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Research Article

COMPARATIVE STUDIES ON CURCUMIN CONTENT IN FRESH AND STORED SAMPLES OF TURMERIC RHIZOMES

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ABSTRACT
Turmeric or Indian saffron contains curcumins as triterpenoidal derivatives. Turmeric rhizomes are used in India since ancient time for anti asthmatic, antidiabetic, chronic fever, antiseptic, wound healing etc. Rhizomes are mostly cultivated in Sangli district which is today known as saffron city. The cultivated rhizomes are traditionally prepared by and stored in pit holes ('PEV') made in ground near by Haripur village. The pits are made under the ground about 20-25 feet deep and 15-18 feet diameter and smeared with mud. The claims made by traditional suppliers that "pev" stored turmeric fetches more economic prize compare to air or sun dried and normal stored rhizomes. It was important to evaluate the local claim and find out the exact causes for the preservation of turmeric rhizomes in Pev. In the present work we had analyzed the soil sample from Haripur region located on the banks of Krishna river. The samples of rhizomes were collected from Pev owners after every six months and were inspected for insect or fungal attacks and compared with local marketed samples. The total curcumin content was estimated by simple spectroscopic method using methanolic extract of different samples of rhizomes. The soil content of the Haripur region showed presence of common mineral salts along with silicates and selenium which has antioxidant activity. The linearity of calibration was obtained with coefficient 0.9997. It was found that curcumin content varies from fresh to stored rhizomes (3.426 ±1.42 SD to 5. 784 ±1.32 SD) up to 2.5 years. After 3 years samples showed decrease in curcumin content (3.186 ±1.012 SD). This confirms the traditional and local claims about storage conditions and also the prevention of the rhizomes from the microbial attacks.
KEYWORDS: Turmeric rhizomes, Curcumin, Spectroscopic method.

INTRODUCTION

Turmeric (*Curcuma longa*) is a rhizomatous herbaceous plant of the family Zingiberaceae. It is native to tropical South Asia and needs temperatures between 20-30° C and a considerable amount of annual rainfall to thrive. Plants are gathered annually for their rhizomes and re-seeded from some of those rhizomes in the following season. The rhizomes are boiled for several hours and then dried in hot ovens, after which they are ground into a deep orange-yellow powder commonly used as a spice in curries. Turmeric known as Indian saffron, since it is widely used as an alternative to the far more expensive saffron spice. Sangli, a town in the southern part of the Indian state of Maharashtra, is the largest and most important trading centre for turmeric in Asia or perhaps in the entire world. In Ayurvedic practices, turmeric is thought to have many medicinal properties and in many South Asian countries (particularly India) use it as a readily available antiseptic for cuts, burns and bruises. Practitioners of Ayurvedic medicine say it has fluoride which is thought to be essential for teeth. It is also used as an antibacterial agent. It is taken in some Asian countries as a dietary supplement, which allegedly helps with stomach problems and other ailments. It is popular as a tea in Okinawa, Japan. Pakistanis also use it as an anti-inflammatory agent, irritable bowel syndrome. In Afghanistan and North West Pakistan, turmeric is applied to a piece of burnt cloth, and placed over a wound to cleanse and stimulate recovery. Indians, in addition to its Ayurvedic properties, use turmeric in a wide variety of skin creams that are also exported to neighboring countries. It is currently being investigated for possible benefits in Alzheimer's disease, cancer and other disorders. It is only in recent years that Western scientists have increasingly recognized the medicinal properties of turmeric. In the latter half of the 20th century, curcumin was identified as responsible for most of the biological effects. The reports in Wall Street Journal claims the sales of curcumin increased 35% and the U.S. National Institutes of Health had four clinical trials underway to study curcumin treatment for pancreatic cancer, multiple myeloma, Alzheimer's, and

