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## Studies on food value base curcumin extraction for commercial exploration

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### Abstract

Curcumin, a conspicuous Curcuminoid recognized as pharmacological inherent active constituent of turmeric has vibrant potential to explore as nutraceutical in formulation of functional food products. It's non-communicable chronic diseases (Neurodegenerative diseases, cardiovascular diseases, diabetes, obesity, allergies, and certain types of cancer) risk bearer efficacy need to be justified in coordination with nutraceutical food value. Demethoxycurcumin and Bisdemethoxycurcumin as active curcumin associated components, give way to monitor nutraceutical efficacy to conceptualize technological value to its up-gradation through separation process. In present investigation, cultivar diversity data on curcumin separation by thin layer chromatography, recorded highest quotient (Demethoxycurcumi/Bisdemethoxycurcumin) in local cultivar (2.70) justifiable for comparatively inferior nutraceutical efficacy value against Rajapuri (2.1), Salem (1.93) and standard curcumin (2.03) respectively. Review base DMC/BDMC quotient change reflects on functionality deviation value and provides consolidated hypothesis on quotient. Higher the quotient value more is the functional efficacy and viceversa. It also provides a vibrant option to food technologists to standardize curcumin demethylation process exclusively in the interest of functional efficacy. The data base limitations of present investigation generate technological option for further studies on fractionation of curcumin with utmost demethoxy curcumin integration.

**Keywords:** Curcumin, solvent extraction, turmeric, DMC/BDMC quotient

### Introduction

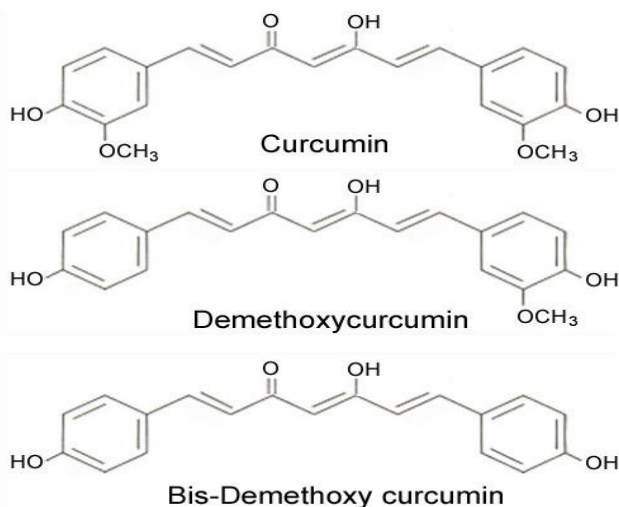
Turmeric (*Curcuma longa* L.) being US FDA GRAS recognized, is a medicinal plant belongs to *Zingiberaceae* family. It is a culinary spice with inherent coloring property, used as an ingredient in formulation of curries and other ethnic meals in Indian dishes. The ayurvedic medicinal characterization of turmeric from years together has underlined its state of art status as pharmaceutical ingredient to develop specialty type of food products (Akram *et al.*, 2010) [2]. Irrespective of nutrient contribution (Protein 6.3%, Fat 5.1%, Carbohydrate 69.4%, and minerals 3.5%) the presence of essential oil (3.5%), oleoresins (5.7%) and curcumin (2.5 to 6%) justified the nomenclature of turmeric as a golden saffron spice (Kamble *et al.*, 2011) [4]. Turmeric extract is an oleoresin comprising of volatile oil, yellow coloured curcuminoids and monoterpenoids and sesquiterpenoids as colourless flavouring compounds. The curcuminoids are characterized by yellow colored tint and three components referred as curcumin, demethoxycurcumin and bisdemethoxycurcumin (Sing and Jain, 2012). Amongst 120 curcuma species the *Curcuma Longa* being rich source of curcumin is notified as a biologically targeted phytochemical. Commercially available "curcumin" is a mixture of curcumin (approx. 77%), demethoxycurcumin (approx. 18%) and bisdemethoxycurcumin (approx. 5%) (Basnet and Basnet, 2011) [3].

