Full Length Research Paper

Comparative study of curcumin extraction from turmeric varieties grown in Maharashtra

Kamble K. J.[1]*, Ingale V. M.[2] and Kaledhonkar D. P.[3]

[1]Mahatma Phule Krishi Vidyapeeth (MPKV) Rahuri, India.
[2]Loak Mangal Agricultural College, Solapur, India.
[3]Department of statistics Statistics, Mahatma Phule Krishi Vidyapeeth (MPKV) Rahuri, India.

The study was under taken in the College of Agricultural Engineering, M.A.U. Parbhani in which curcumin was extracted from Salem, Krishna, Rajapuri and Pratibbha varieties by ethanol, acetone, hexane, and HPLC (high performance liquid chromatography) method. 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 should to be taken to convince farmers to cultivate variety Pratibha having the highest curcumin content. The highest range of curcumin percentage was observed from 3.584 to 7.730% in Pratibha followed by Salem 2.169 to 5.932%, Rajapuri 2.812 to 4.366% and Krishna 1.599 to 3.520%, respectively. The highest percentage of curcumin was extracted by HPLC method in comparison to solvents ethanol, acetone and hexane. The range of curcumin extracted by HPLC method was found as 2.308 to 5.662% from boiled turmeric and 3.520 to 5.932% from steam cooked turmeric, respectively.

Key words: High performance liquid chromatography (HPLC), ASTA-American spices trade association, College of Agricultural Engineering (CAE), College of Food Technology (CFT), Babasaheb Ambedkar Marathwada University (BAMU), Marathwada Agricultural University (M.A.U), nanometer (nm), elute-mixture from which a typical ingredient is to be separated.

INTRODUCTION

Turmeric (*Curcuma Longa L.*) belongs to "Zingiberaceae" family, is a native to South east India and Indonesia. India is the leading producer (90%), exporter and utilizes 80% of it with nearly 1.84 lakh ha of area under cultivation with total production of 8.56 lakh tonnes during the year 2006 to 2007 (www.spices.com and Spices Board of India, 2004). Generally, it contains protein 6.3%, fat 5.1%, minerals 3.5%, COH 69.4%, m.c.13.1%, essential oil 3.5%, curcumin 2.5 to 6%, and oleoresins 5.7%. It is a principal ingredient in food preparations and in medicines etc. It is also used in textile industry, in the preparation of oils, ointments and poultice, in cosmetic product to prepare natural and herbal creams, lotion and hair dye (Negi et al., 1999).

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 to 183°C. It is insoluble in w ater, slightly soluble in alcohol and glacial acetic acid (Narayanan et al., 1981). Curcumin can be extracted by solvent extraction, high performance liquid chromatography (HPLC) and supercritical carbon dioxide extraction method (Balashanmugan, 1991).

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

---

*Corresponding author. E-mail: aaskk@rediffmail.com.

MATERIALS AND METHODS

In the study four turmeric varieties (V_1- Salem, V_2- Krishna, V_3-Rajapuri and V_4 -Pratibbha) grown in Maharashtra were used for curcumin extraction by four methods (M1.ethanol, M_2 -acetone, M_3-hexane, and M_4-HPLC) and two curing methods (C_1-boiling and C_2-steam cooking) were followed.

The curcumin extraction 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 was analyzed by Split-split plot design (Gomez and Gomez, 1984; Panse and Sukhatme, 1976; Nigam. and Gupta, 1969).

Solvent extraction method

ASTA (1958) gave the method of curcumin extraction by solvents (M_1, M_2, and M_3) was followed in this study. Curcumin was quantitatively extracted by refluxing the material in alcohol and was estimated spectrometrically at 425 nm. Turmeric powder samples below 300 mesh 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 was used to introduce the sample which contains the components intended for separation. Elute from the column passes through a detector which generates signals that get recorded on a recorder and processed in data processor.

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

Chromatographic condition

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 10 ml 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 2 ml filtered extract was transferred into 50 ml volumetric flask and diluted up to 10 ml volume with methanol.

The elution was carried out with gradient solvent system with a flow rate 1 ml/min at 40°C temperature. The mobile p hase 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 μl loop. The sample was injected at 20 μl/loop. An HP 2000 series ultra-violet detector was used at wavelength 425 nm, 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 Figures 1 and 2.

