A REPORT ON
EXTRACTION TECHNOLOGIES FOR POLYPHENOLS

BY

Vijay Ravisankar 2010A1PS324H

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Hindustan Unilever Research Center, Bangalore

A Practice School-II station of

BIRLA INSTITUTE OF TECHNOLOGY & SCIENCE-PILANI

20th June, 2014
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Practice School II

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Designation: Research Scientist

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Project Areas: Extraction Technologies for polyphenols using non-thermal methods

Abstract: The use of conventional methods of extraction generally involves the use of heat as a source of extraction. This method can cause structural degradation to polyphenols and also loss of volatiles that might be responsible for the flavor and aroma.

The main aim of this project is to determine the possible alternatives to conventional extraction by identifying various other non-thermal methods which can be used for extraction of polyphenols. The various methods can also be aided by the use of pre-treatment which also have to be studied by experimentation and analysis of the polyphenol content.

Signature of the Student
Date:

Signature of the Faculty
Date:
Acknowledgment

I would like to express my sincere thanks to Hindustan Unilever Research Center, Bangalore for providing me with the opportunity and resources to carry out the project work. I would like to specifically thank Dr. Sreejit Nair for being a constant source of motivation. I am deeply indebted to Mr. B. Indreesh for his immense help, co-operation and invaluable suggestions. I am grateful to other members of HURC for reviewing my work, providing constant support and encouragement.

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1. Introduction

1.1 About the Company

1.1.1 Hindustan Unilever Limited (HUL)

Hindustan Unilever Limited (HUL) is an Indian consumer goods company based in Mumbai, Maharashtra. It is owned by Anglo-Dutch company, Unilever which owns a 67% controlling share in HUL. HUL’s products include foods, beverages, cleaning agents and personal care products.

HUL was established in 1933 as Lever Brothers India Limited and, in 1956, became known as Hindustan Lever Limited, as a result of a merger between Lever Brothers, Hindustan Vanaspati Mfg. Co. Ltd. and United Traders Ltd. It is headquartered in Mumbai, India and employs over 16,500 workers, whilst also indirectly helping to facilitate the employment of over 65,000 people. The company was renamed in June 2007 as “Hindustan Unilever Limited”.

Hindustan Unilever’s distribution covers over 2 million retail outlets across India directly and its products are available in over 6.4 million outlets in the country. As per Nielsen market research data, two out of three Indians use HUL products.
1.1.2 Products

a. Food & Drink

b. Home Care
c. **Personal Care**

![Unilever Products](image1)

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d. **Water Purifier**

![Water Purifier](image2)

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**Fig 1 Unilever Products**
1.2 Project Background

1.2.1 Introduction to Tea

Tea chemistry has led consumers and researchers to debate numerous issues and to probe for a deeper understanding of the nature of this beverage. Various kinds of this beverage can be formed by varying the processing procedures. For example, frying the leaves before drying will prevent the fermentation step from happening and result in the formation of green tea. Brewing techniques can impact flavor and chemistry of brew significantly.

The fermentation of black tea is an endogenous process and is catalyzed by the enzymes within it.

**Green Tea:** Green tea is produced by pan frying it so as to deactivate the enzymes and prevent it from fermenting. Fermentation will lead to the formation of oolong/black tea.

**Oolong Tea:** Leaves are allowed to wither to moisture content of 55 to 72% of leaf weight. This increases the concentration of polyphenols which deteriorates the leaf structure. The leaves are then left for fermentation for a certain period of time before being stopped midway through the process and pan fried so as to prevent it from converting to black tea. Withering is an important factor for aroma development.

Fermentation (Oxidation) converts simple polyphenols to more complex condensed polyphenols that give bright red colour and the astringent flavor.

**Black Tea:** Leaves are allowed to wither for a certain amount of time to a moisture content of about 70% so as to increase the concentration of polyphenols in the matrix. The leaves are then left for fermentation for a long period of time. Leaves are then fired at a high temperature to halt the fermentation process.
1.2.2 Tea Processing

![The Tea Process](image)

**Fig 2: Tea processing**

**Withering:** Withering is the first processing step in the factory and is a process in which freshly plucked leaf is conditioned physically, as well as, chemically for subsequent processing stages. Withering is used to remove excess water from the leaves (reduce moisture content from the initial level of around 75%) and allows a very slight amount of oxidation. The leaves can be either put under the sun or left in a cool breezy room to pull moisture out from the leaves.

