The Investigation of the Effect of the Infusion Time on Antioxidant Effect of *Rosa canina* Tea

Extended Essay (Biology)

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Abstract:

Plant tea are consumed in society in order to have natural antioxidant. As the free radicals in our body attack and harm our body, the antioxidants react with free radicals and scravenge them. They decrease the negative effect of free radicals to our body. The idea of all tea having antioxidant effect is known by literature but there is not an exact literal information about the factors affecting the antioxidant activity of tea. The focus of this study is the effect of infusion time on antioxidant effect of tea. It is impossible to test all tea kinds that the infusion time has an effect on antioxidant activity, so *Rosa canina* is chosen. So the aim of this study is to investigate whether infusion time has an effect on the antioxidant activity of *Rosa canina* by measuring its light absorbance value, after mixed with a free radical source called DPPH, in spectrophotometer. In the experiment different infused timed *Rosa canina* tea were prepared by same volumed hot water and same mass of tea. The tea extracts were mixed with a free radical source called DPPH and the change in the absorbance of the light of the mixtures were observed. As the antioxidant effect of the tea increases, the light absorbance value of the tea and DPPH mixture in spectrophotometer decreases.

In this investigation it was found that the infusion time has an effect on the antioxidant effect of *Rosa canina.* The maximum antioxidant effect is observed in 6 minutes infused tea. On the other hand the minimum antioxidant effect is observed in 60 minutes infused tea. Overall, the results of this study justifies the hypothesis that time of infusion affects the antioxidant activity of *Rosa canina* but there can not be seen a direct relation between time of infusion and antioxidant activity.

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Introduction

Humans are influenced by highly reactive molecules called free radicals and this causes oxidative damage in the organisms. Radiation, pollution and cigarette smoking can be considered as the causes of the free radicals. During aerobic respiration reactive oxygen species (ROS) are produced and this also harms organisms.[1] These free radicals include superoxide, hydroxyl and peroxyl radical and these radicals are unstable and have an unpaired electron. The radicals react aggressively with the compounds because they tend to pair their unpaired electrons. Free radicals are damaging cell membrane and they have effect on every organ and tissue. When antioxidants react with free radicals, they do not tend to react with the compounds in the body[2]. Therefore there will be no effect of free radicals any more. In organisms this process is disrupted by antioxidants. Inspite of developing an antioxidative system, they still need substances that have antioxidant effect[3]. Some antioxidant compounds were used in the food industry and added in the food. An example of it is BHT (butylated hydroxytoluene). These antioxidants are synthetic and might show toxic effect. Because of this people are using natural sources of antioxidants recently. Plant originated foods can be considered as natural antioxidant sources. So people are in need of finding natural sources of antioxidants. Tea is one of the most common consumed source of antioxidants. And this yields the increase of the consumption of plant teas[4]. The raw materials of the plant teas are its flower, leaf, root and fruit. By infusion, plant tea includes raw materials of the plants. When people drink these prepared tea, they show antioxidant effect in body and prevent the diseases that are caused by free radicals.

The topic of my research is to find whether infusion time, which changes the amount of raw materials like antioxidants that passes from tea leaves to water, affects the antioxidant

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1 http://medallionlabs.com/Downloads/Antiox_acti_pdf (Accessed on 25 September 2010) Available from World Wide Web

2 http://iopscience.iop.org/09673334/28/4/R01;jsessionid=2F2098F531E366675AFCC9E7AC92D109.c3 (Accessed on 25 September 2010) Available from World Wide Web

3 http://www.pjoes.com/pdf/14.6/861-867.pdf (Accessed on 25 September 2010) Available from World Wide Web

4 Turkey 10. Food Congress 21-23 Mayıs 2008,Erzurum Özlem Çağındı

effect of the *Rosa canina* tea (Appendix 3, Picture 4). The reason why I chose this topic is my curiosity about the antioxidant effect of tea. I knew that all tea have an antioxidant effect but I didn't know if the infusion time of the tea has an effect on antioxidant effect or the optimum infusion time for a tea. I think infusion time is an effective factor on antioxidant effect of a tea. We should now the optimum infusion time of each tea. The roseship tea is my favourite tea and it is one of the most consumed tea in the world. That is why I chose this topic "How does infusion time affect the antioxidant activity of *Rosa canina*?"

