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Critical study of curcumin extraction

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SUMMARY : The study of curcumin extraction from turmeric varieties grown in Maharashtra was under taken in the College of Agricultural Engineering, M.A.U.Parbhani. Curcumin was extracted from Salem, Krishna, Rajapuri and Pratibbha varieties by ethanol, acetone, hexane, and HPLC (High performance liquid chromatography) method. From the analysis it was found that to extract more curcumin percentage it is better to go for variety Pratibha, curcumin extraction method HPLC and steam cooking method. The turmeric variety Salem is widely grown and well established in Maharashtra, now efforts have to be taken to convince farmers to cultivate turmeric variety Pratibha having the highest curcumin content. The range of curcumin percentage in Pratibha was from 3.584 to 7.730 per cent followed by Salem 2.169 to 5.932 per cent, Rajapuri 2.812 to 4.366 per cent and Krishna 1.599 to 3.520 per cent, respectively. The highest percentage of curcumin was extracted by HPLC method as compared with the solvents ethanol, acetone and hexane. The highest percentage of curcumin was found in variety Pratibha (7.730%) and minimum in Krishna (2.308%) by HPLC method. The range of curcumin extracted by HPLC method was found as 2.308 to 5.662 per cent from boiled turmeric and 3.520 to 5.932 per cent from steam cooked turmeric, respectively.

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Turmeric (*Curcuma longa* L.) belongs to "Zingiberaceae" family and is a native to south east India and Indonesia. India is the leading producer (90%) and exporter, of the world and utilizes 80 per cent of it. India has nearly 1.84 lakh ha of area under turmeric with total production of 8.56 lakh tonnes during the year 2006-07 (Anonymous, 2004). Generally it contains protein 6.3 per cent, fat 5.1 per cent, minerals 3.5 per cent, COH 69.4 per cent, m.c.13.1 per cent, essential oil 3.5 per cent, curcumin 2.5 to 6 per cent, and oleoresins 5.7 per cent. It is a principal ingredient in food preparations and medicines. It is also used as dye in textile industry, in the preparation of oils, ointments and poultice, in cosmetic product to prepare natural and herbal creams, lotions and hair dye (Negi *et al.,* 1999).

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Curcumin is yellow colour pigment, most valued constituent of turmeric consists of 1,7 - bis (4 - hydroxy, 3-methoxy phenyl) hepta-1, 6-diene 5-diene (www.turmeric.co.in). In the pure isolated state, curcumin separates as an orange yellow crystalline powder having melting point of 180-183°C. It is insoluble in water, slightly soluble in alcohol and glacial acetic acid curcumin can be extracted by solvent extraction, HPLC (High performance liquid chromatography) and supercritical carbon dioxide extraction method (Balashanmugan, 1991).

The study was conducted at the Dept. of Agricultural Process Engineering, C.A.Engg, and Dept. of Food Engineering, C.F.Tech. (M.A.U. Parbhani), and Dept. of Food Technology, Institute of Chemical Technology, Bhimrao Ambedkar Marathawada University, Aurangabad to know the best turmeric variety, curcumin extraction method and turmeric cooking method helpful to farmers, businessmen and industrialist.

EXPERIMENTAL METHODS

The study of curcumin extraction from four turmeric varieties (V), V_{1}- Salem, V_2 -Krishna, V_3-Rajapuri and V_4 Pratibbha mostly grown in Maharashtra were used for four

curcumin extraction methods, namely M_1-ethanol, M_2 - acetone, M_3-hexane, and M_4-HPLC method and curing methods; C_1-boiling and C_2-steam cooking were taken in the study.

The curcumin extraction study was undertaken in a Split-Split Plot Design. Turmeric varieties were taken in a main plot and extraction methods in sub plot and cooking methods in sub- sub plot with three replications. The data generated were analyzed by Split-Split Plot Design (Gomez and Gomez, 1984, Panse and Sukhatme, 1976, Nigam and Gupta, 1969).

Solvent extraction method :

ASTA in 1958 gave the method of curcumin extraction, was followed in this study. Curcumin was quantitatively extracted by refluxing the material in alcohol and was estimated spectrometrically at 425 nm.

