

How Sweet it is to be Naturally Brewed

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Summary: Coffee ranks among one of the most popular beverages in the United States, with estimates suggesting that approximately 75% of Americans aged 20 and above indulge in its consumption. Coffee has been associated with many health benefits including studies showing the consumption of coffee has an inverse relationship with reduced risk for depression, Parkinson's disease, cognitive decline, certain cancers, and type II diabetes. Due to the nature of their disease, individuals diagnosed with type II diabetes are recommended to reduce their intake of refined carbohydrates including sugar. Because coffee has a naturally bitter taste, many people add external sweeteners to make their cup of coffee more palatable. This study focuses on varying different factors involved in the preparation of a cup of coffee to naturally brew a sweeter cup of coffee. Specifically, we are interested in finding out how different roasting levels of coffee beans and extraction times impact individuals' taste perception of sweetness in naturally brewed cups of coffee during French press preparation. To achieve this, we manipulated the roasting level- light, medium, and dark- and the extraction time- ranging from one to five minutes- to create nine distinct coffee combinations. We predict that varying roasting levels and extraction times will change the perception of the sweetness of our naturally brewed coffee. In addition, we are interested in quantifying the caffeine level within each of the nine coffee combinations. We hypothesize that the caffeine levels will vary across the various combinations of roasting levels and extraction times. To investigate these hypotheses, we chose to use a split plot design in which we used three bags of coffee for each roasting level as the whole plot, and each of the whole plots corresponding split plots were three cups of coffee at our different extraction times under the same roasting level. Choosing this design allowed us to optimize the study's efficiency, enhance statistical power, and provide a detailed analysis of the interactions between coffee roasting levels and extraction times. The data analysis involves utilizing mixed-effects models for interaction effects, to uncover the nuances of taste perception and caffeine concentration within varying combinations. Additionally, a power analysis was conducted to determine an optimal sample size ensuring the study's ability to detect meaningful differences in taste and caffeine content with adequate statistical power, thus contributing

valuable insights into crafting naturally sweeter coffee blends. By examining both taste perception and caffeine concentration, our aim is to offer insights into crafting a naturally sweeter cup of coffee, thereby potentially reducing reliance on external sweeteners.

Background and Literature Review

Coffee is a popular beverage with estimates stating approximately 75% of individuals in the United States over the age of 20 drink coffee (Rehm et al., 2020). Studies have shown coffee is linked to many health benefits including an inverse relationship with risk for depression, Parkinson's disease, cognitive decline, certain cancers (e.g., colorectal cancer, endometrial cancer, liver cancer, and melanoma), and diabetes mellitus II. Type II diabetes is a chronic condition in which the pancreas does not produce enough insulin or the body does not use the insulin the pancreas produces effectively. Insulin is a hormone found in the body that regulates blood glucose. Type II diabetes is a serious health issue and when uncontrolled can lead to damage to many of the body's systems. Specifically, type II diabetes can damage nerves, cause problems with tactile sensation, damage blood vessels and increase the risk of heart attack, stroke, chronic kidney disease, and vision loss. Individuals diagnosed with type II diabetes need to follow a healthy diet that is low in refined carbohydrates (including sugar), saturated fat, and processed foods (Lane, 2011).

Several studies have explored the relationship between coffee consumption and risk for type II diabetes. A systematic review completed by Van Dam & Hu (2005) identified nine cohort studies examining coffee consumption and risk for type II diabetes. In a total of 193,473 participants, 8394 cases of type II diabetes were identified, and a calculated summary relative risks (RR) using a random-effects model was completed. The RR of type II diabetes was 0.65 for the highest (≥ 6 cups per day) and 0.72 for the second highest (4-6 cups per day) category of coffee consumption compared with the lowest consumption category (≤ 2 cups per day). The authors found this association did not differ substantially by sex, obesity, or region (United States and Europe).

