
EXTENDED ESSAY

- Chemistry -

Topic:

**Investigating the effects of changing temperature and time on tea
polyphenol concentration in green tea**

Research question:

**How will changing the temperature and time of the cold brewing process
affect the tea polyphenol concentration in green tea water?**

Word Count: 3999

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1.0 Introduction

Tea was initially for medicinal and edible use before people started drinking it. Currently, many people believe drinking tea is beneficial for their health, like releasing heart attack, cancer, diabetes and other serious risks, etc. According to Barnes and Magee (1956), dimethylnitrosamines caused liver tumours in experiments on rats. Meanwhile, drinking tea can reduce the risk of liver cancer associated with the consumption of nitrite in meat or preserved foods as tea leaves are full of tea polyphenols which have anti-inflammatory and antioxidant characteristics.

Tea polyphenol (TPs) is composed of about 30 phenolic substances, including flavanols (catechins), flavonols (flavonols), flavonoids (anthocyanins), hydroxy-4-flavanols, anthocyanins, phenolic acids and other polyphenols. The highest content is catechin, also known as “tannin”, “tea acid” or “tea shoe”, which accounts for 20% to 35% of the dry matter content in tea (Zhang Xiufen et al., 2019). According to the molecular structure characteristics of catechin, it can be divided into four substances: epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG), and its structural formula is shown in *Figure 1*.

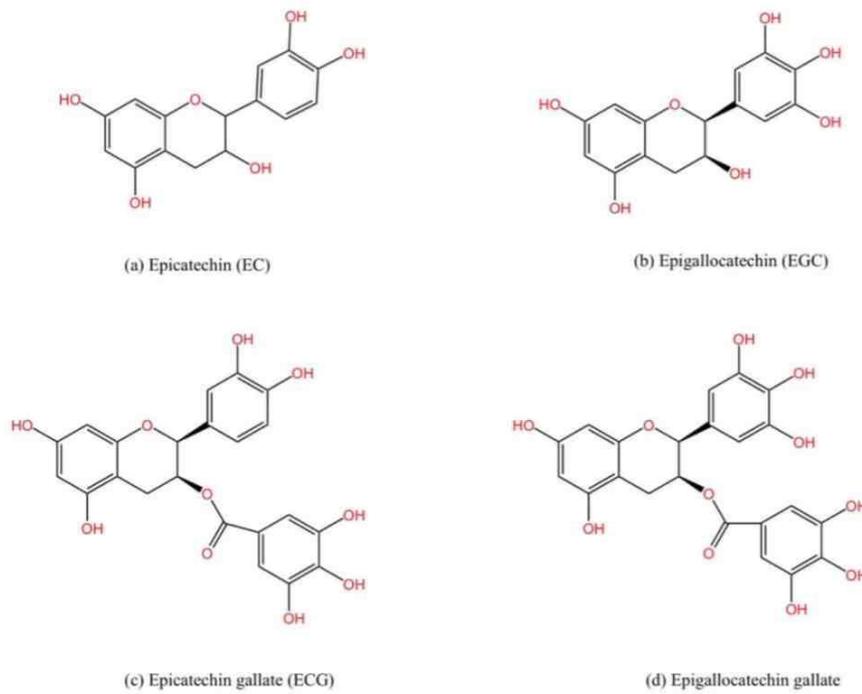


Figure 1. The structures of four major components of tea polyphenol

Furthermore, green tea acts as an antioxidant, phenol group in the structure indicates that TPs can scavenge the free radical. It functions by engaging in a propagation reaction with a kind of free radical. As a result of its numerous resonance structures, the new free radical product (in the benzene ring) is less reactive.

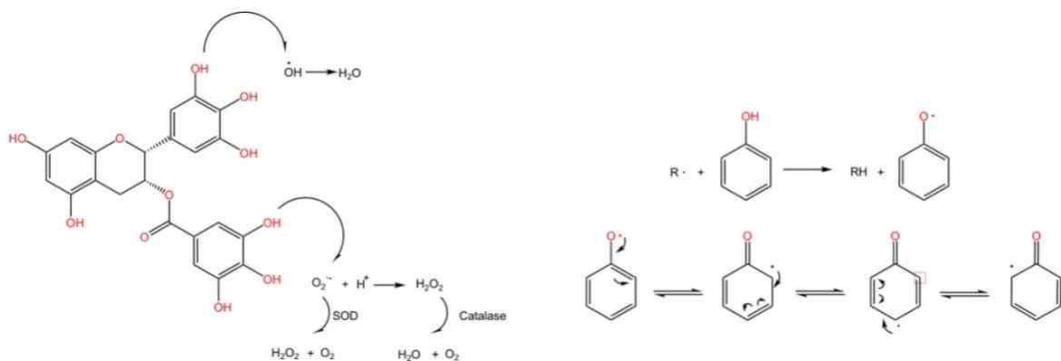


Figure 2. TPs act as antioxidants to scavenge the radical oxygen species

Hence, the extraction of polyphenols from tea has great research value and significance for health, the beverage industry and so on. Normally, people use hot water to brew tea in their daily life. The research was initially set to investigate the extraction of tea polyphenols from green tea leaves focusing on higher temperature ranges (50 °C to 80 °C). However, in the era of "fast food culture" prevailing, the pace of modern life is fast, and tea culture has gradually adapted to the new mode. Consumers choose cold tea with a simple brewing method, short brewing time and guaranteed taste and nutrition. Furthermore, using the cold-brewing method can save electricity and not easily break the structure of tea polyphenol. It is appealing that there is such a lack of investigation on tea polyphenol diffusion in the cold brewing process. Therefore, in this study, the effects of temperature at 0°C, 5°C, 10°C, 15°C, 20°C and time at 10 min, 20 min, 30 min, 40 min, 50 min and 60 min of cold-brewing processes on the concentration of TPs in tea water were finally investigated, and the behavior of cold-brewing tea was evaluated to offer guidance to cold-brewing in daily life and the food industry.

1.1 Research Question

How will changing the temperature and time of the cold brewing process affect the tea polyphenol concentration in green tea water?

