



SETB 3812-02
UNDERGRADUATE PROJECT 2

COMPARISON OF SPENT COFFEE GROUND CRUDE EXTRACTS AND FRACTIONS ON POLYPHENOL AND ANTIOXIDANT CONTENT

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1.0 INTRODUCTION



1.1 BACKGROUND OF STUDY

Spent Coffee Grounds

According to the SCA, the “golden ratio” for brewing coffee is 55g per litre. This works out at just under 14g for a 250ml cup



Damage to soil

Coffee grounds contains oil and other compounds that make soil more acidic



Global Coffee Consumption

Expected to grow by 3.3% to 170.3 million 60kg in 2022/2023



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Time Decomposing

Used coffee thrown directly without being separated or treated , need at least **three month** before the coffee grounds start decomposing in the landfill's anaerobic environment



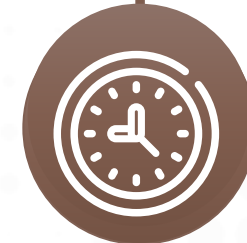
Generate greenhouse gases

Create methane which is the greenhouse gas



Potential

Spent coffee grounds have a high potential, both in terms of organic composting and in terms of energy recovery and the production of new materials



1.2 PROBLEM STATEMENT

- From the previous study, phytochemical compounds such as chlorogenic acid is still remained about 25% in the spent coffee ground compare to fresh coffee beans
- Therefore, spent coffee ground **still contain significant amount of valuable phytochemical** content that can be further exploited.
- The selection of an **appropriate extraction method is crucial for maximizing the yield of polyphenols** from spent coffee grounds, as these compounds are known for their antioxidant potential and various health benefits.
- In order to determine the best extraction technique for enhancing the yield of polyphenols from spent coffee grounds, it is crucial to **compare the effectiveness of solid-phase extraction and ultrasound-assisted extraction.**
- UAE has been noted as an **efficient method for extracting phenolic compounds** from various sources and has been reported to ensure **faster and better extraction of polyphenols** with minimized breakdown of compounds.
- SPE has been successfully applied for **the extraction and characterization of phenolic compounds**, with studies reporting **higher total polyphenol content in SPE fractions** compared to conventional extraction methods.
- Furthermore, the SCG extracts and SCG fractions must retains the bioactivity.
- Hence, in this study we will investigate the antioxidant activities in the SCG extracts and SCG fractions.



1.3 OBJECTIVE

To Compare the Spent Coffee Grounds Crude Extracts and Fractions on Polyphnhenol and Antioxidant Content

1.4 RESEARCH SCOPE

01



Extraction of SCG is carried out using ultrasound assisted extraction and ethanol as solvent based on the optimum parameters proposed by (Caballero-) Galván et al., 2018).

Fractionation of SCG extract will be carried out using reverse phase C18 solid phase extraction (SPE) with the independent variables are ratio of eluent system 60%, loading concentration of the SCG extract (5-25 mg/mL), and the volume of the eluent 30mL



02

Evaluation of Total Phenolic Content in SCG Crude extracts and SCG Fractions.



03

04



Evaluation of the antioxidant activity of the extracted and fractionated polyphenols using DPPH analysis.

2.0 LITERATURE REVIEW



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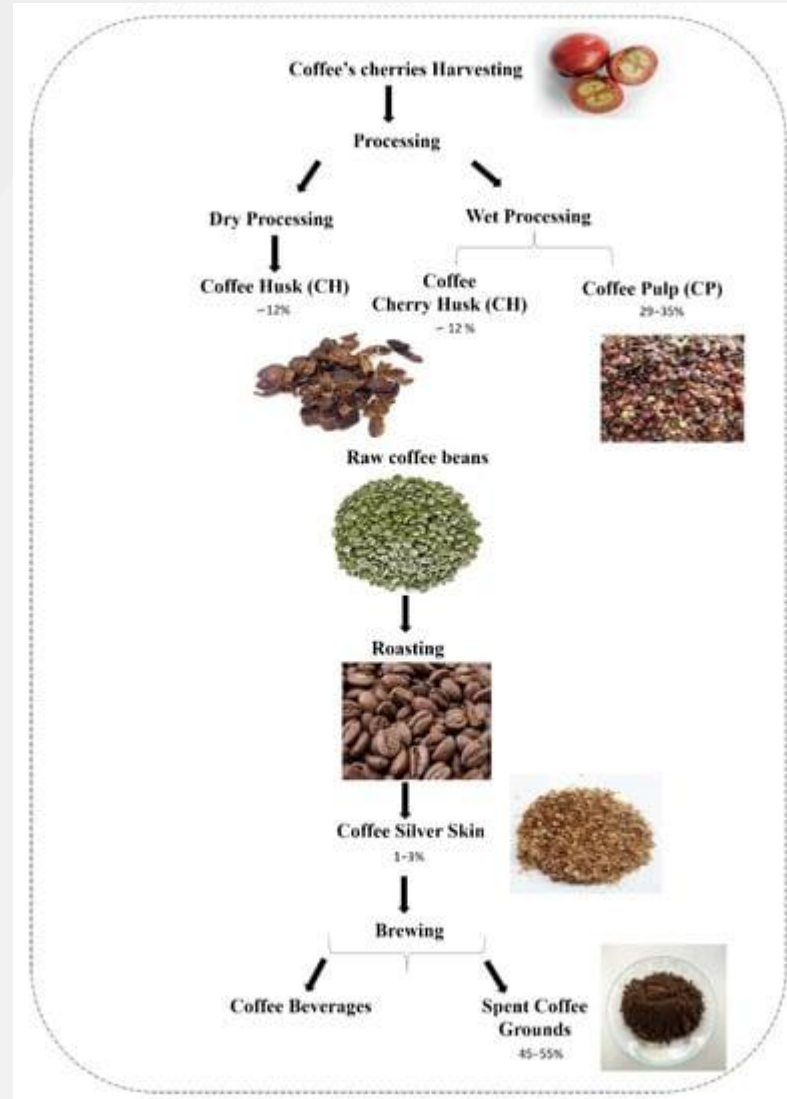
SPENT COFFEE GROUNDS

COLOUR

Cold-brewed coffees tended to be redder and **hot-brewed** coffees to be blacker.

CHEMICAL COMPOSITIONS

The active substance in the spent coffee grounds were found to **chlorogenic acid** 0.16%, while in coffee ground coffee before brewing it was obtained 1.50% chlorogenic acid.