The topic of this research is a very common investigation subject. The most common investigation subject is the effect of infusion time on green tea's antioxidant effect[5]. The antioxidant effect of the roseship tea has been proved by some experiments[6]. But I was not able to find an investigation about the effect of infusion time on antioxidant effect of roseship tea. According to my researches the infusion time also effect the antioxidant effect. So people have to know if there is an effect of infusion time on the antioxidant effect of *Rosa canina* tea.

So my reasearch question is "**Does the infusion time of *Rosa canina* tea affect the antioxidant effect of that tea which is measured by observing the change in light absorbance of roseship tea having different infusion times by using a device called spectrophotometer?**".

Hypothesis

[5] http://altmedicine.about.com/cs/3/a/GreenTea.htm (Accessed on 20 September 2010) Available from World Wide Web
[6] D.A. Daels-Rakotoarison Phototherapy Research 26 Mar 2002

Tea is the most consumed drink after water across the world[7].The scavenging effect of the plant tea's is a proved so plant tea's prevent so many diseases that are caused by free radicals. Our body has an antioxidant system. This can be enough for a healthy person but natural antioxidants are supportive to the antioxidant system and protect people from having disease such as cancer[8]. This can show the importance of consuming antioxidants like tea in daily life.

There are many factors that effect the antioxidant effect of plant teas. In this experiment the type of the plant and the concentration of the plant tea are kept constant so that the effect of the infusion time can be observed. As we know from the researches[9] that *Rosa canina* has an antioxidant effect, the aim of the experiment is to see if there is an effect of the infusion time on the antioxidant effect of the *Rosa canina* tea.

As teas are infusing, the amount of the matters in the plant that is infused to the tea changes. These matters are the cause of the radical scavenging effect.[10] In light of this information **it can be hypothesized that as the time of the infusion changes, the colour of the mixture and the absorption of light of the tea ,when it is put in spectrophotometer, changes which means the antioxidant effect of the tea changes.**

Method Developement and Planning

[7] http://pubs.acs.org/doi/abs/10.1021/jf000877h (Accessed on 5 October 2010) Available from World Wide Web

[8] http://www.healthierharvest.com/news_articles/nutritional_ information/free_radicals.htm (Accessed 0n 15 September 2010) Available from World Wide Web

[9] Department of Nutrition and Dietetic School of Health, University of Erzincan, 24100, Erzincan, Turkey

[10] http://www.coffee-tea.co.uk/infusion-benefits.php (Accessed on 20 September 2010) Available from World Wide Web

To test the reseach question “Does the infusion time of *Rosa canina* have an effect on the absobtion of light of the roseship tea when it is placed in spectrophotometer which shows the antioxidant effect of the tea?” same concentrated *Rosa canina* tea should be used.

In order to observe the antioxidant effect, we should mix the antioxidant solution which is in our experiment roseship tea with a solution which has free radicals and has high light absorbance. By doing this, we can observe the scravenging effect of antioxidant solution. As I was doing my researches I found a method that can be used in my experiment called the DPPH Method. The DPPH method was found by Marsden Blois nearly 50 years ago. (at Stanford University 1958) The DPPH (1,1-diphenyl-2-picryl-hydrazyl) (Appendix 1) molecule is a free radical because of the effect of delocalisation of the spare electron over the molecule. The decolisation makes the dark violet color of the DPPH when it dissolves in ethanol. When a hydrogen donator substance, which is in our experiment roseship tea, is mixed with DPPH solution, it reduces the DPPH and it loses its violet color which decreases its light absorbance in the light spectrophotometer (Appendix 2 and Appendix 3 Picture 2). As I decided to use the spectrophotometer in my experiment and try to find how does the infusion time changes the antioxidant effect, the Blois method is suitable.

As I was searching about the method that I will going to use, I could not find any information about the molarity of the DPPH which will be used in the experiment. So I tried to find the suitable molarity of the DPPH solution. At first trial I dissolved 5 mg of DPPH in 5 mL methanol and the molar concentration of the DPPH that disolved in methanol was so much that the roseship tea that is prepared by one pochette (Appendix 3, Picture 3) can not vanish the violet color. After two trials trials I found that the suitable mass of DPPH is 1mg for 5 mL of methanol. When I prepared this concentration, the results were obtained.

In order to find the effect of the infusion time of roseship tea on its antioxidant effect that is consumed in daily life, one pochette of tea which contains 1,5 gram of *Rosa canina* leaves and one glass of tap water (approximately 200 mL) is used to give more useful results because the tea is prepared by this in daily life. Not only this but also the tap water is heated

in the kettle so that the temperature of the tea will be 100°C and it will be the same for all the tea that is used in the experiment.