1.2 Background information

1.2.1 The introduction of fermented tea

The fermented tea is generally defined as tea that has undergone oxidative fermentation by biological enzymes or microbial fermentation in the production of tea leaves (Xu Zhenggang et al., 2019). Meanwhile, fermented teas can be divided into lightly fermented, semi-fermented, fully fermented and post-fermented teas. Green tea is a kind of unfermented tea which do not through fermented tea. Most tea polyphenols are not be eliminated without the fermented process.

1.2.2 Three types of methods of extracting tea polyphenol

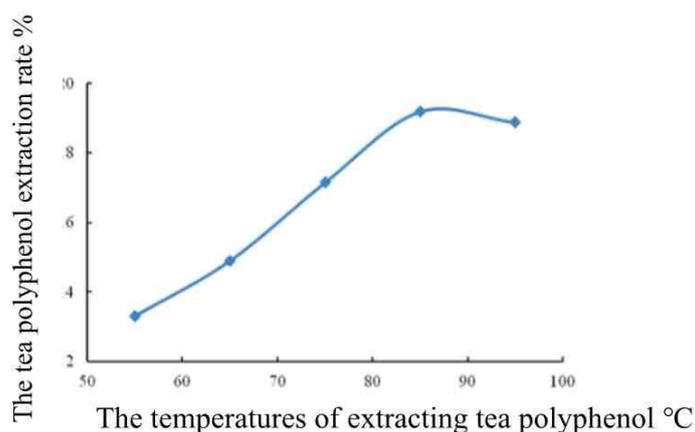
Currently, there are three types of methods of extracting TPs, which are the physical method, chemical method and biological method (Yin Peng et al., 2017). The following table shows the function of the different extracting processes:

Table 1. The function of each method

The function of each method		
Physical method	Chemical method	Biological method
The main purpose is to break down the cellular fragmentation rate of tea leaves by increasing the number of extended milling and crushing steps.	The interaction between the TP and the extractant is increased, mainly through the addition of solvents and other substances, to promote the diffusion of the active ingredient effluent.	Increase the concentration of TP by enzymatic treatment or microbiological techniques.

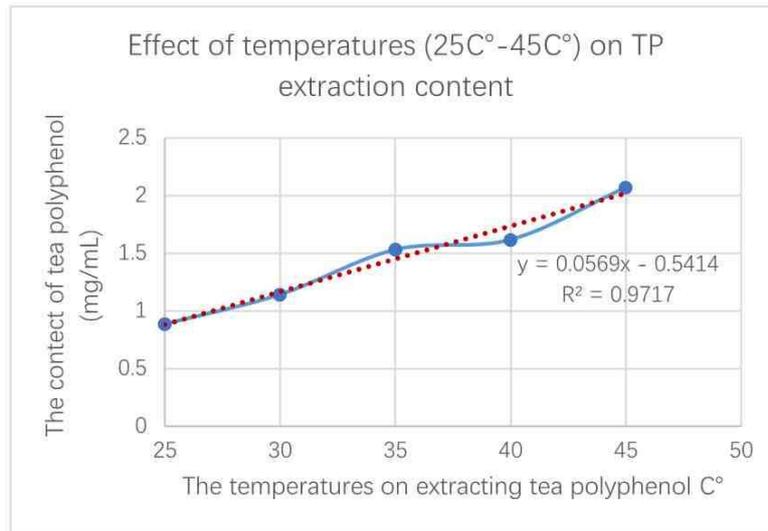
1.2.3 Effect of temperatures on TPs extraction rates

In the high-temperature zone (55°C-95°C) (Li Kexin, 2017), the influence of the extraction temperature on the extraction rate of tea polyphenols tended to increase first and then decrease. The first increase was due to the increased movement of water molecules, which led to the enhanced interaction between water molecules and tea polyphenols and a rapid increase in extraction rate. However, as the temperature continued to increase, the tea polyphenols might be destroyed by the high temperature and therefore the extraction rate decreased.



Graph 1. Effect of temperatures (55°C-95°C) on TPs extraction rate (Li Kexin, 2017)

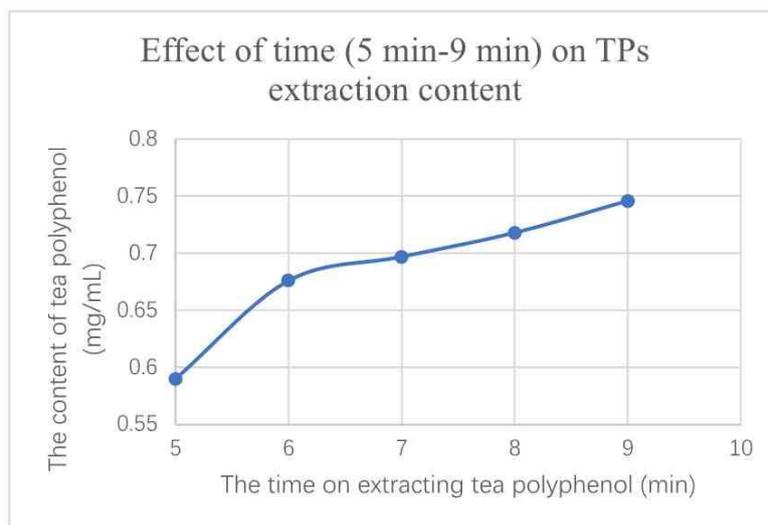
In the medium temperature zone (25°C-45°C) the effect of infusion temperature on the extraction rate of tea polyphenols was uniformly elevated, but the taste became worse. This is because the rate of solubilization of astringent substances such as catechin increases at higher temperatures.



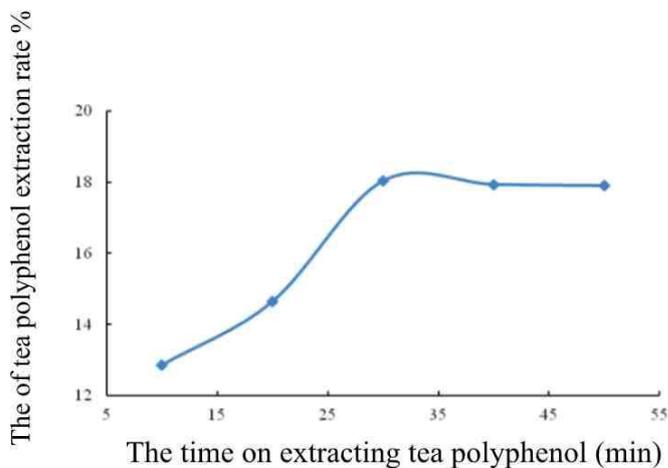
Graph 2. Effect of temperatures (25°C-45°C) on TPs extraction content (Ma Xinyu et al., 2021)

1.2.4 Effect of time on TPs extraction rates

The TP extraction rate increases as the brewing time increases. Meanwhile, the concentration rises quickly at the beginning and then gradually levels off. This is due to the gradual equilibration of the osmotic pressure.