MOISTURE CONTENT

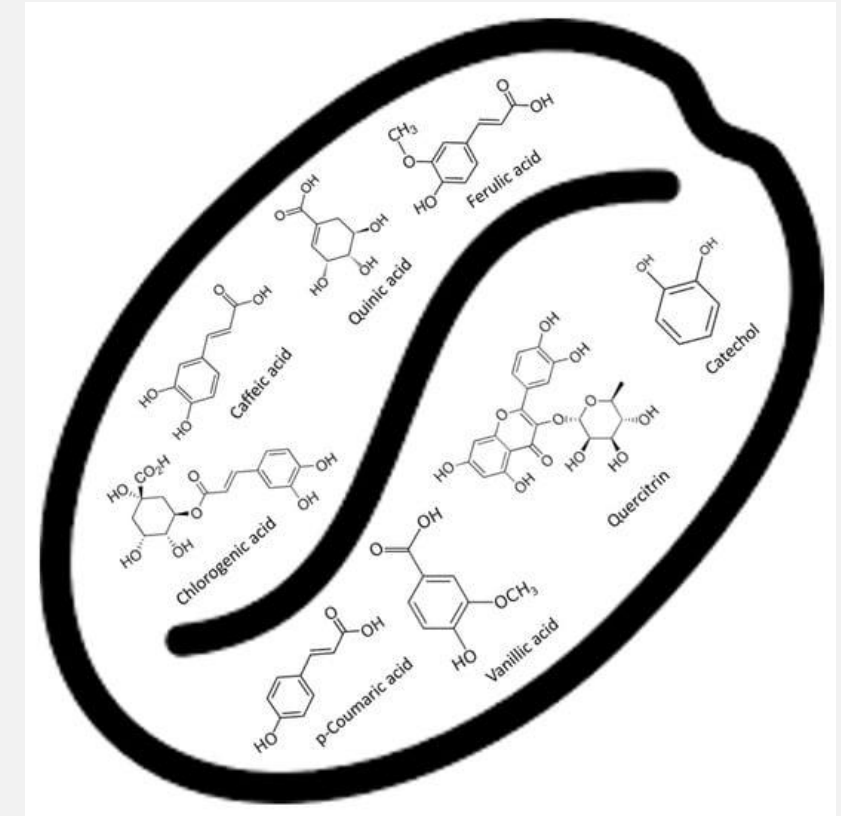
When collected, used coffee grinds contain **40.5% moisture**. Dry spent coffee grounds have high compressibility and as their moisture content rises and does their compressibility.

PARTICLE SIZE

90% of the analysed spent coffee grounds **particles size between 320 and 420 micrometres**. The particles of used coffee grinds are porous and contain several gaps within them

Phenolic Compound in SCG

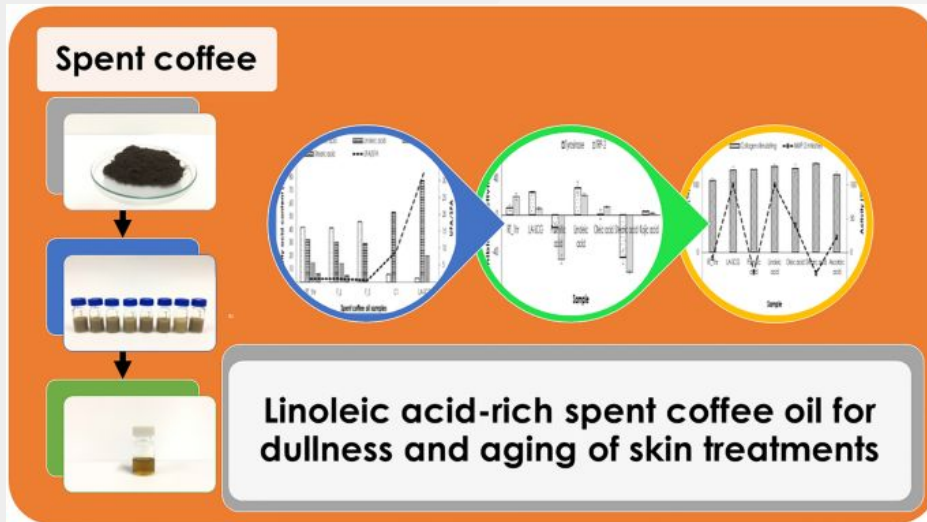
- Spent coffee grounds (SCG) are a rich source of phenolic compounds which are known for their antioxidant properties.
- Chlorogenic acid** is the **main phenolic compound** found in SCG with other compounds such as caffeic acid, ferulic acid, and quinic acid also present in smaller amounts (Zuorro & Lavecchia, 2012)



Main phenolic compounds of coffee beans
(Solomakou et al., 2022)

Biological of Spent Coffee Grounds (SCG)

- The cost-effective valorization of specialty ingredients from spent coffee grounds for cosmetics is sparsely presented.
- Linoleic acid-rich spent coffee oil as a specialty material for skin lightening and antiaging cosmetics, is objectively to be presented.



(Bevilacqua et al., 2023)

(Kanlayavattanakul et al., 2021) indicates that SCG are a valuable source of bioactive compounds, including antioxidants, chlorogenic acid, trigonelline, polyphenols, and melanoidins, which have been associated with various health benefits and potential applications in the pharmaceutical, cosmetic, and food industries