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

$$\text{Concentration of sample} = \frac{\text{Area of sample}}{\text{Area of curcumin}} \times \text{Conc. of curcumin}$$

$$Cs = \frac{As}{Ac} \times Cc \tag{1}$$

Where, Cs = Concentration of sample, μg/ml, Cc = Concentration of std. curcumin, 200 μg/ml, As = Area of sample, μv/sec and Ac = Area of std. curcumin, μv/sec. The concentration of curcumin in turmeric sample was determined in mg/100 mg converted into percentage.

RESULTS AND DISCUSSION

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 are statistically significant from each other in respect of curcumin extraction. Variety V_4 – Pratibha (4.980%) is statistically superior over all. The variety V_1 – Salem (3.328) is statistically inferior in curcumin extraction. Same type of result was observed in curcumin extraction method and method M_4 – HPLC (4.938%) is statistically superior and M_3 –Hexane (3.5814%) is inferior. Curcumin retained in turmeric rhizomes by steam cooking (C_2) is statistically superior to boiling (C_1). The same trend is observed from Figures 1 and 2 that s, Pratibha variety contains the highest curcumin percentage than all varieties taken for study, detected by the HPLC from the boiling method and steam cooked method, respectively.

It is observed from Tables 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 are statically significant.

In turmeric variety x curcumin extraction method there is dependence in turmeric variety and curcumin extraction method. It is observed that curcumin extraction method HPLC (M_4) is statistically superior method and curcumin extraction by solvent hexane (M_3) is inferior. It is also observed from the Figure 3.