Graph 3. Effect of time (5 min-9 min) on TPs extraction content (Ma Xinyu et al., 2021)



Graph 4. Effect of time (10 min-50 min) on TPs extraction content (Li Kexin, 2017)

1.2.5 The method used to extract and filter the tea water



Graph 5. The Büchner funnel equipment (Three degrees Chinese net, 2010)

The filter equipment that I use is the Büchner funnel, filter flask and an electric pump.

The equipment units are very efficient and simple to implement.

1.2.6 Potassium permanganate titration method used to measure the amount of TPs

The methods used to measure TPs included the Folin-Ciocalteu method, the UHPLC-UV method, titration and so on. The KMnO_4 oxidative titration method was used

because of the limitations of the conditions.

The content of tea polyphenols can be determined using the potassium permanganate (KMnO₄) titration method. The titration approach is based on the idea that potassium permanganate serves as a strong oxidizing agent and tea polyphenols are extremely reductive. Potassium permanganate and tea polyphenols might directly react without an indicator. There are various reaction mechanisms for the oxidation of tea polyphenols by potassium permanganate. However, studies have demonstrated a quantitative relationship between tea polyphenols and potassium permanganate (Wan Xiaochun et al., 2018)

According to Wei Pingyang (2010), the conversion factor is that 1mL of 0.318g/mL potassium permanganate consumed equals 5.82mg of tea polyphenols in the test solution. This is true if 0.318g/mL potassium permanganate solution is used to titrate tea polyphenols. Thus, formula 1 may be used to determine the concentration of tea polyphenols and convert the total amount of tea polyphenol content.

Formula 1:

$$X = \frac{(A-B) \times \omega \times 0.00582 / 0.318}{m \times V_1 / V_2}$$

X----Concentration of tea polyphenols, %

A----Consumption of potassium permanganate, mL

B----Consuming of potassium permanganate in blank test

ω ---Concentration of potassium permanganate, %

m ---Mass of tea leaves, g

V_1 ---Volume of test liquid in titration, mL

V_2 ---Total volume of test liquid, mL

2.0 Methodology

2.1 Variables

2.1.1 Independent Variables

1. The temperature used in tea extraction (0°C, 5°C, 10°C, 15°C, 20°C)

As these five temperatures are all lower than room temperatures, the control of low temperature may consider fixing the temperature of the tea soup by adjusting it to the desired temperature in the refrigerator at 0°C, 5°C, 10°C, 15°C, and 20°C.

2. The time for brewing tea in water (10 min, 20 min, 30 min, 40 min, 50 min, 60 min)

Using the stopwatch to brew the tea for 10 minutes at each interval with a total of six intervals. Each interval indicates the extraction of the same volume of tea water from the beaker.

2.1.2 Dependent Variables

1. The percentage content of tea polyphenol (%)

Recording the volume of potassium permanganate consumed for titration in each trial and then calculate the final percentage content of tea polyphenol under different temperatures and times

2.1.3 Controlled Variables

Table 2. Controlled Variables

Controlled variables	Reason to control	How to control
The brand of tea leaves	Different brands of tea leaves may contain different amount of tea polyphenol in it.	I brought tea bags for whole experiment are from the same brand.
The mass of tea leaves	Different mass of tea leaves have different amount of tea polyphenol diffuse out. Dissolution can only be compared with same mass of tea leaves.	The tea bags are all from one product with same brand. And use the electronic balance for weighting to make sure there are approximately no difference and error.
The ratio value between tea bag and water	The ration value of cold-brewing need to be same. Because the concentration for same trials need to be controlled.	The volume of water that I use for brewing is 250mL. And the mass of the tea bag is 2g.

Temperature of refrigerator	The refrigerator is important to control the lower brew temperature (independent variable). Unstable or inaccurate refrigerator temperature may bring some error on the final result	Setting the refrigerator temperature achieved the ideal. Putting the beaker of water without tea bag in the refrigerator to fit the temperature and keep the same with the inside temperature of refrigerator which at 0°C, 5°C and 10°C.
The oxygen content in the air	Tea polyphenols have phenolic hydroxyl groups and are susceptible to oxidation. Non-enzymatic oxidation generally occurs easily when exposed to air.	Extracting process under same environment. On the other hand, extract the tea from the beaker as quickly as possible.

2.2 Materials, Reagents and Equipment

Table 3. Equipment used

Equipment		
Apparatus	Characteristic	Number
Measuring cylinder	250±0.1mL	3
Volumetric flasks	500±0.1mL	2
	1000±0.1mL	1
Conical flask	250mL	5
Stopwatch	±0.01s	1

Brown glass burette	25.00±0.05mL	1
Refrigerator	-20°C-10°C±0.1°C	1
Pipette	20±0.01mL	1
Electronic scale	200.000±0.001g	1
Magnetic stirrer	50~1500r/min	1
Büchner funnel	Size:60mm Volume: 125mL Uncertainty: 0.1mL	1
Filter paper	Size: 60mm	10
Thermometer	0°C-100°C±0.5°C	1

Materials and reagents

Table 4. Materials and reagents used

Materials and reagents		
Reagent	Concentration	Volume
Potassium permanganate	0.001mol/L	1000mL

2.3 Procedures

Preparation of 0.001mol/L Potassium Permanganate

Calculation:

$$n(\text{KMnO}_4) = C_{\text{Final}_{\text{KMnO}_4}} \times V_{\text{Final}_{\text{KMnO}_4}}$$

$$n(\text{KMnO}_4) = 0.001 \text{ mol/L} \times 1 \text{ L} = 0.001 \text{ mol}$$

$$m(\text{KMnO}_4) = n(\text{KMnO}_4) \times M(\text{KMnO}_4)$$

$$m(\text{KMnO}_4) = 0.001 \text{ mol} \times (39.10 + 54.94 + 16.00 \times 4) \text{ g/mol} = 0.158 \text{ g}$$

1. Using the electronic balance measure 0.158g of solid potassium permanganate
2. Use distilled water to dissolve the solid potassium permanganate in the beaker
3. Transfer the potassium permanganate into the 1000mL volumetric flask and rinse the beaker several times
4. Add distilled water into the 1000mL volumetric flask nearly the 1000mL mark of the volumetric flask and start dilution.
5. Stopper the flask and invert it several times to ensure a homogeneous solution.
6. Label the flask.