While the tea are infused, they are kept in dark. If we keep them under light, the mixture will absorb the light and the free radicals in the tea will react with light. If they react with light, the light absorbance values will decrease and this can change the light absorbance values of tea in spectrophotometer.

After the infusion, 100 µL of the roseship tea is mixed with 900 µL of DPPH. The mixture is kept 10 minutes in dark in order to make them react with each other.

The light absorbance values of the mixtures in the spectrophotometer is measured with respect to methanol because the DPPH solution is prepared with methanol and the effect will be observed with respect to methanol.

The method will be a constant procedure for different infusion time.

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Method

Materials Used In The Experiment

- Spectrophotometer (CM-2700d)
- Magnetic mixer
- DPPH (2,2-diphenyl-1-picrylhydrazyl) 1mg
- Tap Water 5 L
- Pipette (900µg & 100µg)
- Glass tube x25
- Methanol 5 mL
- Roseship tea (*Rosa canina*) (25 pochette of tea which is totally 37,5 g)

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1. Preparation of the DPPH

1mg DPPH (2,2-diphenyl-1-picrylhydrazyl) is dissolved in 25 mL Methanol by using the magnetic mixer.

2. Preparation of Roseship Teas

1 L of tap water is heated in the kettle to 100°C. 5 beakers are taken. 200 mL of hot tap water is added in each beaker. The beakers are labelled as 3, 6, 10, 40, 60 by showing their infusion time. One pochette of roseship tea is added in each beaker. The beakers are kept in dark.

3. The tea in the first beaker is infused 3 minutes .

4. 100 mL of it is taken. 900 µL of DPPH solution is added to it and the mixture is kept 10 minutes in dark.

5. Methanol is put into the glass tube and the tube is put in spectrophotometer and spectrophotometer is rezerod by methanol. (in 517 nm)

6. After 10 minutes the reaction mixture which is prepared in step 4 is put in different in glass tube and put in spectrophotometer (in 517 nm) and measured the light absrbance value.

7. The tea in the second beaker is infused 6 minutes. The tea in the third beaker is infused 10 minutes. The tea in the fourth beaker is infused 40 minutes. The tea in the fifth beaker is infused 60 minutes.

8. Steps 4 to 6 is repeated for each tea for 6, 10, 40, 60 minutes infused tea.

9. Steps 2 to 8 is repeated 5 times with the prepared DPPH in order to have 5 trials for each infused time tea.
10. Calculate mean values of light absorbance of roseship tea that is infused in different times. According to the results make an anova single factor test to the results.

Results:

Type of tea that is used	Number of Trials	Light Absorbance (nm)	Mass of infused tea (1 pochette) (/g)	The temperature of water (°C) ±0,5 °C	Volume of water that tea is infused (/mL) ±0,1mL	Time of infusion (min) ± 0,1 min
Roseship tea (*Rosa canina*)	1	0,150	1,5	100,0	200,0	3,0
	2	0,150				
	3	0,151				
	4	0,152				
	5	0,153				
Roseship tea (*Rosa canina*)	1	0,141	1,5	100,0	200,0	6,0
	2	0,141				
	3	0,142				
	4	0,143				
	5	0,149				
Roseship tea (*Rosa canina*)	1	0,162	1,5	100,0	200,0	10,0
	2	0,162				
	3	0,162				
	4	0,164				
	5	0,166				
Roseship tea (*Rosa canina*)	1	0,166	1,5	100,0	200,0	40,0
	2	0,168				
	3	0,169				
	4	0,170				
	5	0,171				
Roseship tea (*Rosa canina*)	1	0,231	1,5	100,0	200,0	60,0
	2	0,233				
	3	0,233				
	4	0,233				
	5	0,234				

Table 1: This table shows the light absorbance of the different time infused roseship teas which are prepared by the same mass of tea and same volume of water.