**Oxidation / Fermentation:** For teas that require oxidation, the leaves are left on their own in a climate-controlled room where they turn progressively darker. This is accompanied by agitation in some cases. In this process the chlorophyll in the leaves is enzymatically broken down, and its tannins are released or transformed.

**Firing:** This is done to stop the tea leaf oxidation at a desired level. This process is accomplished by moderately heating tea leaves, thus deactivating their oxidative enzymes and removing unwanted scents in the leaves, without damaging the flavour of the tea. Traditionally, the tea leaves are pan fried or steamed.
**Drying**: This can be done in many ways including panning, sunning, air drying, or baking. Baking is usually the most common. Great care must be taken to not over-cook the leaves. The drying of the produced tea is responsible for many new flavour compounds particularly important in green teas.

**1.2.3 Chemical Composition** [5]:

Tea consists of a large number of different chemical compounds, some very complex in nature too. Some of them are:

1. **Polyphenols**: 30-40% wt/wt of the extracted solids. About 180-240mg is present per cup of tea. Polyphenols have been found to provide the astringency to tea.

2. **Theobromine**: These are present in much lower quantities than caffeine. They are present in more quantities if the methylation of caffeine is absent.

3. **Proteins & Amino Acids**

   18 amino acids have been found to be present in tea. The free amino acid content increases during the withering process but decreases during the fermentation process. Theanine is an amino acid that has been found to be unique to tea.

4. **Aroma**

   Hundreds of compounds in trace quantities. Mostly glycoside derivatives which are freed during the fermentation process. Attaching bound glycosides by glycosidase treatment offers the possibility of increase in the quality.
1.2.4 Polyphenols
Polyphenols refer to millions of natural and synthetic aromatic molecules that are substituted with multiple hydroxyl groups. These are principally responsible for the colours and astringency and partially for the flavour of the tea.

Chemical Classification:

Degree of complexity of polyphenol increases as it gets oxidized into much complex compounds. Differentiating these complex compounds is much more difficult than the simple polyphenols. Flavonoids are a dominant class of polyphenols. Flavonoids are divided into two groups, flavonols and flavanols.

1. Green Tea Polyphenols: These are mainly subdivided into catechins and flavonols.

2. Catechins and Gallocatechins: There are four most common types of catechins that are present in tea.

They are Epigallocatechin gallate (EGCG) , Epigallocatechin (EGC), Epicatechin gallate (ECG) and Epicatechin (EC)

Fig 3 Polyphenol- Catechin
3. **Other polyphenols:** Flavones and their glycosides.

4. **Black Tea Polyphenols:** Extent and conditions of fermentation determines the degree to which the green tea polyphenols are converted.

5. **Residual Green Tea Polyphenols:** Unconverted even after fermentation
   
   a. **Catechin:** Survive the fermentation process.
   
   b. **Flavonols:** Oxidized after fermentation

6. **Theaflavins:** Key distinction of black and green tea. They contribute to around 3 to 5% wt/wt of extracted solids. Provide a bright red colour making it easily distinguishable.

   ![Molecular Structure: Theaflavin](image)

   **Fig 4: Polyphenol - Theflavins**

7. **Thearubigins.** Though not yet clearly defined, these are divided into three sub categories, SI, SIIa, SIIb. Separation of thearubigins on normal phase chromatography and using this technique of counter current chromatography have shown some promise.
Fig 5: Flavonol distribution in green & black tea

Tea leaves consist of various kinds of polyphenols in various compositions Green tea contains higher amount of lower molecular weight polyphenols (catechins) whereas black tea contains majorly higher molecular weight oxidized polyphenols. Black tea in addition to the catechins, also contain thearubigins (TRs) and theaflavins (TFs). The figure above shows the composition of various polyphenols in green tea and black tea.