Extraction of tea polyphenol

1. Using a cylinder measure 250mL distilled water and pouring into the 250mL beaker.
And put the beaker into the refrigerator at 0 °C. After a long time of waiting, use a thermometer to ensure the temperature of the water in the beaker.
2. Putting a tea bag into the beaker. Starting the stopwatch for 10 minutes at each

interval.

3. When the time reached 10 minutes, use the 20mL pipette to transfer 40mL of tea water into the bottle with the label.
4. Repeat Step 3 for six-time intervals which are 10min, 20min, 30min, 40min, 50min and 60min.
5. Repeat Step 2 to step 4 under 5 different temperatures which are 0°C, 5°C, 10°C, 15°C and 20°C.
6. Repeat Step 1 to step 5 for five times.

Titration :

1. Measuring 20mL of tea water and transferring to the 100mL conical flask by 20mL pipette
2. Pour the 0.001mol/L of potassium permanganate into the brown glass burette through the plastic funnel.
3. Pre-start the titration and eliminate the bubble under the brown glass burette.
4. Start titration and see the colour change of the tea water. Recording the volume of potassium permanganate that is consumed.



Figure 3. The cold brewing process implement in the refrigerator

2.4 Risk Assessment

1. Potassium manganate has the potential to cause eye damage in addition to causing severe skin and eye irritation and burning. Shortness of breath, coughing, and/or lung irritation can result from breathing potassium permanganate. Wearing protective eyewear, gloves, and a lab coat is therefore crucial.
2. The majority of the equipment was constructed of glass, which was brittle and would scratch the skin. Do an experiment while wearing gloves.
3. Because the reactants in this experiment were powder, they needed to be stored carefully to prevent injury to our eyes if they accidentally got into our eyes. By putting on safety glasses, it can be properly avoided.

3.0 Data

3.1 Qualitative observations

As the titration was processed, the solution changed colour from light yellow to light pink and finally dark brown.



(Initial)



(Process)

Figure 4&5. Color of the solution when titration is processed

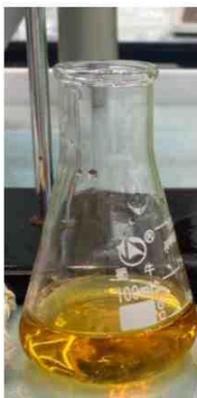


Figure 6. Color of the solution at the end of the titration

3.2 Quantitative data

Raw data (the temperature and time changes) of Table 5 of 0°C within 10 to 60 minutes as shown in the below. Meanwhile, Table 9., Table 10., Table 11., and Table 12. in the appendix which shows the volume of magnesium permanganate consumed in the titration of the tea polyphenol in the tea water.

Table 5. The volume of potassium permanganate consumed on measuring tea polyphenol of 0°C within 10 to 60 minutes

No	The temperature of tea water ±0.1°C	The time on making tea ±0.005s	The volume of potassium permanganate±0.05mL				
			Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
1	0	10	6.0	5.5	5.5	5.7	5.6
2		20	6.9	9.6	5.6	6.5	6.3
3		30	11.2	9.6	9.5	9.8	14.2
4		40	10.2	9.4	10.4	9.7	10.1
5		50	12.1	12.3	12.6	12.7	12.4
6		60	18.0	17.3	17.7	11.7	17.3

4.0 Data analysis

4.1 Sample calculation

Using 20°C and 60min as an sample

Average volume of potassium permanganate consumed:

$$\frac{13.8 + 14.0 + 13.5 + 13.9 + 14.1}{5} = 13.86\text{mL}$$

Uncertainty:

$$\frac{14.1 - 13.5}{2} = 0.3$$

The percentage content of tea polyphenol:

$$X = \frac{13.86 \times 1.00 \times 0.00582 \div 0.318}{2 \times 20 \div 250} = 1.56\%$$

4.2 Experiment Uncertainty

$$\text{Uncertainty for Measuring cylinder} = \frac{1\text{ml}}{250\text{ml}} \times 100 = 0.4\%$$

$$\text{Uncertainty for electronic balance CX-12000 } (\pm 0.01\text{g}) \times 1 = \frac{0.005}{0.158} \times 100 = 3.16\%$$

$$\text{Uncertainty for burette (brown)} = \frac{0.1\text{ml}}{50\text{ml}} \times 100 = 0.2\%$$

$$\text{Total uncertainty for measurements} = 0.4\% + 3.16\% + 0.2\% = 3.76\%$$

Percentage uncertainty of volume of KMnO_4 (Using 10min, 5°C as sample) =

$$\frac{0.1\text{mL}}{5.66\text{mL}} \times 100 = 1.77\%$$

$$\text{Percentage uncertainty of mass of tea bag} = \frac{0.1}{2} = 0.05\%$$

$$\text{Percentage uncertainty of test liquid} = \frac{0.01}{25} = 0.04\%$$

$$\text{Percentage uncertainty of total volume of liquid} = \frac{1}{250} = 0.4\%$$

