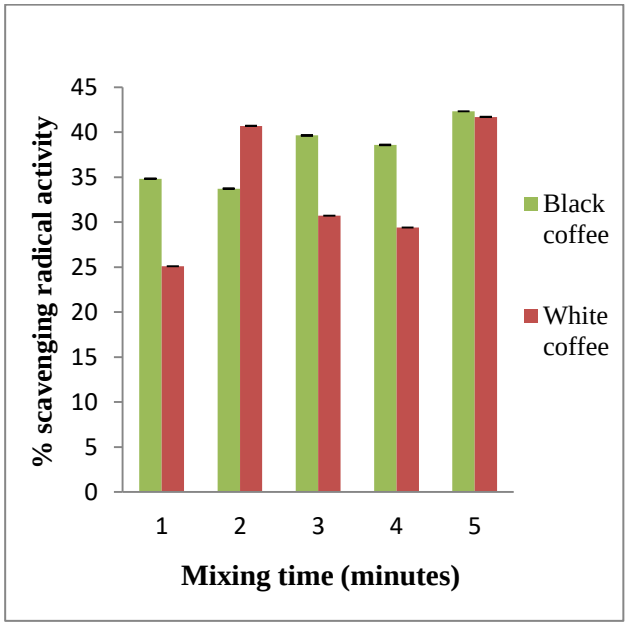


Figure 5.4: Graph of antioxidant activity of black and white coffee; (a) At different temperature with mixing time fixed at 2 minutes and without adding *Nigella sativa*; (b) Coffee mixed with different amount of *Nigella sativa* with temperature fixed at 100 ℃ and mixing time fixed at 2 minutes; (c) At different mixing time with temperature fixed at 100 ℃ and amount of *Nigella sativa* added fixed at 0.5g

5.5 Screening process

5.5.1 Caffeine content pareto chart, ANOVA table and interaction graph

After analysing the results of both coffee caffeine content analysis, it was found that three parameters (temperature, ratio of *Nigella sativa* to coffee and mixing time) had contributed a very significant effect separately to the results. According to table 5.5.1(a) and table 5.5.1(b), the p values for these three parameters were less than 0.05 which means they are significant for white and black coffee. No significant inetractions were found. For black coffee the R^2 value was 0.9930 and implied that less than 1% of of total variations were not being explained in this model. The final equation was as equation 2:

Caffeine
= -20.99340 + (-1.55334*A) + (+119.75100*B) + (-4.66944*C) + (- 2.61251* A * B)

(Equation 2)

As for white coffee, the R^2 value was 0.9576 and implied that less than 1% of of total variations were not being explained in this model. The final equation was as equation 3:

Caffeine = +70.16945+ (-0.48042*A) + (-57.04163*B) +(-8.90278*C)

(Equation 3)

where,

A = temperature

B = ratio of *Nigella sativa* to coffee

C = mixing time

Table 5.5.1(a): ANOVA model for black coffee caffeine content analysis

Response 1 Caffeine

ANOVA for selected factorial model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	1985.79	4	496.45	106.19	0.0015	significant
A-Temperature	*473.81*	*1*	*473.81*	*101.35*	*0.0021*	
B-Ratio of N.S	*1064.91*	*1*	*1064.91*	*227.79*	*0.0006*	
C-Mixing Time	*392.47*	*1*	*392.47*	*83.95*	*0.0027*	
AB	*54.60*	*1*	*54.60*	*11.68*	*0.0419*	
Residual	14.02	3	4.67			
Cor Total	1999.81	7				

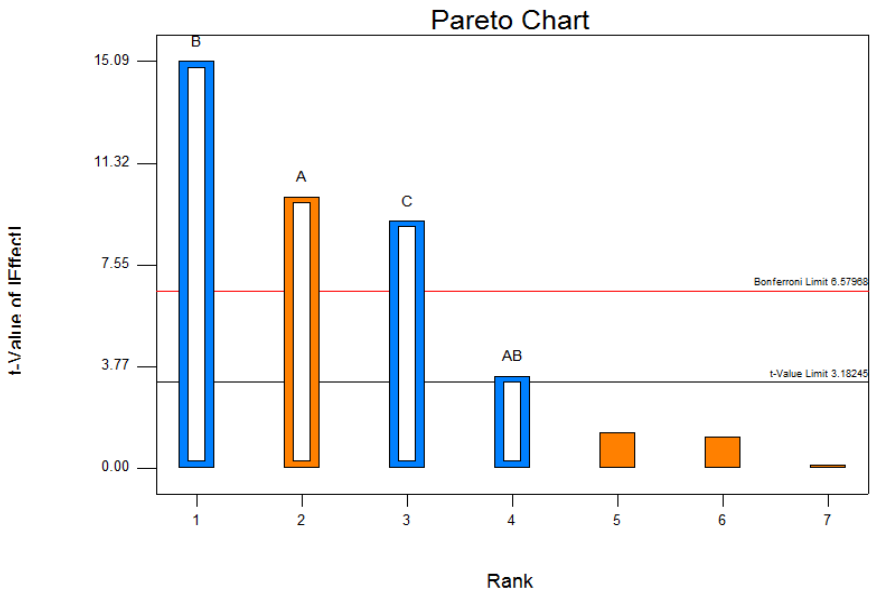


Figure 5.5.1(a): Pareto chart of black caffeine content analysis

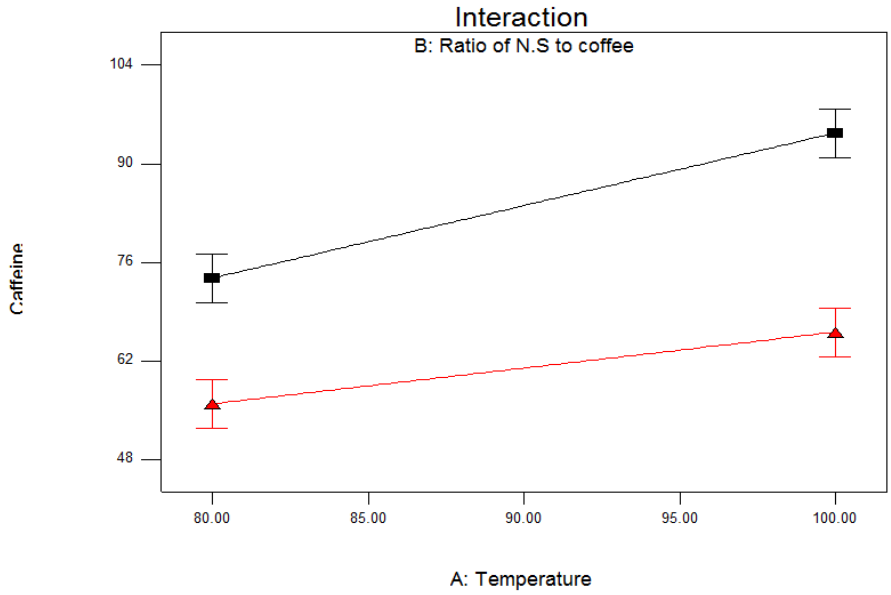


Figure 5.5.1 (b): The interaction graph showed that there was no significant interactions between parameters on caffeine content analysis.