Data Analysis

The following formulas were used to obtain the corresponding values:

Mean:

$$\bar{x} = \frac{1}{n} \cdot \sum_{i=1}^{n} x_i$$

where;
n is the number of trials (fort his experiment n is 5)
xi is the light absorbance value for trial number i
Standard Deviation:

$$\sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \overline{x})^2} = \sqrt{\frac{1}{N}\left(\sum_{i=1}^{N} x_i^2\right) - \overline{x}^2}.$$

where;
n is the number of trials (fort his experiment n is 5)
xi is the light absorbance value for trial number i
$\bar{x}$ is the mean value of corresponding group
Standard Error;

$$SD_{\bar{x}} = \frac{\sigma}{\sqrt{n}}$$

where;

n is the number of trials (fort his experiment n is 5)

$\bar{x}$ is the mean value of corresponding group

σ is the standard deviation of corresponding groups

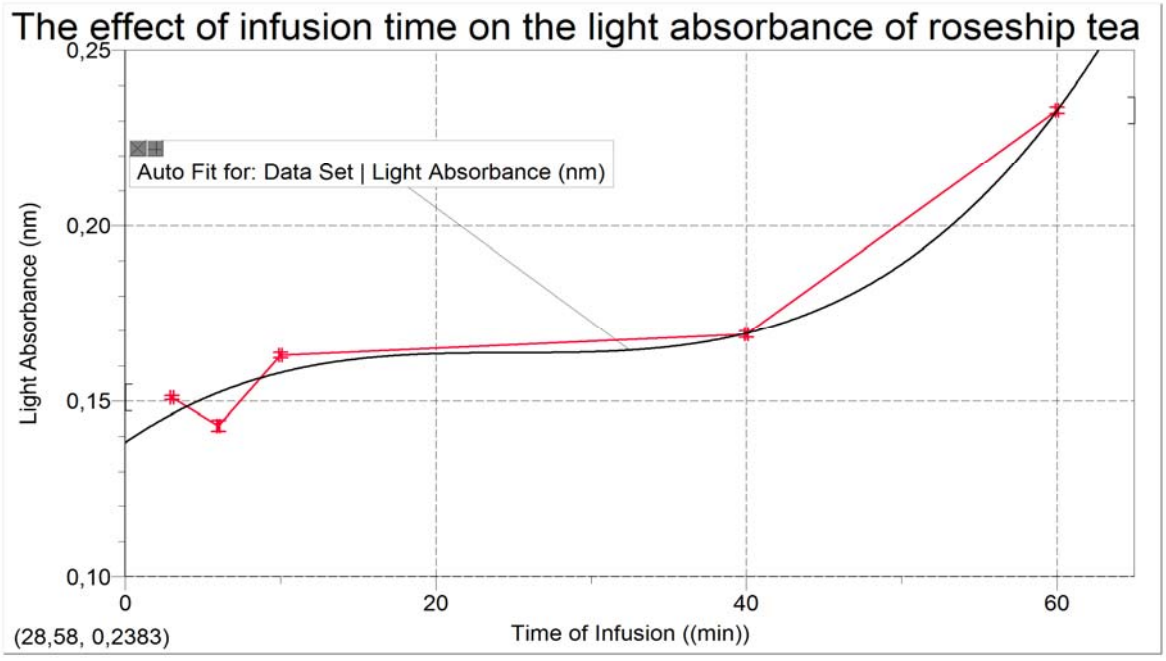
time of infusion	3 minutes	6 minutes	10 minutes	40 minutes	60 minutes
Trial 1	0,150	0,141	0,162	0,166	0,231
Trial 2	0,150	0,141	0,162	0,168	0,233
Trial 3	0,151	0,142	0,162	0,169	0,233
Trial 4	0,152	0,143	0,164	0,170	0,233
Trial 5	0,153	0,149	0,166	0,171	0,234
mean	0,151	0,143	0,163	0,169	0,233
median	0,151	0,142	0,162	0,169	0,233
range	0,003	0,008	0,004	0,005	0,003
variance	0,0000017	1,12E-05	3,2E-06	3,7E-06	1,2E-06
standard deviation	0,0013038	0,003347	0,001789	0,001924	0,001095
standard error	0,0005831	0,001497	0,0008	0,00086	0,00049
t	2,7764451	2,776445	2,776445	2,776445	2,776445
95% CI	0,0016189	0,004155	0,002221	0,002388	0,00136

Table 2: This table shows the descriptive statics for each experimental group . The data above are obtained using Microsoft Office Excel 2003.

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
3 minutes infused	5	0,756	0,1512	1,7E-06		
6 minutes infused	5	0,716	0,1432	1,12E-05		

10 minutes infused	5	0,816	0,1632	3,2E-06		
40 minutes infused	5	0,844	0,1688	3,7E-06		
60 minutes infused	5	1,164	0,2328	1,2E-06		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0,025231	4	0,006308	1501,867	1,77E-24	2,866081
Within Groups	8,4E-05	20	4,2E-06			
Total	0,025315	24				

Table 3: This table shows the Anova statistical calculation for all groups.