1.2.5 Extraction of tea solids:

The quality and the characteristic of a tea extract is dependent on the type of solids extracted from the tea matrix, for example, polyphenols are known to be responsible for giving the characteristics like astringency, bitterness, colour etc. Green tea is known to show characteristics of bitterness whereas black tea shows reduced characteristics of the astringency however showing improved colour characteristics. This difference is mainly attributed to the difference in the kind of solids that are extracted from the tea leaves. Catechins are present in the green tea
leaves have shown to cause more bitterness, astringency and TF’s & TR’s have been proved to cause the color characteristics of black tea.\(^{[5]}\)

**1.2.6 Challenges faced during extraction**

The extraction of the polyphenols has become essential in order to provide the right characteristic for tea extract and there are a lot of challenges that are encountered during the extraction process. Different kinds of tea systems have been studied for the extraction of polyphenols and the problems were attributed to the binding of polyphenols within the matrix, the mass transfer resistances caused by the cell membranes and the cell walls during the extraction process thereby not allowing maximum extraction yield of solids from the system. Previous literature work on tea systems (green tea systems, black tea systems) on overcoming these difficulties provided with possibilities of using different extraction technologies that use external force field to overcome these barriers and assist in higher yield of solids and polyphenols out of the leaf matrix. The different extraction technologies were used for systems that were similar to the one to be tested and the pros and cons of the same were analyzed before selecting the technology for better extraction.

**1.3 EXTRACTION TECHNOLOGIES**

**1.3.1 Conventional Methods:**

The most conventional methods of extraction are hot/cold infusion. This is generally observed when a tea bag is dipped inside a cup of hot or cold water. The conventional method of extraction has been found to involve the use of a larger amount of heat. The use of heat for this extraction has been found to have a significant effect on the structure of the polyphenol and loss of polyphenols and other volatiles as well.
The basic steps involved while performing a conventional method of extraction are as follows:

1. Boundary Layer Resistance
2. Diffusion of water into leaf
3. Diffusion within tea pores
4. Solubilizing polyphenols
5. Diffusion of polyphenol solution out of the tea matrix
6. Partitioning of polyphenols at interface
7. Bulk Resistance

Fig 6: Extraction process

There are various other problems apart from heat which are also associated with the extraction process. They may be classified as:

**External Mass Transfer**

Can be improved with the help of agitation or stirring to increase the turbulence and thus aid in higher mass transfer rates.

**Solubility of solvent (mostly water)**

Allowable amount of heat can be applied to increase the solubility of water without the degradation of polyphenols in the process. Heating to temperatures that are high but not close to boiling point of water

**Internal Mass Transfer**

The resistances to internal mass transfer are generally caused by the cell walls/cell membranes. Possible ways of decreasing this resistance includes the weakening of the cell matrix/wall as a pre-treatment before using alternative extraction methods for better mass transfer rates.
Extraction however is not restricted the use of conventional methods and various other methods could also be used to perform the extraction process. Some of the processes which do not use heat as a parameter are as follows:

- Ionic Liquids
- Ultrasound Assisted extraction
- Microwave Assisted Extraction
- Super-critical fluids
- Ultra high pressure extraction

A combination of any of the above processes could also be used to perform the extraction process.

A brief look into the pros and cons of each of the above mentioned process gives an idea about the best process which could be performed on a lab scale to test the extraction before up-scaling the same.

1.3.2 Ionic Liquids

Ionic liquids can be described in simple terms as salts in liquid state. They can act as very powerful solvents due to the presence of short lived ion pairs. The use of this has been tested for the extraction of flavonoids along with the help of microwave assisted extraction.\[^{12}\]

- **Pros**: A much greener alternative to solvent usage, ionic liquids provide a much larger amount of extraction when compared to solvents. Low volatility eliminates the release of gaseous substances into the environment. Lower solvent consumption.
• **Cons:** In extraction of tea polyphenols, water is used as solvent. Water being much less volatile than other solvents is a better and safer bet than ionic liquids (environmental-wise as well). Careful handling must be done as these liquids are combustible in nature.

### 1.3.3 Super-Critical Fluids

A supercritical fluid is any substance at a temperature and pressure above its critical point, where distinct liquid and gas phases do not exist. The application of ultrasound during such supercritical extraction processes has been proposed recently as a mechanism both for rate acceleration and yield improvement. Supercritical fluids like carbon di-oxide can be used in addition to ultrasound for extraction process. [2]

- **Pros:** The solubility can be increased to our convenience by changing the pressure of the fluid. Increasing it makes it more soluble. Much lesser solvent consumption.
- **Cons:** Pressure also varies with temperature and can vary very sharply near critical point. Therefore there is a decrease in solubility near critical point. All fluids have to be first brought up to the critical point and beyond to act as supercritical fluids. **No formation of bubbles** also due to lack of phase boundaries thereby removing the possibility of cell damage due to cavitations

### 1.3.4 Ultra High Pressure

Ultra high pressure technique involves the application of high pressure for performing the extraction process. [6]

- **Pros:** Short extraction time, mild extraction condition, high extraction yield, less impurity, high reproducibility at shorter times, simplified manipulation, and lowered
energy input, as well as solvent consumption. Can also be performed at ambient temperature thus reducing the structural damage due to high temperature.