Table 5.5.1(b): ANOVA model for black coffee caffeine content analysis

Response 1 Caffeine

ANOVA for selected factorial model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	1871.61	3	623.87	30.08	0.0033	significant
A-Temperature	*184.64*	*1*	*184.64*	*8.90*	*0.0406*	
B-Ratio of N.S	*260.30*	*1*	*260.30*	*12.55*	*0.0240*	
C-Mixing Time	*1426.67*	*1*	*1426.67*	*68.78*	*0.0012*	
Residual	82.97	4	20.74			
Cor Total	1954.58	7				

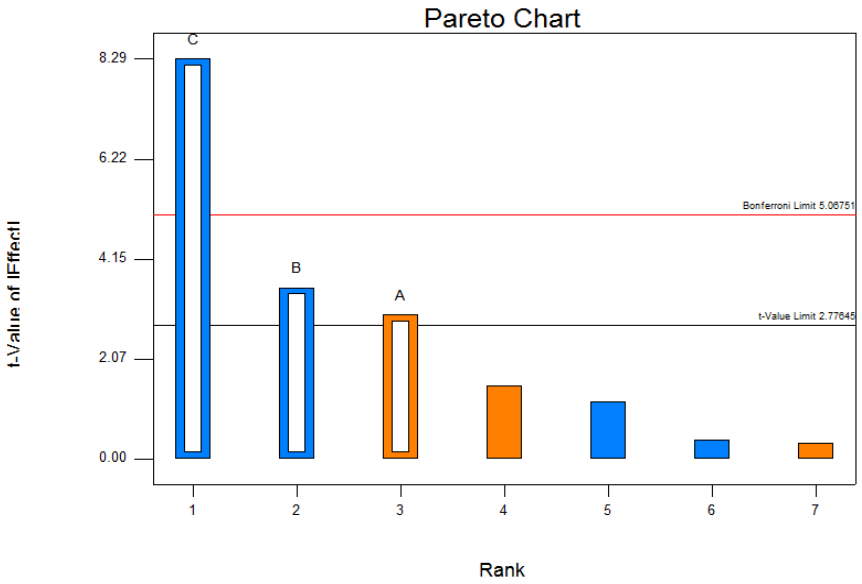


Figure 5.5.1(c): Pareto chart of white coffee caffeine content analysis

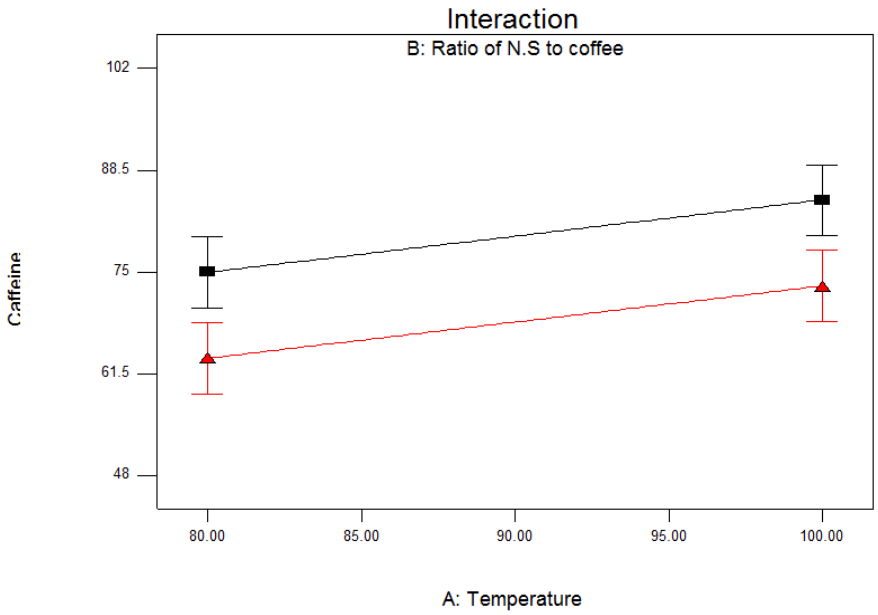


Figure 5.5.1 (d): The interaction graph showed that there was no significant interaction between parameters on caffeine content analysis.

5.5.2 Total phenolic content pareto chart, ANOVA table and interaction graph

After analysing the results of both coffee total phenolic content analysis, it was found that temperature, ratio of *Nigella sativa* to coffee and mixing time contributed a significant effect to the results of total phenolic content of black coffee with p value less than 0.05 as shown in table 5.5.2(a). Besides, according to figure 5.5.2(b) and figure 5.5.2(c), there were significant interactions found between temperature and ratio of *Nigella sativa* to coffee and between ratio of *Nigella sativa* to coffee and mixing time. The combination of either two parameters contributed significant effect to overall results. The R^2 value was 0.9983. This implied that almost all the variations were being explained in this model. The final equation was as equation 4:

Total phenolic content

= +55.41006 -（0.16312*A）- (160.68288*B) – (1.21878*C) + (1.83461*A*B) + (8.45075*B*C)

(Equation 4)

As for white coffee, the significant parameters were the ratio of *Nigella sativa* and mixing time only with p value less than 0.05. The interaction between parameters did not contribute any significant effect to final results. The R^2 value was 0.9711 and implied that around 2.9% of variations were not being explained in this model. The final equation was as equation 5:

Total phenolic content

= +5.46288 -（0.032125*A）- (11.74725*B) + (0.33031*C) + (0.15999*A*B)

(Equation 5)

where,

A = temperature

B = ratio of *Nigella sativa* to coffee

C = mixing time

Table 5.5.2(a): ANOVA model for black coffee total phenolic content analysis

Response 2 Total Phenolic Content

ANOVA for selected factorial model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	243.22	5	48.64	230.82	0.0043	significant
A-Temperature	*119.98*	*1*	*119.98*	*569.32*	*0.0018*	
B-Ratio of N.S	*52.26*	*1*	*52.26*	*247.99*	*0.0040*	
C-Mixing Time	*31.19*	*1*	*31.19*	*148.02*	*0.0067*	
AB	*26.93*	*1*	*26.93*	*127.77*	*0.0077*	
BC	*12.85*	*1*	*12.85*	*61.00*	*0.0160*	
Residual	0.42	2	0.21			
Cor Total	243.64	7				