Mechanism Ultrasound Assisted Extraction

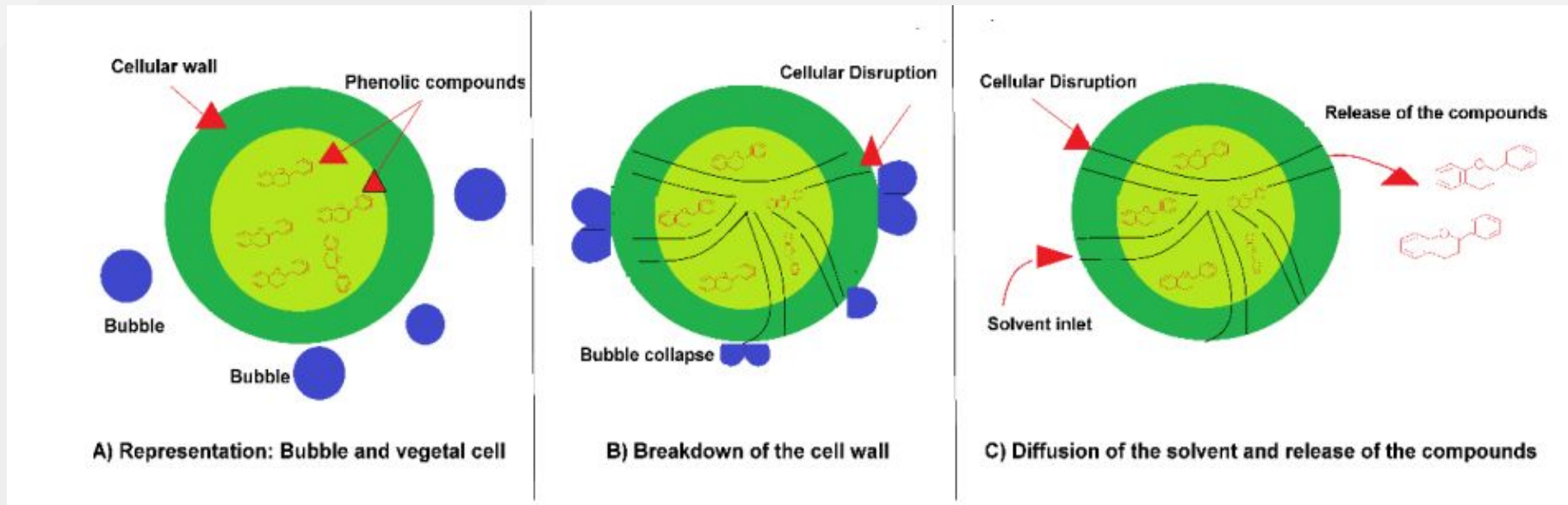
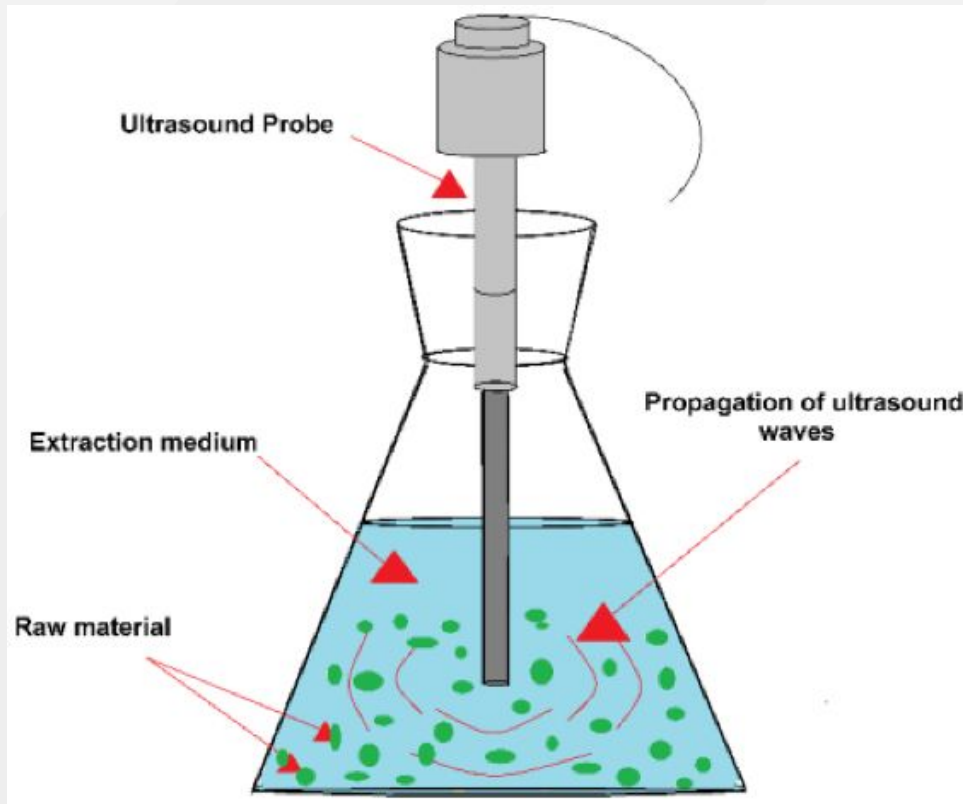


Figure above show a graphical representation of cavitation-bubble collapsing and releasing plant material in three steps.

Phenomenon of cavitation

Where collapsing bubbles generated by ultrasound waves create shockwaves that leading to fragmentation of the cellular matrix, erosion of plant tissues, and enhanced absorption and swelling index

FACTOR OF UAE



LIQUID SOLVENT TO SOLID RATIO

The **LSR increases**, the concentration and viscosity of the **extraction medium decrease**

TIME OF SONIFICATION

Enhanced cavitation impact of ultrasound, which **promotes various processes** such as swelling, hydration, fragmentation

ETHANOL CONCENTRATION

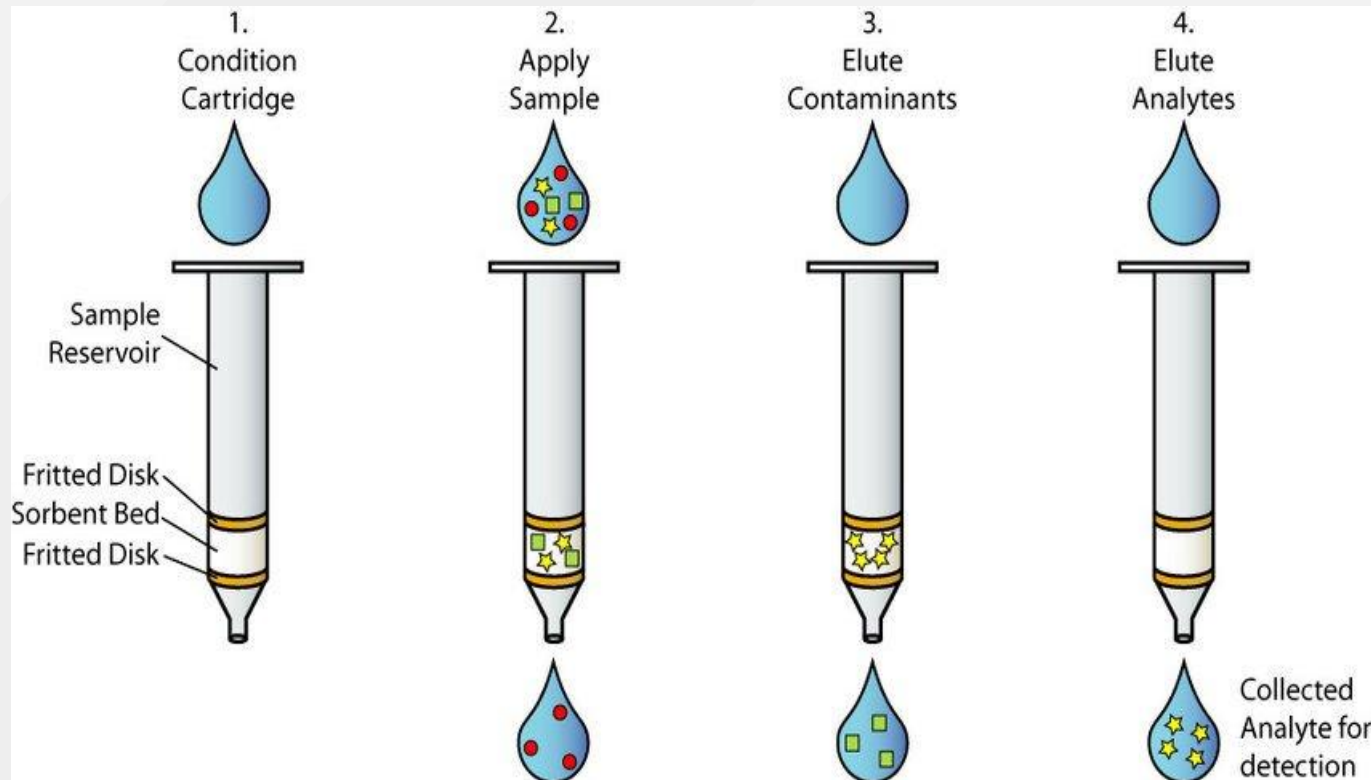
Preferred solvent due to its **high affinity** for these compounds, low cost, renewable supply, and designation as a generally recognized as safe solvent.