Procedure :

– Turmeric powder 0.20 g was filled into conical flask.
– Then 40 ml of ethyl alcohol was dropped in it and refluxed for 2 hr 30min.
– Then it was cooled and filtered quantitatively into 100 ml volumetric flask. Then the extracted residue was transferred to the filter wash thoroughly and diluted with 100 ml ethanol.
– Pipetted 1 ml of filtered extracted and transferred into a 100 ml volumetric flask and diluted up to 50 ml volume with ethanol.
– The absorbance of the extract and standard solution of 425 nm, using 1cm cells against an alcohol blank was measured by spectrophotometer.

$$\text{\% curcumin by weight} = \frac{\text{0.00025xabs.of sample x val.madeup}}{\text{Abs.of stad. x wt.of sample x dilution factor}} \text{x100}$$

$$= \frac{\text{0.00025 x A 425 x 50}}{\text{0.42 x wt.of sample (g) x 1}} \text{x 100}$$

Since, 0.42 absorbance at 425 nm = 0.00025 g curcumin

Turmeric powder samples below 300mesh were used for curcumin extraction (IS -2446, 1963). The curcumin obtained by this method in grams of powder form was converted into percentage of curcumin extracted by particular solvent and turmeric variety. The results obtained were tabulated for further analysis.

High performance liquid chromatography (HPLC) method :

HPLC system operates under high pressure but the efficiency of separation is not related to pressure. In this system a solvent in reservoir is in mobile phase which passes through filter and gets pumped by a solvent pump so that it can flow through a column in which the stationary phase is packed. An injector located in between column and the pump is used to introduce the sample which contains the components intended for separation. The elute from the column passes through a detector which generates signals that get recorded on a recorder and processed in data processor.

Chromatographic condition		
Column	:	Grace smart RP C_{18} 5 micron. (250 mm x 4.6 mm i.d.)
Mobile phase	:	Acetonitrile : Methanol : water : Acetic acid 41 : 23 : 35 : 1
Detector	:	UV detector
Wavelength	:	425 nm
Temperature	:	40^0C
Flow rate	:	1 ml/min
Retention time	:	5.3 min
Pressure	:	1000 Psig

The HPLC model, HP 2000 series fitted with C_{18} column (250 x 4.6mm i.d.), Millipore swinnex type filter (pore size = 0.45 mm) was used for filtration of sample. The injection system was used as 20 ml sample loop.

Standard curcumin sample of 0.1 g was added in and diluted up to 100 ml methanol in volumetric flask. Then 2 ml extract was taken from stock solution and made up volume with 10ml methanol. Turmeric powder samples (0.1 g each) were weighed accurately and transferred into 100 ml volumetric flask. Then 100 ml methanol was dropped in it. Then 2ml filtered extract was transferred in to 50 ml volumetric flask and diluted up to 10ml volume with methanol.

The elution was carried out with gradient solvent system with a flow rate 1ml/min at 40^0C temperature. The mobile phase consisted of methanol (23%), acetonitrile (41%) water (35%) and acetic acid (1%) (v/v) basis. A chromatographic analysis was done on HPLC system consisting of 7225i Rheodyne injector with 20 ml loop. The sample was injected at 20 ml/loop. An HP 2000 series ultra-violet detector was used at wavelength 425nm, for detection of curcumin. Then the chromatograms were processed by chromeleon chromatography management system and the curcumin in different sample were quantified using HP chemistation software. The concentration of curcumin in different varieties of turmeric

was determined by using peak area of sample and area of standard curcumin, which is obtained from peak report of HPLC chromatogram shown in Fig 1 and 2.

$$\frac{As}{Ac} = \frac{Cs}{Cc}$$

$$\therefore \text{Concentration of Sample} = \frac{\text{Area of sample x Conc. of curcumin}}{\text{Area of curcumin}}$$

$$Cs = \frac{As}{Ac} x Cc \qquad -(1)$$

where,

Cs – Concentration of sample, mg/ml

Cc – Concentration of std. curcumin, 200 mg/ml

As - Area of sample, µv/sec

Ac – Area of std. curcumin, µv/sec

The concentration of curcumin in turmeric sample was determined in mg/100 mg converted into percentage.

EXPERIMENTAL FINDINGS AND ANALYSIS

Note:-Treatment means with common super subscript are statistically non-significant and without common super subscript they are statistically significant.

From Table 1 it is reveled that main effects in curcumin extraction study due to different turmeric varieties grown in Maharashtra, curcumin extraction methods and cooking methods were statistically significant.