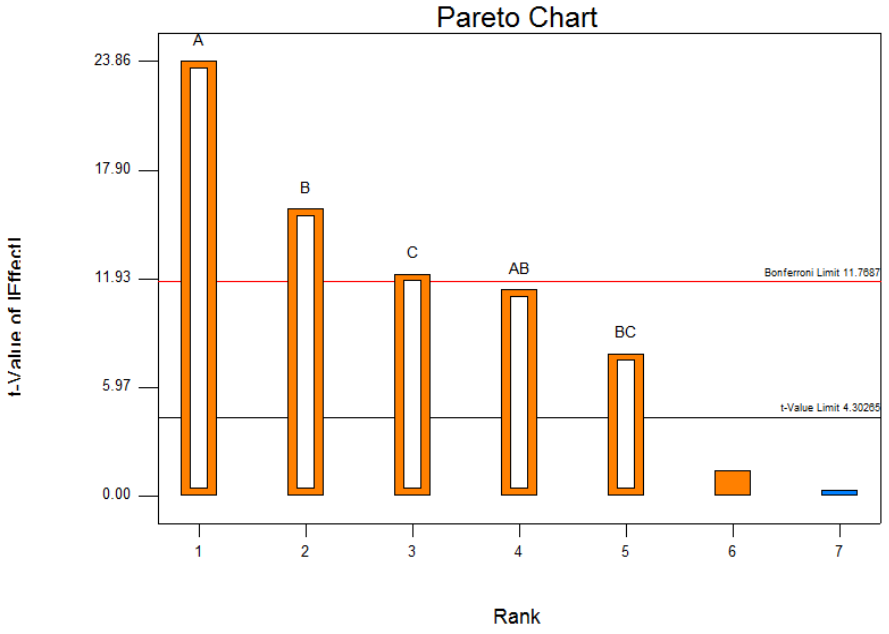


Figure 5.5.2(a): Pareto chart of black coffee total phenolic content analysis

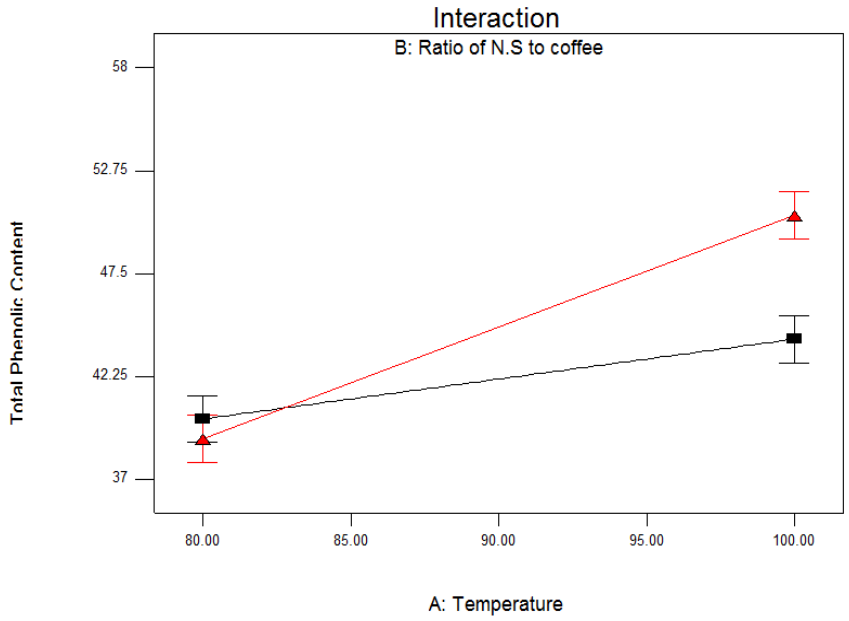


Figure 5.5.2 (b): The interaction graph showed there was interaction between temperature and ratio of *Nigella sativa* to black coffee .

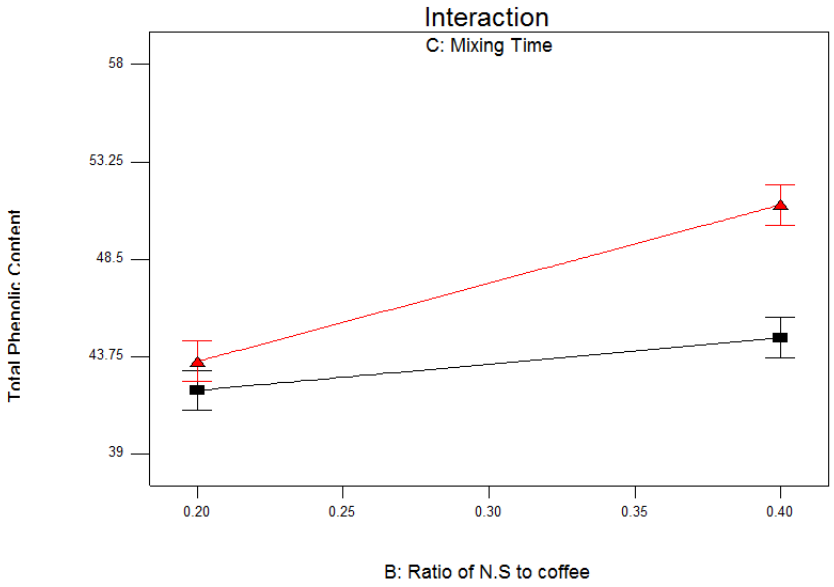


Figure 5.5.2(c): The interaction graph showed that there was interaction between ratio of *Nigella sativa* to black coffee and mixing time.

Table 5.5.2(b) ANOVA model for white coffee total phenolic content analysis

Response 2 Total Phenolic Content

Hierarchical Terms Added after Manual Regression

A

ANOVA for selected factorial model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	2.93	4	0.73	25.23	0.0120	significant
A-Temperature	*0.20*	*1*	*0.20*	*6.94*	*0.0781*	
B-Ratio of N.S	*0.56*	*1*	*0.56*	*19.36*	*0.0218*	
C-Mixing Time	*1.96*	*1*	*1.96*	*67.59*	*0.0038*	
AB	*0.20*	*1*	*0.20*	*7.05*	*0.0767*	
Residual	0.087	3	0.029			
Cor Total	3.02	7				