Curcumin extraction by solvent ethanol (M_1) and acetone (M_2) are statistically at par. The combination of variety Pratibha (V_4) and curcumin extraction method
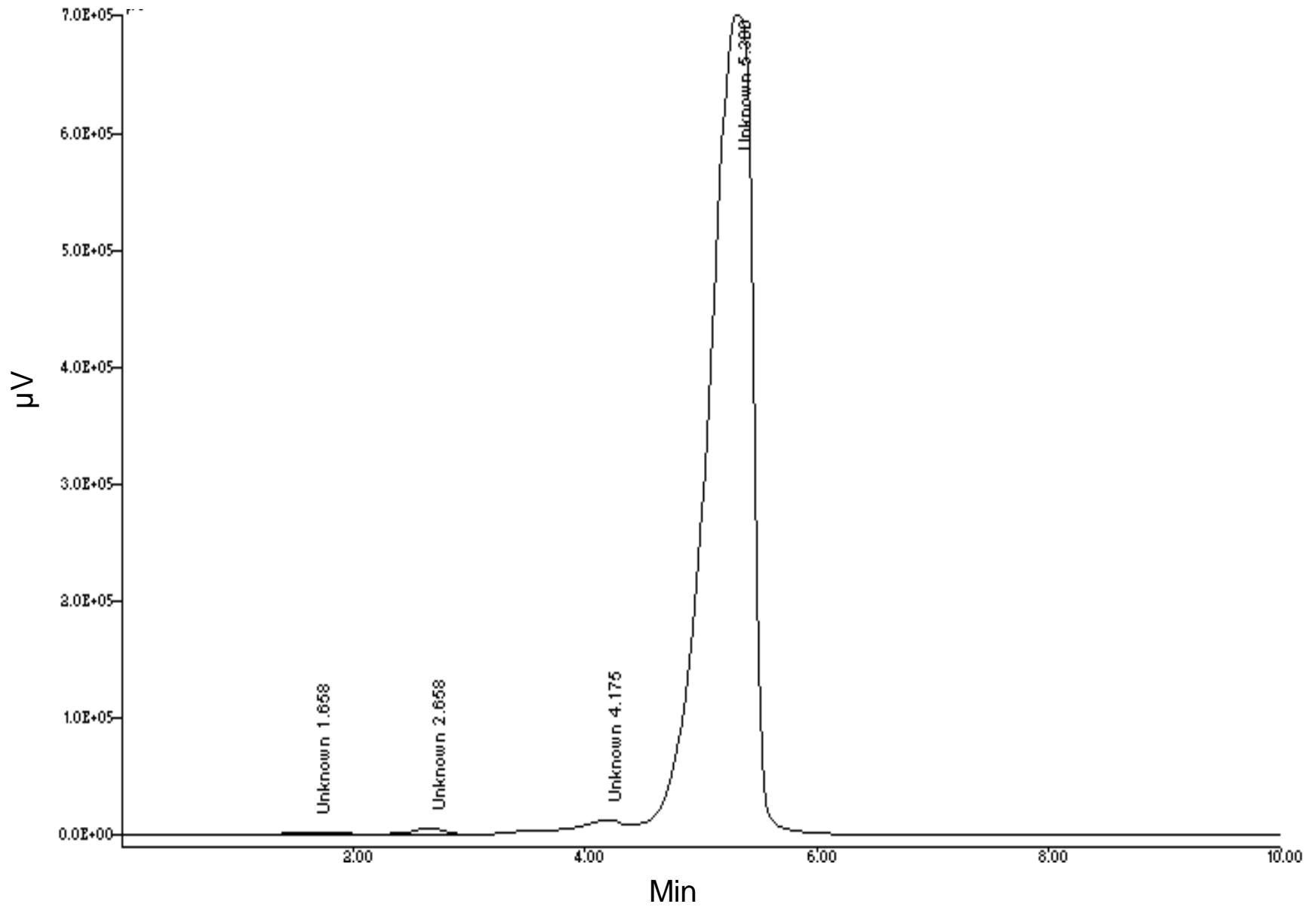
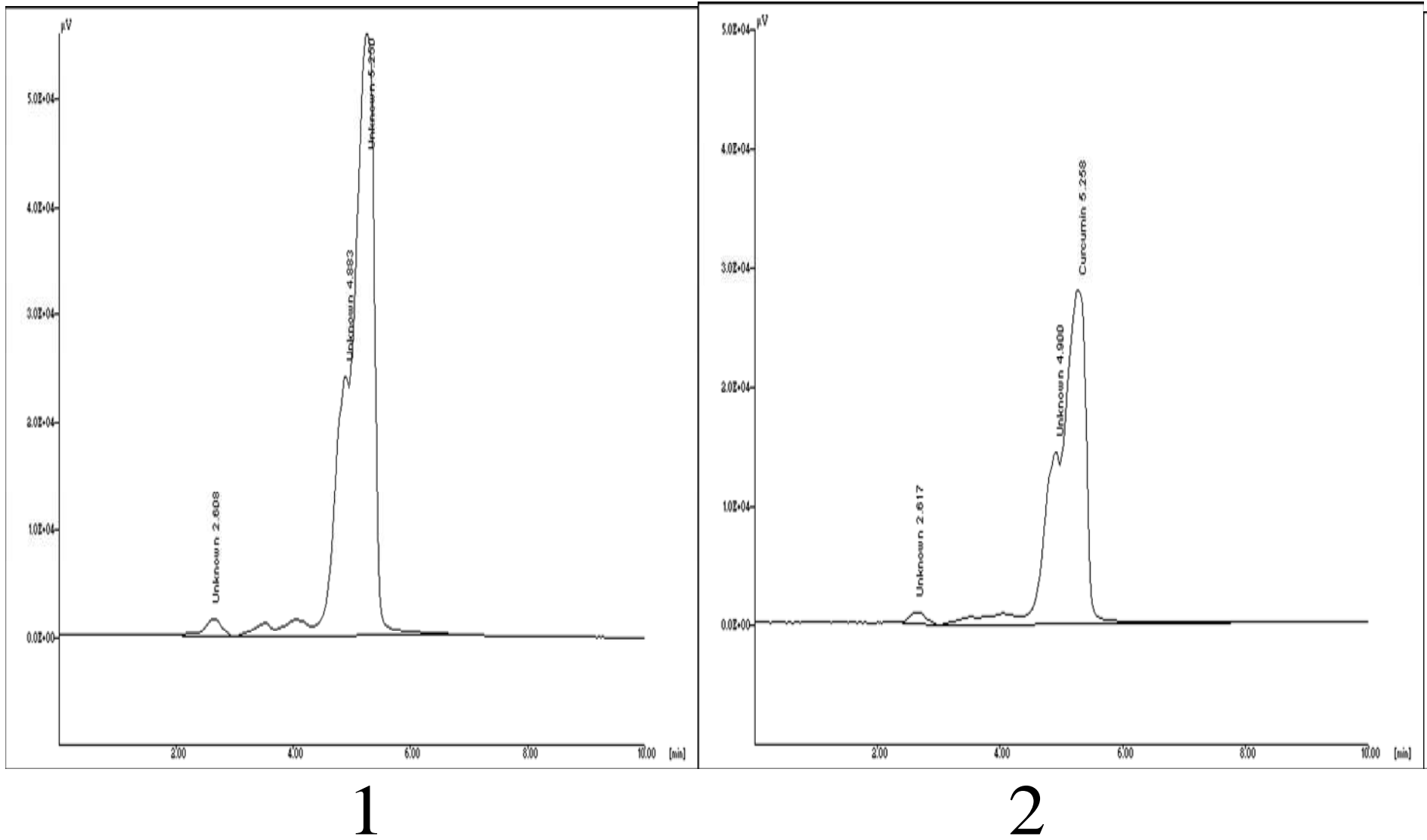