In a prospective study of 17,111 Dutch men and women aged 30 – 60 years, researchers found individuals who drank at least seven cups of coffee a day were 0.50 times as likely to develop type II diabetes than those who drank two cups or less a day (van Dam & Feskens, 2002). In another large prospective cohort study of 38,176 men and women examining demographic characteristics, food-frequency information, and risk factors for the presence of chronic disease (van Dieren et al., 2009), it was found that consuming at least three cups of coffee a day was associated with a lower risk of type II diabetes. These results are in line with previous studies. In addition to the inverse association of coffee with risk of type II diabetes, caffeine intake slightly attenuated the relation between coffee consumption and type II diabetes, although the correlation between coffee and caffeine made it difficult to distinguish the effects of caffeine from coffee.

These epidemiological findings stand in contrast to controlled experimental studies, which suggest that caffeine, a common component of coffee, may exacerbate insulin resistance in individuals diagnosed with type II diabetes. Specifically, caffeine affects how the body responds to insulin by lowering sensitivity to it. This means cells will not react as much to the presence of the hormone which causes the body to make more insulin. Several studies investigating the impact of caffeine on glucose tolerance in patients with type II diabetes have shown an exaggerated rise in blood glucose in response to the presence of caffeine. This effect was replicated in a study of women with gestational diabetes where in addition to exaggerating the glucose and insulin response to a carbohydrate challenge, caffeine also decreased the whole-body insulin sensitivity. Results from these studies confirm that the presence of caffeine in the body increases insulin resistance in type II diabetes patients (Lane, 2011).

Moreover, considerations extend beyond caffeine content, as the addition of sweeteners to coffee poses challenges for individuals managing type II diabetes. Coffee has a naturally bitter taste which causes coffee consumers to add external sweeteners to their coffee to make it more palatable. The external sweeteners added to coffee can include milk, coffee creamers, natural sugar, and artificial sugar. All of these external sweeteners would be contraindicated for use by individuals diagnosed with type II diabetes who are recommended to significantly reduce their intake of refined carbohydrates such as sugar. In addition, taste perception can vary in individuals based on cultural background and ethnicity. Studies have found sweet taste

perception is greater in non-Hispanic Black adults than in non-Hispanic White adults (Bowser et al., 2019). Non-Hispanic Black adults also have one of the highest prevalence of type II diabetes compared to other ethnic groups according to the Centers for Disease Control and Prevention.

In this study, we investigate two questions about how the preparation of coffee can impact taste perception and caffeine level. Specifically, in our first research question, we are interested in finding out how different roasting levels of coffee beans and extraction times impact individuals' taste perception of sweetness in naturally brewed cups of coffee using French press preparation. To address this, we varied the roasting level: light, medium, and dark, and the extraction time to one minute, three minutes, and five minutes to prepare nine different combinations of coffee. We predict that varying roasting levels and extraction times will change the perception of the sweetness of naturally brewed coffee.

In our second research question, we are interested in examining the caffeine level in these nine combinations of varied roasting levels and extraction times in naturally brewed cups of coffee. We predict that the caffeine level of each combination of roasting level and extraction time will vary among the different combinations brewed. We ask these questions to identify the sweetest naturally brewed cup of coffee with the least amount of caffeine present. Through our investigation, we seek not only to understand the nuances of coffee preparation but also to offer insights that can help non-Hispanic Black adults, make informed choices about their coffee consumption to mitigate health risks associated with added sweeteners.

Research Questions and Hypotheses

Research Question 1: How do varying combinations of different roasting levels (i.e., light, medium, and dark) and extraction times (i.e., under extraction- after 1 minute, balanced- 3 minutes, and over-extraction- 5 minutes) impact participants' ratings of sweetness in a cup of coffee?

Subjective Outcome: Individuals will use a Likert scale to rate the sweetness of the cup of coffee on a scale of 1 (not sweet) – 5 (very sweet).

Hypothesis:

H0: Roasting level and extraction time do not affect participants' ratings of sweetness in a cup of coffee.

HA: Roasting level and extraction time do affect participants' ratings of sweetness in a cup of coffee.

Research Question 2: How do varying combinations of different roasting levels (i.e., light, medium, and dark) and extraction times (i.e., under extraction- after 1 minute, balanced- 3 minutes, and over-extraction- 5 minutes) impact the caffeine level in a cup of coffee?

Objective Outcome: A spectrophotometer will be used to measure the caffeine level (in milligrams) in each cup of coffee brewed.