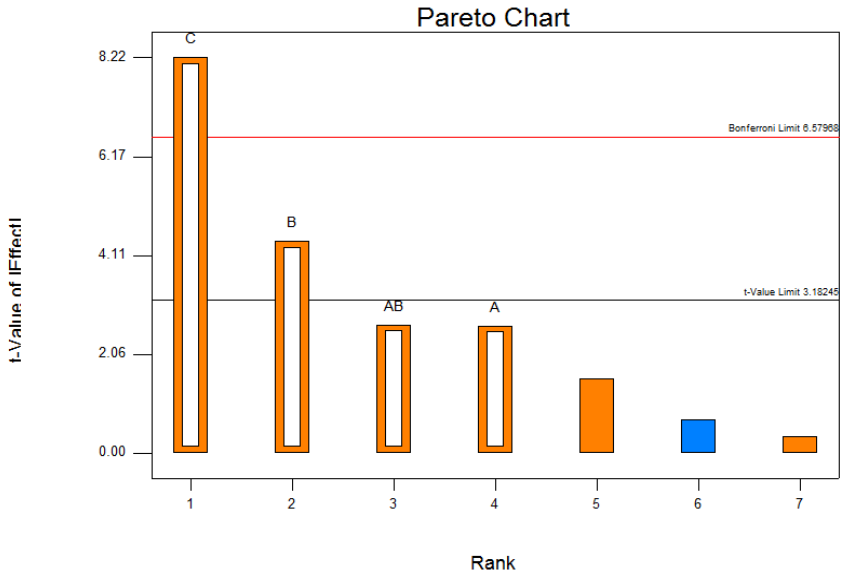


Figure 5.5.2(d): Pareto chart of white coffee phenolic content analysis

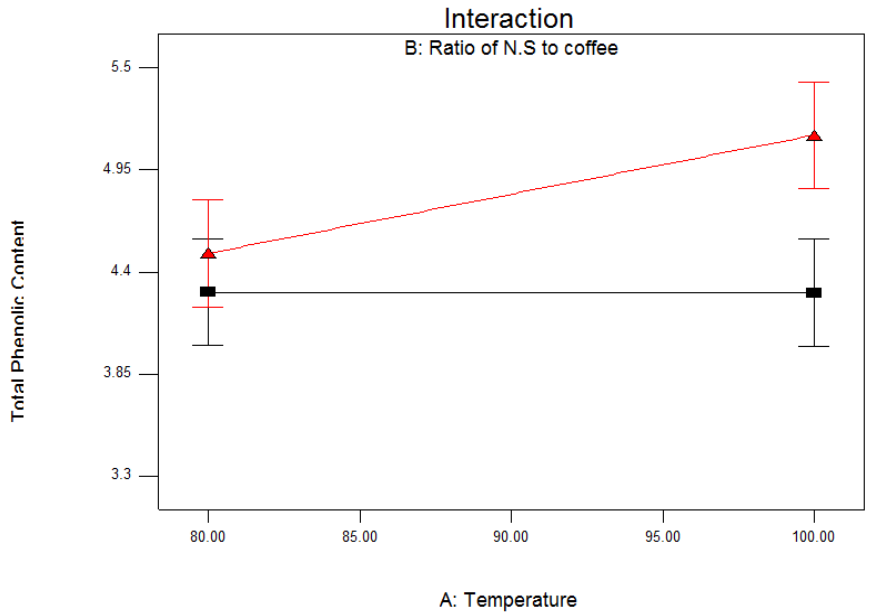


Figure 5.5.2(e): The interaction between parameters did not contribute significant effect to the overall results.

5.5.3 Antioxidant activity pareto chart, ANOVA table and interaction graph

After analysing the results of black and white coffee antioxidant activity analysis, it was found that mixing time contributed significant effect to the results of both coffee's antioxidant activity . For black coffee, mixing time and temperature were the parameter which are significant with p value equaled to 0.0258 and 0.1929. Ratio of *Nigella sativa* to coffee itself did not contribute any significant results. However, according to figure 5.5.3(b), the interaction between ratio of *Nigella sativa* to coffee and temperature made a significant contribution to the results with p value less than 0.05. This may be due to the the amount of *Nigella sativa* added was not significant enough to make significant contribution independently. The R^2 value was 0.9434 and implied that around 5.66% of variations were not being explained in this model. The final equation was as equation 6:

Antioxidant of black coffee

= +2.44438 -（0.43960*A）- (93.27225*B) + (0.78575*C) - (1.08945*A*B)

(Equation 6)

As for white coffee, only mixing time and ratio of nigella sativa to coffee contributed significant effects to the results with p value equaled to 0.0038 and 0.0218. No significant effect was contributed by interaction between parameters. This could be seen from figure 5.5.3(d). The R^2 value was 0.9584 and implied that around 4.16% of variations were not being explained in this model. The final equation was as equation 7:

Antioxidant of white coffee

= +3.92553 + （0.23118*A）+ (11.34108*B) + (3.66305*C) - (0.016840*A*C) – (1.15883*B*C)

(Equation 7)

where,

A = temperature

B = ratio of *Nigella sativa* to coffee

C = mixing time

Table 5.5.3(a): ANOVA model for black coffee antioxidant activity analysis

Response 3 Antioxidant

ANOVA for selected factorial model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	32.61	4	8.15	12.50	0.0325	significant
A-Temperature	*10.17*	*1*	*10.17*	*15.59*	*0.0290*	
B-Ratio of N.S	*1.83*	*1*	*1.83*	*2.80*	*0.1929*	
C-Mixing Time	*11.11*	*1*	*11.11*	*17.03*	*0.0258*	
AB	*9.50*	*1*	*9.50*	*14.55*	*0.0317*	
Residual	1.96	3	0.65			
Cor Total	34.57	7				

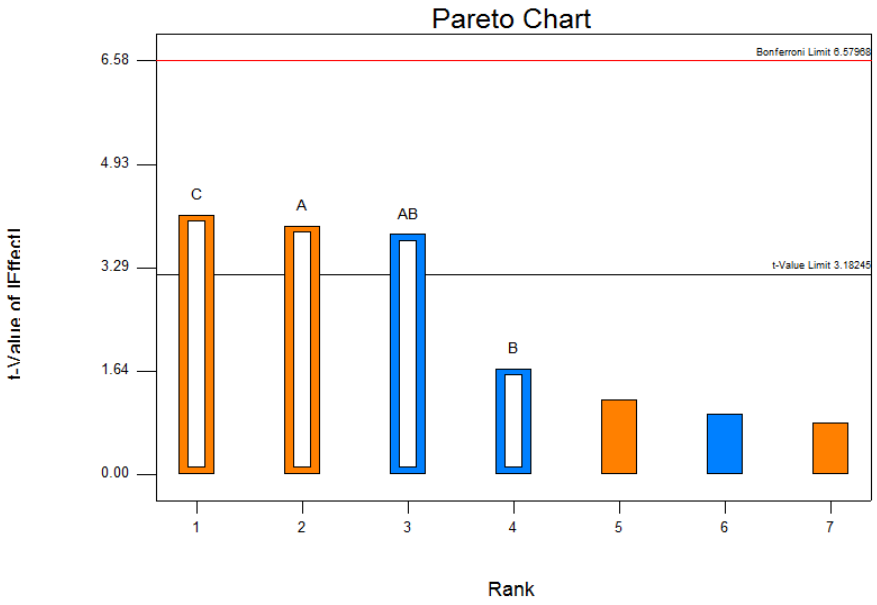


Figure 5.5.3(a): Pareto chart of black coffee antioxidant activity.

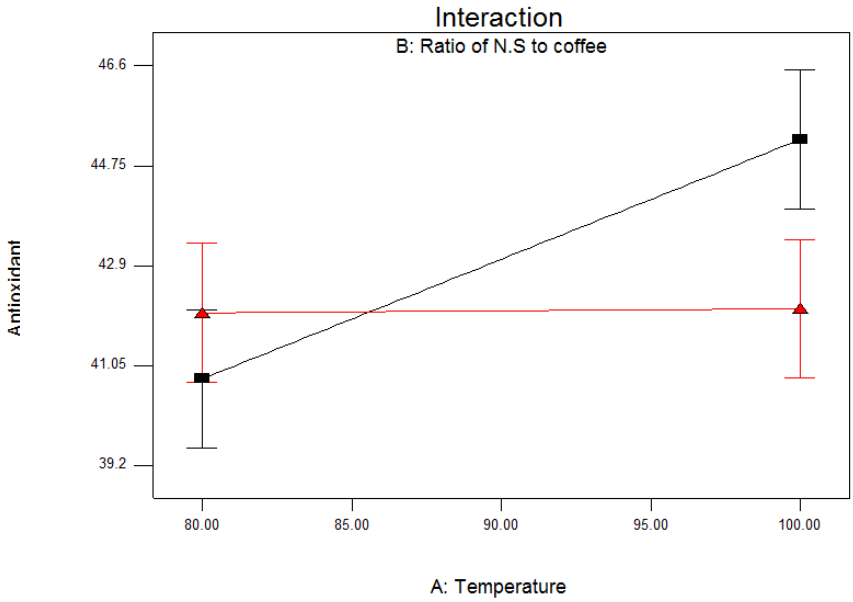


Figure 5.5.3(b): The interaction graph showed interactions between temperature and ratio of *Nigella sativa* to coffee.