SOLID PHASE EXTRACTION



Solid-phase extraction (SPE) is a sample preparation technique that involves the use of a solid adsorbent to isolate, separate, and purify compounds from a liquid sample.

FUNDAMENTAL STEPS



1. Conditioning

- SPE disk prepared by washing with a solvent to wet the sorbent.

2. Sample Loading

- Sample is percolated through the solid phase
- Allowing the target analyte retained on the sorbent while other compounds pass through

3. Washing

- Sorbent is washed to remove impurities
- Ensuring the target analytes remain retained on the sorbent

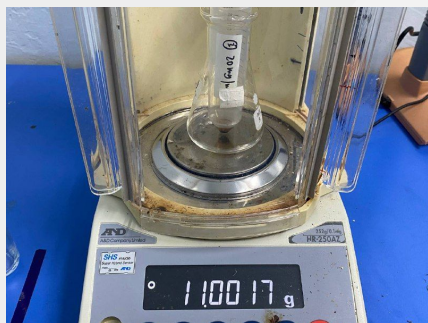
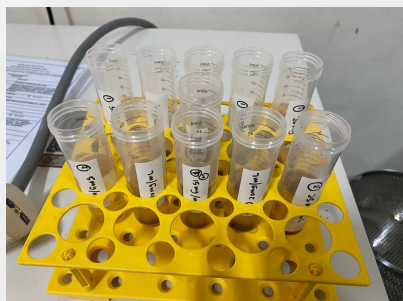
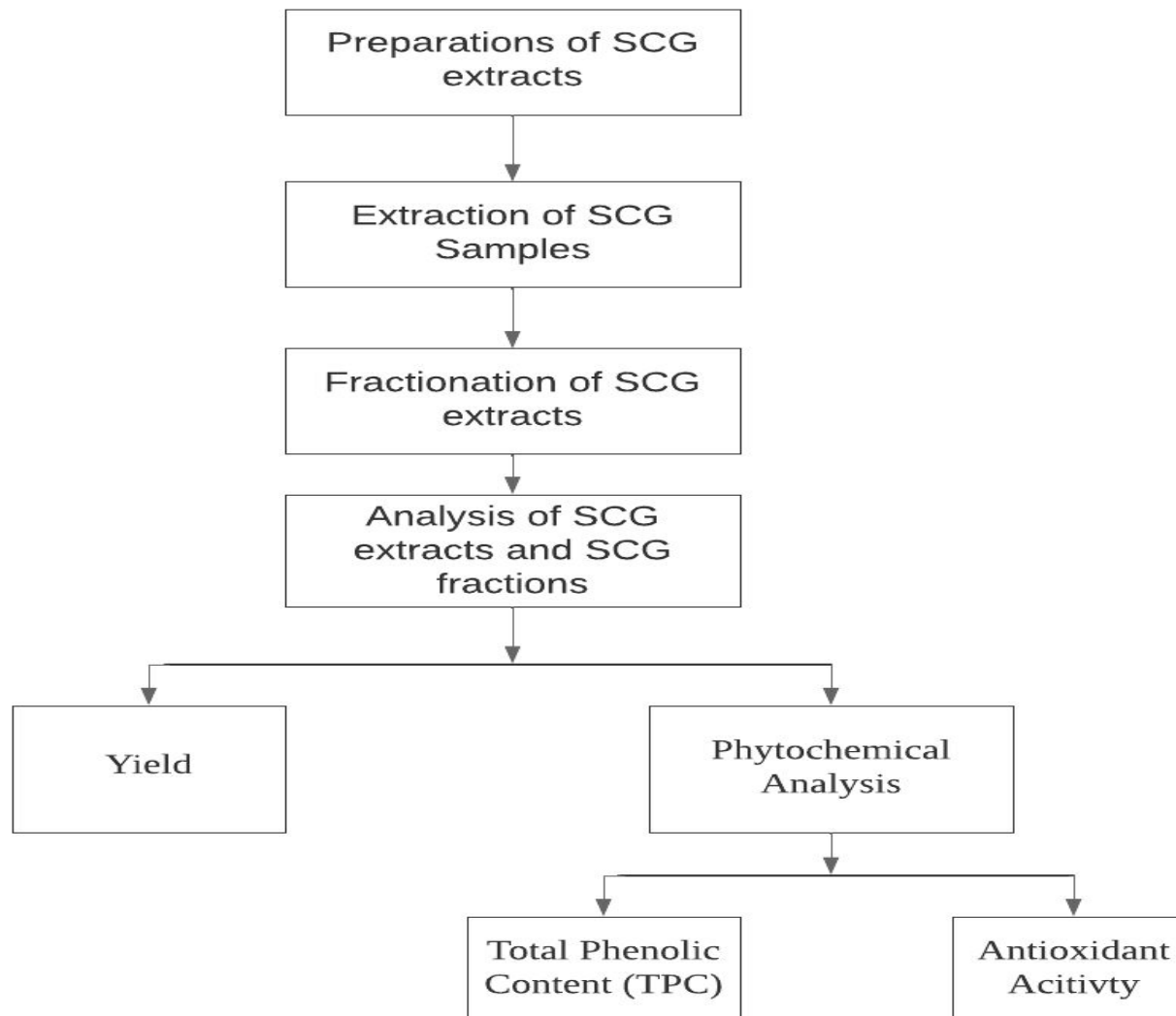
4. Elution

- Retained analytes released from the sorbent material by eluting with suitable solvent