Hypothesis:

H0: Roasting level and extraction time do not affect the caffeine level in a cup of coffee.

HA: Roasting level and extraction time do affect the caffeine level in a cup of coffee.

Experiment Design

Our first experiment employs a Factorial Design with roasting levels (Light, Medium, Dark) and extraction times (1 minute, 3 minutes, 5 minutes) as factors. The key aspect of this design is that each participant typically tests only one combination of conditions, requiring separate groups of participants for each combination of roasting and extraction times. To obtain data for all combinations, we need to first recruit a sufficient number of participants to cover all combinations, ensuring a sufficient number to guarantee the reliability of the statistical results.

We plan to recruit 270 participants, assigning ten participants to each combination. Subsequently, using a random number generator, participants are assigned a number from 0 to 270 and distributed into nine groups to ensure each combination has an equal number (30) of evaluation samples. Afterward, following the extraction standards mentioned above, nine groups of coffee are extracted for the 3x3 factorial design, including three levels of roasting and three levels of

extraction times (the order of extraction is not crucial but should ensure that the transition time between them is as short and similar as possible). Coffee is then distributed to participants based on the previously divided groups. Participants are instructed to immediately evaluate upon receiving the coffee and are educated to stir the coffee before each tasting to ensure the accuracy of the evaluation. We use a previously established method to quantify subjective factors, asking them to rate the coffee's sweetness on a Likert scale from 1 to 5; a certain amount of coffee liquid is reserved (ensuring a consistent amount of coffee is retained) for caffeine content measurement using a spectrophotometer.

Our second experiment is a Random Block Design, based on literature that suggests that different age groups perceive coffee taste differently. Therefore, we design this experiment to create homogeneous experimental groups to reduce the impact of individual differences on experimental results. To create these homogeneous groups, we divide participants into three age groups: young, middle-aged, and old, recruiting 90 people for each group, totaling 270 participants. In the experiment design, the 90 participants in each age group are further randomly assigned (using a random number allocation method similar to the first experiment) to nine different coffee preparation combinations, each including three roasting levels (Light, Medium, Dark) and three extraction times (1 minute, 3 minutes, 5 minutes). This setup ensures that each combination has ten participants for testing in each age group, ensuring sufficient data for effective analysis. Moreover, the uniform sample size allows for the direct application of many standard statistical tests, such as ANOVA (analysis of variance), without the need for complex weighting adjustments or sample size normalization. During the coffee preparation and evaluation process, we ensure that each batch of coffee is prepared under the same conditions, including roasting temperature, extraction time, and the amount of coffee used, to ensure consistency in the experiment. Participants immediately evaluate the coffee upon receipt, rating its sweetness on a Likert scale from 1 to 5. Additionally, a portion of the coffee sample is retained for subsequent measurement of caffeine content using a spectrophotometer to obtain data on caffeine content.

Our third experiment is a split plot design, which is slightly more complex compared to the first two experiment designs. For each roasting level, we purchase three bags of coffee beans (of the

same roasting level from the same manufacturer and region), totaling nine bags of coffee beans. Each bag of coffee beans serves as a whole plot, with each whole plot's corresponding split plots being three cups of coffee at different extraction times under the same roasting level. To complete this experiment, 13 participants are needed for each bag of coffee, i.e., each whole plot, with each participant tasting three different extraction times of coffee corresponding to their assigned whole plot. This requires a total of 117 participants, each testing three cups of coffee, generating a total of 351 observational data points. The number of participants is based on a power analysis conducted using G*Power, which initially suggested 111 participants. This number was adjusted to allow for an even distribution of participants across our nine whole plots. The details of this will be explained more thoroughly in the subsequent analysis section. This experiment is designed to be completed within one day to control other potential variables such as environmental humidity, temperature, and weather conditions on the day. Considering we have nine whole plots, each with three split plots corresponding to three different extraction times of coffee, a total of 27 cups of coffee need to be extracted, and coffee is distributed according to the previously mentioned insulation measures and distribution requirements. Participants immediately evaluate the coffee after receiving and tasting it, ensuring the accuracy and timeliness of the evaluation. The assessment method previously introduced uses a Likert scale to evaluate the sweetness of the coffee. Additionally, a portion of each extraction is reserved for subsequent measurement of caffeine content using a spectrophotometer to obtain data on caffeine levels.