Table 5.5.3(b): ANOVA model for white coffee antioxidant activity analysis

Response 2 Total Phenolic Content

Hierarchical Terms Added after Manual Regression

A

ANOVA for selected factorial model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	2.93	4	0.73	25.23	0.0120	significant
A-Temperature	*0.20*	*1*	*0.20*	*6.94*	*0.0781*	
B-Ratio of N.S	*0.56*	*1*	*0.56*	*19.36*	*0.0218*	
C-Mixing Time	*1.96*	*1*	*1.96*	*67.59*	*0.0038*	
AB	*0.20*	*1*	*0.20*	*7.05*	*0.0767*	
Residual	0.087	3	0.029			
Cor Total	3.02	7				

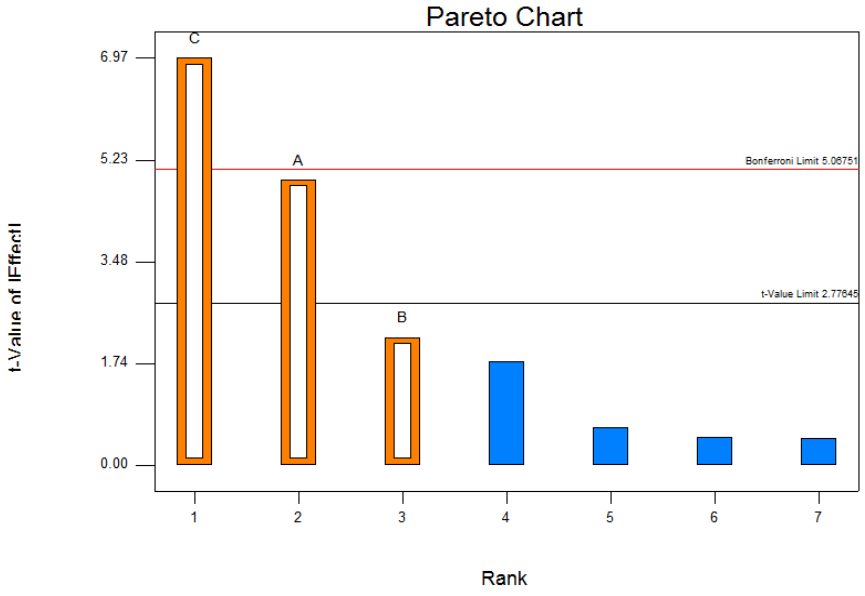


Figure 5.5.3(c): Pareto chart of white coffee antioxidant activity analysis

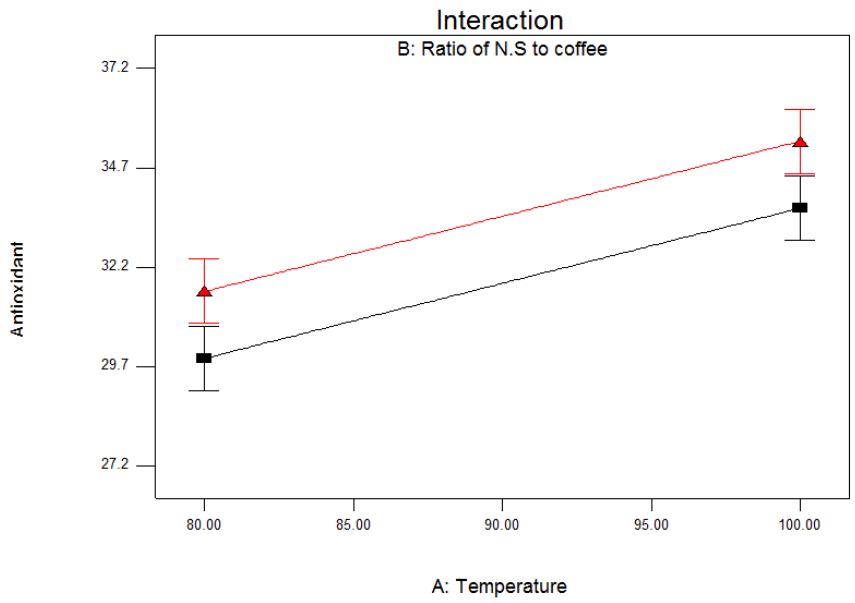


Figure 5.5.3(d): The interaction graph showed that there was no significant interaction between parameters under white coffee antioxidant activity analysis.

5.5.4 Correlation of caffeine content, total phenolic content and antioxidant activity

For both coffee caffeine content analysis, it was found that three parameters which were temperature, ratio of *Nigella sativa* to coffee and mixing time contributed significant effects on the overall caffeine content. However, there were no significant interactions between the parameter itself. Caffeine's solubility and volatility was high under higher temperature (Brones, 2015). It was believed that under higher temperature between 80 ℃ to 100 ℃, caffeine content changed easily and when parameters were manipulated separately, significant effects will be caused.

However, for total phenolic content and antioxidant activity analysis, for black coffee, three parameters (temperature, ratio of *Nigella sativa* to coffee and mixing time) contributed a significant effect to overall total phenolic content. The results were similar to caffeine content and there were interactions between temperature and ratio of *Nigella sativa* to coffee and also between ratio of *Nigella sativa* to coffee and mixing time.

Similarly, for antioxidant activity, temperature and mixing time contributed to the overall results and interaction between temperature and ratio of *Nigella sativa* to coffee was found. This implied that black coffee actually worked well with *Nigella sativa* under the current temperature and mixing time condition. However, amount of *Nigella sativa* added was not so significant for antioxidant activity analysis, but it showed interactions.

As for white coffee, for total phenolic content and antioxidant activity, there were only ratio of *Nigella sativa* to coffee and mixing time contributed significant effect to the overall results. However, temperature did not show significant effect. Light roasted coffee bean was used for white coffee. Due to the low roasting level, there was not big changes in chemical and physical properties of white coffee bean. Therefore, white coffee bean was more stable if compared to black coffee bean (Wang & Advisor, n.d.). Therefore, there was a possible that *Nigella sativa* could not bring much significant effect to white coffee due to the stability of the coffee bean. However, further analysis should be conducted to analyze the changes in components of coffee under the influence of these three parameters.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

From the results, it could be seen that *Nigella sativa* contributed a more significant effect to black coffee if compared to white coffee. Three parameters which were temperature, ratio of *Nigella sativa* and mixing time contributed significantly to black coffee with p value less than 0.05, however, only ratio of *Nigella sativa* and mixing time brought significant effect to overall results of white coffee with p value less than 0.05. This probably due to the different roasting level of coffee beans. However, it was suggested that further studies on the changes in components of coffee beans to be done in order to analyze the effect of these three parameters Through screening, the significant parameters were determined and it served as a good indication for further improvement or further studies such as optimization process. Overall, the objective of this research which was to study the effect of significant screening parameters of coffee beans mixed with *Nigella sativa* in terms of biological compounds was achieved. It was concluded that *Nigella sativa* possessed high potential to be mixed with coffee to be a nutritional beverage with lower caffeine content but higher total phenolic content and antioxidant activity.

