ExpDesign Group Final Project:

Analyzing Factors on the Taste of Tea among Young Generation

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Outline:

I. Summary

Tea has become the second most popular drink in the world and is consumed by 2/3 of the world's population. There are various types of tea with their unique steeping process, and each can give people a completely different taste. Hence, to discover more secrets behind the tea, especially green tea, we came up with two types of research questions (one subjective and one objective). The questions are: "How will water temperature and amount of tea leaves affect the caffeine level in a cup of tea?" and "How will water temperature and the amount of tea leaves affect the taste of tea among the young generation". Based on those two scientific research questions, we designed three types of experiments (factorial design, block design, and split-plot design) to examine our research questions formally. For each design, we carefully discussed how we are going to conduct them in detail, such as the randomization plan and recruitment plan. After comparing the strengths and weaknesses of each design, we finally chose to implement the factorial design due to its outstanding advantages. Finally, we also built some statistical models and tests to furtherly examine our experiment and research questions. For example, we conduct a power analysis which helps us to determine the total sample size that we need to recruit for the experiment. We also run ANOVA tests to examine the main effect and the interaction effects of how the steeping process affects the taste as well as the caffeine level of a cup of tea.

II. Background & Introduction

Next to water, tea is the second most popular drink in the world and is consumed by two-thirds of the world's population (Statista, 2020). It comes with more than a thousand variations and low prices. Popular tea types in the market are black tea (fermented), green tea (non-fermented), and oolong tea (semi-fermented), which are made due to different processes of drying and fermentation that determine their chemical composition. While being cheap, tea is widely consumed because it contains beneficial components good for our health. The main polyphenolic compounds of green and black tea are catechins and theaflavins respectively, responsible for most of the physiological effects of tea. Encouraging data has shown that tea consumption has preventive effects for cancers and cardiovascular diseases (Khan & Mukhtar, 2014). Drinking tea is also anti-aging, antidiabetic, and associated with a lower risk of Parkinson's (Hu et al., 2007).

For some people, making tea can be as easy as pouring hot water into a cup with tea leaves. Even easier, ready-to-drink tea, mostly black or green, sold in supermarkets is also many people's choice if they ever want to drink tea without having to brew it first. However, it is worth noting that many bottled tea drinks have added sugar which cancels some healthy benefits of tea. There is also a comparison between using loose tea leaves or tea bags. Conceivably, when steeping loose-leaf tea, there is more room for tea leaves to absorb water, expand, and then release a wide range of vitamins, minerals, flavors, and aromas than using tea bags (Goodwin, 2019). When the tea bag is steeped, the size of the tea bag limits the infusion process and thus cannot extract the full flavor from the tea leaves. Therefore, to optimize the journey of drinking tea, loose-leaf tea is the best choice. Made from the processed leaf of Camellia sinensis, there are approximately 600 traces of aroma compounds in tea leaves. As tea brews, chemicals such as tannins, amino acids, aromas, and flavors slowly diffuse into the water and contribute to different tastes of tea. Similar to cooking, which requires us to use the right amount of ingredients and seasoning, optimizing the taste of tea also needs us to pay attention to a number of relevant factors. In this study, we will conduct an experiment and explore how two factors, water temperature and the amount of tea leaves, affect the taste and caffeine level of a cup of tea.

III. Literature Review & Research Question

Water Temperature

When water is added to a cup with tea leaves inside, it starts to flow through the leaves and extract aromas that are responsible for smell and flavor from tea leaves, followed by compounds related to mouthfeel and texture, and lastly the heaviest compounds such as heavier polyphenols, flavanols, and tannin. The water temperature controls how fast those chemical components are released. If the water brings too much heat, compounds might be dissolved too quickly and then create an imbalance; an opposite effect may happen if there is too little heat, generating a tasteless flavor (Willett, 2020). Thus, in order to make the most out of tea leaves, the key is to use the most suitable brewing temperature.

Caffeine, as one of the components in tea leaves, is also affected by water temperature. As the water temperature increases, the dissolution rate of caffeine increases and thus increases the caffeine level while holding the brewing time the same. According to a study conducted by a Taiwan tea research agency, the amount of caffeine in a cup of tea brewed at 100℃ (212°F) was at least double the amount when brewed at 80℃ (176°F) regardless of the type of tea (Huang, 2018).