Total percentage uncertainty of experiment (Using 10min, 5°C as sample)

$$= 1.77\% + 0.05\% + 0.04\% + 0.4\% + 3.16\% = 5.42\%$$

4.3 Processed data

Table 6. The average volume of potassium permanganate in titration

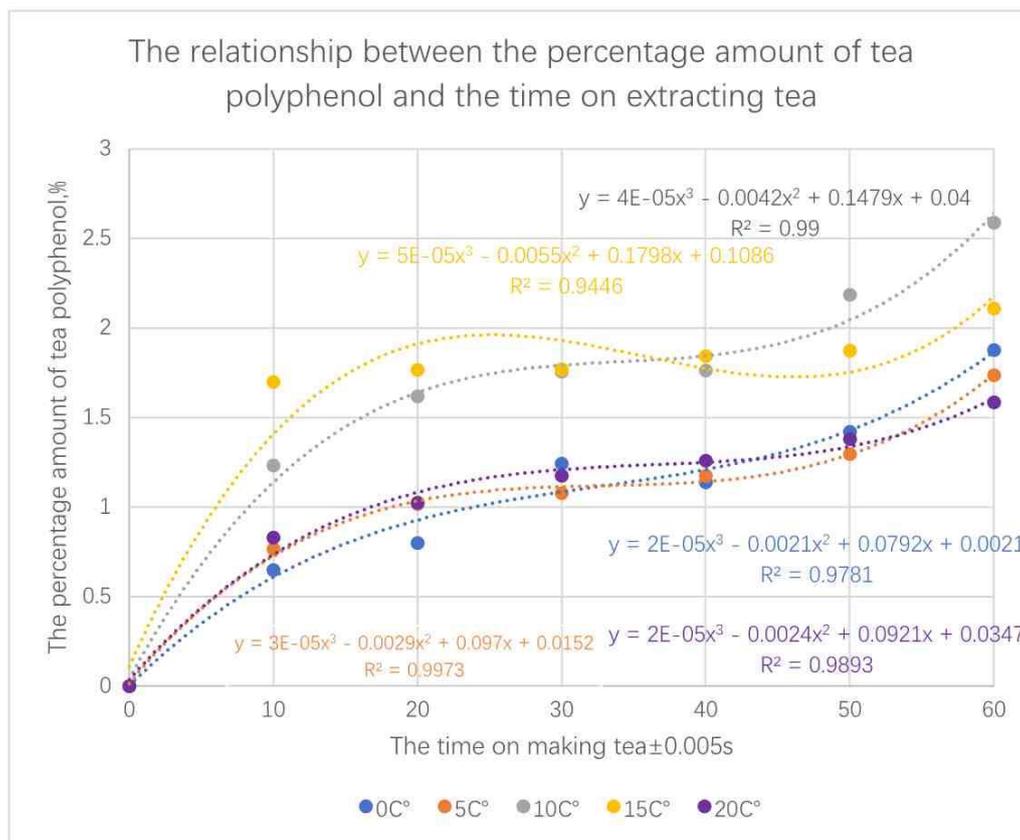
The temperature of tea water $\pm 0.1^\circ\text{C}$	The average volume of potassium permanganate $\pm 0.05\text{mL}$					
	The time on making tea $\pm 0.1\text{s}$					
	10min	20min	30min	40min	50min	60min
0	5.660 $\pm 1.77\%$	6.980 $\pm 1.43\%$	10.86 $\pm 0.92\%$	9.960 $\pm 1.00\%$	12.42 $\pm 0.81\%$	16.40 $\pm 0.61\%$
5	6.700 $\pm 1.49\%$	8.880 $\pm 1.13\%$	9.420 $\pm 1.06\%$	10.26 $\pm 0.97\%$	11.32 $\pm 0.88\%$	15.18 $\pm 0.66\%$
10	10.76 $\pm 0.93\%$	14.14 $\pm 0.71\%$	15.34 $\pm 0.65\%$	15.42 $\pm 0.64\%$	19.10 $\pm 0.52\%$	22.64 $\pm 0.44\%$
15	14.84 $\pm 0.67\%$	15.44 $\pm 0.65\%$	15.46 $\pm 0.65\%$	16.12 $\pm 0.62\%$	16.38 $\pm 0.61\%$	18.44 $\pm 0.54\%$
20	7.260 $\pm 1.38\%$	8.960 $\pm 1.11\%$	10.26 $\pm 0.97\%$	11.02 $\pm 0.91\%$	12.06 $\pm 0.83\%$	13.86 $\pm 0.72\%$

Table 7. The content of tea polyphenols in tea water

The temperature of tea water $\pm 0.1^{\circ}\text{C}$	The content of tea polyphenols, %					
	The time on making tea $\pm 0.005\text{s}$					
	10min	20min	30min	40min	50min	60min
0	0.64 $\pm 5.42\%$	0.79 $\pm 5.08\%$	1.24 $\pm 4.57\%$	1.14 $\pm 4.65\%$	1.42 $\pm 4.45\%$	1.88 $\pm 4.26\%$
5	0.77 $\pm 5.14\%$	1.02 $\pm 4.77\%$	1.08 $\pm 4.71\%$	1.17 $\pm 4.62\%$	1.29 $\pm 4.53\%$	1.74 $\pm 4.26\%$
10	1.23 $\pm 4.58\%$	1.62 $\pm 4.36\%$	1.75 $\pm 4.30\%$	1.76 $\pm 4.30\%$	2.18 $\pm 4.17\%$	2.59 $\pm 4.09\%$
15	1.70 $\pm 4.32\%$	1.76 $\pm 4.30\%$	1.77 $\pm 4.30\%$	1.84 $\pm 4.27\%$	1.87 $\pm 4.26\%$	2.11 $\pm 4.19\%$
20	0.83 $\pm 5.03\%$	1.02 $\pm 4.77\%$	1.17 $\pm 4.62\%$	1.26 $\pm 4.56\%$	1.38 $\pm 4.48\%$	1.56 $\pm 4.37\%$

4.3.1 The relationship between the time and the content of tea polyphenol

From the data, the highest percentage content of tea polyphenols happens at 10°C and 60 minutes, while the lowest one happens at 0°C and 10 minutes.



Graph 6. The relationship between the percentage amount of tea polyphenol and the time on extracting tea

Graph 6. illustrates the relationship between the percentage amount of tea polyphenol and the time on extracting tea. And some outliers and fluctuations will be focused on during the analysis.