Graph 1: This graph shows the relation between the infusion time of roseship tea and its antioxidant effect by looking its light absorbance. The error bars indicate Standard error for each group.

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Conclusion and Evaluation

In this experiment, the effect of infusion time of the roseship tea on its antioxidant activity is investigated. DPPH solution is used as a free radical source and the ability of the roseship tea extracts to scravenge them is observed by measuring the change of light absorbance of roseship tea extracts. As the DPPH is prepared by dissolving it in methanol, the spectrophotometer is rezerod with methanol. So the results of the absorbance values of tea and DPPH mixtures are with respect to the light absorbance value of methanol.

The tea extracts are prepared by the same mass of tea which is one pochette in my experiment and same volume of water which is 200 mL of tap water. The temperature of the water samples are kept constant too in order to measure only the effect of infusion time. I placed the tea samples in the dark in a water bath which contains 100°C tap water in order to make the temperature constant.

The tea are infused in dark because they can absorb the light that can effect the light absorbance values of the tea extracts. After that same volume of DPPH mixture which has free radicals is added to each tea extracts having different infusion time. After they react the light absorbance values are measured in spectrophotometer with respect to methanol.

During the experiment, the antioxidant effect of roseship tea are observed by looking the colour change of the tea and DPPH mixtures. By observation, it was obvious that 6 minutes infused roseship tea has the greatest antioxidant effect because it has vanished the dark violet colour of the DPPH mixture the best. (See Table 1)

In this experiment I used Blois method because Blois method is an excepted and most useful method to measure the antioxidant effect. Not only this but also I did my experiment with daily used values in order to obtain more realistic results. I used one pochette of roseship tea which is infused in 200 mL of hot tap water. These are the properties of the tea that we consume in our daily life. One glass of water is approximately 200 mL and we use generally one pochette of tea for a glass of water. By doing this, the results of the experiment will be

more realistic and useful.

The calibration of the spectrophotometer is done before the experiment by the technicians of Ankara University Pharmacy Faculty Toxicology Department.

The results of the experiment shows us that the highest antioxidant effect is observed in 6 minutes infusion. The mean value of light absorbance of 6 minute infused tea is 0,143. As the light absorbance decreases, the antioxidant effect increases. The lowest antioxidant effect is measured in 60 minutes infusion. The mean value of light absorbance of 60 minutes infused tea is 0,233. As the light absorbance values increases, the antioxidant effect will decrease. So it can be said that in our experiment the optimum infusion time for roseship tea is 6 minutes. (See Table 2)

When the results are considered it is seen that they support the hypothesis that infusion time changes the antioxidant activity of roseship tea. In each infusion time we observe antioxidant effect of roseship tea but we can not find a direct relation between time of infusion and antioxidant activity. From the comparison of different infused time tea, we can say that as the minimum light absorbance value is obtained by 6 minutes infused tea, the optimum infusion time for roseship tea is 6 minutes.

The anova results also shows that our hypothesis is true because the p value is 1,77E-24 which is much more smaller than 0,05(See Table 3). So that we can say that as the time of infusion changes, the colour of the mixture and the absorbtion of light of the tea, when it is put in spectrophotometer, changes which means the antioxidant effect of the tea changes.

The Error and Uncertainity

In this experiment digital devices like spectrophotometer are used. This minimizes the uncertainities of the results. Digital weigher, digital pipette and spectrophotometer are used. After doing the anova test to our results of the experiment, we can see that our standard errors

are very small and the errors are smaller than %10. This shows that the results of this experiment is accurate. 6 minutes infused tea's light absorbance value has highest standard error which is 0,001497 and 60 minutes infused tea's light absorbance has lowest standard error which is 0,00049. (See Table 2)

Also the standard deviation of the results of the experiment is low too. This is because the measurements are done by spectrophotometer which is a digital device. It has minimum standard deviation. This shows that the results of the data are precisezed. As our experiment results are accurate and precised, we can say that our experiment is reliable. We tried to minimise the cause of error and improve our experiment. We try to obtain it by following means :

- The volume and molarity of DPPH mixture is kept constant in each trial so that the amount of free radicals in each trial is kept same. The DPPH solution is prepared by dissolving 1 mg DPPH dissolved in 25 mL of methanol and this solution of DPPH is used in all trials.
- The mass of tea leaves that is infused in each trial kept constant. In each trial one pochette of roseship tea is used.
- The volume, source and temperature of the water that is used in infusion are kept constant. 200 mL of 100°C tap water is used in each trial. It prevents the changes on results due to temperature and density of the tea used in each trial.
- The tea extracts and DPPH mixtures are kept in dark in order to make them not to react with light. Reacting with light decreases its light absorption and it will change the results of the experiment.
- The time that is required for each tea to react with the same volume of DPPH is kept constant.

