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Influence of climatic conditions on curcumin content and colour values of turmeric

Abstract

In this work, Aleppey finger turmeric rhizomes are collected from the Spices Board distribution center Kozhikode, Kerala and are planted in the five selected villages of central Kerala having different climatic conditions. Similar manuaring conditions are employed. After harvesting the collected rhizomes are dried under similar conditions and methods. All samples are extracted using solvents Acetone, Ethyl Acetate and Methanol using Soxhlet extraction. The moisture content of all the samples is determined using Dean-Stark apparatus and are compared. The colour value is determined using UV spectrophotometer. The yield and color values are compared. The total curcumin content and the percentages of Curcumin, Dimethoxy curcumin and Bisdemethoxy curcumin are determined using HPLC (High pressure liquid chromatography) and are compared.

Keywords: spectrophotometer, curcumin, physiological, flavor, antimicrobial effect, turmeric fingers, yellow colour

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Introduction

Spices are a group of esoteric food adjuncts, which have been in use for thousands of years. Some of the spices are also known to possess several medicinal properties[1] and are effectively used in the indigenous systems of medicine. The physiological effects of spices in most instances have been attributed to the main spice active principles present in them. Among the physiological influences the spices are documented to exhibit, their hypolipidemic and antioxidant properties have far-reaching health implications.[2] Turmeric is a perennial herb having 60 to 90cm height and it belongs to the family Zingebereceae and genus Curcuma. Curcuma has 42 species of which Curcuma longa is commercially cultivated.[3] Turmeric is grown for its essential oil and yellow colour. Essential oil (turmerol) imparts aroma and flavour, whereas the yellow colour is due to curcumin. Turmeric has been prized as a dye for centuries.[4] Total production of turmeric in India was 3, 03166 metric tonnes during 2004-2005.[5] Turmeric (Curcuma longa L.) belongs to the Zingiberacea family reported to possess numerous medicinal properties including antioxidant activity, anti-protozoal activity, anti-tumour activity, anti-inflammatory activity and antivenom activity.[6–10] Due to the presence of curcumin as an active constituent and the volatile oils turmeric is widely used as a medicine for the treatment of many ailments. It is vastly used as an anti-inflammatory agent.[11] And hence also shows antioxidant property which is even better than the vitamin and E.[12] The rhizome extract had been studied for its anticancer activity. The other activities shown are antimicrobial effect (against bacteria, fungi).[13] Turmeric lowers the cholesterol level and inhibits platelet aggregation and hence shows cardiovascular effects.[14] Turmeric has also proven to be effective for the brain conditions like Alzheimer's and therefore India could be considered to be resistant or having less percent of Alzheimer's patients.[15] In the present work Aleppey finger turmeric rhizomes are collected from the Spices Board distribution center Kozhikode, Kerala and are planted in the five selected villages of central Kerala having different climatic conditions. The soil samples are analyzed and the various soil parameters are compared. Then, effect of climate on curcumin content and its color values are studied in detail.

Materials and methods

Aleppey turmeric fingers are collected from the spices board Kozhikode. Turmeric fingers are planted in all the five selected villages by the flat bed method. (Sample1: From Aikkaranadu village of Kunnathunadu taluk, Sample 2: From Valakam village of Muvattupuzha taluk, Sample 3 : From Mannamkandam village of Devikulam taluk, Sample 4 : From Konnathady village of Idukki taluk, Sample 5: From Thekkoy village of Meenachil taluk). Immediately after planting mulching finished. Mulch is a cover to the soil surface. Mulching was done by coconut leaves, jack tree leaves, plant residues etc. The effect of mulch is complex. Reduction in soil water loss occurs not only because the mulch acts as barrier preventing loss, but also because the soil radiation balance and its thermal regime are usually altered, thus influencing the evaporation rate at the surface. The soils are frequently irrigated and the weeds are removed by hand by plucking. Cow dung and wood ash are used as the additional manures. Harvesting from all the cultivated areas was done. Before harvesting the soil is first loosened with a small digger, and clumps are manually lifted. Rhizomes are cleaned by soaking in water. Curing and drying of Turmeric Rhizomes:-Turmeric rhizomes are cured before drying. Curing involves boiling fresh rhizomes in water until soft before drying. Boiling destroys the vitality of fresh rhizomes, reduces drying time and yields a uniformly colored product.[16] Turmeric rhizomes collected from all the five places are separately boiled in copper vessels with water just enough to soak them and is according to the traditional method. Boiling is stopped when froth comes out, with release of white fumes having typical turmeric aroma. The cooking lasts for about 40 minutes. Then the rhizome's are tested by pressing with fingers. Optimum cooking is attained when the rhizome's yields to finger pressure and can be perforated by a blunt piece of wood. The cooked rhizomes are allowed to cool gradually and spread out to dry in the open in 5-6 cm thick layers on uncoated plain bamboo mats. The rhizomes from all the five places are dried separately. The rhizomes are turned over intermittently to ensure uniformity in drying. During night time they are heaped and are covered with plastic sheet. It takes twelve days for the rhizomes to become completely dry.