It is also observed that all varieties were statistically significant from each other in respect of curcumin extraction. Variety V_4 – Pratibha (4.980%) was statistically superior over all. The variety V_1 – Salem (3.328) was statistically inferior in curcumin extraction in comparison to Pratibha. Same type of result was observed in curcumin extraction method M_4 – HPLC (4.938%) which was statistically superior and M_3 –Hexane (2.5814%) was inferior. Curcumin retained in turmeric rhizomes by steam cooking (C_2) was statistically superior to boiling (C_1). The same trend was observed from Fig.1 and 2 *i.e.* Pratibha variety contained the highest curcumin percentage than all varieties taken for study, detected by the HPLC from the boiling method and steam cooked method, respectively.

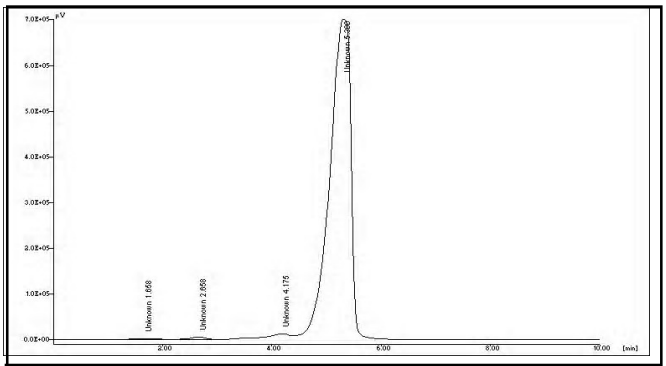

Fig. 1 : HPLC chromatogram of standard curcumin

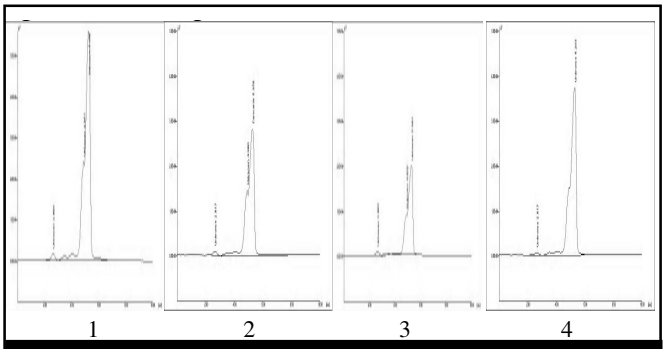

Fig. 2 : HPLC chromatogram of boiling treatment (1) Pratibha (2) Krishna (3) Rajapuri and (4) Salem

It is observed from Table 2, 3 and 4, that all single factor interaction namely turmeric variety x curcumin extraction method, turmeric variety x cooking method and turmeric variety x curcumin extraction method x cooking method were statically significant.

Table 1 : Main effect due to turmeric variety, curcumin extraction method and cooking method

Variety	Mean	Curcumin Extraction Method	Mean	Cooking Methods	Mean
Salem (V_1)	3.328[b]	Ethanol(M_1)	3.2949[c]	Boiling (C_1)	3.370[a]
Krishna(V_2)	2.237[a]	Acetone(M_2)	3.1801[b]	Steam Cooking(C_2)	3.628[b]
Rajapuri(V_3)	3.451[c]	Hexane(M_3)	2.5814[a]		
Pratibha(V_4)	4.980[d]	HPLC(M_4)	4.938[d]		
Results	Significant	Significant		Significant	
SE	0.00001	0.0013		0.0004	
CD	0.0214	0.1035		0.0608	
CD at 5% L.S.					

Table 2 : Interaction due to turmeric variety x curcumin extraction method

Curcumin extraction method				
Variety	Ethanol (M_1)	Acetone (M_2)	Hexane(M_3)	HPLC (M_4)
Salem (V_1)	2.738[b]	2.571[b]	2.206[a]	5.797[c]
Krishna(V_2)	2.180[b]	2.149[b]	1.705[a]	2.914[c]
Rajapuri(V_3)	3.601[b]	3.482[b]	2.849[a]	3.873[c]
Pratibha(V_4)	4.661[b]	4.518[b]	3.567[a]	7.176[c]
Result	Significant			
Particular	SE	CD		
Variety x CE Method (at fix variety level)	0.0050	0.2069		
Variety x CE Method (at fix extraction method level)	0.0038	0.1805		