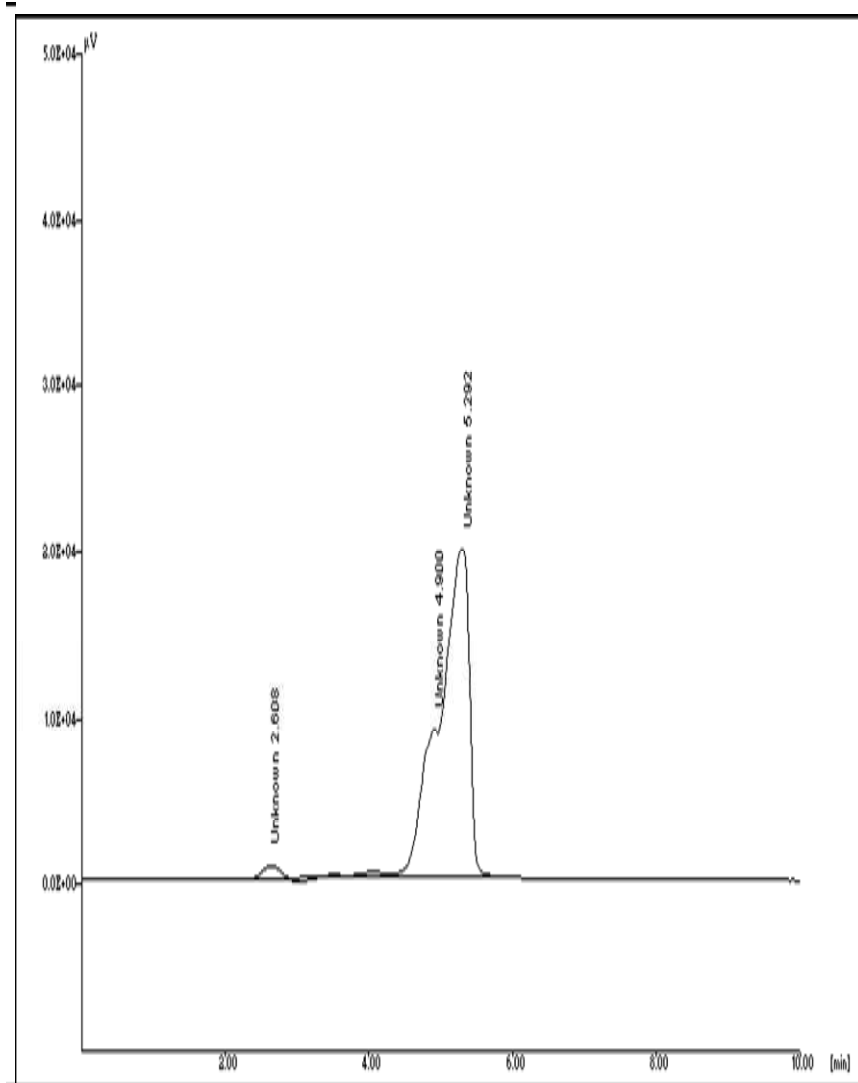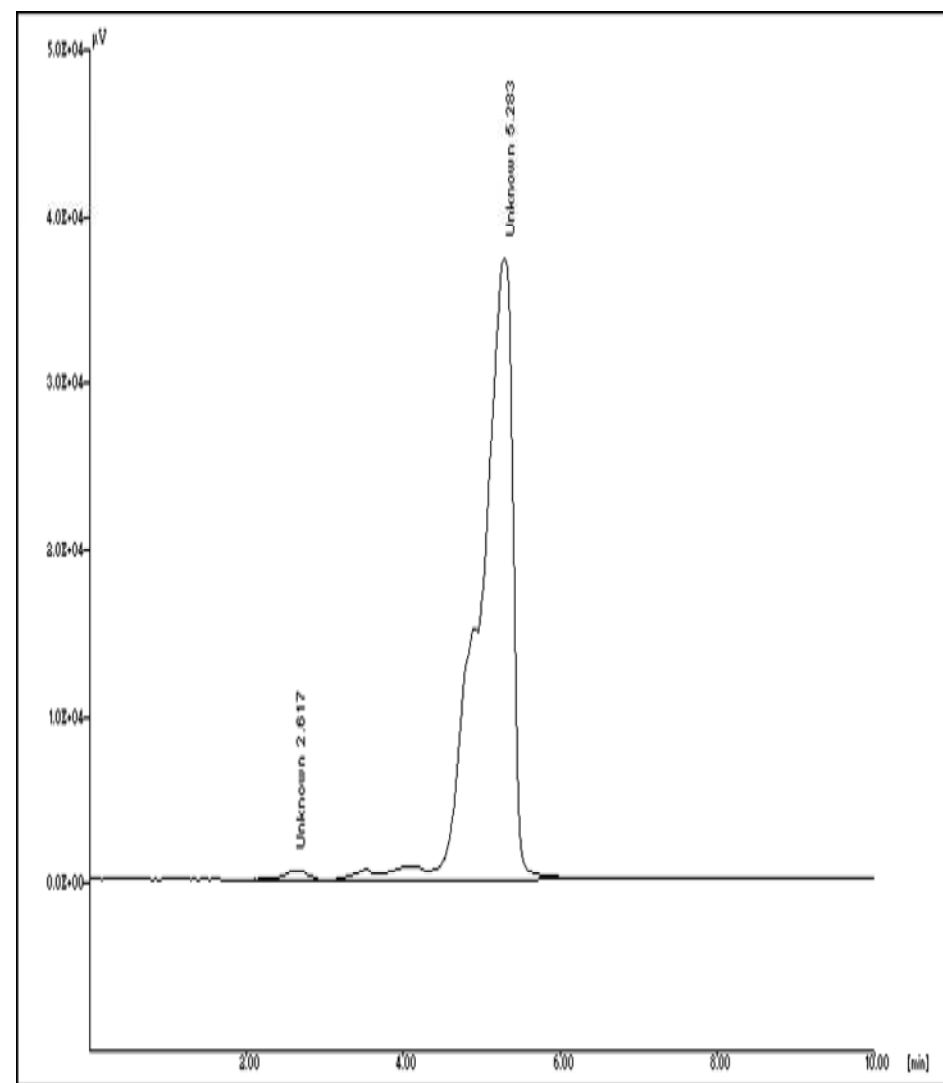**Figure 1.** HPLC Chromatogram of Standard Curcumin.