Amount of Tea Leaves

The final taste of tea is also directly linked to the amount of tea leaves in a single cup. Same as choosing an appropriate water temperature, adding the right amount of tea leaves is also important to avoid imbalance. According to Willett, a moderate amount can contribute to the richness of flavor, but too much might start to generate bitterness and become over strong (2020). However, if you find the taste to be weak and the tea seems watery, you should add more tea leaves next time. The industry standard is to use 1 measuring teaspoon per 6 oz. of water or 1 heaping measuring teaspoon per cup (8 oz.), but an optimized outcome should also depend on the size of tea leaves (Sweetsteep, 2019). In addition, it is reasonable to assume that more tea leaves contain more caffeine, and thus the amount of tea leaves should also affect the caffeine level inside a cup of tea.

Based on the previous studies, we aim to design an experiment and address the following research question in our study:

> **RQ1**: How will water temperature and amount of tea leaves affect the caffeine level in a cup of tea?

We are also particularly interested in the young generation's tastes, thus the following question is asked:

RQ2: How will water temperature and amount of tea leaves affect the taste of a cup of tea among the young generation?

IV. Design of Experiment (FD, RBD, Split-Plot)

Participants

Participants will be the current students and faculties of New York University. Researchers will send out the recruitment information through the "@nyu.edu" email and select the first N respondents as the participants for this experiment. We will also ask them to provide some personal information, such as age and gender to ensure they will meet our inclusion criteria. This study will apply for approval from New York University Human Subjects Committee IRB. All participants will be provided written informed consent prior to participating.

Materials

Xihu Green tea leaves will be used in the experiment. The amount of tea(factor 1) that we plan to test in this experiment will range from 10%, 30%, and 50%.

Temperature-controlled boiling kettles will be employed to adjust the temperature of water(factor 2) to 65℃, 85℃, or 95℃. **Teapots with scales** will be used to measure the amount of boiling water and tea leaves. **Sealed opaque tea cups** will be provided to the participants so that they will not have expectations when seeing the color of the liquid.

Confounding variables

1. Water quality: Since different types of water may have an influence on the taste of the tea, we decided to use filtered tap water.

2. Type of teapot/maker: Several identical glass teapots with scales will be used to make the tea.
3. Type of teacup: To prevent participants from knowing what kind of tea we give to them, we will use sealed opaque cups and deliver them via conveyor belt
4. Water volume: The amount of water we inject into the teapot may directly change the taste of the tea, so we fix the amount of water at 80% of the teapot.
5. Heating Method: Same as what we discussed in the Materials section, temperature controlled boiling kettle will make sure all tea is heated in the same way
6. Steeping time: The final taste of tea heavily depends on how long you steep it. After doing some research, we found that 2 mins is the best for making green tea.
7. Gender: According to our research, there is some difference between males and females in terms of tea preference. To avoid any confounding issue caused by gender, we will recruit the same number of female and male participants
8. The person who makes tea: we will hire several well-trained people to make the tea so that each cup of tea made by that specific person will taste nearly identical.

Evaluation

Participants will be provided with a tea evaluation form (*Index A*) modified from SCA Cupping Form (Speciality Coffee Association, 2022) to rate the taste of tea. Each cup of tea will be evaluated from five dimensions: Fragrance, Flavor, Body, Aftertaste, and Balance, where Fragrance, Flavor, Body, Aftertaste will be rated from 1-10 and Balance will be rated from -5 to 5.

Fragrance refers to the aromatic aspects of the tea, as detected by an initial smell before tasting in the mouth. A better fragrance receives a higher score. **Flavor** is described as “bright” when favorable, “bitter” when unpleasant, or “dull” or “flat” when missing. Flavor contributes to the liveliness, sweetness, and fresh-fruit character of the tea. A lack of flavor (“dullness” or “flatness”) receives a low score, and too much bitterness receives a low score; a “bright”, lively flavor receives a high score. **Body** refers to the tactile feeling of the tea liquid in the mouth, highly related to the “viscosity” of the liquid. A “watery” tea can have good flavor but lacks body, while a “thick” tea can have strong body but bad flavor. Tea with a pleasant body receives higher scores. **Aftertaste** refers to the length or duration of positive flavor emanating from the back of the palate and remaining after the tea is swallowed. If the aftertaste is either short or unpleasant, a lower score should be given. **Balance** refers to the overall impression of the tea. Ideally, a tea is balanced between all of the above attributes, with none dominating over any other. Positive scores should be given with an overall positive experience. A total score will be calculated for each cup of tea.