In selecting the split plot design for our coffee research, we aimed to optimize our study's efficiency, enhance statistical power, and provide a detailed analysis of the interaction between coffee roasting levels and extraction times, while also effectively managing resource utilization. This decision was informed by the limitations observed in the other two experimental designs we considered—factorial and block designs.

Factorial designs, although comprehensive in testing interactions between multiple factors, do not accommodate the hierarchical structure of our experiment as efficiently as split plot designs. They typically require a larger sample size to achieve the necessary power to detect meaningful interactions, which could escalate costs and complicate logistics in a study that involves various

coffee preparation techniques. Furthermore, factorial designs treat each factor independently, which could lead to oversimplified conclusions that fail to capture the nuanced interactions present in coffee brewing, potentially resulting in biased estimates of treatment effects.

Block designs, on the other hand, are adept at controlling for known sources of variation among participants, such as age or other baseline characteristics. However, they do not adequately address other demographic variables like ethnicity that might influence taste perception, which could limit the generalizability of the findings. Moreover, while block designs control individual differences, they may not effectively manage the interactions between the treatments within each block, thus not fully exploiting the data's hierarchical structure.

The split plot design addresses these issues by incorporating a hierarchical structure that allows for detailed analysis of interactions within and across different levels of treatment. By designating the roasting level as the whole plot and the extraction times as the subplots, this design facilitates a rigorous examination of how these two factors interact within the same roasting batch, enhancing the reliability and relevance of the results. Each participant tasting three different extraction times under the same roasting condition significantly reduces variability both within and between participants. This repeated measures setup not only boosts the statistical power of the study but also controls for potential confounding factors more effectively.

Statistically, the split plot design introduces random effects for both main plots and subplots, allowing us to detect smaller yet meaningful differences in taste perception that may arise from various treatment combinations. This enhancement of statistical power is crucial for drawing reliable conclusions and making informed decisions based on the experimental results.

Moreover, the efficiency of resource use is a significant advantage of the split plot design. It requires fewer coffee beans and less repetitive setup for different extraction times under the same roasting conditions, reducing both waste and operational complexity. This design not only makes the experiment more cost-effective but also simplifies the logistical aspects of coffee preparation and distribution to participants.

In summary, the split plot design's ability to provide a comprehensive examination of variable interactions, its enhanced statistical power, and efficient use of resources make it the ideal choice for our study. It strikes a balance between experimental rigor and practical implementation, ensuring that we can explore the intricate effects of coffee preparation on taste perception and caffeine levels effectively and efficiently. This design choice aligns with our goal of analyzing the impact of coffee roasting levels and extraction times on coffee sweetness, thereby helping to minimize the use of external sweeteners among coffee drinkers, particularly those managing conditions such as type II diabetes.

Data Analysis and Sample Size Determination

Model Specification

Our data analysis will employ a split plot model to examine the influence of roasting level and extraction time on participants' perception of sweetness in coffee and the caffeine levels. The model will encompass fixed effects for roasting level, extraction time, their interaction, as well as random effects for participant ID and the whole plot (roasting level) to account for the nested structure of the experiment.

For our primary aim, the split plot model can be represented as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + (\alpha\gamma)_{ik} + \epsilon_{ijk}$$

Where:

- Y_{ijk} represents the response variable (sweetness perception).
- μ is the overall mean.
- α_i denotes the effect of roasting level (whole plot).
- β_j signifies the effect of extraction time (split plot).
- $(\alpha\beta)_{ij}$ refers to the interaction effect between roasting level and extraction time.
- γ_k captures the random effect of participant ID.

- $(\alpha\gamma)_{ik}$ accounts for the random effect of whole plot (roasting level).
- ϵ_{ijk} represents the error term.