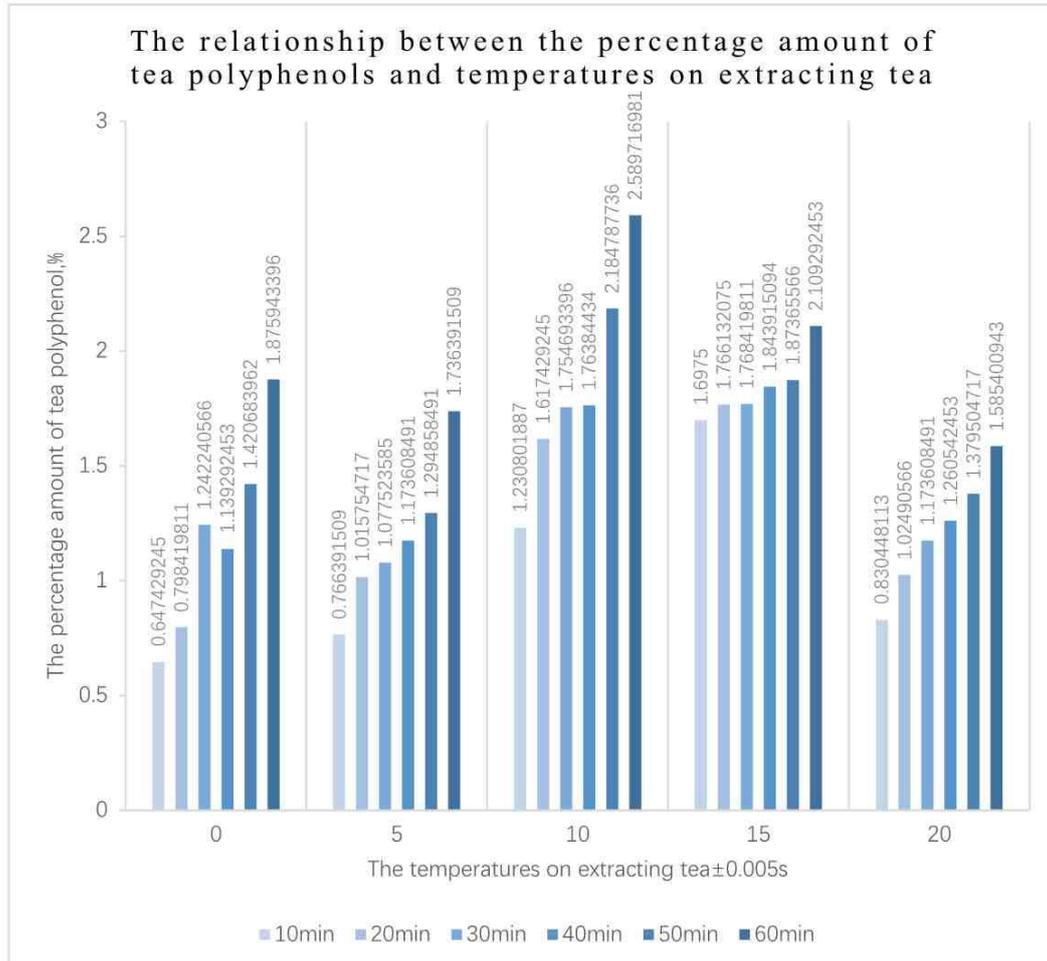
First of all, the R² value of each trend from 0min to 60min is 0.9511, 0.9975, 0.9835, 0.9807 and 0.9992 respectively which are all strong positive correlations. It is obvious that the TPs concentration increases with increasing cold soak time at the same temperature. The rate of increase is fastest from 0-10 minutes, followed by a plateau

from 10-50 minutes. After 50 minutes, the rate of increase picks up again and becomes faster from 50-60 minutes.

At 10 minutes, the percentage amount of tea polyphenol was the highest at 15°C (1.70%) which represents by the yellow point, while the percentage amount of tea polyphenol at the highest temperature (20°C) is less than 15°C (yellow line) and 10°C (grey line). On the other hand, at 60 minutes, the percentage amount of tea polyphenol dissolved most under 10°C of 2.59% (grey line), while the percentage amount of tea polyphenol dissolved under 0°C (blue line) and 5°C (orange line) are all exceed than 20°C (purple line) which are 1.88, 1.74 and 1.56 respectively. For the grey line and blue line, there are some points such as 40 min under 10°C, 20 min, 30 min and 40 min under 0°C.

According to trends and the comparison of specific points, there are two factors that affect the diffusion rate of tea polyphenols. Firstly, osmotic pressure affects the diffusion rate. Due to diffusion is particles move from an area of higher concentration to an area of lower concentration until equilibrium reached. However, another factor is the extent to which the tea tissue is damaged. The rate of extraction into water is faster when the difference in osmotic pressure between the water and the tea leaves is greatest at 0-10 minutes. As the osmotic pressure continues to decrease, the tea tissue structure is basically stable and the diffusion rate decreases. After 50 min, the tea tissue is damaged by water infiltration and the diffusion rate is elevated.

4.3.3 The relationship between the temperatures and the content of tea polyphenols



Graph 7. The relationship between the percentage amount of tea polyphenols and temperatures on extracting tea

Graph 7. illustrates the relationship between the percentage amount of tea polyphenol and temperatures on extracting tea.

It is obvious that trends of TPs concentration under six time periods are increases with increasing temperatures. However, all trends after reaching the peak at 15°C saw a

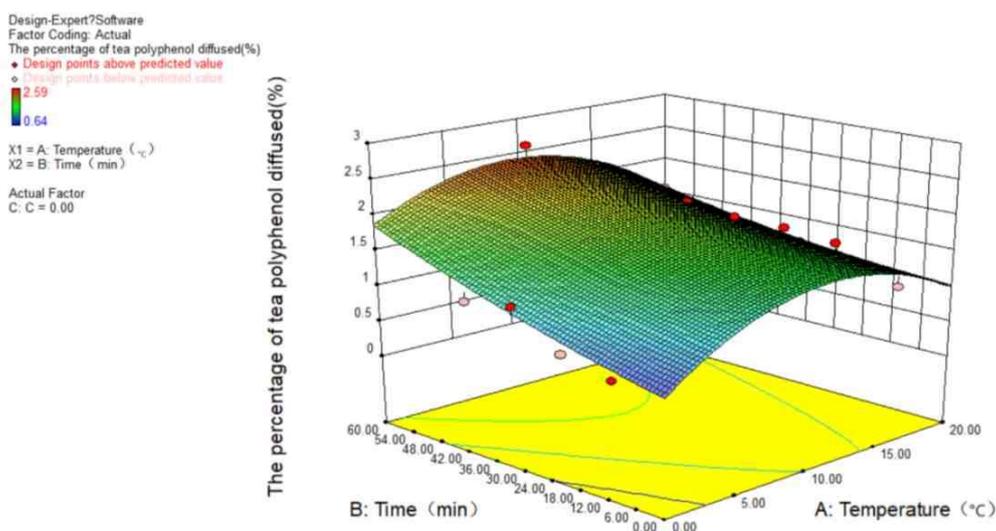
decrease. The rate of decrease is from 0°C to 5°C, followed by the fastest rises from 5°C to 15°C. When reached 15°C, the rate of falling again at 20°C.