But this does not show that it has no errors. As the experiment consists of measurements, we could not obtain impeccable results. There can be some unpredictable error sources like:

- In one pochette of roseship tea, there is different parts of the tea plant. In each

pochette there can be different masses of different parts of tea. So the mass of organic compounds that one pochette of tea is different from each other and it changes the antioxidant effect independent from infusion time.

This could be the only error source that can change the results of the experiment since the other variables are tried to be kept constant by using digital devices which have minimum error percentages. Each pochette of the tea can be consist of different masses of different parts of roseship plant. The antioxidant effect of roseship tea is originated from its polyphenol content. Polyphenols have the ability to scravenge the free radicals. The polyphenol antioxidants in plants mostly found in their leaves so it is better for us to use only the leaves of the plant in the experiment in order to see the maximum antioxidant effect of tea.[11] In one pochette tea, it is not possible to know which part of the plant and the percentage of the parts are included. This will change the polyphenol content which directly change the antioxidant effect of the plant.This aspect is neither experimented nor controlled in the experiment. So it can be an error source. To overcome this source of error, inspite of using prepared pochettes, we should use the same number of roseship plant leaves, which is taken from the same roseship plant, in each trial of this investigation.

After observing the results and acquiring an answer to the research question "**Does the infusion time of *Rosa canina* tea affect the antioxidant effect of that tea which is measured by observing the change in light absorbance of roseship tea having different infusion times by using a device called spectrophotometer?"** a new question arises: Does the infusion time affects the antioxidant activity of all plant tea? Some researches can be done to investigate the effect of infusion time on other plant tea like green tea.

Also it would be interesting to investigate the optimum infusion time of each plant tea. By investigating this we can get maximum antioxidant effect from all types of tea. There are many other plant tea that is commonly consumed in the world that can be experimented.

In our daily lives all kinds of tea are used because of their antioxidant effect. As we try

[11] http://en.wikipedia.org/wiki/Polyphenol_antioxidant Accessed on 10 October 2010

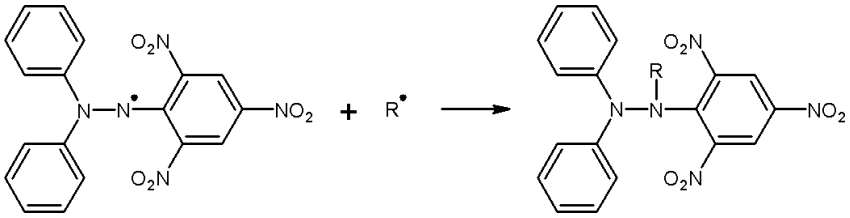
to obtain the antioxidant effect of tea, it is beneficial to know that are there any factors that can increase the antioxidant effect of plant tea. In this research it is found that infusion time affects the antioxidant effect. As the optimum infusion time for each plant tea are found, people will consume more antioxidant from tea.

Appendix

Appendix 1:

The Use of DPPH (2,2-diphenyl-1-picrylhydrazine)

The DPPH, a free radical stable which has dark violet colour, was found by Goldschmidt and Renn in 1922. It proved to be used in some investigations like polymerization inhibition or radical chemistry and the determination of antioxidant properties of amines, phenols and natural compounds. DPPH is insoluble in water but soluble in methanol. [12]So in the experiment the DPPH solution is prepared by methanol.



Picture 1: This picture shows how DPPH accepts hydrogen from a hydrogen donator.

Appendix 2:

Spectrophotometer

A spectrophotometer consists of two parts called spectrometer and photometer. Spectrometer is the part which produces light and photometer is the part which measures the intensity of light. The glass tubes which contains a liquid which will be measured is placed between the spectrometer beam and photometer. The amount of light that passes through the glass tube is measured by photometer. [13]

[12] http://www.chempap.org/papers/591a11.pdf Accessed on 18 November 2010
[13] http://www.ruf.rice.edu/~bioslabs/methods/protein/spectrophotometer.html Accessed on 18 November 2010

Appendix 3:

Picture 2: This is the spectrophotometer that was used in the experiment.

Picture 3: This picture shows the pochette of roseship tea that is used in this study.

Picture 4: This picture shows the roseship plant.

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