• **Cons:** High equipment costs and limitations on floor space for placing the equipments for testing purposes. Maintenance is also difficult for huge equipments. Other sources like lipids/proteins could be co-extracted in the process due to large pressure being applied in this process. Extraction of these might lead to the **formation of tea cream.**

### 1.3.5 Microwave Assisted Extraction

Dielectric heating is caused due to microwave radiation. This dielectric heating can help in better extraction of the polyphenols that are present in the leaf matrix by improving the solubility of the solvent as well as improving the diffusion of substances from within the cell matrix.\(^{[8,10]}\)

• **Pros:** Lower energy consumption. Increased solubility of solvent due to increase in temperature. Reduced environmental burden and also lesser solvent consumption. Easy to handle and shorter extraction times.

• **Cons:** **Loss of volatiles** might take place due to application of heat. A certain amount of structural loss of compounds might also take place due to the same reason. Efficiency might be poor when target compounds for extractions are volatile/temperature sensitive.

### 1.3.6 Ultrasound Assisted extraction

Ultrasound assisted extraction involves the use of a sonicator that is used to create cavitation inside a medium so as to cause the explosion of small bubbles in the medium. In the process of explosion, the effect is profound and causes enough damage to the cell wall and thereby reduce the resistance that are created by the cell wall/membrane.\(^{[2]}\) Some parameters that could be varied...
while testing the effect are ultrasonication are temperature, solid : liquid ratio, duration of exposure to ultrasonication

- **Pros:** Lower energy consumption. Lesser heat/thermal effect compared to conventional methods, thereby reducing structural damage due to heat. Comparatively shorter extraction times. Lower investments compared to other methods like supercritical extraction/ ultra high pressure extraction.

- **Cons:** Filtration step might be involved. Larger solvent extraction as compared to MAE. Multiple extractions might be required for larger yield.

![Fig 7: Cavitation – How it happens](image)

### 1.3.7 Infra Red Assisted Extracation:

The extraction is performed with the help of IR Lamps which provide the infra red rays. The infra red rays help in weakening the binding of solids with the proteins/lipids of the cell matrix and thus aid in better extraction of substances from the tea matrix.

- **Pros:** Large energy transfer within small space. Very Low solvent consumption therefore reducing environmental burden to large extents

- **Cons:** Infra Red radiation must be controlled as heating might lead to structural damage of compounds/loss of volatiles.
1.3.8 Pre-Treatment methods:

The application of the external force fields may also be accompanied or preceded by certain pre-treatments that might help in an increased amount of extraction. This increase can be done by weakening the cell wall thus allowing a larger amount of extraction.\[^13\] The weakening of cell wall however can be performed by various methods. Some of the examples of the pre-treatment processes are:

- Bead Beating
- Osmotic Shock
- EDTA as chelating agent

**Bead Beating**

- This method involved the use of glass/ceramic beads that can cause mechanical shear on the cell wall of the plant particles thus helping in larger amount of mass transfer. This method has been found to yield a 50% breakage even for particles of small size. The beads collide with the cellular sample, cracking open the cell to release intercellular components. The beads are initially suspended in the aqueous media. \[^14\]

- Since there is already the use of cutting, the use of another mechanical process as a pre-treatment wouldn’t have a considerable effect on the weakening of the cell matrix.

**Osmotic Shock**

**Hypotonic Solution**: Massive movement of solvent from outside the cell to inside causing swelling of the cell which eventually bursts and leads to the release of components inside the cell.
. The drawback associated with this method is the possibility of creating a large concentration gradient enough to cause the solvent to transfer in such massive quantities inside the cell.