According to *Graph 7.*, there are more fluctuations and value differences of under 0°C to 20°C between 50 min and 60 min. At 5°C and 15°C, the value of the concentration of tea polyphenols at 50 min and 60 min are all lower than the standard trend which are 1.29 (5°C), 1.74 (5°C) and 1.87 (15°C), 2.10 (15°C) respectively. Meanwhile, the temperature reached 10°C, and the percentage amount of tea polyphenol diffusion under 50 min and 60 min is significantly higher than their standard trends which are 2.18 (10°C) and 2.59 (10°C) respectively. However, the percentage amount of tea polyphenol is similar from 0°C to 20°C under 10 min to 60 min.

As a result, trends as shown in *Graph 7.* can be illustrated for some reasons. First of all, the time of extraction tea is longer means that the tea polyphenol particles in tea leaves can be dissolved more completely. Because time is one of the most significant factors that affect particle collision and rate of reaction. On the other hand, due to tea polyphenol has many hydrogen bonds. So it is more stable and not easy to break. 60 minutes means there is enough time to break the structure of tea polyphenol. And actually, there are many structures of tea polyphenol compounds, so the components of tea polyphenol can diffuse at different time intervals.

4.4 Discussion about proceeded data and the literature

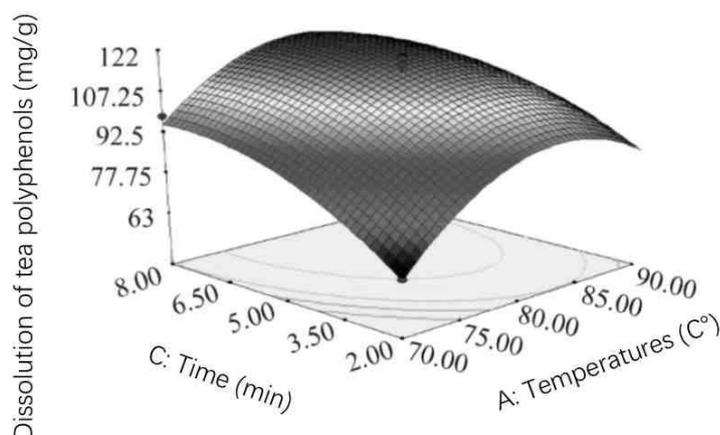
4.4.1 Response surface analysis



Graph 8. My experiment results

As shown in *Graph 8.*, Using the Design-Expert Software, I set the independent variables (temperatures and times) as well as the response (the percentage of tea polyphenol diffused). After typing in my experimental data, the software provides me with the three-dimensional diagram.

Design-expert software was used to visualize the impact and the trend of the percentage amount of tea polyphenol dissolved in different temperatures and times in three-dimensional diagram. From the diagram, the peak point and the optimal conditions can be deduced which is 60 minutes under 10°C (2.59%).



Graph 9. The dissolution of tea polyphenol under different temperatures and times

(Chen Zhenghan, 2012)

To ensure the accuracy and reliability of the result, it's necessary to make a comparison with the other research. There is a research article on discovering the effect of the percentage amount of tea polyphenol under different temperatures and times separately. In the study, as shown in *Graph 9.*, the trend of temperature and times affecting the percentage amount of tea polyphenols are all increasing. The maximum amount of tea polyphenol is under 81.42°C and 5 minutes (Chen Zhenghan et al., 2012). For this reported research experiment, two independent variables are carried out simultaneously. So compare to the research the time carry out the increasing trend, which matches my experiment result. However, the trend of different temperatures in the study does not perfectly match the experiment results. Because the study did not carry out the temperatures that were chosen in this reported research experiment. Furthermore, the optimal conditions in the study may also not match and compare with the reported results due to less evidence.

5.0 Conclusion

In the study, temperature and time were the major factors affecting the percentage amount of tea polyphenol dissolved in the tea water.

Nowadays, people may prefer cold water to make tea more than hot water, because cold water may be more convenient and quick. Global concern about the real phenomenon of making tea under different temperatures and times. Longer time in making tea can help the major components in tea leaves dissolve completely. This investigation focused on two of the above-discussed factors on dissolved tea polyphenol, the temperature and time, and the result shows that the maximum percentage amount of tea polyphenol occurs when the temperature is 10°C and time of 60 minutes. It was found to be the optimum value to achieve the maximum amount of tea polyphenol in cold water mode. Comparing with the result from Chen Zhenghan 's research (the optimum temperature 81.42°C and time 5 minutes), it can be seen that the time is the same, it is matched, but the optimal temperature is not, due to the difference between cold and hot water.

Related to human health, using cold water to make the tea can promote the stable thermal ability of major components in tea leaves that dissolved in the water (ECG and EGCG). And these components cannot easily be oxidised by cold water, which can maintain the value of tea water for human health. So the optimal temperature (10°C) and time (60 min) can be realised in the real life.

6.0 Evaluation

6.1 Strength and limitation

6.1.1 Strength

The first strength of this study is that an easy method was applied to determine tea polyphenol content in green tea. The simple titration by KMnO_4 method was applied to determine the TPs content rather than the Ferrous tartrate spectrometric method which requires a delicate instrument, saving time and is more convenient.

In order to gain a more reliable and accurate result, each variable is repeated five times, having smaller standard derivation, which can highly increase the reliability of the experiment or prevent some possible errors, and the result was highly constituent with the literature data.

Also, the use of the Response Surface Methodology is an innovative trial. Although the experiment has only two variables, it provided a new investigating direction and analyzed or modelled the interaction system mathematically. Different from the linear analysis, a three-dimensional diagram succeeded in visualizing the relationships more vividly and thus helped to investigate deeply.

6.1.2 Limitation

The investigation is effective and meaningful because it only focuses on green tea leaves and temperatures under room temperatures from 0°C to 25°C . So the

investigation will be meaningless if the brand, type of tea leaves, and different temperatures changes.