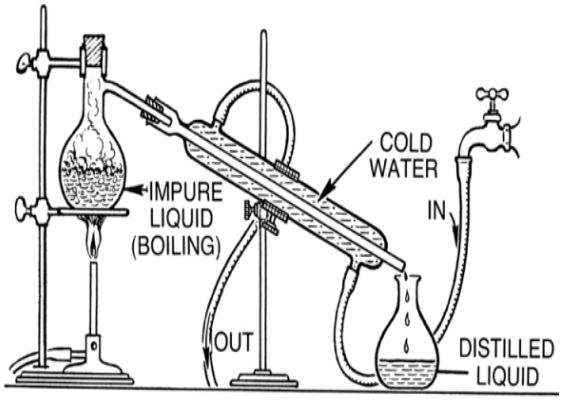

Figure 1 Isolation of oleoresin by distillation method.

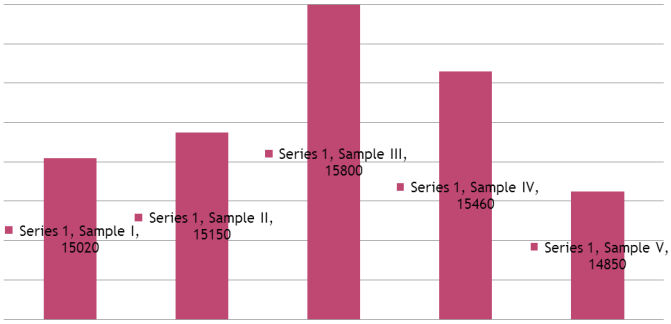

Figure 2 Curcumin colour values of the various samples.

Characterization

The following quantitative and qualitative analysis are carried out using dried turmeric rhizomes collected from various villages:

I. Sample Preparation: Dried turmeric rhizomes are powdered in a mixer grinder without undergoing excessive heating. The sample is then mixed well for 3-5 minutes until the sample is uniform.

II. Determination of Moisture Content of Turmeric Samples by Dean- Stark Method: 10g of sample is accurately weighed out in to a 250ml standard flask.

Add 75ml of toluene sufficient to cover the sample and add 3-4 boiling chips into it. Assemble the apparatus and fill the trap with toluene by pouring it through the condenser until it just fills the trap. Insert a non-absorbing cotton wool into the top of the condenser to prevent condensation of atmospheric moisture in the condenser. Give water connection through the condenser. Heat to boil and reflux at the rate of 2 drops per second for about 1 hour until all the water has been collected in the trap. Towards the last stage the rate of drops increased to four drops per seconds. Stop heating and rinse the condenser carefully with 5ml of toluene. Continue refluxing for 5 minutes, stop heating, allow the trap to cool to room temperature and note the volume of water.[17]

Moisture % = Volume of water in ml X100 / Weight of sample in g

Extraction of turmeric oleoresin

About 10g of the sample is taken in the extraction thimble containing a piece of cotton at the bottom. It is then placed in the inner tube of the Soxhlet apparatus. The apparatus is then fitted to a 250ml round bottom flask containing about 150ml methanol and boiling chips, and to a reflux condenser. The assembly is then placed in a heating mantle and started heating. Reflux continuously for 6hours. When the methanol starts boiling, the vapors' rises through the left side arm into the condenser where it liquefies. The condensed solvent drips into the thimble. The hot solvent begins to fill the thimble and extracts the oleoresin from the sample. Once the thimble is filled with solvent, the side arm on the right acts as a siphon and the solvent which now contains the oleoresin drains back into the round bottom flask. The process is repeated automatically for 6 hours continuously. The extraction is also carried out using solvents Ethyl Acetate and Acetone.[18]

Isolation of oleoresin by distillation method

The round bottom flask containing the solvent along with the extracted oleoresin is attached to a condenser. The whole assembly is then placed in a heating mantle and started heating. The solvent starts boiling and the vapor's begin to rise through the neck of the round bottom flask. As the vapors pass through the condenser, they will condense and drip into the collection receiver. The process is continued until almost all the solvent is removed from the oleoresin mixture. Then the remaining mixture is quantitatively transferred to a previously weighed petridish. It is then placed a hot air oven until all the solvent is vaporized. It is then again weighed. The difference weight gives the yield of oleoresin extract.[19] Determination of the colour value of the Turmeric