Figure 2. HPLC Chromatogram of Boiling treatment (1) Pratibha (2) Krishna (3) Rajapuri and (4) Salem.

Figure 2. Contd.

Table 1. Main effect due to turmeric variety, curcumin extraction 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 (V3)	3.451^c	Hexane (M_3)	2.5814^a		
Pratibha (V4)	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.					

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

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

Variety	Curcumin extraction method			
	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 (V3)	3.601^b	3.482^b	2.849^a	3.873^c
Pratibha (V4)	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.

S/N	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 (V3)	3.286^a	3.616^b
4	Pratibha (V4)	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

HPLC (M_4) is superior over all and variety Krishna and curcumin extraction method by solvent hexane (M_3) is inferior. It is also observed from Figure 4.

In turmeric variety and cooking method, curcumin percentage extracted from Pratibha by the steam cooking method is statistically superior. Therefore, to extract maximum curcumin from any variety it is to go for the steam cooking method (C_2). It is also observed from Figure 5. In curcumin extraction method and cooking method interaction the results obtained by M_4C_2 that is, HPLC x steam cooking method combination is 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 are at par. Method M_4 is superior and M_3 is inferior. It is also observed from Figure 6.
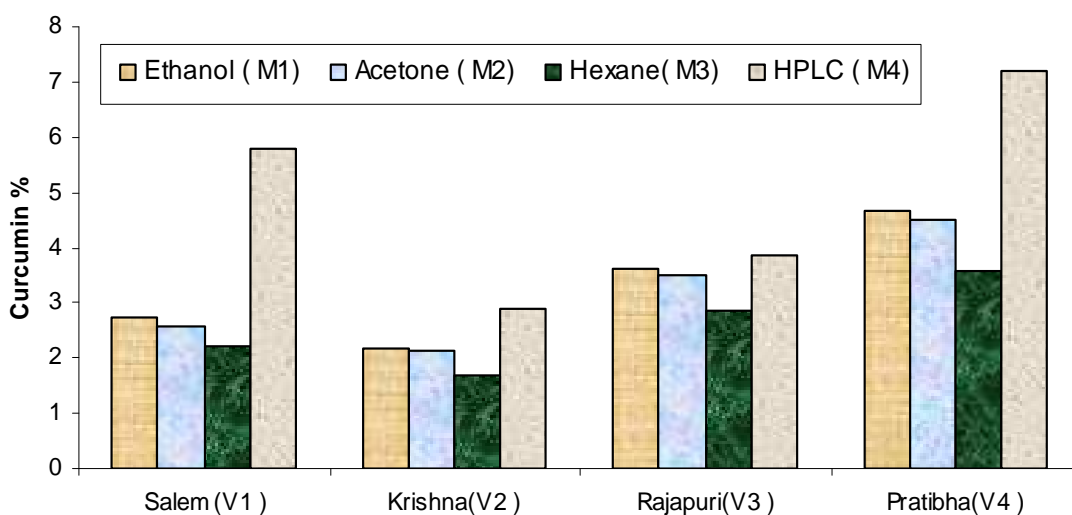
From the Table 5, it is reveled that interaction turmeric variety x curcumin extraction method x cooking method is

Table 4. Interaction due to Curcumin extraction method x Turmeric cooking method.
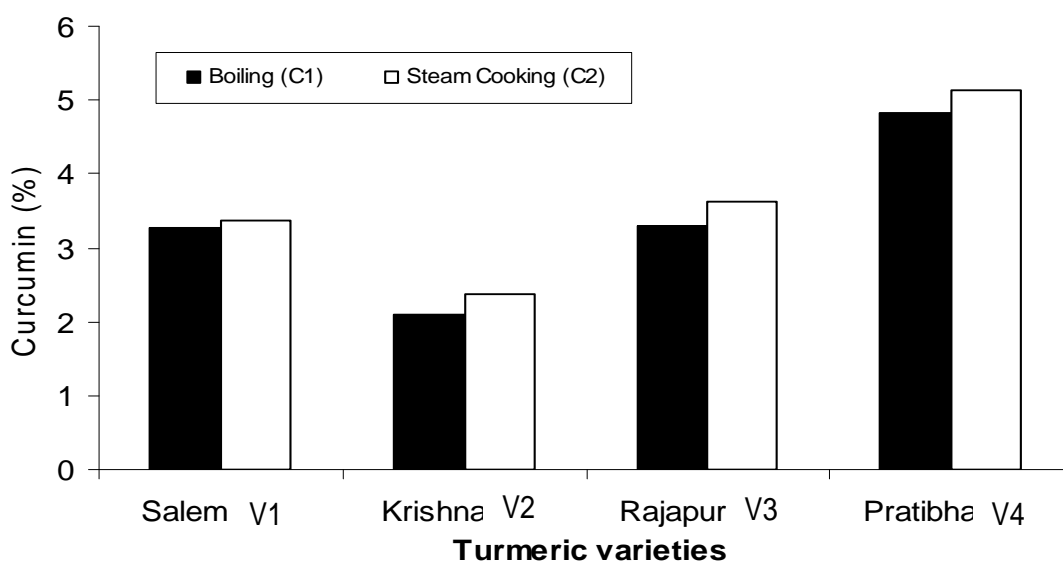
Curcumin extraction method	Turmeric cooking method	
	C_1	C_2
M_1	3.246^b	3.344^b
M_2	3.146^b	3.214^b
M_3	2.594^a	2.569^a
M_4	4.493^c	5.387^c
Results	Significant	
Particular	**SE**	**CD**
Curcumin extraction method x Cooking Method (at fix Cooking method)	0.0018	0.1216
Turmeric extraction method x Cooking method (at fix Cooking Method)	0.0021	0.1345

Figure 3. Turmeric variety x curcumin extraction methods interaction.