In R, the syntax appears as follows:

```
model <- lmer(sweetness ~ roasting * extraction + (1 | participant) + (1 | roasting), data = coffee_data)
```

For our secondary aim, the split plot model can be represented as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + \epsilon_{ijk}$$

Where:

- Y_{ijk} represents the response variable (caffeine level).
- μ is the overall mean.
- α_i denotes the effect of roasting level (whole plot).
- β_j signifies the effect of extraction time (split plot).
- $(\alpha\beta)_{ij}$ refers to the interaction effect between roasting level and extraction time.
- γ_k accounts for the random effect of whole plot (roasting level).
- ϵ_{ijk} represents the error term.

In R, the syntax appears as follows:

```
model <- lmer(caffeine ~ roasting * extraction + (1 | roasting), data = coffee_data)
```

Software and Packages

Our data analysis will be conducted using R statistical software. We will utilize the lme4 package for fitting linear mixed-effects models and the emmeans package for post hoc comparisons and effect size estimation.

Power Analysis

A power analysis was conducted using G*Power software to determine the required sample size. We selected the ANOVA: Fixed effects, special, main effects and interactions in the F-tests. The analysis was based on an estimated effect size of 0.3, numerator degrees of freedom of 2 (representing the subplot effect in the split plot design), an alpha level of 0.05, and a desired power of 0.80. These parameters were chosen based on existing literature in similar studies (Faul et. al 2009; Ong et al. 2018).

The formula used for sample size calculation in G*Power is:

$$N = \frac{2*(Z\alpha+Z\beta)*\text{numerator df}}{d^2}$$

Given the effect size of 0.3 and numerator degrees of freedom of 2, the calculated sample size required to detect the effects in our split plot design was determined to be 111 participants.

Since we plan to have all participants complete the coffee drinking in one day, we don't expect any attrition. Therefore, we aim to recruit at least 117 participants for the study to ensure we have a sufficient number of complete and valid data sets for analysis.

From a statistical perspective, the hierarchical structure of the split-plot design enhances our study's statistical power. By incorporating random effects for main plots and subplots, we can detect smaller yet meaningful differences in coffee taste due to different treatment combinations. This statistical power is essential for drawing reliable conclusions and making informed decisions based on our experimental results. Moreover, the efficient use of resources is a significant advantage of the split-plot design. Additionally, factorial designs typically require a larger sample size to detect interaction effects with sufficient power, which may not be feasible or practical in a coffee brewing study. In comparison, the split-plot design optimizes resource utilization while maintaining experimental rigor and precision.

Alternative designs, such as factorial and blocked designs, may not be as effective for our study. Factorial designs, while versatile, may oversimplify the interactions between factors and fail to capture the hierarchical relationships present in our experiment. Treating factors as independent entities could lead to biased estimates of treatment effects and miss important interactions that impact coffee taste.

Similarly, blocked designs, which are effective for controlling known sources of variation, may not adequately address the nested structure and interactions between main plot and subplot treatments. This limitation could result in a loss of experimental control and reduced efficiency in capturing the nuanced effects of different treatment combinations on coffee taste.

Implementation Plan

To examine the impact of varying roasting levels and extraction times on the perceived sweetness and caffeine content of coffee, we decide to use a split-plot design, the whole plot factor is the three bags of roasting levels (Light, Medium, Dark), and the subplot factor is the extraction time (1 minute, 3 minutes, 5 minutes). In exploring the impact of optimal coffee roasting and extraction times on coffee quality, we have designed a series of meticulous experimental setups.

In all experiments, we strictly use single-origin coffee beans from high altitudes in Ethiopia to ensure consistency of raw materials, and uniformly grind them to a medium coarseness before the experiment to ensure consistency in grinding. To standardize the experiment, we use a Stainless Steel French press (34oz) for coffee extraction, which facilitates the management of extraction times. We ensure all equipment is thoroughly cleaned and functioning properly before each brewing session to avoid any residual factors affecting the outcomes. Controlled variables in the experiment include: a constant extraction water temperature maintained at 95°C, achieved using a temperature-controlled electric kettle and a heating pad placed at the bottom of the French press, use of filtered pure water to ensure consistent water quality, and a fixed coffee-to-water ratio of 1:15. Once the coffee extraction is complete, we swiftly stir the coffee with a non-metal stirrer to ensure an even distribution of particles. The coffee is then quickly transferred from the French press to a thermos with a sealed lid (to ensure temperature stability

and reduce oxidation during transfer). Before distribution, the coffee in the thermos is stirred again swiftly, and a wide-mouth spout ensures uniformity of the coffee as it is poured. We strive to minimize the time from coffee preparation completion to distribution to participants, ensuring consistent speed in each distribution.