**Hypertonic Solution:** Continuous extraction of substances from inside the cell eventually leading to the weakening of the cell membrane/ cell wall. Must be performed multiple times to effect an appreciable amount of weakening of the cell membrane

![Diagram of solvent movement in hypertonic and hypotonic solution](image)

**Fig 8: Solvent movement in hypertonic and hypotonic solution**

**EDTA as chelating agent**

- EDTA could be used as a chelating agent that can be used to remove the calcium and magnesium ions that bind the layers of lipids to each other. EDTA can chelate with these ions thus weakening the link between the different layers of lipids and thus improving the permeability of the cell wall and thus improving the mass transfer rate. [13]

These methods have proven to be very useful in the breakage of cell wall, use of these as pre treatments to ultrasound can definitely help in a larger yield of breakage of cell wall and thus allowing a larger extraction of the intercellular components and thus increasing the yield without affecting the components.
2. Materials & Methods

From the above methods for the use of force fields for the extraction of polyphenols from tea and the possible use of pre-treatments, the use of ultra sound assisted extraction or microwave assisted extraction with or without any pre-treatment would be idea for testing the extraction efficiency on a lab scale to increase the extraction yield of solids from the leaf matrix.

2.1 Ultrasound Assisted Extraction (UAE)

2.1.1 Materials

- Tea Dhool [macerated tea leaves]
  - Chemicals Required: Sodium Carbonate (7.5%), Gallic Acid (10%), Folin-Ciocalteau Reagent

2.1.2 Experimental setup & procedures

The procedure for UAE involves the use of a sonicator (ICW LTD, Pune, 250 W) bath which helps in creating ultrasound waves through a medium. This creation of waves through a medium (water) helps in creating cavitations in the medium and thus causing the bursting of water bubbles with high intensity. The bursting of water bubbles thus help in weakening of the cell walls and thus help in improving the extraction of the solids from the cell matrix.

![Flowchart – Experimental Procedure-UAE](image)

**Fig 9: Flowchart – Experimental Procedure-UAE**
2.1.3 Analytical procedure

**Total Polyphenol Analysis:** For Polyphenolic and Non-Polyphenolic fraction in the tea, analysis was done using UV spectrophotometer with a method called as Colorimetric method using Folin-Ciocalteu reagent (Ultraspec-7000 GE) (Appendix A).

**Solids Measurement:** Solids Measurements was done by gravimetric analysis (Appendix A)

**Catechin Measurement:** Catechin measurements were done with the help of a High Performance Liquid Chromatography (HPLC) (Appendix A)

**Color Measurements:** Color Measurements were performed using a Hunter Lab Equipment (Appendix A)
2.2 Infra Red Assisted Extraction (IAE)

2.2.1 Materials
- Tea Dhool [macerated tea leaves]
- **Chemicals Required**: Sodium Carbonate (7.5%) , Gallic Acid (10%) , Folin-Ciocalteau Reagent

2.2.2 Experimental setup & procedures
The procedure for IAE involves the use of infra red lamps which produce infra red rays. The production of these infra red rays help in weakening the binding between the solids which are attached with the lipids/proteins in the cell matrix thus helping in higher extraction of solids.

![Flowchart – Experimental Procedure- IAE](image1)

![Experimental Setup for Infra Red Radiation](image2)
2.2.3 Analytical procedure

**Total Polyphenol Analysis:** For Polyphenolic and Non-Polyphenolic fraction in the tea, analysis was done using UV spectrophotometer with a method called as Colorimetric method using Folin-Ciocalteu reagent (Ultraspec-7000 GE) (Appendix A).

**Solids Measurement:** Solids Measurements was done by gravimetric analysis (Appendix A)
3. Results & Discussions

3.1 Ultrasound Assisted Extraction (UAE)

3.1.1 Experiment 1 – Effect of solid:liquid ratio (Ambient Temperature, 15’)

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Weight</th>
<th>Water Added</th>
<th>Ratio</th>
<th>Ultrasound (Y/N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>200</td>
<td>240</td>
<td>1:4</td>
<td>Y</td>
</tr>
<tr>
<td>Control 1</td>
<td>200</td>
<td>240</td>
<td>1:4</td>
<td>N</td>
</tr>
<tr>
<td>Sample 2</td>
<td>200</td>
<td>600</td>
<td>1:10</td>
<td>Y</td>
</tr>
<tr>
<td>Control 2</td>
<td>200</td>
<td>600</td>
<td>1:10</td>
<td>N</td>
</tr>
</tbody>
</table>

Table 1: Sample Data for Experiment 1 – UAE – Effect of Solid : Liquid ratio

Solids Measurement

Chart 1: Solids Measurement – Effect of solid:liquid ratio - UAE
Based on the result obtained above, further experimentation was carried out to check the effects of other parameters that can aid the extraction process. The parameters tested were temperature & duration of exposure to ultrasonication.