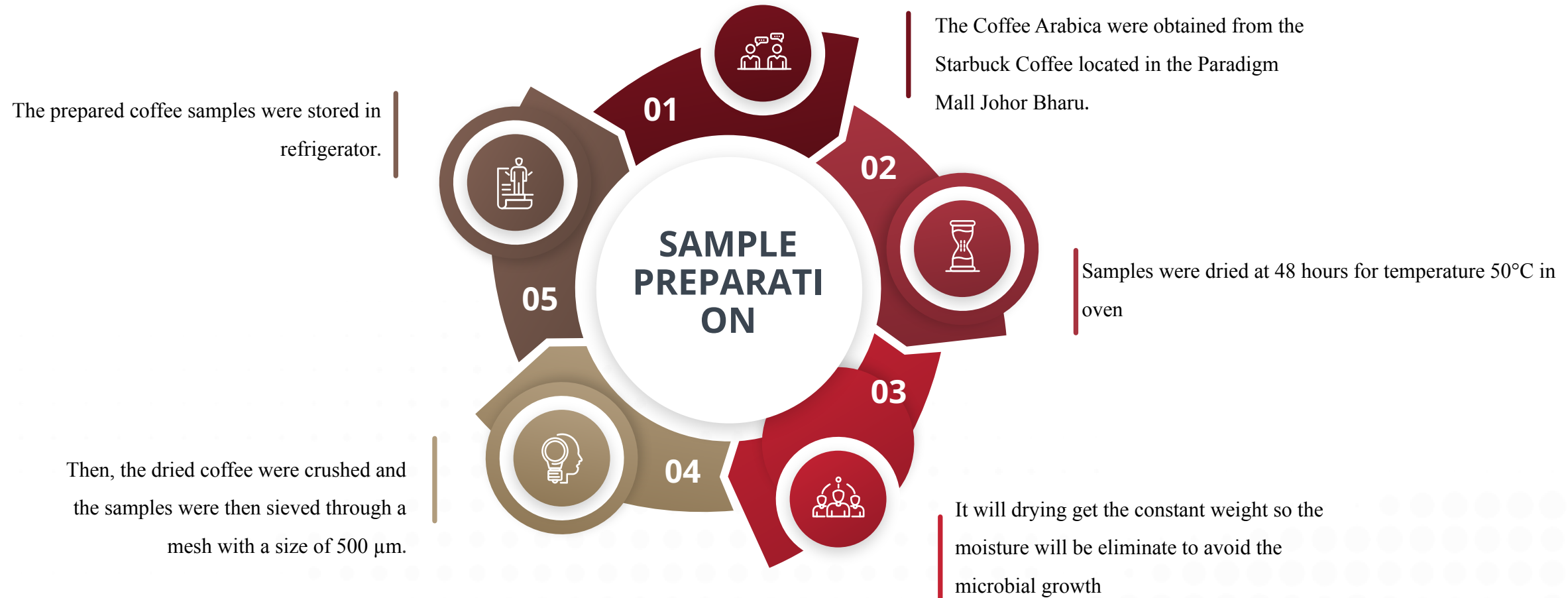
3.0 METHODOLOGY



FLOW PROCEDURE METHODOLOGY



SAMPLE PREPARATION

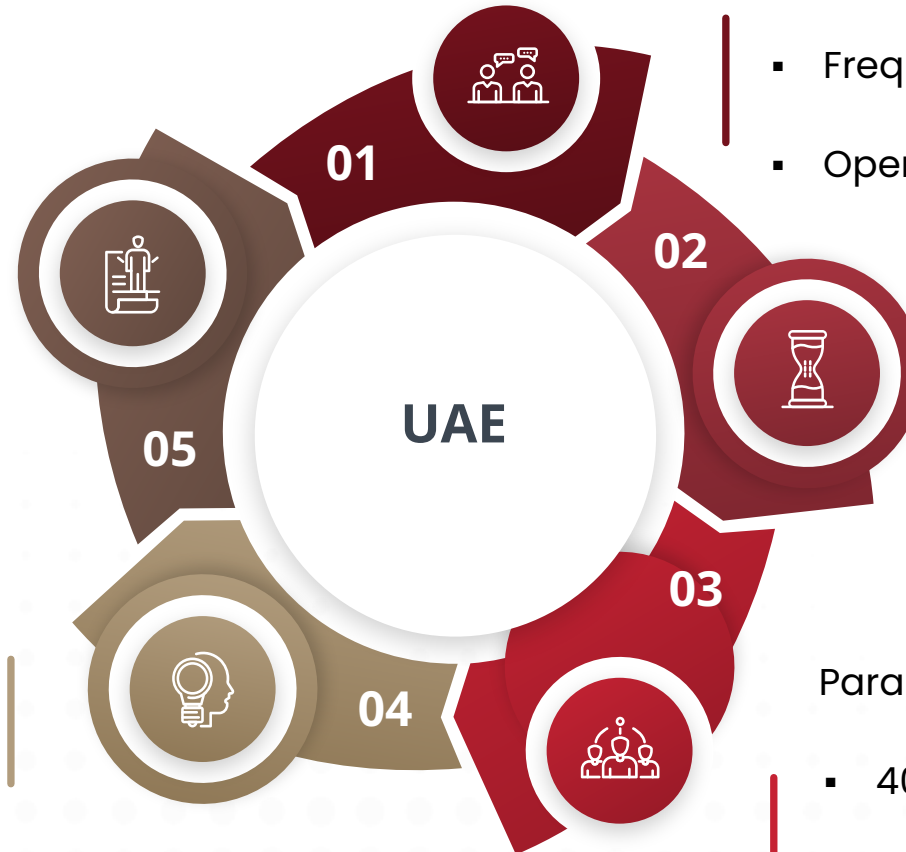


ULTRASOUND ASSISTED EXTRACTION

Proposed by this article with slight modification (Caballero-) Galván et al., 2018).

The resulting solution the filtered under vacuum and the extracts stored in a test tube at 5°C and storage in fridge.

After the sonication, the mixtures were centrifuged at 4000rm for 20 minutes to separate the solid and liquid phase.