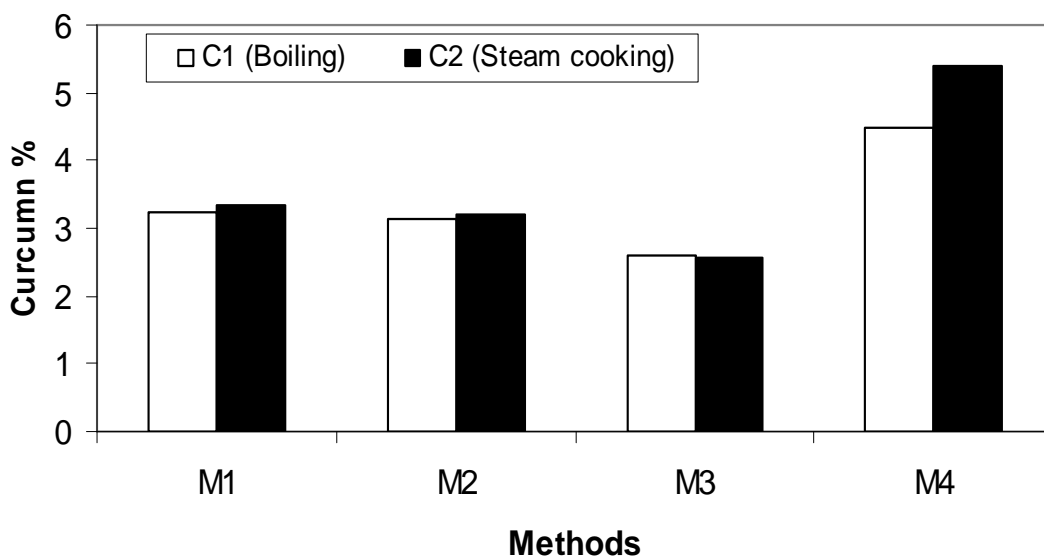**Figure 4.** Turmeric variety x cooking methods interaction.

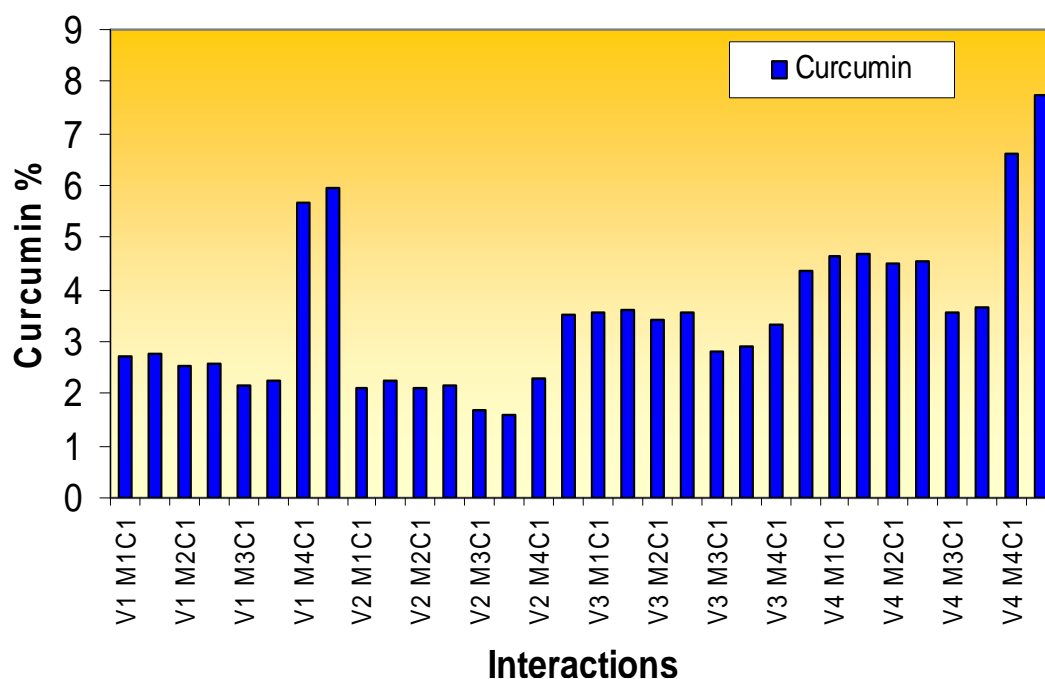**Figure 5.** Method curcumin extraction x turmeric cooking methods interaction.

Figure 6. Turmeric variety x curcumin extraction method x cooking methods interaction.

statistically significant. Grand mean and standard deviation of curcumin percentage is 2.9956 and 1.1007, respectively. The combination $V_4M_4C_2$ that is variety x curcumin extraction method x cooking method is statistically superior and $V_2M_3C_1$ is inferior.