6.2 Recommendations

Throughout the whole research, spectrophotometer was used to determine the total phenolic content and antioxidant activity. However, from the absorbance, we cannot observe the changes of components of coffee under the influence of various parameters. Therefore, it was suggested to further the study by using high liquid

performance chromatography or mass spectrometry to analyse the changes of components of coffee in details.

In this research, purified caffeine was extracted and caffeine content was determined by measuring the weight of caffeine obtained through liquid-liquid extraction. This method was time-consuming and it hard to get the real mass of caffeine since there were losses throughout the whole extraction and purification process. Therefore, it was suggested to make research on a better way in measuring the caffeine content, such as measuring the caffeine content directly from the chloroform. Besides, the equipment used for caffeine extraction should be ensured in a good condition before starting the experiment because the malfunction of equipment can cause the failure in getting a good results.

Lastly, from the experiment, it seemed that the amount of *Nigella sativa* played a role in reducing the caffeine content and increasing the total phenolic content as well as antioxidant activity. However, the performance was not really stable. This could be seen from the results of antioxidant activity in which significant effect cannot be seen when *Nigella sativa* was added to white coffee. This may be either due to the amount of Nigella sativa added was not that significant or the coffee itself. Thus, it was suggested that in future studies, sensory test can be carried out to find out the range acceptance level of public towards the coffee mixed with *Nigella sativa*. From the survey, amount of *Nigella sativa* to be added to coffee can be determined for further study.

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APPENDIX

A.1 Caffeine content analysis

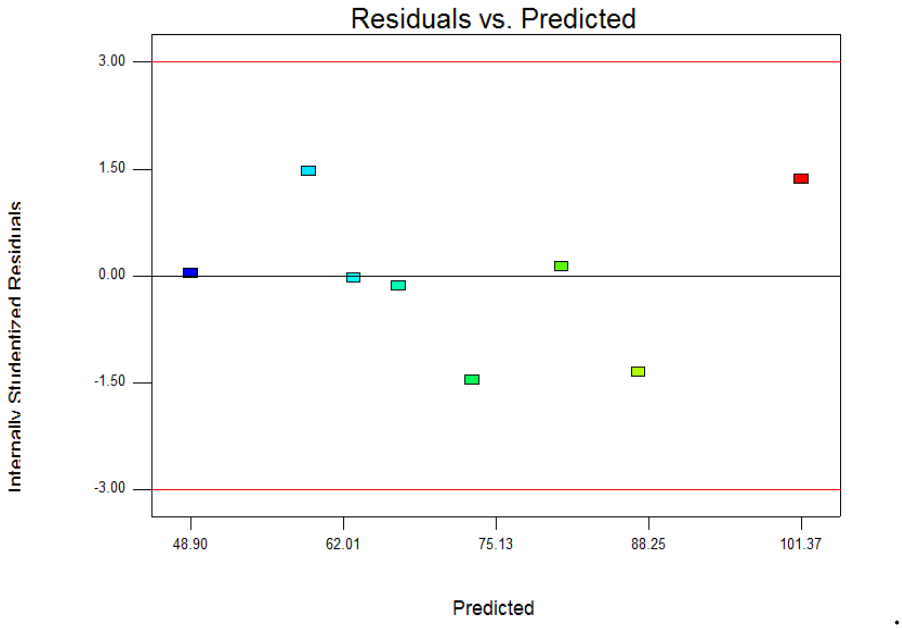


Figure A-1: Graph of residuals versus predicted black coffee caffeine content analysis.

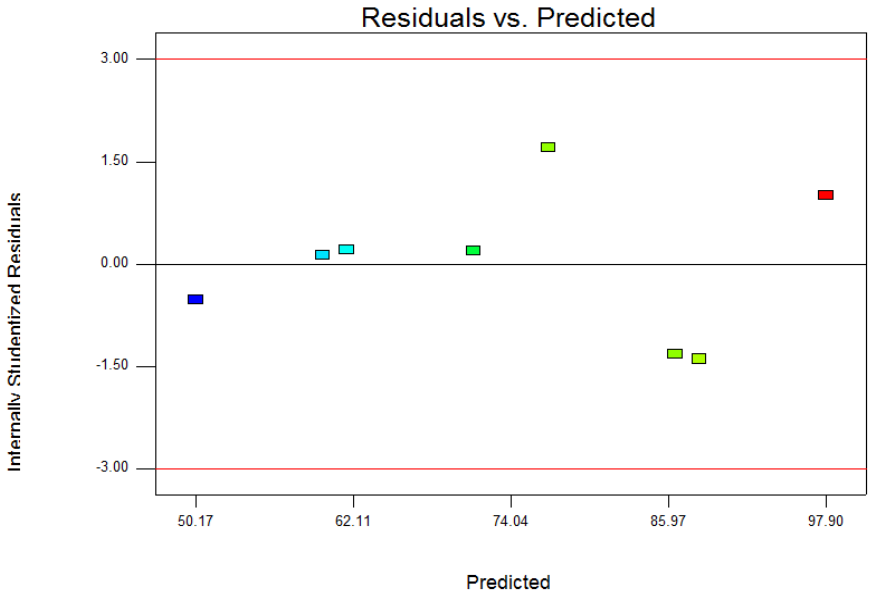


Figure A-2: Graph of residuals versus predicted white coffee caffeine content analysis.

A.2 Total phenolic content analysis

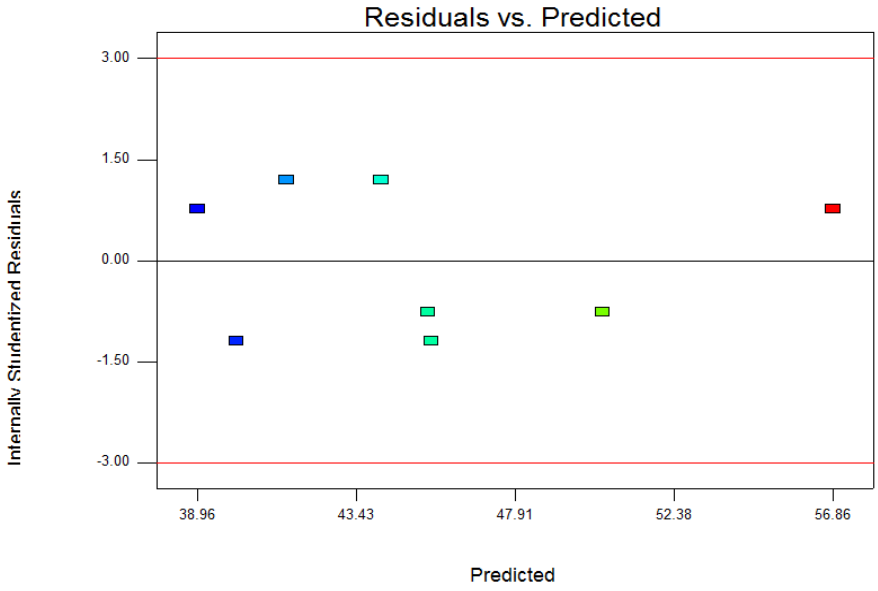


Figure A-3: Graph of residual versus predicted black coffee total phenolic content analysis.