For randomization and blocking, as for the whole plot randomization, within the same roasting level, we randomly assign the bags to be prepared. Within each roasting level (whole plot), we randomly assign the tasting order of extraction times coffee to the subjects, we will provide 2 oz of coffee per cup.

For the environmental controls, we control the environment by using a central air conditioning system to minimize variability in brewing and tasting conditions for temperature and humidity. We also will use filtered water with a consistent mineral content for all brewing. As for the coffee grinding, we will use a uniform grind size (medium coarseness) with the same coffee grinder to ensure consistency across brews. And for each time grinding, we will make sure the grinder is properly cleaned.

For the subjects (tasters) recruitment, our subject population is non-Hispanic Black adults aged 18 to 65 years. Participant exclusion criteria includes allergy or sensitivity to coffee, individuals with medical conditions affecting taste perception, pregnant/nursing women, history of or currently smoking, and no more than five alcoholic beverages consumed per week. For the recruiting strategy, we will advertise our project by sending flyers to certain neighborhoods and community centers in New York. We will randomly select 117 people in our experiment from all the signup subjects. We will ensure that both researchers and participants are blind to the specific conditions (one roasting level (bag) and three extraction times) to prevent bias. As for the subject pre-training, we will educate participants on how to use the Likert scale for sweetness rating.

For data collection protocol, we use a 1-5 Likert scale immediately post-tasting for sweetness measurement. We offer water in between coffee cups to refresh the taste buds of subjects. We collect samples from each brewing session for subsequent caffeine analysis using a spectrophotometer for caffeine measurement.

For the budget consideration, we estimate our budget to be around 3000 dollars, including the equipment, coffee beans, and a 5-dollar encouragement for each subject's participation.

The total duration of the experiment will be approximately one month. We plan to use three weeks for subject recruiting and preparation, one day for conducting the experiment, and approximately two hours, including preparation, brewing, tasting, and data collection. The data analysis and final report will be finished within one week.

For the logistical considerations, we will have to secure a place that can accommodate the controlled environment requirements. We will be trained to procure adequate French press units, thermoses for sample retention, and a spectrophotometer. We will have five staff members to prepare coffee, manage the tasting sessions, and perform laboratory analysis. We will also have backup equipment available, especially for critical components like the spectrophotometer.

For participant safety and ethics, we will obtain approval from an institutional review board (IRB) from New York University, and we will ensure written informed consent is obtained from all participants, outlining the study's scope and their rights. All of the experimenters have received certification from the Collaborative Institutional Training Initiative (CITI). Certificates can be found [here](#). Experimenters will guarantee that all data collected will be kept confidential and stored securely to protect the privacy of the subjects.

Limitations

While meticulous planning and rigorous experimental protocols have been put in place to ensure the reliability and validity of the project's findings, several limitations need to be acknowledged. First, the taste perception variability. As for the subjects' tasting, it can vary due to factors such as age, health status, and even daily fluctuations in sensory sensitivity, which will affect the measurement of our sweetness taste outcome. Although we have attempted to minimize these effects by randomizing the order of coffee combinations presented to participants, inherent variability in sensory perception among individuals could still influence the results. Second, focusing the study on a specific demographic group (non-Hispanic Black adults) enhances

internal validity but may limit the generalizability of the findings to the broader population. Taste preferences and perceptions can vary widely across different cultures and demographic backgrounds. Third, although we tried to standardize brewing conditions and environmental factors, slight variations in factors such as air quality, brewing equipment performance, and water composition could introduce uncontrolled variability into the experiment. Fourth, due to the budget constraints, we could only test three bags of coffee beans for each roasting level, it limited us to further test variability in the bags of roasting level. Also, we only recruited 117 subjects for this study. With more budget, we could have increased our sample size to yield more power and effect size with more generalizability.

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