Using ultrasound probe

- Frequency 60kHz
- Operated at 230 V

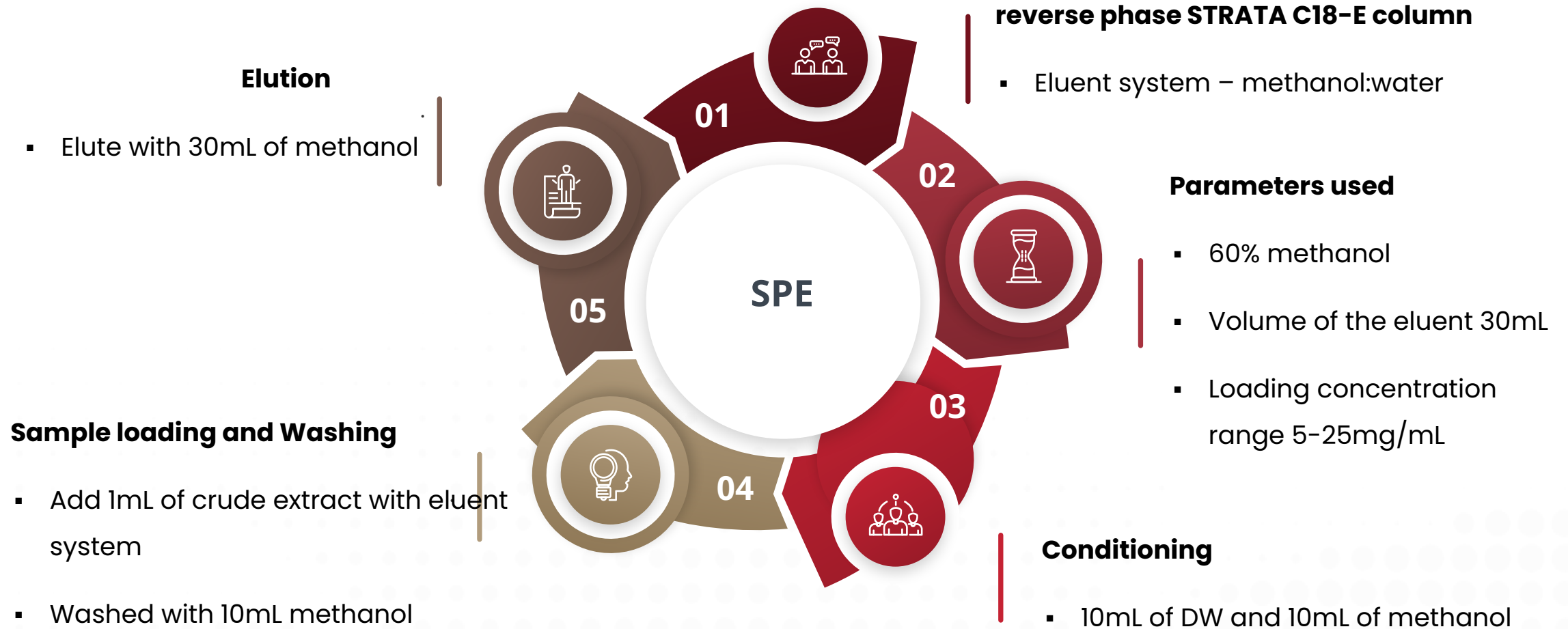
Sample Preparation

- 4mg of powdered sample and combining with 40 mL of the 60% ethanol solvent

Parameter UAE probe

- 40 mins
- 20s 20s pulse
- 60 amplitude

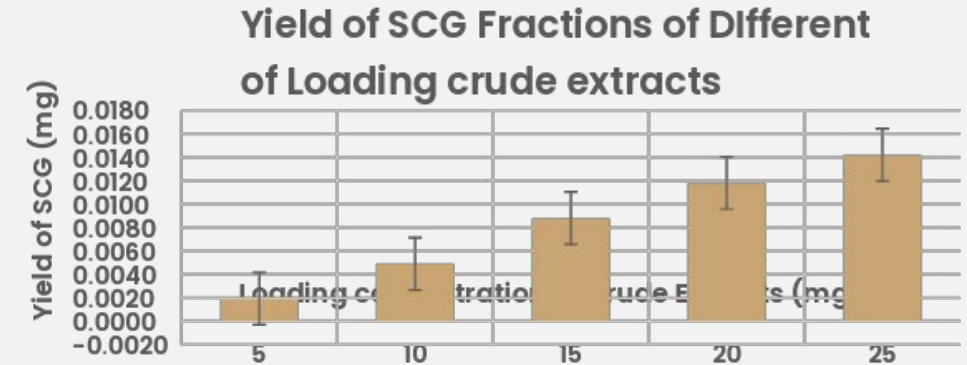
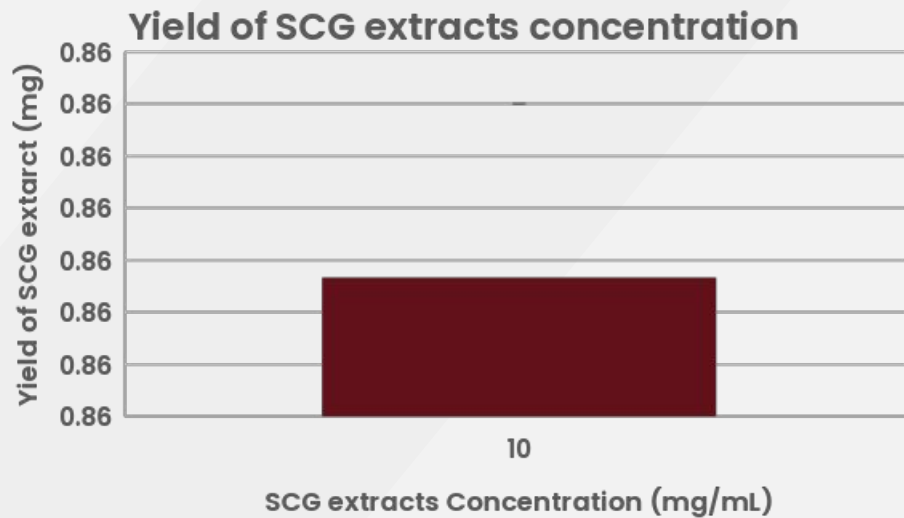
SOLID PHASE EXTRACTION



4.0 RESULT



4.1 Yield of SCG extracts and SCG fractions

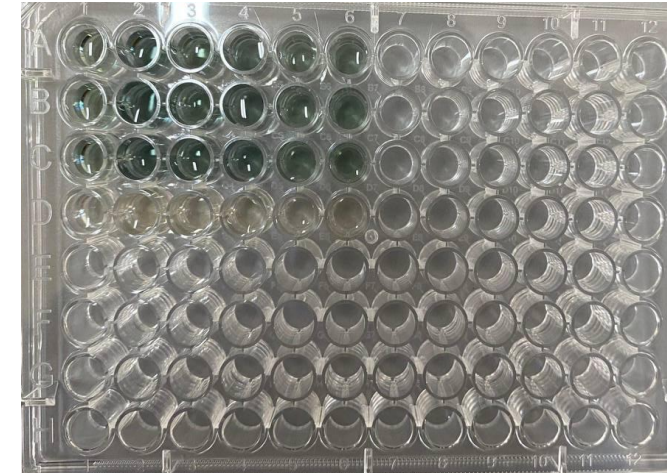
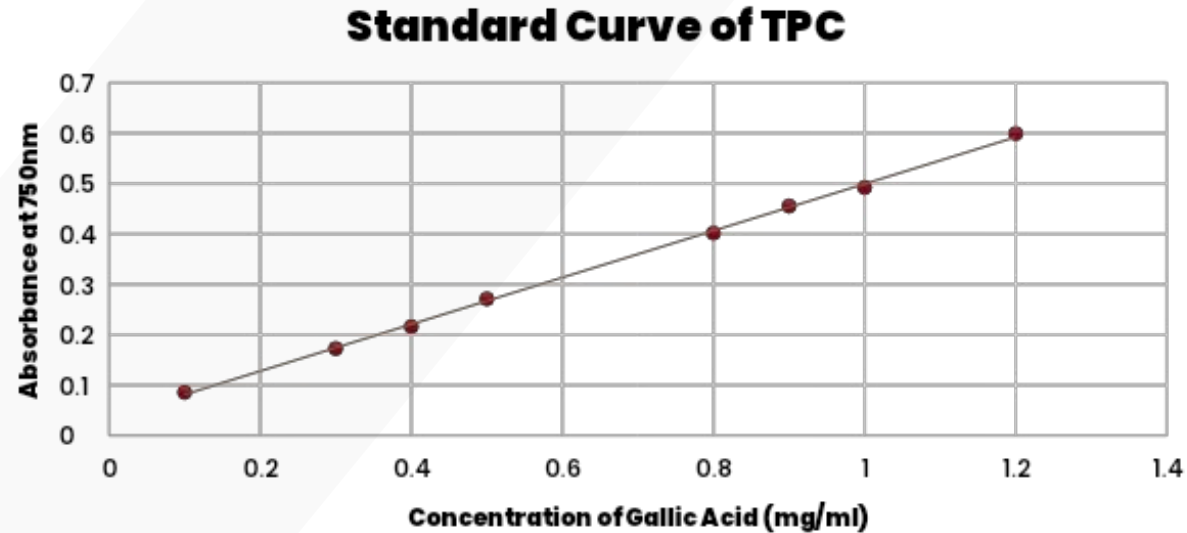


Yield is important to determine the efficiency of the extraction process and to optimize the process parameters

- SCG **extract has higher yield** in than SCG fractions.
- SCG extracts has 0.0881mg higher than SCG fractions with loading crude concentrations 25mg/mL with 0.0142mg
- Determined by calculating the **constant weight** of the samples
- For 24 hours, **the moisture loss is 60%.**
- Crude extract **contains a mixture of various compounds**, including bioactive substances and other components present in the raw materials.

