

Optimization of Polyphenol Content in Green Tea and studying its Antioxidant property

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Summary

The aim of my project is to optimize the level of Polyphenol content present in Green Tea at various temperatures. During the project various combinations of temperatures and time intervals were used to obtain the Green Tea extract and the polyphenol content of all these extracts were estimated. Both unfermented and artificial fermentation conditions were used to obtain the extract. The extracts were further used to screen the various contents of the extract both normally and after purifying the sample with PVP (Polyvinyl pyrrolidone). The extract with the highest content of polyphenols were further used to estimate the antioxidant property after purification with column chromatography.

I. Introduction

Green Tea is obtained from the plant *Camellia sinensis*. This plant is mostly grown in China. The green tea has found its origin in china and japan and have been used since ages by the people out there as a medicinal drink for refreshment as well to treat basic ailments of human body. These days there is an intense research going on in the area of green tea's activity for the treatment of various ailments like high cholesterol level, high blood pressure, atherosclerosis, anti bacterial, anti inflammatory, anti ageing , antioxidant activity etc. It has been found that the polyphenols are the major component of green tea and are responsible for its activity as a medicinal drink. The polyphenol content of green tea is found to be highest as compared to other tea types like oolong, black tea, white tea, yellow tea etc.

It is due to very less oxidation of polyphenols during the green tea making process. Black tea obtained after partial or full fermentation has very less polyphenol content. Low oxidation due to less exposure to sunlight during the green tea processing is responsible for the highest polyphenol content.

The aim of my project is to find out the temperature and time interval at which highest amount of polyphenol can be obtained in the green tea extract. So that it can be used at an industrial scale to obtain green tea highly rich in polyphenols. It will decrease the amount of green tea consumption per day and will increase the efficiency of green tea.

II. Process Plan

1. Green Tea Extracts obtained at various temperatures and time intervals.
2. Concentration of Green tea extracts using the Vacuum evaporator.

3. Estimation of polyphenols by using Folin Ciocalteu's reagent and UV spectrophotometer.
4. Screening of various components present in the green tea extract.
5. Screening of various components present in the green tea extract after treating it with PVP (Polyvinyl pyrrolidone).
6. Purification of green tea extract having highest polyphenol content using column separation.
7. Measuring of antioxidant property using FRAP assay and phosphomolybdenum assay.

Objectives

1. To obtain the green tea extract with highest amount of polyphenols after optimization of temperature and time interval.
2. To purify the green tea extract with highest amount of polyphenols using column separation.
3. To measure the antioxidant activity using FRAP assay and phosphomolybdenum assay.
4. To screen the various components present in the green tea extract.
5. To provide with a method that can be used at an industrial scale to obtain green tea extract with highest amount of polyphenols without much loss.

III. Materials and Methods

1. Green Tea Extract (unfermented):

Materials required: Glass beaker, Magnetic stirrer, thermometer, filter paper, aluminium foil (to cover the beaker), weighing balance, 5gram green tea leaves and distilled water.

Method:

1. 5grams of green tea leaves are weighed and dissolved in 100 ml of distilled water in a beaker.

- The solution is kept for stirring on a magnetic stirrer with around 400 rpm.
- The temperature and time interval are fixed accordingly.
- The extract is then filtered using filter paper.

- The supernatant is separated from the pellet.
- To the pellet 10 ml of 70 % acetone is added and the pellet is dissolved.
- The supernatant and the acetone dissolved sample is estimated and screened.

Various temperatures and time intervals used are as follows:

Temperature (in °C)	Time Interval (in hours)
60	1
70	1
50	1
55	1
55	2
Room temperature	Overnight

Estimation of polyphenols in various green tea extracts:

SOP used: SOPA0016

2. Green tea extract(Artificially fermented):

Materials used: Beaker, yeast broth, 5 grams green tea leaves, shaker.

Method:

- 5 grams of green tea leaves are weighed and are added to 100 ml of distilled water (sterilized).
- 1 ml of yeast broth is added to the sample in the laminar flow.
- The solution is kept in shaker overnight.
- Next day optical density is measured using UV spectrophotometer.