Colour value of turmeric is determined by spectrophotometric method (IS :10925: 1984).Weigh accurately 0.1 g of turmeric powder in to a small beaker. Transfer in to a 100ml standard flask and made up to the mark with 95% ethyl alcohol. Pipette 10ml of the solution into another 100ml flask and made up with ethyl alcohol. Measure the absorbance of the extract at 425nm in 1cm cells against ethyl alcohol blank.[20]

Colour value = absorbance x 1000

Crystallization of curcumin

50 ml of turmeric sample are extracted with 250ml of ethyl acetate in a column. Solvent is distilled off. Then again washed with little ethyl acetate. Concentrate the solution and kept for 3 days. Curcuminoids crystallizes. Filtered using vacuum pump. Washed with little solvent. Dried in an air oven. It is repeated for all the samples.[21]

Determination of colour value of curcumin

0.05g of curcumin is accurately transferred in to a 100ml stand flask. Made up to a 100ml stand flask. Made up to the mark using ethyl alcohol. From the solution 1ml is pippeted out and transferred to a 100ml standard flask. Again made up using ethyl alcohol. The absorbance of the solution is determined at 425nm using alcohol blank in a spectrophotometer.

Determination of curcumin content in turmeric samples

The curcumin content in turmeric samples are determined spectrophotometrically (ASTA method, ASTA 4th M 18.0). Accurately 1gm of the sample is dissolved in 100ml acetone. Reflux in a water condenser for 1 hour. Cool and transferred quantitatively to a 200ml flask and made up to the mark.1ml of this solution is pipette out in to a 100ml flask and made up to the mark. The absorbance is then measured at 425nm.

% of curcumin content = 200 x absorbance x100/wt. of sample x1650

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Determination of colour values of turmeric oleoresin spectrophotometrically

Weigh accurately 0.1 g of the turmeric oleoresin sample prepared into a small beaker transfer into a 100 ml volumetric flask with 95% ethyl alcohol. Made up to the mark. Pipette 10 ml of this solution into another 100 ml volumetric flask. Made up to the mark with 95% ethanol. Measure the absorbance of the extract at 425 nm in 1 cm cells against alcohol blank.

Colour value of turmeric oleoresin = absorbance x 1000

Estimation of curcuminoids by HPLC (high pressure liquid chromatography)

The HPLC analysis was carried at Neogen Food and Animal Security (India) Private limited Ltd, Ernakulam. The HPLC analysis was performed using model Agilent 1260 with the UV detector. A CAPCELL (C-18) HPLC packed column (4.6mm I.D. x250mm), type MG 5µm number AKAD/05245 was used for chromatographic separations. The mobile phase consist of acetonitrile and a buffer 0.05M KH_2PO_4 in the ratio 60:40 .The pH is 7. The separation was performed using isocratic elution (0-10 min) with a flow rate of 0.8 ml/min and the column temperature is 30^0C.Tge injection volume was 20µl. UV detection was carried out at 424nm. Standard curcumin was obtained from sigma chemicals Germany. All other chemicals and reagents used were from E-Merck and of analytical grade.

Results and discussions

The Soil collected from various villages are analyzed for pH, salinity, moisture content, carbonates and bicarbonates, calcium and magnesium, organic carbon, nitrogen, phosphorous and potassium content. The data obtained are given below. The pH of the soil is highest in Kunnackal village and lowest in Aikkaranadu village. The soil collected from Aikkaranadu village has high moisture content and is lowest in the soil collected from Thekkoy village. The calcium and magnesium contents are highest in Kunnackal village and is lowest in Thekkoy Village. The percentage of organic carbon is highest in Konnathady village and lowest in the soil collected from Mannamkandam village. The nitrogen content is almost same in all the villages, but a little bit high in Konnathady village. The available Phosphorus content is highest in the soil collected from Thekkoy village. Potassium content is highest in the soil collected from Aikkaranadu village .Of the above villages Mannamkandam and Konnathady are having the average temperature less than 25^0C and are situated in the Western Ghats. Both the places are above 2000mts above sea level. The soil parameters have only little effect on the colour and curcumin content of turmeric. It may affect the yield of turmeric. The colour values of turmeric cultivated in Mannamkandam and Konnathady villages are higher. The amount of demethoxy and bisdemethoxy curcumins are also higher in the samples collected from these villages. The colour value of curcuminoids are also higher in the samples from these places. The low temperature and higher altitude in these regions may affect the colour value and the composition of curcuminoids.[22–24]

Table 1 Moisture percentage of various samples

Turmeric sample	Moisture %
Sample 1	13.214
Sample 2	15.354
Sample 3	12.057
Sample 4	14.398
Sample 5	12.008