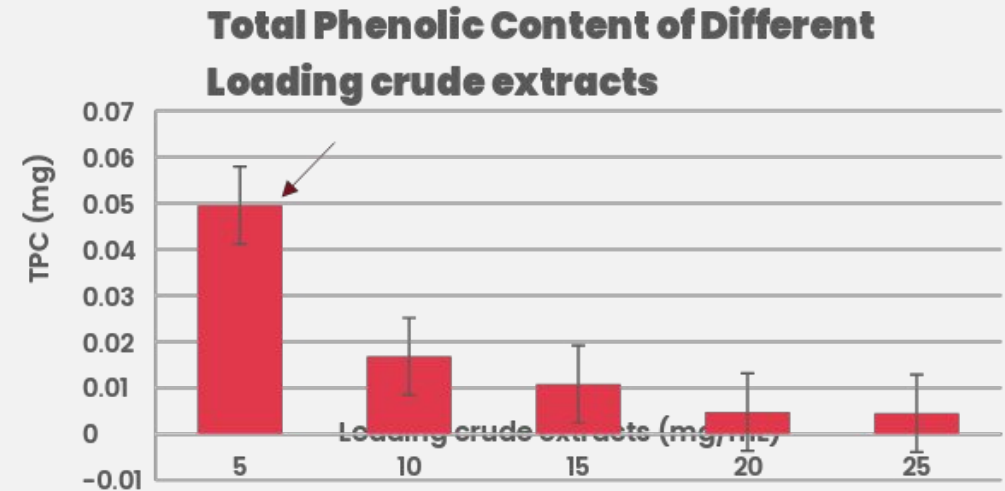
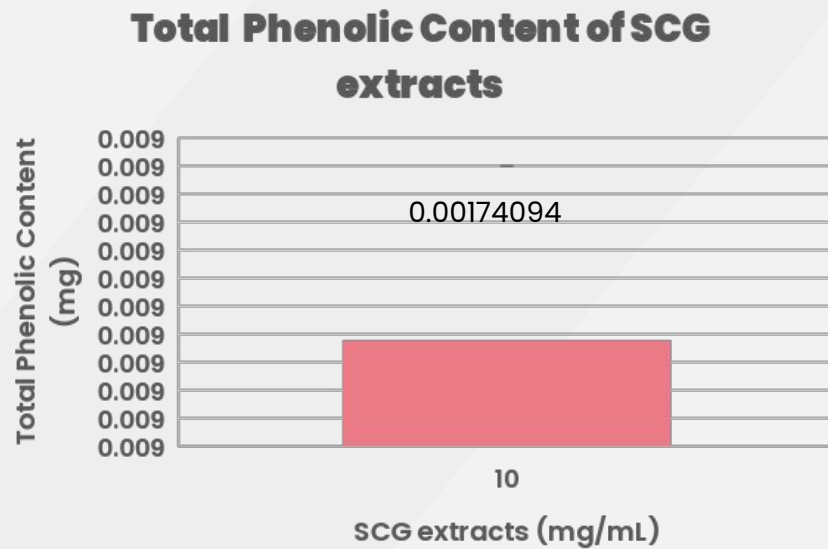
- This graph shows a clear trend of **increasing yield with higher loading concentrations of the SCG fractions.**
- Determined by calculating the **constant weight** of the samples.
- This suggests a **proportional relationship** between the loading concentration and the yield of polyphenols, indicating the potential for **enhanced extraction efficiency at higher concentrations.**

4.2 Total Phenolic Content of SCG extracts and SCG Fractions



- **Folin-Ciocalteu method involves the redox reaction** between the Folin-Ciocalteu reagent (FCR) and phenolic compounds. When the FCR is added to a solution containing phenols in basic conditions, it is **reduced by the phenolic compounds** resulting in the formation of **a blue-colored complex**
- Total phenolic content (TPC) of each fraction of spent coffee grounds (SCG) was **determined using the Folin-Ciocalteu method** with slight modification as suggested by Lawag et al. (2023).

4.2 Total Phenolic Content of SCG extracts and SCG Fractions



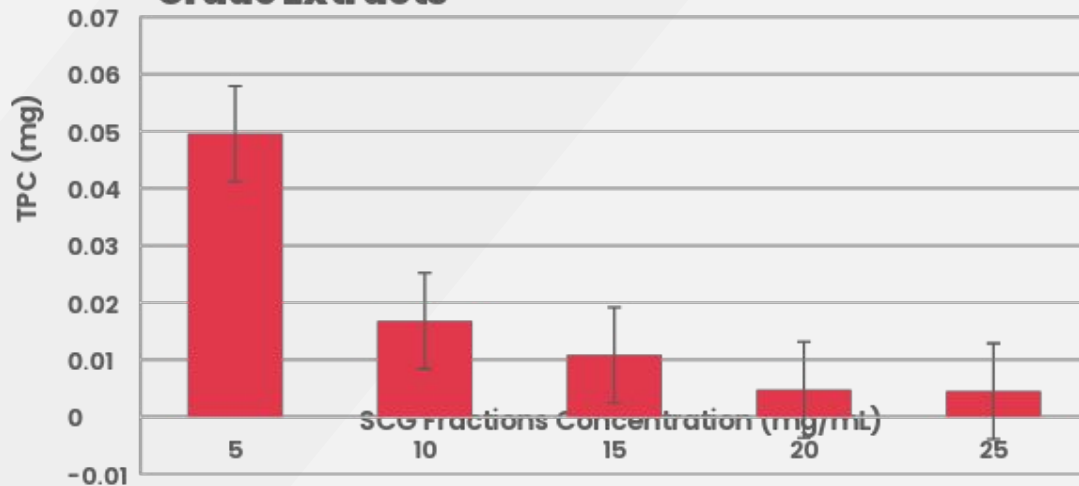
- This two figure compare the SCG extracts and SCG fractions with different loading crude extracts
- SCG fractions of loading crude concentrations **of 5mg/mL has the highest result of TPC** which is the **0.049558665mg** than the SCG extract only contain **0.001741mg**.
- So, it shows that the **SCG fractions has higher TPC value than SCG crude extracts**.
- This show that **SPE method can enriched the TPC than UAE method**.
- This is because the **selectivity** of the SPE method in isolating the phenolic compounds that enhanced the TPC value while the UAE method not be selective in isolating phenolic compounds leading to lower concentration of the phenolic compounds in crude extracts

TPC (mg GAE/g DW) vs Loading Crude Concentration (mg/mL)