In addition, we will also need to evaluate the caffeine level of the tea. This chemical measurement will be conducted by ATAGO PAL-COFFEE(BX/TDS), a caffeine detector made by the Japanese brand ATAGO. Its measurable caffeine concentration ranges from 0 to 25%, so we can get a relatively objective outcome variable by calculating our caffeine levels. For example, if we choose the factorial design, we will have 9 teapots because there are two covariates (water temperature and amount of tea), and each has three levels. Hence, each teapot will contain a unique combination of water temperature and amount of tea, and we can use the BX/TDS to measure the caffeine level of each teapot.

Experimental Design

Aim one: Objective measurement

Although the two aims that we are interested in are two different types of outcomes(one objective, one subjective), we can still test them simultaneously. The general idea is that for each teapot with a combination of water temperature and amount of tea before we spread them into teacups and deliver them to participants, we will measure its caffeine level through the way which we talked about previously in the evaluation section. Then, after completing the entire study, we will build some statistical models, such as ANOVA, to further analyze the steeping effects on the caffeine level of the tea.

Aim two: Subjective measurement

1) Factorial Design

Factorial design enables researchers to test the effects of more than one factor at a time. With the factorial design, participants will be randomly assigned to different combinations of factor levels.

In this experiment, the water temperature has three levels (65℃, 85℃, or 95℃), and the amount of tea has three levels (10%, 30%, 50%). A 3 x 3 factorial design will be conducted with 8 degrees of freedom. Each subject will be assigned to one combination of three levels of water temperature and three levels of amount of tea. The experimental unit will be participants drinking tea. There will be N/9 participants in each combination and N/9 replicates for each combination.

Participants will be numbered from 1 to N and randomly assigned into 9 treatment groups using R. Each tea maker will make N/9 cups of tea according to the specific instructions on varying water temperatures and amounts of tea for respective participants using the identical green tea leaves, boiling kettles, teapots, and other tea utensils in the same room. After steeping for 2 minutes, tea makers will pour the tea into N/9 sealed opaque cups and put them on the conveyor belt (to prevent tea makers' expectations). Participants then will smell, drink, and taste the tea and fill out the tea evaluation form. In the end, researchers will calculate the score for each treatment group.

Factorial design has relatively high internal validity. By using random assignment, the characteristics of the participants will be balanced out, so researchers can make causal inferences about the effects of the two factors. In terms of data analysis, this design is computationally easy and interpretable. Researchers can use the ANOVA model in R to figure out whether there are main effects for factors or interaction effects between factors. Moreover, this design is easy to implement and participants-friendly in a way that each participant only experiences one treatment and the procedure of making tea is simple. However, it is worth mentioning that all the designs will face the issue of sampling error that NYU students and faculties are not representative enough of the whole population. The first N email respondents might have some traits and characteristics that would affect the results of the experiment, and this design does not strategically correct the sampling bias. In this case, this design has certain limitations regarding generalizability. In other words, researchers cannot infer the whole young generation's drinking preferences with the conclusions of this experiment.

	10% tea	30% tea	50% tea
65°C	Combo 1	Combo 2	Combo 3
85°C	Combo 4	Combo 5	Combo 6
95°C	Combo 7	Combo 8	Combo 9