<table>
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<tr>
<td>1:10</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>
3.1.2 Experiment 2 – Effect of Time (Ratio 1:10, Ambient Temperature)

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Weight</th>
<th>Water Added</th>
<th>Ratio</th>
<th>Ultrasound (Y/N)</th>
<th>Time</th>
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</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>200</td>
<td>600</td>
<td>1:10</td>
<td>Y</td>
<td>15’</td>
</tr>
<tr>
<td>Control 1</td>
<td>200</td>
<td>600</td>
<td>1:10</td>
<td>N</td>
<td>15’</td>
</tr>
<tr>
<td>Sample 2</td>
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<td>600</td>
<td>1:10</td>
<td>Y</td>
<td>60’</td>
</tr>
<tr>
<td>Control 2</td>
<td>200</td>
<td>600</td>
<td>1:10</td>
<td>N</td>
<td>60’</td>
</tr>
</tbody>
</table>

Table 2: Sample Data for Experiment 2 – UAE – Effect of Time

Chart 3: Solids & Polyphenols Measurement – Effect of time - UAE
3.1.3 Experiment 3 – Effect of Temperature (Ratio 1:4, 60’)

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Weight</th>
<th>Water Added</th>
<th>Ratio</th>
<th>Ultrasound (Y/N)</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>200</td>
<td>240</td>
<td>1:4</td>
<td>Y</td>
<td>25ºC</td>
</tr>
<tr>
<td>Control 1</td>
<td>200</td>
<td>240</td>
<td>1:4</td>
<td>N</td>
<td>25ºC</td>
</tr>
<tr>
<td>Sample 2</td>
<td>200</td>
<td>240</td>
<td>1:4</td>
<td>Y</td>
<td>50ºC</td>
</tr>
<tr>
<td>Control 2</td>
<td>200</td>
<td>240</td>
<td>1:4</td>
<td>N</td>
<td>50ºC</td>
</tr>
</tbody>
</table>

Table 3: Sample Data for Experiment 3 - UAE – Effect of Temperature

<table>
<thead>
<tr>
<th>% increase w.r.t control</th>
<th>%PP/SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>25ºC</td>
<td>12.680</td>
</tr>
<tr>
<td>50ºC</td>
<td>7.100</td>
</tr>
</tbody>
</table>

Chart 4: Solids & Polyphenols Measurement – Effect of Temperature - UAE
Cost Analysis

(Energy usage of Ultrasonicator not added)

Chart 5: Cost Analysis

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>UAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:10</td>
<td>148.57</td>
<td>153.06</td>
</tr>
<tr>
<td>1:04</td>
<td>121.15</td>
<td>115.92</td>
</tr>
</tbody>
</table>

kJ/g of solids

Energy Utilized
**Color Analysis**

**Chart 6 : Color Analysis**

- **UAE Sample : US Assisted Extraction ; Solid : Liquid 1:10 ; Time = 60’ ; Temperature : 25°C & 50°C**

**Polyphenols Profile Analysis**

- **TR Profile**
  - **Solid : Liquid 1:10 ; Time = 60’; Temperature : 50°C**

**Chart 7 : TR Profile - UAE**
Heat Map Analysis for TR Profile

Chart 8: Heat Map Analysis - UAE
Catechin Analysis

Solid : Liquid 1:10 ; Time = 60'; Temperature : 50°C

Chart 9 : Catechin Profile - UAE
3.2 Infra Red Assisted Extraction (IAE)

3.2.1 Experiment 1 – Effect of Intensity of radiation (Solid : Liquid ratio 1:1, Temperature 30°C, Leaf Type : Fine pluck, Time : 20 min)

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Weight</th>
<th>Water Added</th>
<th>Ratio</th>
<th>IR Radiation(Y/N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>200</td>
<td>56</td>
<td>1:1</td>
<td>Y</td>
</tr>
<tr>
<td>Sample 2</td>
<td>200</td>
<td>56</td>
<td>1:1</td>
<td>Y</td>
</tr>
<tr>
<td>Control 1</td>
<td>200</td>
<td>56</td>
<td>1:1</td>
<td>N</td>
</tr>
</tbody>
</table>

**Table 4: Sample Data for Experiment 1 - IAE – Effect of Intensity of radiation**

![Effect of intensity of radiation](image)

**Chart 10: Solids & Polyphenols Measurement – Effect of intensity - IAE**
3.2.2 Experiment 1 – Effect of leaf type (Solid : Liquid ratio 1:1, Temperature 30°C, Intensity of radiation: 15%, Time: 20 min)