Result TPC of SCG Fractions

- The graph show a **decreasing trend as the loading crude concentration increases.**
- The **highest TPC value** was obtained at the lowest loading crude concentration of **5 mg/mL with a value of 0.049559 mg GAE/g DW.**

Total Phenolic Content of Different Loading Crude Extracts

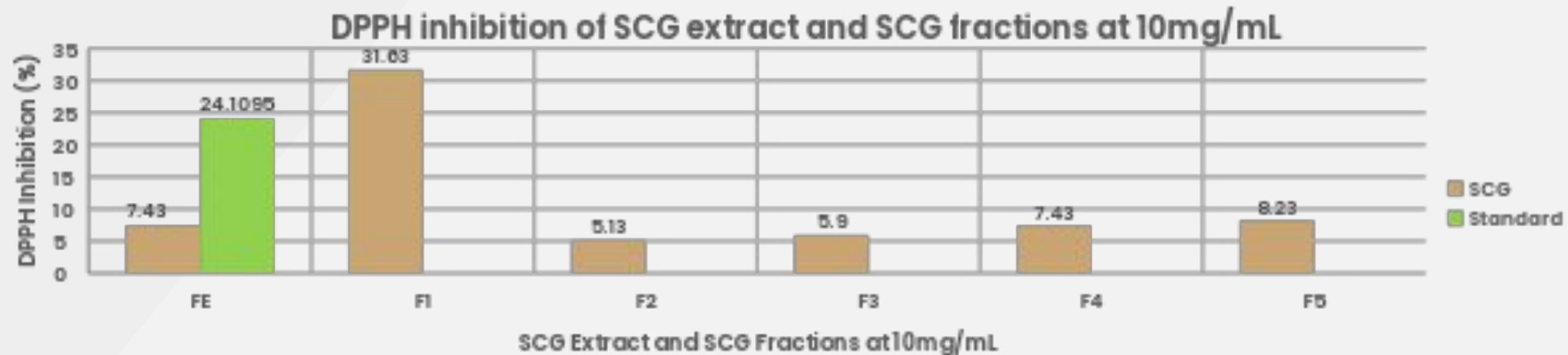


Discussion TPC of SCG Fractions

- The decreasing trend in TPC values with increasing loading crude concentration may be **due to the saturation of the extraction capacity of the SPE method.**
- At higher loading crude concentrations, the extraction efficiency may decrease **due to the saturation of the sorbent material** that leading to lower TPC values.

4.3 Graph DPPH inhibition (%) against Sample Concentration (mg/mL)

4.3 Result DPPH assays

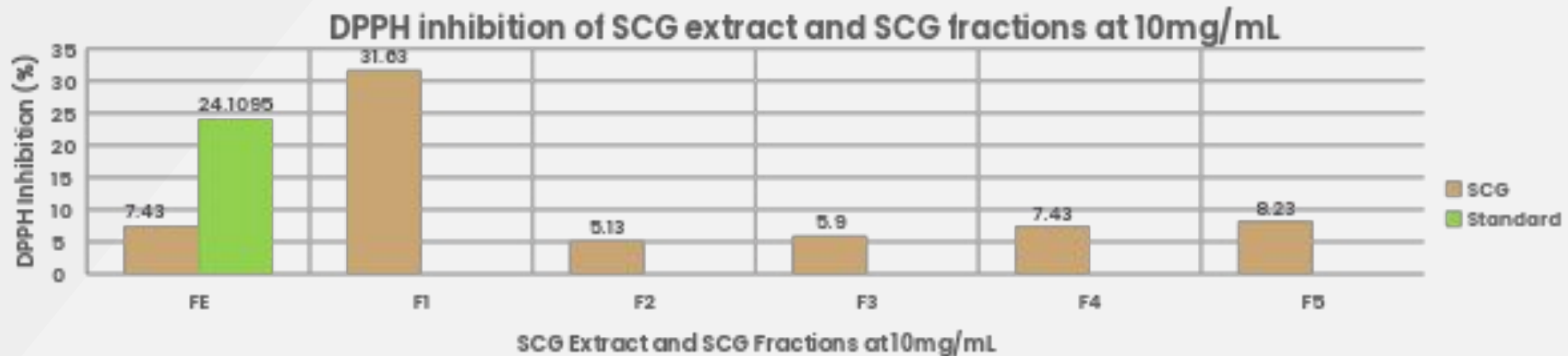


- The figure above show DPPH inhibition of SCG extracts (FE) and SCG fractions(F1-F5).
- As above, the **DPPH inhibition (%) of ascorbic acid is 24.1095%** and when comparing to **FE that only got 7.43%**.
- While for F1, from 5mg/mL, shows that is has **the highest values which is 31.63%** which is more higher than ascorbic acid and FE.
- F2,F3,F4 and F5 **not shows significant different** while comparing to F1
- This shows that loading crude concentration **5mg/mL as F1 has the highest values than other SCG fractions with different loading crude extracts concentration and also SCG extract.**

4.3 Graph DPPH inhibition (%) against Sample Concentration (mg/mL)

4.3 Discussion DPPH assays

- The DPPH assay is based on the ability of antioxidants to **reduce the DPPH radical**, resulting in a **decrease in absorbance**.
- **The higher the percentage of inhibition, the greater the antioxidant activity of the sample**



- The higher the percentage of inhibition, the **higher the antioxidant activity** of the sample
- So, **F1 has the higher antioxidant activity**.
- This shows that **SPE method can enhanced** the concentration of phenolic compounds and antioxidants present than UAE method.

5.0 CONCLUSION



Result	Loading Crude Extract (at 10mg/mL)	SCG Fractions (5–25mg/mL)	Justification
Yield	0.860067mg	0.0142mg At F5	Crude extract contains a mixture of various compounds , including bioactive substances and other components present in the raw materials.
TPC	0.00174mg	0.04956mg At F1 (at 5mg/mL)	SPE ensures selective isolation and purification , then result in higher concentration of phenolic compounds .
DPPH assays (Scavenging activity)	7.43%	31.63% At F1 (at 5mg/mL)	The SPE method allowed for the isolation and

- The **solid-phase extraction (SPE) method** was found to be more selective in **isolating phenolic compounds** because **it resulting in higher TPC values in the SCG fractions** compared to the ultrasound-assisted extraction (UAE) method used for crude extract.
- The **yield values for SCG fractions were also higher** than the yield value for the crude extract.
- The **DPPH inhibition for SPE methods also higher than UAE methods** in loading crude extracts.
- The **valorization of SCG as a source of valuable compounds with potential applications** in the pharmaceutical, cosmetic, and food industries is an interesting example of waste valorization in the agriculture food industry.

5.0 RECOMENDATION



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- Can do comparison with other Coffee By-Products so can compare the polyphenol and antioxidant content of SCG crude extracts and fractions with other coffee by-products, such as coffee pulp or coffee silverskin.
- Can do identification of specific polyphenols so can identify the specific polyphenols present in the spent coffee ground (SCG) crude extracts and fractions. This could involve using analytical techniques such as HPLC or LC-MS to quantify the concentration of individual polyphenols.

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