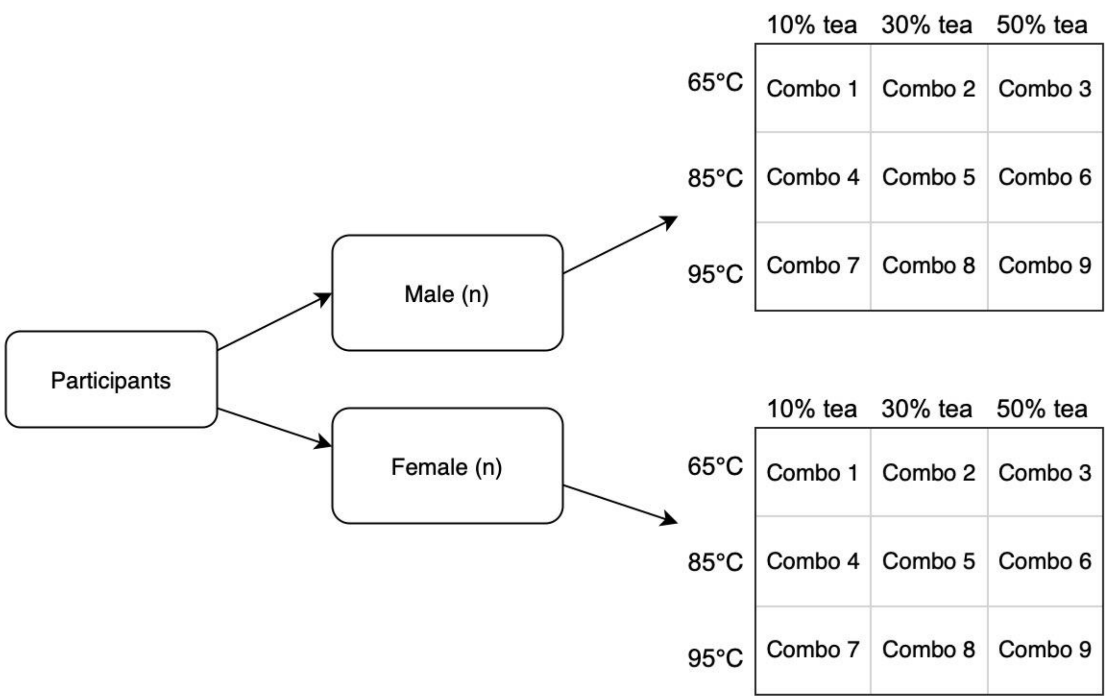
2) Randomized Block Design

Blocking is the process by which a confounding variable's effects are filtered out. Similar groups, called blocks, are created. All treatments are implemented within each block. When blocking is incorporated in an experiment, the random assignment to treatments occurs after the blocks have been formed and is done separately for each block (Devore and Peck).

In this study, researchers will use gender as a blocking variable. Researchers will make sure that for each gender, participants will be randomly assigned to 9 combinations of water temperatures and amounts of tea. The experimental unit will be participants drinking tea. With this design, if researchers see a difference in the tastes of tea for different combinations, the extraneous variable gender can be ruled out as a possible explanation because participants of both genders will be presented in each combination.

In this design, the ratio of male and female participants is controlled to be 1:1 (i.e., each gender has n participants) and participants will be divided into two blocks. Within each block, participants will be numbered from 1 to n and randomly assigned into 9 treatment groups using R. Randomization process and experimental procedures will be the same as mentioned in the factorial design within each block. At the end, researchers will calculate the score for each participant.

Compared to factorial design, randomized block design has higher internal validity. In order to average out the characteristics of the participants, a random assignment will be employed; furthermore, blocking the participants by gender will help the researchers to determine the factors that truly affect the taste of tea and reduce within group variance. In addition, this design will protect participants well by having them only drink one cup of tea during the whole process instead of having repeated measures, which will also reduce the measurement error. Regarding the disadvantages of this design, it will be harder to implement (in terms of dealing with blocks and managing participants) and it might cost more time and money. Also, the data analysis process will be more complicated, especially when building models with interaction effects involved. Considering that the characteristics of participants would be more complicated and, thus, more blocks will be needed, the difficulties of understanding and interpreting the model will also increase. Finally, similar to the factorial design, the external validity would not be high enough to generalize the results to the whole population.



