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Research Article

BIO DECAFFEINATION-A STUDY ON THE EFFECTS OF *BREVIBACTERIUM* ON DIFFERENT SAMPLES OF COFFEE, TEA AND COLA CONTAINING CAFFEINE

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Abstract

HPLC analysis of caffeine was performed in SHIMADZU LC 20 – AD system, and the caffeine compounds were separated on a C18 column under isocratic conditions with 40% methanol in water at a flow rate of 1.0 ml/min. Compounds eluting from the column were detected and the peak areas were compared with those obtained with standards of known concentration. The HPLC analysis of caffeine degradation by *Brevibacterium* is done by injecting the sample volume of about 20µl HPLC analysis is done for the sample at different incubation periods with standard caffeine concentration (Known). The sample is analyzed for every twelve hours of incubation and peak values are obtained. Caffeine concentration is an important parameter to be checked as excessive consumption of caffeine leads to many health hazards.

Keywords: , Biodecaffeination, Brevibacterium, HPLC.

Introduction

Caffeine

Caffeine is found in about a hundred species of plants, but the most highly cultivated sources are the coffee beans, (*Coffea arabica* or *Coffea canephora*, variety *robusta*), the leaves & leaf-buds of tea (*Thea sinensis* or *Camellia sinensis*), cola nuts (*Cola acuminata*) and cocoa beans (*Theobroma cacao*).Coffee and tea plants are the major sources of natural caffeine and related compounds such as theophylline and theobromine [1]. A very large proportion of the non-alcoholic beverages used in social settings contain caffeine. The most important beverages and foods containing caffeine are coffee, tea, cola nuts, cola drinks, cocoa, and chocolate. The present study employs different coffee samples to test the bio-decaffeinating effect. Caffeine is often the primary focus when the negative health effects of coffee are discussed.

Implications of Decaffeination Methods

The decaffeination process itself is not always innocuous. There are three common decaffeination methods: the use of one of two organic solvents, either methylene chloride or ethyl acetate; water extraction known as the Swiss water process or European water process; and supercritical carbon dioxide. Eighty percent of decaffeinated coffee is processed with solvents. The health effects of these solvents as found in decaffeinated coffee are not well known, but studies suggest that methylene chloride (dichloromethane) is shown to be carcinogenic, and the National Cancer Institute's list of chemicals labels it as a possible human carcinogen. In the decaffeination process, the solvents are removed from the coffee beans, but residues have still been found in decaffeinated coffee and tea. When water and carbon dioxide are used to decaffeinate coffee, a measurable residue is not left behind in the remaining beans, but

high acidity and other phytochemicals found in coffee remain. Additionally, in the process of water extraction, unique flavor characteristics of coffees from different origins are blended and blurred water extraction due to the intermingling of the flavors from various types of beans in the water bath. Among the decaffeination methods, methylene chloride extraction retains the most flavors but leaves a dry taste in the mouth; both water extraction and CO2 extraction blur the flavor of the beans and ethyl acetate adds a sweet fruit flavor. Additionally, inferior beans that may be old or moldy are often used for decaffeination because the process can remove off flavors and mask the age or condition of the beans.

Biodecaffeination: A natural route of Decaffeination

Current studies suggest that, for people who are sensitive to coffee's effects, decaffeinated brews may still exacerbate their health problems Biodecaffeination can be defined as the removal of caffeine from coffee, tea and other caffeine containing materials by the action of externally added microbial cells or enzymes [2]. The concept of biodecaffeination is a relatively new area of decaffeination and there is a growing interest in this area of biotechnology due the advantages it offers like being environmentally safe, economical and in preserving the quality of the beverages [3]. Development of biological or enzymatic methods of decaffeination demands a deep understanding of the caffeine metabolism in microbial systems. Detailed information on different enzymes involved in the degradation of caffeine in different organisms could help in developing an enzymatic process for caffeine removal [4].

According to FDA guidelines, decaffeinated coffee must have 97% of the caffeine removed. In actuality, the caffeine content of coffee beans varies widely; therefore the caffeine content of decaffeinated coffee also fluctuates, and can be 10mg or more per 12 ounce cup. Other than biodecaffeination, alternative methods fail to live up to the standards.

The present investigation is a preliminary attempt to check the effect of the isolated strain of *Brevibacterium* on different coffee samples with reference to caffeine degradation. An HPLC analysis of standard caffeine is checked out to compare the effect of caffeine degradation by *Brevibacterium* on different coffee samples. 10 different samples are screened for caffeine degrading efficiency by *Brevibacterium.*

Materials and Methods

Amplification of the caffeine tolerant bacteria

Solid screening medium (SSM) for amplifying the caffeine-tolerant bacteria was prepared by mixing the mineral solution with caffeine (2.5 g/ L) and agar (1.5%) and autoclaved at 121°C for 10 min. Liquid amplifying medium (LAM) was obtained after addition of caffeine (0.5 g /L) and sucrose/glucose (5.0 g /L) in the mineral solution and disinfection. The amplified bacteria were used for further studies of biodecaffeination.