colorectal cancer. Turmeric is currently used in the formulation of some sunscreens. Turmeric paste is used by some Indian women to keep them free of superfluous hair. Turmeric paste is applied to bride and groom before marriage in some places of India, Bangladesh, and Pakistan, where it is believed turmeric gives glow to skin and keeps some harmful bacteria away from the body. The government of Thailand is funding a project to extract and isolate tetrahydrocurcuminoids (THC) from turmeric that might have antioxidant and skin-lightening properties and might be used to treat skin inflammations, making these compounds useful in cosmetic formulations. Turmeric contains up to 5% essential oils and 3% curcumin. It can exist at least in two tautomeric forms, keto and enol. The keto form is preferred in solid phase and the enol form in solution. Curcumin [1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] is the major yellow pigment extracted from turmeric a commonly used spice, derived from the rhizome of the herb Curcuma longa Linn[1]. It is a naturally occurring polyphenolic phytochemical currently being examined in preclinical trials for cancer chemo protective drug development, with pharmacological actions that including antioxidant[1,2] anti-inflammatory[3,4] and cancer chemo preventive actions.[5–7] In the present work we had analyzed the soil sample from Haripur region located on the banks of Krishna river. The samples of rhizomes were collected from Pev owners after every six months and were inspected for insect or fungal attacks and compared with local marketed samples. The total curcumin content was estimated by simple spectroscopic method using methanolic extract of different samples of rhizomes.

MATERIAL AND METHODS

The turmeric rhizomes were collected from pev owners of sangli and also from the local market. Samples were collected after every six months and authenticated from botany department Shivaji University Kolhapur for correct species. Collected samples were powdered using electric blender every time and fine powder (# 180) was used for extraction of curcumin. Extracted sample were estimated for total curcumin content by simple spectroscopic method using double beam spectrophotometer (Jasco V-530). All the reagents and chemicals used for extraction and estimation were of A.R. grade (Loba Chem.)

Extraction of curcumin from turmeric powder

50g of each sample turmeric powder was extracted with 95% alcohol in soxhlet assembly until all the coloring matter was extracted completely. Alcoholic extract was distilled of to a semisolid brown colored mass (about 4.5%). The crude extract was then dissolved in 50 ml of benzene and extracted twice with equal volume of 0.1% sodium hydroxide solution. The alkaline extracts were combined and acidified with dilute hydrochloric acid. A yellow colored precipitate was formed. It was allowed to settle for 15 min. After setting of precipitate the extract was concentrated by boiling on water bath and at the same time dissolving the ppt. in boiling water. During this process of boiling the resinous material was agglomerated and lumpy mass was formed. The solution was filtered in hot condition and the filtrate was concentrated to very small volume and finally cooled to get curcumin (1.5%). The product was recrystallized using 95% alcohol[8].

Spectrophotometric estimation

Preparation of sample 1g of powder sample was extracted with methanol (25mlx4) over steaming water bath separately for each sample. The extracts were filtered and volume was made up to 100 ml with methanol[9].

Preparation of standard 0.10 mg /ml solution of curcumin reference standard (Yucca Enterprises, Mumbai) was prepared in methanol. 1ml of this stock solution was diluted to 10ml which gives 10µg/ml of working standard solution.

Calibration curve A UV-Visible spectrophotometer (Jasco V-530) was employed in the assay procedure. Standard curve was obtained using 1.4 – 2.4ml of working standard solution was further diluted to 10ml with methanol. Methanolic extracts of different test samples were diluted and absorbance at 430nm was recorded against methanol as blank (Table.No.1). Dilutions were made suitably so that the absorbance lies between 0.1-1.0. Curcumin content in different sample was calculated using linear regression equation of calibration graph (Fig.No.1) plotted between concentration and absorbance. The equation for curcumin was Abs = A + B * Conc. Where A= 0.0055 and B= 0.1379 with correlation coefficient 0.9997 (Table No.2). Curcumin content in test sample was expressed as % of total curcumin considering dilution factors (Table No.3 and Fig.No.2)

Different dilutions were made for each sample solution and concentrations were adjusted within the beer limit and concentration of curcumin was determined using standard curve and dilution factor. All the samples were obtained freshly and marketed samples were obtained as per requirement.