3) Split plot Design

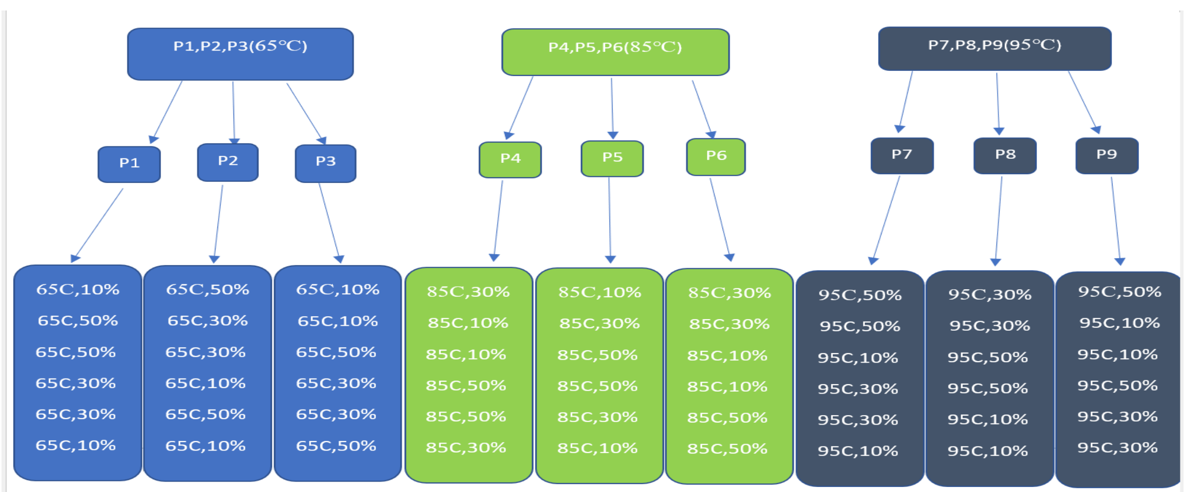
After considering the pros and cons of factorial design and randomized block design, we decided to introduce the last experiment method which is called the split plot design, to further help us better understand the secret behind the effects of brewing factors on the final taste of tea. In general, the rationale of the split plot design is that there are two levels of randomization that will be used to assign experimental units to the treatment factors. The first stage of randomization is called the whole plot randomization, where researchers randomly assign experimental units to treatment factor A. In the second stage of randomization, researchers furtherly split the whole plot into several subplots and randomly assigned the experimental units to the treatment factor B. Finally, based on the collected results, researchers will build statistical models and make further analyses. Based on this idea, we can divide the entire study into four steps in order to answer our research questions by using a split plot design.

Step 1: We first control all the confounding variables by using the method that we mentioned at the beginning of the design section so that the only remaining factors that will eventually affect the taste of water are water temperature (65℃, 85℃, 95℃), and amount of tea leaves (10%, 30%, 50%).

Step 2: We do not know the actual sample size priorly, but to better illustrate, let's assume we recruit nine people to participate in our study. At the whole plot level, we randomly assign three of them to receive the tea with steeping water at 65℃, where another three participants receive the tea steeping at 85℃, and the rest three receive tea with steeping water at 95℃. At this whole plot stage, the experimental unit is participants, and the experimental factor is water temperature.

Step 3: The idea of subplot randomization is that since our second experimental factor (amount of tea) has three levels and there are nine participants, we need at least fifty-four cups of tea(replicates) in order to test the main effects and interaction effects of water temperature and amount of tea. Based on that, we say for each student with a fixed temperature, we randomly assign six out of fifty-four cups of tea with the same amount of tea to each participant. Thus, each individual will have two cups per amount of tea, which is a total of six cups. At this split plot stage, our experimental unit is the cup of tea, and the experimental factor is the amount of tea.

The following chart is a visualization of how the split plot design works, where we set Participants: P1-P9, Water temperature: 65℃, 85℃, 95℃, Amount of Tea: 10%, 30%, 50%



Compared to factorial and block designs, we only need to recruit a small number of participants and experimenters to conduct the study. For instance, in the previous example, only nine participants are enough to test the main effects and interaction effects of the steeping factors of tea, which can largely save our budget and time. In addition, the taste result which we collect from participants would be less biased if the sample size is small. Nonetheless, split plot design still has some drawbacks which are hard to handle and overcome. For example, one of the lethal trackbacks is that the results we collect might not be generalizable and representative due to the small sample size. In our example, we only have nine samples at the whole plot level (water temperature). We do not want the result that we collect to be unable to represent the whole population. Also, it might be hard to implement the study and conduct the later data analysis because we have two-stage randomizations.

Finally, after judging and comparing the Pros and Cons of all three designs, we decided to choose the factorial design to support us to test the research hypotheses since it has relatively good generalizability and it is easy to implement as well as to conduct the data analysis.

V. Data Analysis and Sample Size Determination

As mentioned earlier, we decided to use factorial design to discuss the two aims and to analyze the experimental data. First, for the first aim, according to the Taiwan Tea Research Institute (2016), caffeine is easily soluble in hot water because the dissolution rate of caffeine increases with increasing water temperature. Therefore, we hypothesize that the caffeine will be increased when the brewed tea is hot, and the amount of tea leaves is large.