3. Screening of components present in green tea extract :

SOP No for flavonoids: SOPA0059

SOP No for alkaloids: SOPA0057

SOP No for Phenols: SOPA0053

SOP No for Saponins: SOPA0081

SOP No for Phytosterols : SOPA0085

SOP No for Phlobatannins: SOPA0087

5. Screening of components present in green tea extract purified with PVP :

SOP No for flavonoids: SOPA0059

SOP No for alkaloids: SOPA0057

SOP No for Phenols: SOPA0053

SOP No for Saponins: SOPA0081

SOP No for Phytosterols : SOPA0085

SOP No for Phlobatannins: SOPA0087

6. Extraction of polyphenols using PVP:

Method:

- The aqueous green tea extract is concentrated using vacuum evaporator.(final volume 10 ml)
- To the concentrated extract 3 % PVP is added and dissolved.
- The sample is then centrifuged at 5000 rpm for 10 mins.

7. Purification of green tea extract using column separation:

Materials required: Separating column, 3 grams of silica gel, 30 ml solution containing ethanol and water (1:1) and 2 ml of green tea extract.

Method:

- 3 grams of silica gel is weighed and added to the separating column.
- Around 5 ml of ethanol and water solution is added to pack the column.
- 2 ml of sample is added followed by the addition of 2 ml of mobile phase.
- Four fractions each containing 4 ml are obtained by the subsequent addition of mobile phase to the column.

8. Estimation of antioxidant property of the four fractions obtained:

SOP No for FRAP assay: SOPA0050

SOP No for Phosphomolybdenum assay: SOPA0093

IV. Results and Interpretation

1. Polyphenol content in various green tea extracts:

Temperature(in°C)	Time (in hours)	Polyphenol %
50	1	66
60	1	51
70	1	49
55	2	72
Room Temp	Overnight	72
Fermented	Overnight	27
Room temp	Overnight(with PVP)	64

2. Screening of components of green tea extract(without PVP):

Component	Result
Flavonoids (NaOH test)	Positive
Flavonoids (lead acetate test)	Positive
Saponins	Positive
Phytosterols	Negative
Phenols	Positive
Phlobatannins	Negative
Alkaloids	Negative

3. Screening of components of green tea extract (with PVP):

Component	Result
Flavonoids (NaOH test)	Positive
Flavonoids (lead acetate test)	Positive
Saponins	Negative
Phytosterols	Negative
Phenols	Positive
Phlobatannins	Negative
Alkaloids	Negative

4. Estimation of antioxidant property of green tea extract fractions using FRAP assay:

FRAP assay was done but satisfactory results were not obtained. It was followed by Phosphomolybdenum assay.

5. Estimation of antioxidant property of green tea extract fractions using Phosphomolybdenum assay:

The first two fractions were found out to contain large amount of tannins and catechins as compared to the other two fractions. It shows that the purification resulted in the accumulation of tannins and catechins in the first two fractions.

V. Conclusion or future perspectives CONCLUSION

The optimization of temperature and time interval for maximum polyphenol content in the Green tea extract was achieved. It was observed that the maximum polyphenol content is achieved when the extract is obtained at 55°C for 2hrs and at room temperature for overnight. Further investigation is required to get more accurate results.

The green tea extract showed antioxidant activity which was confirmed by Phosphomolybdenum assay. Further investigation is required to separate out the compounds responsible for the antioxidant activity.

FUTURE PERSPECTIVE:

Green tea extract is found out to provide vast range of therapeutic benefits due to the presence of wide range of polyphenols. Further investigation is required to find out the activity of various polyphenols and the minimum amount of polyphenols required to achieve the therapeutic effect. Formulations containing a discrete amount of polyphenols are needed to be produced at an industrial scale.

The anticancer activity of polyphenols need to be studied in detail.

Appendix

No. of Tables: 4

No. of Figures: 0

No. of Words:500

No. of SOP followed: 8

No. of SOP Prepared: 2

No. of Chemical Safety Date Sheet referred: 2

References

Articles:

- [1.] Tea polyphenols: prevention of cancer and optimizing health1–3
- [2.] HasanMukhtar and Nihal Ahmad
- [3.] Green Tea Polyphenols and Metabolites in Prostatectomy Tissue: Implications for Cancer Prevention

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- [1.] Evans, John C. *Tea in China: The History of China's National Drink*. Greenwood Press, 1992. ISBN 978-0-313-28049-8
- [2.] Lam, K.C./Lam, K.S. *The Way of Tea: The Sublime Art of Oriental Tea Drinking*. Barron's Educational Series, 2002. ISBN 978-0-7641-1968-2

External links:

- [1.] [Green Tea \(an overview from the University of Maryland Medical Center\)](#)
- [2.] [Green Tea's cancer fighting potential \(audio interview\)](#)
- [3.] [WebMD review about the health benefits of green tea](#)