Table 3: Interaction due to turmeric variety x cooking method

Sr.No.	Variety	Cooking method	
		Boiling (C_1)	Steam cooking (C_1)
1.	Salem (V_1)	3.273[a]	3.382[a]
2.	Krishna(V_2)	2.088[a]	2.386[b]
3.	Rajapuri(V_3)	3.286[a]	3.616[b]
4.	Pratibha(V_4)	4.832[a]	5.129[b]
Result		Significant	
Particular		SE	CD
Variety x cooking method (at fix variety level)		0.0018	0.1216
Variety x CE method (at fix cooking method method level)		0.0009	0.0886

Table 4 : Interaction due to curcumin extraction method x turmeric cooking method

Curcumin extraction method	Turmeric cooking method	
	C_1	C_2
M_1	3.246[b]	3.344[b]
M_2	3.146[b]	3.214[b]
M_3	2.594[a]	2.569[a]
M_4	4.493[c]	5.387[c]
Results	Significant	
Particular	SE	CD
Curcumin extraction method x Cooking method (at fix cooking method)	0.0018	0.1216
Turmeric extraction method x cooking method (at fix cooking method)	0.0021	0.1345

In turmeric variety x curcumin extraction method there was dependence in turmeric variety and curcumin extraction method. It is observed that curcumin extraction method HPLC (M_4) was statistically superior method and curcumin extraction by solvent hexane (M_3) was inferior.

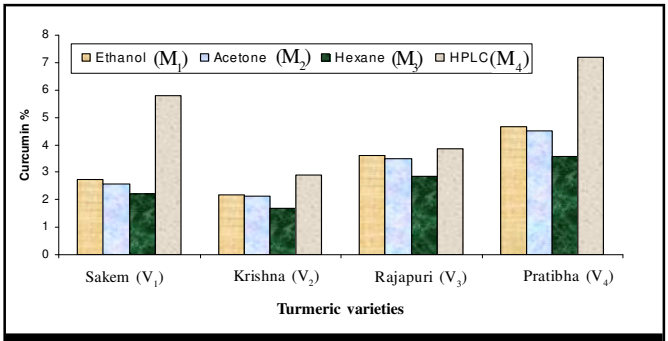

Fig. 3 : Turmeric variety x curcumin extraction methods interaction

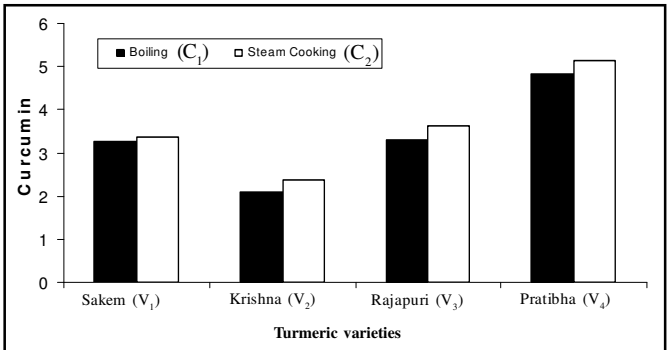

Fig. 4 :Turmeric variety x cooking methods interaction

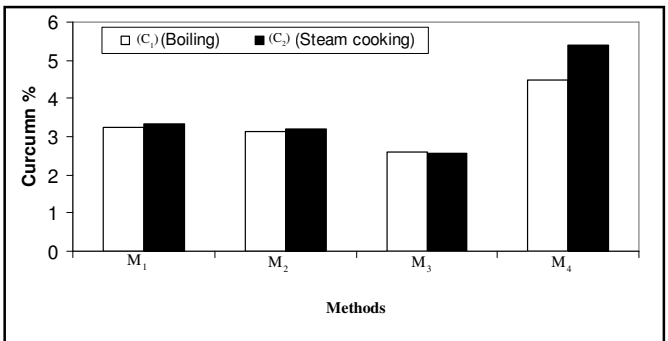

Fig. 5 : Method curcumin extraction x turmeric cooking methods interaction

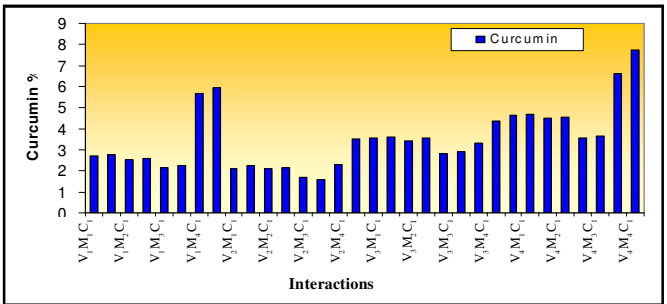

Fig. 6 : Turmeric variety x curcumin extraction method x cooking methods interaction