Then, for the second aim, we know that the feelings towards a cup of tea is very personal and subjective, and it is difficult to declare a clear relationship between this feeling and the amount of tea or the temperature of hot water. Based on the research of Yoshan Tea (2021), all kinds of tea have an optimal combination in terms of tea leaves dosage and water temperature. From this research, we know that 85 to 100 degrees Celsius may be the temperature for brewing the best flavor of tea. And for the amount of tea leaves, based on the research, it could be the best way to brew tea to have tea leaves make up 40% of the teapot 40% to just laying the tea leaves on the bottom of the pot. Therefore, we chose to conduct a 3x3 factorial design, which will measure 9 different combinations of tea. These combinations were created based on 3 levels of water temperature (65°, 85°, 95°) and 3 levels of the amount of tea leaves (10%, 30%, 50%).

One of the big advantages of a factorial design is that it allows researchers to look for interactions between independent variables. An interaction is a result in which the effects of one experimental manipulation depends on the experimental manipulation of another independent variable.

The first model we will use for aim one is the ANOVA model:

Model_1 = aov(Caffeine ~ Amountoftea * Temperature, data=data)

The outcome variable **Caffeine** means the caffeine level we detected from each combination of tea. **Amountoftea** indicates the amount of tea leaves we put into the teapot, **Temperature** means the water temperature we use for brewing tea.

The second model we will use for aim two is ANOVA model:

Model_2 = aov(Score ~ Amountoftea * Temperature, data=data)

The outcome variable **Score** indicates the evaluation score for tea of individual participants. After every participant fills out the evaluation form, the final score will be obtained by summing each score of **Fragrance, Body, Flavor, Aftertaste,** and **Balance** of the cup of tea they taste.

Besides, in order to find the best combination of tea out of these 9 treatments, we will utilize Tukey' HSD method to figure out which combination of tea has a significantly higher average performance compared to the other combinations:

PostHocTest(Model_2, method="hsd")

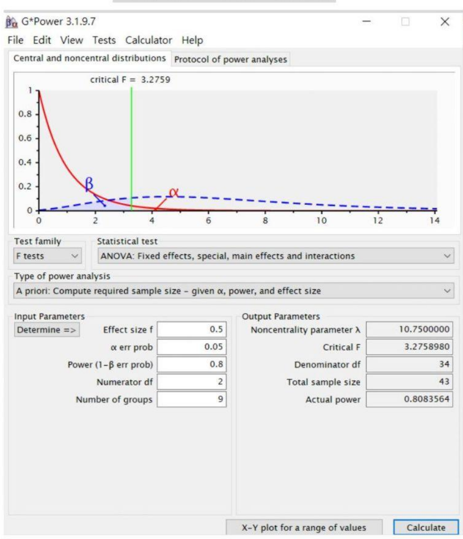
When it comes to determining our effect size, according to recent research, effect size can be used at the planning stage to find the sample size required for sufficient power for researcher's study (Sullivan and Feinn, 2012). Therefore, it is crucial for us to determine the effect size first. Based on Cohen's method, he classified effect sizes as small ($d = 0.2$), medium ($d = 0.5$), and large ($d \geq 0.8$). In the words of Cohen, "a medium effect of 0.5 is visible to the naked eye of a careful observer" (Carson, 2012).

Therefore, our team chose 0.5 to be our effect size. To achieve 80% of power while controlling the type I error at 5%, we decided to use G*Power for conducting analysis. By choosing the Test family to be F tests, we selected the statistical test to be ANOVA: Fixed effects, main effects and interactions, and the Type of power analysis to be A priori: Compute required sample size - Given α, power, and effect size.

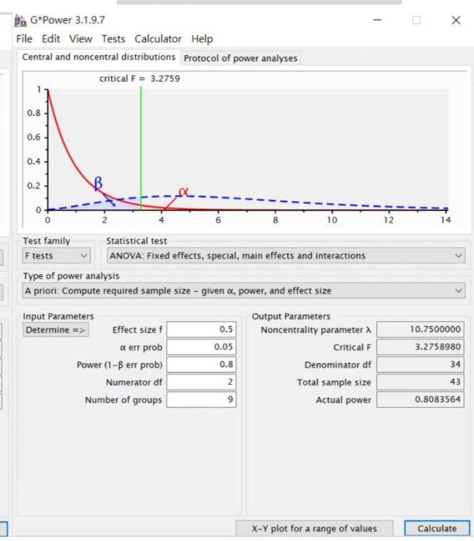
In order to obtain the required sample size for our study, we made our effect size to be 0.5, made the α err prob to be 0.05, and made power to be 0.8. And because we will have 9 combinations of tea (3 water temprature * 3 amount of tea leaves), our number of groups will be 9. Then, because we want to test two main effects and their interaction effect, so the numerator degree of freedom will (3-1) * (3-1), which is 4.