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Weight</th>
<th>Water Added</th>
<th>Ratio</th>
<th>IR Radiation(Y/N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>200</td>
<td>56</td>
<td>1:1</td>
<td>Y</td>
</tr>
<tr>
<td>Sample 2</td>
<td>200</td>
<td>56</td>
<td>1:1</td>
<td>Y</td>
</tr>
<tr>
<td>Control 1</td>
<td>200</td>
<td>56</td>
<td>1:1</td>
<td>N</td>
</tr>
</tbody>
</table>

Table 5: Sample Data for Experiment 2- IAE – Effect of Leaf Type

![Effect of Leaf Type]

<table>
<thead>
<tr>
<th>% increase in solids w.r.t control</th>
<th>%PP/SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fine Pluck</td>
<td>0</td>
</tr>
<tr>
<td>Factory Pluck</td>
<td>7.775</td>
</tr>
</tbody>
</table>

Chart 11: Solids & Polyphenols Measurement – Effect of leaf type - IAE
Discussion

Ultrasound assisted extraction:
Various parameters were tested to see the effect of ultrasound assisted on the extraction of solids from the leaf matrix. The effects of the parameters were also verified on the extraction capabilities of selective solids like polyphenols. The parameters that were tested were solid:liquid ratio, temperature and duration of exposure to ultrasonication.

Effect of parameters on solids content on polyphenols content

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Effect on solids</th>
<th>Effects on %PP/SS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Solid: Liquid Ratio</strong></td>
<td>UAE helped in better extraction of solids at lower limit and upper limit of solid : liquid ratio</td>
<td>Changing the ratio did not have any effect on selective extraction of solids</td>
</tr>
<tr>
<td><strong>Exposure to Ultrasonication</strong></td>
<td>Longer Exposure to Ultrasonication helped in more extraction of solids</td>
<td>Helped in increasing extraction of polyphenols from the cell matrix</td>
</tr>
<tr>
<td><strong>Temperature</strong></td>
<td>Increasing the temperature did not help in improving the extraction.</td>
<td>The presence of heat led to the decrease in the polyphenol content in the ultrasound assisted sample w.r.t control.</td>
</tr>
</tbody>
</table>
**Cost Analysis**

An analysis on the cost effectiveness of the process showed that the use of UAE process as an alternative only for solid extraction was not cost efficient compared to the conventional process. It was found that UAE process used the same amount of energy (kJ) as that of conventional process for every gram of solids extracted. To check if UAE had any added benefit in the sensory area of tea, the color analysis was performed.

**Color Analysis**

The color analysis showed that the UAE sample helped in the improvement of the color by 3 units of a* value (redness measurement using Hunter Lab Equipment). The effect of the color change was verified by doing a polyphenol profiling of the sample.

**Polyphenol Profile Analysis (TR & Catechin Profile)**

TR & Catechin analysis were performed for the samples that showed a color change. From the analysis, it showed that the profile of the catechins and the TR were very similar to the sample with and without ultrasound. There wasn’t much difference in the type of polyphenol that was extracted from the tea matrix with the help of ultrasound.

**Infra Red assisted extraction:**

Various parameters were tested to see the effect of infra red on the extraction of solids from the leaf matrix. The effects of the parameters were also verified on the extraction capabilities of selective solids like polyphenols. The parameters that were tested were intensity of radiation, type of leaf used.
Effect of parameters on solids content on polyphenols content

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Effect on solids</th>
<th>Effects on %PP/SS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intensity of Radiation</strong></td>
<td>Did not help in improving the extraction of solids from the cell matrix</td>
<td>No effect on selective extraction of polyphenols from the cell matrix</td>
</tr>
<tr>
<td><strong>Leaf Type</strong></td>
<td>Better extraction was found to happen in factory pluck over fine pluck</td>
<td>No effect on selective extraction of polyphenols from the cell matrix</td>
</tr>
</tbody>
</table>
4. Conclusions & Recommendations

Conclusions:

- The effect of ultrasound assisted extraction help in better yield of solids and polyphenols than conventional extraction at a specific set of parameters.

- The cost analysis proved that the energy utilized per gram of solids extracted is same with and without ultrasound assisted extraction and therefore better yield using ultrasound assisted extraction was not energy effective.

- Sensory analysis was analysed and ultrasound assisted samples provided an improvement in the color of the samples.