6.2 Improvement of experiment

Table 8. Improvement of experiment

Systematic error Description	Improvement
<p>The oxygen content in the laboratory have fluctuations. Tea polyphenols have phenolic hydroxyl groups and are susceptible to oxidation. Non-enzymatic oxidation generally occurs easily when exposed to air.</p>	<p>There are two way can solve this error. Firstly, do the experiment under the environment full of helium gas. On the other hand, do experiments in vacuum glove boxes. Because there is only noble gas in the box. So it can sequestered oxygen.</p>
<p>The tea leaves in tea bag were not complete purity. The green tea leaves that I used in each tea bag may include some other components exclude tea polyphenol. These impurities substances which also affect the result of experiment.</p>	<p>I would like to choose the tea bags with high quality green tea leaves which can minimize the effect of experiment results by impurity substances.</p>
<p>The tea water with some impurity substance after filter process in each experiment. The tea water can affect the final result of titration. The impurity substances in tea water may affect the final value with some fluctuations.</p>	<p>I use the Büchner funnel to filter the tea water.</p> 

	<p>But this method also remain some impurity substance in the tea water. So I would like to choose some effective and high quality electronic machines to filter the tea water, which make sure the tea water is maximize pure.</p>
<p>The refrigerator temperature was sometimes inconsistent. The low temperature of refrigerator need to manage before and then making tea in it. The fluctuation of temperature in refrigerator will have some effect on the dissolved of each beaker on making tea.</p>	<p>I use pipette to extract the tea water out of the refrigerator temporarily. So there are some effects on the final value. Next time, I would like to extract the tea water in the refrigerator or use the refrigerator with larger space.</p>
<p>The color indicator is vague. So the value may not very precise. In the experiment, I do not use the pH indicator. Because potassium magnesium can react with tea polyphenol directly and have color change. But the real tea water color are not the same, some are a bit transparent, some are green, dark green and yellow. So the vague color change has affect the final results.</p>	<p>There are some methods can use high quality machines to determine the amount of tea polyphenol instead of potassium magnesium titration. For example, Ferrous tartrate spectrophotometry is use direct ultraviolet spectrophotometry and I can find the peak value at 540nm.</p>

6.3 Extension of the investigation

Apart from the variant of temperature and time, the variation of the ratio of tea leaves and water and the quality of water will also affect the TP extraction rate. This is because water is very important when making tea and also affects the value of tea polyphenols dissolved in tea water. Meanwhile, for further experiments, I can choose more kinds of tea such as Black tea, Pu-erh tea, Tieguanyin, etc. There may have a big difference in the percentage amount of tea polyphenol dissolved from different tea leaves. In addition, I can discuss the property of TPs of antioxidant activity and free radical removal rate.

Furthermore, there are some methods that can improve controlled variables in further experiments. For the controlling of oxygen content in the laboratory. There are two ways to solve this error. The first is to conduct the experiment in an environment filled with helium gas, which will prevent the oxygen from reacting with the sample. The second approach is to perform the experiment in a vacuum glove box, where only noble gases are present, effectively sequestering any oxygen.

On the other hand, there are some methods that are more effective to filter tea water such as Ultrasonic extraction, Supercritical fluid extraction, etc. Furthermore, the error occurs on reading the volume of potassium magnesium that consumed in titration can be instead by some accuracy and high-quality electronic machines such as High-Performance Liquid Chromatography, and ultraviolet spectrophotometry to determine the percentage amount of tea polyphenol dissolved in tea water.

7.0 Bibliography

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8.0 Appendix

Table 9. The volume of potassium permanganate consumed on measuring tea

polyphenol of 5°C within 10 to 60 minutes

No	The temperature of tea water $\pm 0.1^\circ\text{C}$	The time on making tea $\pm 0.005\text{s}$	The volume of potassium permanganate $\pm 0.05\text{mL}$				
			Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
7	5	10	6.70	7.50	7.00	6.30	6.00
8		20	10.2	8.30	8.10	8.70	9.10
9		30	9.9	9.60	8.10	9.50	10.0
10		40	10.1	10.1	10.4	10.2	10.5
11		50	10.8	11.1	12.4	11.0	11.3
12		60	19.7	14.5	13.6	14.2	13.9

Table 10. The volume of potassium permanganate consumed on measuring tea

polyphenol of 10°C within 10 to 60 minutes

No	The temperature of tea water $\pm 0.1^\circ\text{C}$	The time on making tea $\pm 0.005\text{s}$	The volume of potassium permanganate $\pm 0.05\text{mL}$				
			Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
13	10	10	10.9	10.4	10.5	11.3	10.7
14		20	14.4	13.6	14.3	13.9	14.5
15		30	15.4	15.4	15.7	15.2	15.0
16		40	16.2	14.5	14.9	15.6	15.9
17		50	18.9	19.3	18.8	19.1	19.4
18		60	22.6	22.8	23.0	22.3	22.5

Table 11. The volume of potassium permanganate consumed on measuring tea polyphenol of 15°C within 10 to 60 minutes

No	The temperature of tea water $\pm 0.1^\circ\text{C}$	The time on making tea $\pm 0.005\text{s}$	The volume of potassium permanganate $\pm 0.05\text{mL}$				
			Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
19	15	10	15.0	14.7	15.1	14.5	14.9
20		20	13.9	16.3	15.8	15.5	15.7
21		30	15.2	15.5	15.0	15.9	15.7
22		40	17.9	16.4	15.7	15.2	15.4
23		50	17.2	15.8	16.6	16.0	16.3
24		60	19.8	18.8	17.5	17.9	18.2

Table 12. The volume of potassium permanganate consumed on measuring tea polyphenol of 20°C within 10 to 60 minutes

No	The temperature of tea water $\pm 0.1^\circ\text{C}$	The time on making tea $\pm 0.005\text{s}$	The volume of potassium permanganate $\pm 0.05\text{mL}$				
			Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
25	20	10	7.50	7.00	7.20	6.90	7.70
26		20	9.00	8.80	9.30	8.90	8.80
27		30	10.2	9.90	10.5	10.3	10.4
28		40	11.1	10.9	11.0	11.3	10.8
29		50	12.2	12.0	12.5	11.7	11.9
30		60	13.8	14.0	13.5	13.9	14.1