After typing these numbers into the blanks of G*Power, we will have a total sample size equal to 54, which could be divided by 9. Thus, finally each combination will have 6 replicates, indicating that each combination of tea will be tasted by 6 different participants, and each participant will only drink a cup of tea of a specific combination.

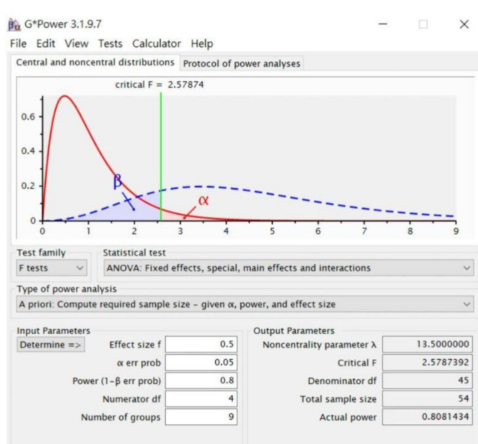
Test one main effect



Test the other main effect



Test two main effects and their interaction at the same time



When it comes to dealing with attrition issues, since we invite professionals to make tea and use professional tea utensils to make 9 large pots of tea for each combination, which will be released to the participants after being evaluated by the electronic detector, so we do not think there will be any attrition issue in the process of brewing tea. On the contrary, we are more worried that the participants will not be able to come as scheduled, resulting in an insufficient total sample size.

Since the total sample size we need is 54, if we only recruit 54 participants, we may face the situation that the participants did not come as scheduled. In order to solve this problem, we decided to recruit 80 participants in total and only collect data from the 54 participants who first arrived on the day. If the number of participants who come to be tested exceeds this number, we will move these participants to another room, and we will still ask a professional tea maker to make a cup of tea for them. But we will not ask these participants to fill out the evaluation form.

VI. Implementation Plan

Randomization

The process of randomization has been explained implicitly in the Factorial Design section.

Environmental Control

The whole experiment will take place in a large laboratory with conveyor belts by considering the possible issues caused by weather, temperature, management, subject protection, etc. In addition, to prevent the potential spread of COVID-19, all participants and experimenters need to show clearance before the beginning of the experiment. All equipment, such as teacups, and teapots, will be disinfected before delivering them to the participants.

Test Subject Training

First, all experimenters will be required to train with some background knowledge of tea in general, such as the origin, cultural background, and steeping process, so that they can answer any question that participants may ask after the experiment. We will also require our experimenters to complete the CITI program and receive a certificate to prevent ethical issues.

Timeline of the Study

Ideally, the entire experiment will take no more than 40 minutes to complete. First of all, during the first 10-15 minutes, we will prepare 54 cups of green tea by using 9 identical teapots (stepped by 9 well-trained tea makers), which includes a unique combination of water temperature and amount of tea. As we discussed previously, before pouring the tea into small teacups and handing them to participants, we will also measure the caffeine level of each teapot using caffeine detectors. This measurement process should take no more than 2 minutes. Then, each cup of tea will be delivered to all 54 participants via a conveyor belt at the same time to avoid any interaction between experimenters and participants. Each participant needs to drink it immediately to avoid getting cool, which should take less than 1 minute. Finally, they will have additional 10-15minutes to complete the evaluation forms and share any feedback with us.

Contribution

Member	**Contribution**
Siyun	Literature review (2 pages), Research questions and hypothesis (2-3 pages)
Yu & Yifei	Experiment design (6-7 pages), Implementation plan (1+ pages)
Chialing (Charlene)	Data Analysis (3-4 pages)
All	Summary (1 page), Slides

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Cupping Evaluation
Grading Form

Name: ______________ Date: ______________
Group #: ____________

Fragrance/Aroma: (aromatic complexity)

Very Poor Outstanding

1	2	3	4	5	6	7	8	9	10

Flavor: (pleasing characteristics of the tea)

Very Flat Very Bright

1	2	3	4	5	6	7	8	9	10

Body: (mouthfeel of the tea)

Very Thin Very Heavy

1	2	3	4	5	6	7	8	9	10

Aftertaste: (pleasing sensations on the palate)

Very Poor Outstanding

1	2	3	4	5	6	7	8	9	10

Balance: (overall pleasing characteristic of the tea)

Not Rated Outstanding

–5	–4	–3	–2	–1	0	1	2	3	4	5

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