RESULT AND DISCUSSION

From the above experiment it was confirmed that storage of turmeric in traditional manner helps in prevention of rhizomes from microbial and fungal attack. The soil content from Haripur region might be responsible for preservation of the rhizomes. It was also found that up to thirty months the curcumin content increases (5.784±1.32) compare to fresh rhizomes and after that it was found decreased (3.186 ±1.012) in the curcumin content well below the fresh rhizomes (3.426 ±1.42). Maximum 2.358% increase in curcumin content was observed in three months old rhizomes while minimum 0.448% in six months old rhizomes. Also there was decrease in (-0.240%) curcumin content was observed at 36 months.

CONCLUSION

From the above study it is confirmed that traditional storage of turmeric rhizomes contains higher amount of curcumin compare to fresh rhizomes. It was also confirmed that soil composition also play important role in preservation of rhizomes. The curcumin content was increased up to 30 months and then gets decreased hence it is better to use the rhizomes before thirty months. Further study is necessary to find exact soil parameter responsible for the preservation of rhizomes.

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Table No.1 Absorption of standard curcumin at 430 nm

Sr.no	Concentration in µg	Absorbance*
01	1.4000	0.1972
02	1.6000	0.2270
03	1.8000	0.2544
04	2.0000	0.2827
05	2.2000	0.3080
06	2.4000	0.3360

* Average of six determinations

Table No.2 Optical characteristics for colorimetric method

λ_{max}	430 nm
Beer's law limit (µg ml^{-1})	1-5
Regression equation data	
Slope	0.0055
Intercept	0.1379
Correlation coefficient (r^2)	0.9997

Expression: Abs = A + B * Conc.

Table No.3: Curcumin content in fresh and stored samples

Sr.no.	Sample	% Curcumin content ± SD*	% change in curcumin content
01	Fresh	3.426 ±1.42	--
02	Six months	3.874 ±1.38	0.448
03	Twelve months	4.186 ±1.22	0.760
04	Eighteen months	4.539 ±1.34	1.113
05	Twenty four months	5. 178 ±1.35	1.752
06	Thirty months	5. 784 ±1.32	2.358
07	Thirty six months	3.186 ±1.012	-0.240

* Average of six determinations ± standard deviation.

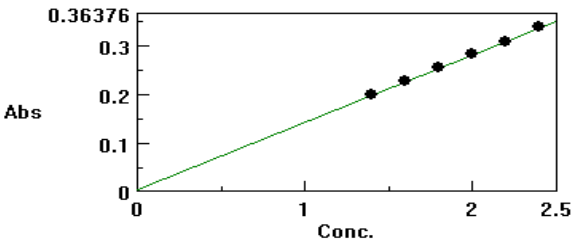

Fig.No.1: Calibration curve for standard curcumin

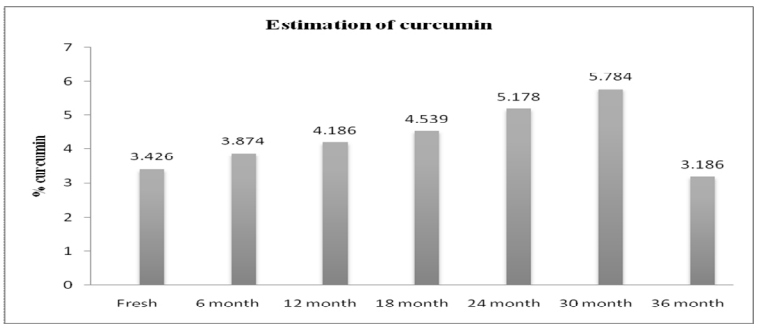

Fig.No.2: % curcumin in fresh and stored samples