% of yield of oleoresin=Weight of extract in g X100 /Weight of sample in g

Turmeric sample	% yield of oleoresin using ethyl acetate solvent	%yield of oleoresin using acetone solvent	% yield of oleoresin using methanol solvent
Sample 1	11.95	12.12	12.52
Sample 2	12.03	12.29	12.52
Sample 3	13.98	14.15	14.63
Sample 4	12.58	12.75	13.28
Sample 5	11.26	11.36	11.91

Table 2 Colour values of various turmeric samples

Turmeric sample	Colour value in colour units
Sample 1	717
Sample 2	742
Sample 3	921
Sample 4	824
Sample 5	698

Table 3 Colour value of curcumin

Sample of turmeric	Colour value of curcumin in c. u
Sample I	15020
Sample II	15150
Sample III	15800
Sample IV	15620
Sample V	14850

Table 4 Percentage of curcumin in various turmeric samples

Turmeric sample	% of curcuminoids
Sample 1	4.34
Sample 2	4.49
Sample 3	5.58
Sample 4	4.99
Sample 5	4.23

Table 5 Colour values in various solvents

Turmeric sample	C.V. in ethyl acetate	C.V.in Acetone	C.V. in methanol
Sample 1	7215	7320	7128
Sample 2	7430	7550	7250
Sample 3	8220	8310	8010
Sample 4	7850	7950	7750
Sample 5	6470	6520	6200

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Table 6 Percentages of various curcuminoids

Turmeric samples	%of curcuminods in turmeric by HPLC	% of Curcumin	%of demethoxy curcumin	%of bisdemethoxy curcumin
Sample 1	4.25	61.9717	19.0085	19.0197
Sample 2	4.61	60.2526	19.5225	20.2248
Sample 3	5.53	58.0773	22.7234	19.1993
Sample 4	5.08	58.8897	24.3488	16.7615
Sample 5	4.19	64.5747	18.3697	17.0555

Table 7 Data analysis of solid samples- pH, salinity, moisture content, carbonates and bicarbonates, calcium and magnesium, organic carbon, nitrogen, phosphorous and potassium content

Soil sample collected from	pH	Conductivity m. s.	Soil salinity Ppm	Soil Moisture %	Bicarbonate meq/l	Ca + Mg in meq/l	% of organic carbon	% of nitrogen	Available P ppm	K in Ppm
Aikkaran-adu	5.21	0.07	1.175×10^3	5.7142	0.404	2.922	0.7850	4.91	14	10
Kunnack-al	7.68	0.127	1.175×10^3	3.9090	0.404	3.896	0.8971	4.86	5.2	2
Mannam-kandam	7.61	0.210	0.175×10^3	3.9162	0.202	1.948	0.3364	5.26	9.2	1
Konnath-ady	6.60	0.182	0.075×10^3	4.412	0.808	1.948	1.6822	5.78	3.4	0.5
Thekkoy	6.05	0.161	0.225×10^3	2.1694	0.404	0.974	1.5701	4.89	20.8	2

Table 8 Colour Value of turmeric, Curcuminoid content sphectophotometrically, Curcuminoid content by HPLC, % of cucumin in curcuminoids, % of demethoxy curcumin in curcuminoids, % of bisdemethoxy curcumin in curcuminoids

Village from which Turmeric collected	Colour Value of turmeric	Curcuminoid content sphectophotometrically (%)	Curcuminoid content by HPLC (%)	% of cucumin in curcuminoids	%of demethoxy curcumin in curcuminoids	%of bisdemethoxy curcumin in curcuminoids
Aikkaranadu	717	4.34	4.25	61.9717	19.0085	19.0197
Kunnackal	742	4.49	4.61	60.2526	19.5225	20.2248
Mannamkandam	921	5.58	5.53	58.0773	22.7234	19.1993
Konnathady	824	4.99	5.08	58.8897	24.3488	16.7615
Thekkoy	698	4.23	4.19	64.5747	18.3697	17.0555

Conclusion

The soil parameters have only little effect on the colour and curcumin content of turmeric. It may affect the yield of turmeric. The colour values of turmeric cultivated in Mannamkandam and Konnathady villages are higher. The amount of demethoxy and bisdemethoxy curcumins are also higher in the samples collected from these villages. The colour value of curcuminoids are also higher in the samples from these places. The low temperature and higher altitude in these regions may affect the colour value and the composition of curcuminoids. The yield of the oleoresin will be higher for the sample collected from Mannamkandam village and then from the sample from Konnathady village. Yield will be higher when the extraction is carried out using methanol as solvent. But the colour value of the oleoresin is higher when acetone is used as the solvent. The conclusion is that, those regions of higher ranges in Kerala were the average temperature is between 20-25 ^{0}C is an ideal area for turmeric with good colour value.

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None.

Conflicts of interest

Authors declared that there is no conflict of interest.

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