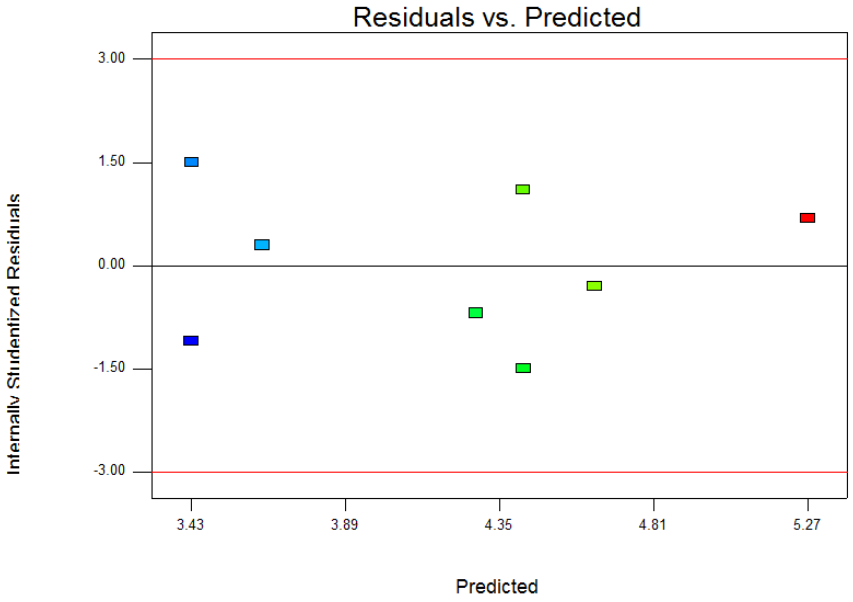


Figure A-4: Graph of residual versus predicted white coffee total phenolic content analysis.

A.3 Antioxidant activity analysis

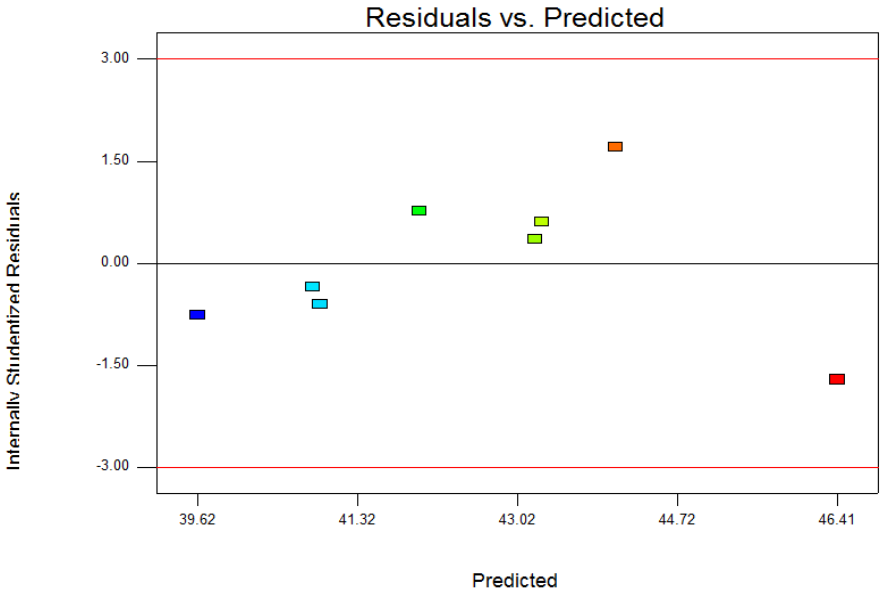


Figure A-5: Graph of residuals versus predicted black coffee antioxidant activity analysis.

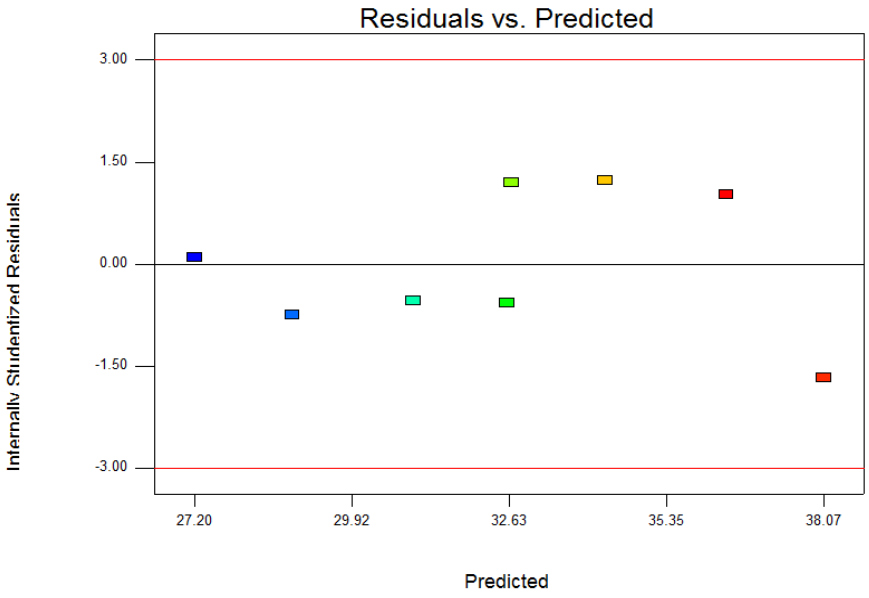
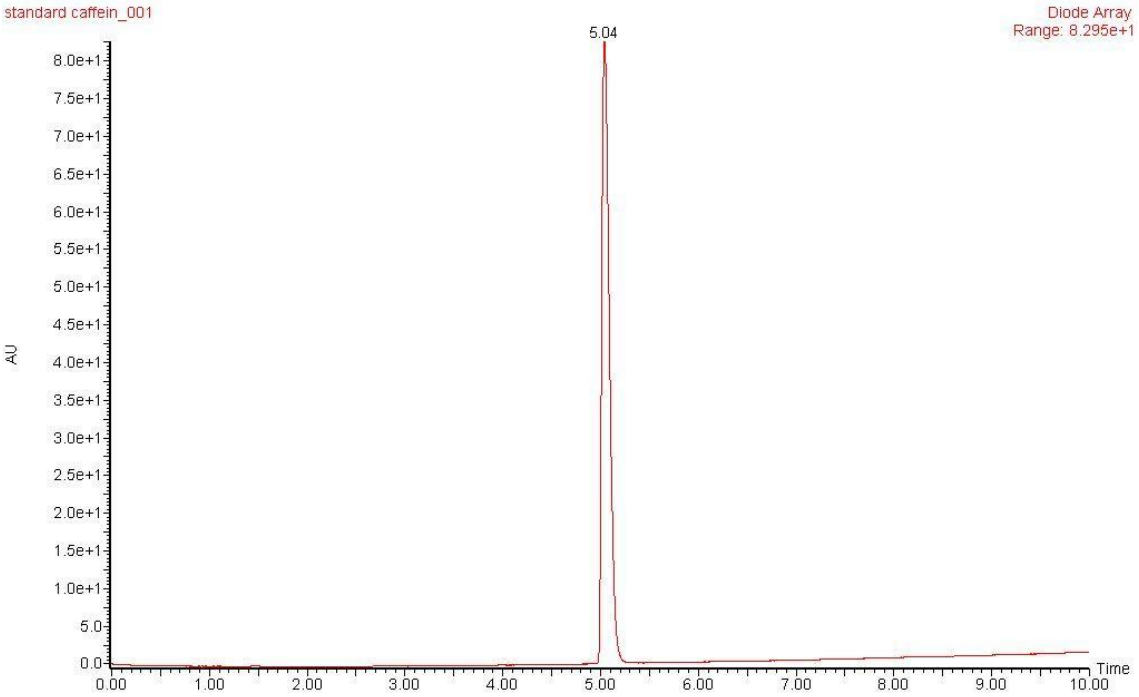