Table 5 : Interaction due to turmeric variety x curcumin extraction method x cooking method

Sr.No.	Treatment	Mean
1.	$V_1 M_1C_1$	2.708
2.	$V_1 M_1C_2$	2.768
3.	$V_1 M_2C_1$	2.554
4.	$V_1 M_2C_2$	2.589
5.	$V_1 M_3C_1$	2.169
6.	$V_1 M_3C_2$	2.242
7.	$V_1 M_4C_1$	5.662
8.	$V_1 M_4C_2$	5.932
9.	$V_2 M_1C_1$	2.113
10.	$V_2 M_1C_2$	2.246
11.	$V_2 M_2C_1$	2.107
12.	$V_2 M_2C_2$	2.179
13.	$V_2 M_3C_1$	1.690
14.	$V_2 M_3C_2$	1.599
15.	$V_2 M_4C_1$	2.308
16.	$V_2 M_4C_2$	3.520
17.	$V_3 M_1C_1$	3.541
18.	$V_3 M_1C_2$	3.601
19.	$V_3 M_2C_1$	3.411
20.	$V_3 M_2C_2$	3.554
21.	$V_3 M_3C_1$	2.812
22.	$V_3 M_3C_2$	2.885
23.	$V_3 M_4C_1$	3.338
24.	$V_3 M_4C_2$	4.366
25.	$V_4 M_1C_1$	4.620
26.	$V_4 M_1C_2$	4.702
27.	$V_4 M_2C_1$	4.501
28.	$V_4 M_2C2$	4.536
29.	$V_4 M_3C_1$	3.584
30	$V_4 M_3C_2$	3.639
31.	$V_4 M_4C_1$	6.622
32.	$V_4 M_4C_2$	7.730
Result	Significant	
Grand mean	2.9956	
STDV	1.1007	
SE	0.0844	
CD	0.1731	

Curcumin extraction by solvent ethanol (M_1) and acetone (M_2) were statistically at par. The combination of variety Pratibha (V_4) and curcumin extraction method HPLC (M_4) was superior over all and variety Krishna and curcumin extraction method by solvent hexane (M_3) was inferior.

In turmeric variety and cooking method, curcumin percentage extracted from Pratibha by the steam cooking method was statistically superior. Therefore, to get curcumin more from any variety it is to go for the steam cooking method (C_2).

In curcumin extraction method and cooking method interaction the results obtained by M_4C_2 *i.e.* HPLC x steam cooking method combination was statistically significant. It is observed that if you go for cooking by boiling or steam cooking method (C_1 or C_2), curcumin extraction by ethanol and acetone (M_1 and M_2) methods were at par. Method M_4 was superior and M_3 was inferior.

From the Table 5, it is reveled that interaction turmeric variety x curcumin extraction method x cooking method was statistically significant. Grand mean and standard deviation of curcumin percentage was 2.9956 and 1.1007, respectively. The combination $V_4M_4C_2$ *i.e.* variety x curcumin extraction method x cooking method was statistically superior and $V_2M_3C_1$ was inferior.

Conclusion:

From the interaction study of turmeric variety, turmeric extraction method and turmeric cooking method following conclusions were drawn.

It is strongly recommended that it is better to use variety Pratibha for better curcumin extraction. Also curcumin extraction method HPLC is recommended for higher percentage of curcumin extraction. The steam cooking method is the best for retaining the maximum curcumin in the rhizomes after processing. It is necessary to take the efforts to increase the area of turmeric variety Pratibha in Maharashtra to get maximum curcumin.

Abbreviation:

HPLC- High Performance Liquid Chromatography, ASTA-American Spices Trade Association, CAE-College of Agricultural Engineering, CFT- College of Food Technology, BAMU-Babasaheb Ambedkar Marathwada University, M.A.U.-Marathwada Agricultural University, nm- nanometer, elute-mixture from which a typical ingredient is to be separated.6

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