Same types of results were obtained by Viasan et al. (1989), Rosnani et al. (2003) Paramasivam et al. (2008) and Lingamullu et al. (2002),

Conclusion

From the interaction study of turmeric variety, curcumin extraction method and turmeric cooking method the following conclusions were drawn: To extract maximum curcumin percentage it is strongly recommended that it is better to go for variety Pratibha, curcumin extraction method HPLC and steam cooking method. The turmeric

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

S/N	Treatment	Mean
1.	$V_1 M_1 C_1$	2.708
2.	$V_1 M_1 C_2$	2.768
3.	$V_1 M_2 C_1$	2.554
4.	$V_1 M_2 C_2$	2.589
5.	$V_1 M_3 C_1$	2.169
6.	$V_1 M_3 C_2$	2.242
7.	$V_1 M_4 C_1$	5.662
8.	$V_1 M_4 C_2$	5.932
9.	$V_2 M_1 C_1$	2.113
10.	$V_2 M_1 C_2$	2.246
11.	$V_2 M_2 C_1$	2.107
12.	$V_2 M_2 C_2$	2.179
13.	$V_2 M_3 C_1$	1.690
14.	$V_2 M_3 C_2$	1.599
15.	$V_2 M_4 C_1$	2.308
16.	$V_2 M_4 C_2$	3.520
17.	$V_3 M_1 C_1$	3.541
18.	$V_3 M_1 C_2$	3.601
19.	$V_3 M_2 C_1$	3.411
20.	$V_3 M_2 C_2$	3.554
21.	$V_3 M_3 C_1$	2.812
22.	$V_3 M_3 C_2$	2.885
23.	$V_3 M_4 C_1$	3.338
24.	$V_3 M_4 C_2$	4.366
25.	$V_4 M_1 C_1$	4.620
26.	$V_4 M_1 C_2$	4.702
27.	$V_4 M_2 C_1$	4.501
28.	$V_4 M_2 C_2$	4.536
29.	$V_4 M_3 C_1$	3.584
30.	$V_4 M_3 C_2$	3.639
31.	$V_4 M_4 C_1$	6.622
32.	$V_4 M_4 C_2$	**7.730**

Result	Significant
Grand Mean	2.9956
STDV	1.1007
SE	0.0844
CD	0.1731

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 highest curcumin content.

REFERENCES

Anonymous (2004). Indian spices. www.spices.com.

ASTA (1958). Official Analytical Methods; American spice Trade Association; Englewood cliffs, NJ, Methods (18) color power of turmeric.

Balashanmugan PV (1991). Processing and curing of turmeric. South Indian Hort., 39 (4): 214-216.

Gomez KW, Gomez AA (1984). Statistical procedures for Agricultural research, John Wiley and Sons.

IS (1963). Specification turmeric powder IS-2446.

Lingamullu J, Mohan R, Kunnumpurath KS (2002). Improved HPLC Method for the Determination of Curcumin, Demethoxycurcumin and Bisdemethoxycurcumin, J. Agric. Food Chem., 50: 3668-3672.

Negi PS, Jayaprakasha GK, Jagan MR, Sakariah KK (1999). Anti bacterial activity of turmeric oil, A by product from curcumin. J. Agric Food Chem., 47(10): 4297-4300.

Nigam AK, Gupta VK (1969). Hand Book on analysis of agricultural experiments IASRI, New Delhi.

Panse V, Sukhatme PV (1976). Statistical method for agricultural workers, ICAR New Delhi.

Paramasivam M, Md. Wasim A, Poi R, Banarjee H , Bandyopadhyay A (2008). Occurance of curcuminoids in *Curcuma longa:* A quality standardization by HPTLC, Bangladesh J. Phermacol., 3: 55-58.

Rosnani HH, Norsaupiah S, Mohamad RS, Ramlan AA, Fikry CP (2003). Extraction of oleoresin from Zingiber zerumbet rhizome: Comparative study on yield, zerumbone and curcumin content, proceedings of International conference on chemical and bioprocess engineering, 27th -29th August 2003, University Malaysia Sabah, Kota Kinabalu.

Viasan AC, Nirmala AM, Madhusudhanna JR, Narayanan CS, Mathew AG (1989). Chemical analysis of some cultivars of curcuma longa linn, J. Fd. Sci. Technol., 26(5): 293-295.