Sample Analysis

The samples of Coffee of different manufacturers, teas and colas were procured from the local market and labelled Caffeine Samples CS3 to CS12. Samples 1 & 2 were standard caffeine procured from Himedia and Aldrich.

The Samples were sterilized and a loopful of actively growing culture was inoculated and incubated on a shaker at 150rpm at a temperature of 30± 2^{o}C for 96 hours. All the above processed samples were drawn at 12 hours intervals and the growth was recorded as an increase in the biomass by weight. Caffeine degradation was followed by HPLC analysis of the residual caffeine present in the medium.

Estimation of methylxanthines by high performance liquid Chromatography (HPLC)

HPLC analysis of caffeine was performed after incubation with *Brevibacterium* for 48 hours in a Shimadzu LC 10 A- HPLC System, and the methylxanthine compounds were separated on a C18 ODS-Luna column under isocratic conditions with 15 % acetonitrile in water at a flow rate of 1.0 ml/min. Compounds eluting from the column were detected at 273 nm, and the peak areas were compared with those obtained with standards of known concentration.

The percentage of caffeine degradation with residual caffeine was obtained by the following calculation.

Area of Sample in chromatogram at RT x Standard weight and dilution X Dilution factor X Sample 0.25 g make up to 200mL X 100/ Area of Standard Caffeine at RT (5.626) X 100 X 100 X 0.25.

Results and Discussion

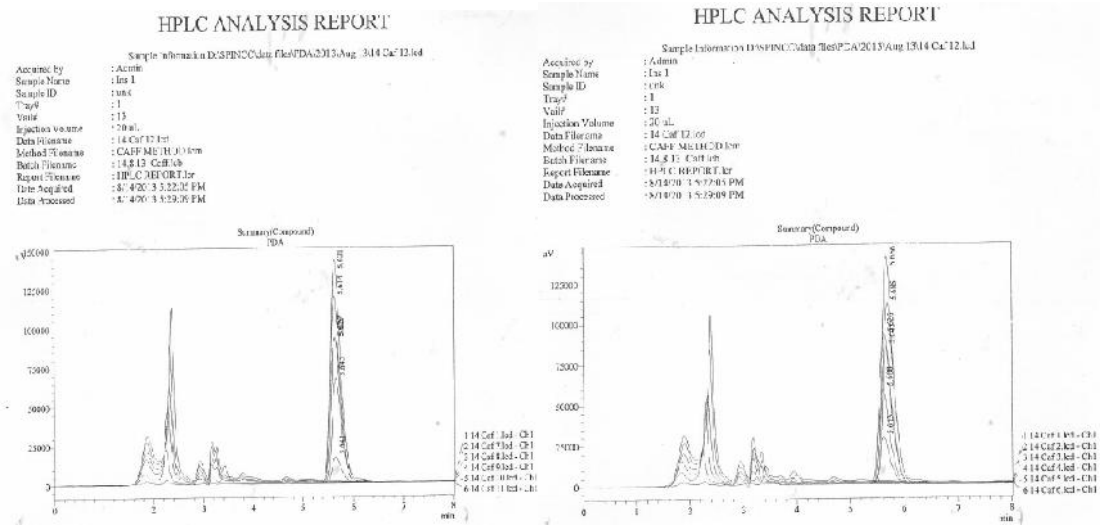
Estimation of methylxanthines by high performance liquid Chromatography (HPLC)

The degradation of caffeine was analyzed and found as 82.6, 64.42, 45.84, 11.06 and 5.92 with coffee

sample (CS) 3 to 7 respectively (Table 1) and the degradation of caffeine was analyzed and found to be 80.07, 59.77, 41.37, 20.30 with CS 8 to 12 respectively.

.**Table 1 HPLC tables showing percentage of caffeine degradation**

SAMPLE NAME	SAMPLE VOLUME	RETENTION TIME	AREA	PEAK HEIGHT	%OF CAFFEINE REDUCED
CAFFEINE STANDARD 1	20µl	5.626	1501220	92197	84
CAFFEINE STANDARD 2	20 µl	5.621	1904197	141389	79
CS 3	20 µl	5.614	1566822	118725	82.6
CS 4	20 µl	5.627	1221664	92319	64.42
CS 5	20 µl	5.645	869130	66704	45.84
CS 6	20 µl	5.641	214755	16605	11.06
CS 7	20 µl	5.637	118936	8239	5.92
CS 8	20 µl	5.685	1574250	111111	80.07
CS 9	20 µl	5.645	1174769	84847	59.77
CS 10	20 µl	5.608	813148	58871	41.37
CS 11	20 µl	5.613	402884	29250	20.30
CS 12	20 µl	5.630	173009	12359	8.81



The Coffee samples responded better than teas and colas as there might be polyphenol interactions from the other two samples. CS 3 and 8 responded better to biodecaffeination with almost 80% caffeine reduced. CS 7 and CS12 were found to respond biodecaffeination the least with 5.92 and 8.81 % caffeine reduced.

Conclusion

The isolate *Brevibacterium* being reported is an efficient caffeine degrader, which may prove useful in the development of an environmental friendly biodecaffeination process.

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