- The profile of the catechins and TR did not vary for the samples with and without ultrasound.

- The intensity of radiation did not help in better extraction of solids from the tea matrix and the leaf type was important in providing better yield of solids. The polyphenol content extracted however remained the same irrespective of the parameters and irrespective of the use of infra red radiation.
**Recommendation**

- A more detailed polyphenol profiling must be performed for the ultrasound samples to check the profiles of the polyphenols extracted due to ultrasonication.

- Other sensory analysis like aroma, taste must be verified to check the sensory benefits of ultrasound assisted extraction.

- A cost analysis similar to that of UAE must be done for Infra red assisted extraction as well.

- A complete profile analysis must also be performed to analyse the type of polyphenols extracted.
5. Appendix

Appendix A

A.1 Folin-Ciocalteau Method for polyphenol measurement

Colorimetric method for measuring Polyphenols in solution using Folin-Ciocalteu reagent:

This method is used to find the total Polyphenolic content in solution by a colorimetric assay using Folin-Ciocalteu phenol reagent.

Principle: The reagent contains phospho-tungstic acids as oxidants, which on reduction by readily oxidized phenolic hydroxy groups yield a blue color with a broad maximum absorption at 765 nm. This is due to the formation of so-called tungsten and molybdenum blues. Gallic acid is used for calibration.

Reagents:

1. Folin-Ciocalteu phenol reagent (10 % volume fraction)
2. Sodium carbonate solution 7.5 % (mass concentration)
3. Gallic acid stock standard solution (100 ppm)

Procedure:

1. For the calibration curve Gallic acid in different concentrations is prepared by adding water.
2. Dilute Folin-Ciocalteu phenol reagent (10%) is added to Gallic acid solutions and the samples which are to be analyzed.
3. After 10 minutes Sodium carbonate (7.5%) solution is added to all the samples.
4. Samples are incubated for 1 hour to allow the reaction to take place between the reagents forming a blue color complex.

5. After 1 hour the samples are analyzed using UV spectrophotometer at a constant wavelength of 765 nm.

6. Calibration curve of concentration of Gallic acid vs. optical density is plotted.

7. The concentration of the Polyphenols is calculated from the optical density values obtained, using the calibration curve of Gallic acid.

A.2 Catechin Measurements using HPLC

High Performance Liquid Chromatography (HPLC)

In liquid chromatography, the separation of analyte molecules is based on their differential partitioning between two non-miscible phases, i.e. the stationary phase and the mobile phase. The stationary phase is either a solid, porous or surface-active material in small-particle form or, more commonly, a viscous liquid immobilized on these particles and is fixed in the system. The mobile phase is a liquid which carries the mixture to be separated.

The method used for calculating the concentration of catechins is called as isocatechin method. The reported values are the peak areas from which concentration can be calculated with the use of the response factor which is calculated keeping caffeine concentration as the standard.

Retention times for different Polyphenols are listed below:
<table>
<thead>
<tr>
<th>Polyphenols</th>
<th>Retention Time (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>4.23</td>
</tr>
<tr>
<td>Epigallocatechin</td>
<td>8.1</td>
</tr>
<tr>
<td>Caffeine</td>
<td>14.9</td>
</tr>
<tr>
<td>Epicatechin</td>
<td>15.9</td>
</tr>
<tr>
<td>Epigallocatechin gallate</td>
<td>17.61</td>
</tr>
<tr>
<td>Epicatechin gallate</td>
<td>21.95</td>
</tr>
</tbody>
</table>

**Table 6: Retention times of different catechins**

**A.3 Gravimetric Method for solids measurement**

A known volume of sample was taken in an empty pan and allowed to evaporate till dryness. The difference in the weight of the pan was measure to calculate the amount of soluble solids in the sample.

1mL of sample was taken in a 2mL eppendorf tube. 1mL of stabilizing solution was added to the sample and then the mixture was centrifuged using a microcentrifuge. The centrifuged samples were then taken in adequate quantities in vials. The vials were placed in the HPLC tray and then tested for catechin content by running the appropriate program in the HPLC.

**A.4 Color Measurements using Hunter Lab equipment**

The hunter lab equipment is set in Total Transmission mode (TTRAN). Standardization was performed by following the instructions provided in the software. Samples containing 0.3g of solids /100mL were prepared by adding the sample to hot water. The samples were then taken inside a cuvette and tested for color (L*, a*, b*). The haze of the sample was also measured in the same process.
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