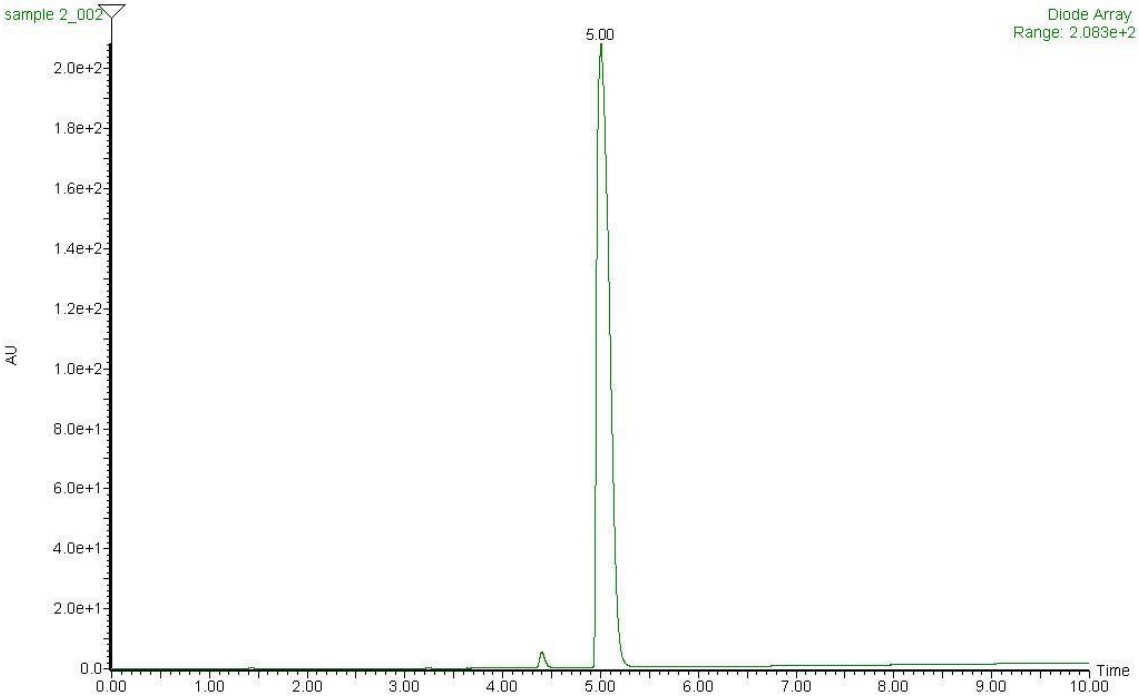
Figure A-6: Graph of residuals versus predicted of white coffee antioxidant activity analysis.

A.4 High performance liquid column test results

(a)



(b)



(c)

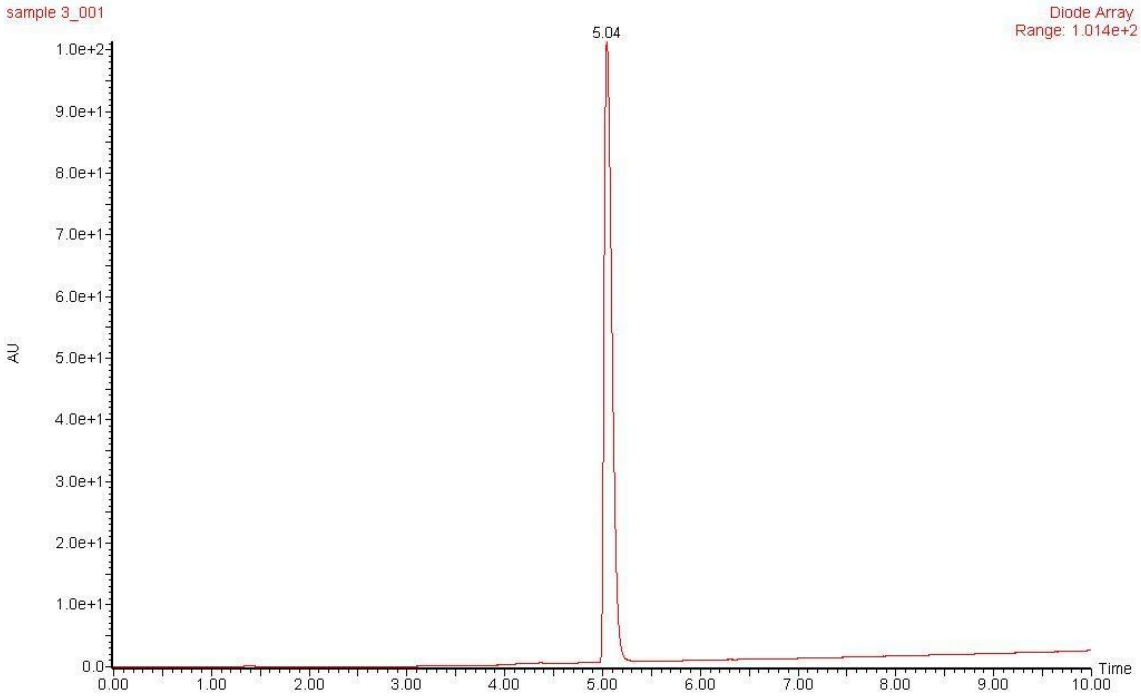


Figure A-7: Results of high performance liquid column test (HPLC) .(a) Sample of standard caffeine (1mg/ml) (b) Sample of extracted caffeine (1mg/ml) (c) Sample of extracted caffeine (10mg/ml)