Curcumin [chemical name: (1E, 6E) - 1, 7 -bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5 Dione) is a bis- $\alpha$ ,  $\beta$ -unsaturated  $\beta$ -diketone. It has a molecular weight of 368.38, a melting point of 179–183°C, and chemical formula as  $C_{21}H_{20}O_6$ . Physiological conditions suffice the equilibrium co-existence status of both an enol and a bis-keto forms. Keto form of curcumin exhibits physical presence domination in solid and liquid phase (acidic and neutral) against enolic form in alkaline conditions. Diversified solubility characterization of lipophilic curcumin also reflects on water insolubility against acidic and neutral pH conditions, over and above the readily soluble status in selected solvents (Dimethylsulfoxide, ethanol, and acetone) and alkali. It has been also recorded that 90% of curcumin is degraded to trans form - 6-(4'-hydroxy-3'-methoxyphenyl) - 2, 4-dioxo-5-hexanal, vanillin, feruloylmethane, and ferulic acid within 30 minutes in liquid/solution form (Basnet and Basnet, 2011; Kulkarni *et al.*, 2012) [3, 5]. The molecular stability is confirmed in acidic condition at high temperatures against relative un-stability in alkaline solutions, in presence of light (Kulkarni *et al.*, 2012) [5]. Anticipated high stability of curcumin at pH 1.2 (highly acidic) in absence of light against its presence is

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specialty feature likely to be considered during formulation of standard recipes of curcumin enriched food products under acidic conditions (Kumavat *et al.*, 2013) [6]. The Joint FAO/WHO Expert Committee on Food Additives, 1996 has granted an acceptable curcumin daily intake level of 0.1–3 mg/kg-BW (Basnet and Basnet, 2011) [3].



**Fig 1:** Structure of three major curcuminoids (Basnet and Basnet, 2011) [3].

The nutraceutical potential of curcumin is synergistically coiling around antioxidant, anti-inflammatory, antiviral, antibacterial, antifungal, anticancer efficacy. Indian medicine system referred status of curcumin has claimed its utility against several non-communicable chronic diseases such as neurodegenerative diseases, cardiovascular diseases (CVD), diabetes, obesity, allergies (Paola *et al.*, 2012; Steffi and Srinivasan, 2014) [7, 10]. Hydroxyl groups of the benzene rings, double bonds in the alkene part and the central  $\beta$ -diketone moiety are suggested to be likely responsible for the high beneficial activities of curcumin (Osawa and Namiki 1985; Ruby *et al.* 1995). Demethoxy curcumin and bis demethoxy curcumin though present in small quantities had a remarkable effect on the activity of the main constituent, i.e., curcumin present in the crude curcuminoid powder (Rege *et al.*, 2014). Human clinical studies have recognized and approved food grade status of curcumin in respect of human threshold tolerance and consumption safety on the basis of admissible dose (High dose: 8–12 g/day). The fundamental hurdle for anticipated *in vivo* concentration efficacy as a therapeutic agent though coiling around low bioavailability, poor absorption, rapid metabolism and systemic clearance needs to be monitored by advance processing technology (Paola *et al.*, 2012) [7].

The present investigation is undertaken to extract and separate the curcumin from different turmeric cultivars. It also reveals DMC/BDMC quotient present in curcuminoid of different cultivars.

## Materials and Method

### Sample collection

Dried rhizome turmeric (*Curcuma Longa*) samples of diverse cultivars (Salem, Rajapuri and local cultivars) were collected from locally recognized market yard, Pune. The admissible solvents of desired purity were also collected from local suppliers. The experimental set up was designed to compensate with objectives notified.

## Equipment used

Experimental set up base required instruments such as open top weighing balance, vernier caliper, soxhron apparatus, hot air oven, kjeldhal apparatus, muffle furnace etc. were used from laboratory of MIT College of Food Technology, Pune

## Method

### 1. Physical Characterization of turmeric

**1) Weight of turmeric rhizome:** Ten pieces of dried and cleaned rhizome were used for average weight determination. The weighing process is facilitated by using an electronic weighing balance having accuracy 0.001.

**2) Size of turmeric rhizome:** Length, width and thickness of turmeric rhizomes (10) were determined for reflecting size by a vernier caliper along with visual shape assessment.

### 2. Proximate Composition

Proximate composition specifying parameters such as moisture content, ash, fat, protein and total carbohydrate of turmeric cultivars were estimated. All the observations recorded are an average of 3 determinations.

**1) Moisture:** Moisture content was determined by distillation method given by Ranganna (2005) [8].

$$\text{Moisture content (\%)} = \frac{\text{Volume of water collected}}{\text{Weight of sample}} \times 100$$

**2) Fat:** Crude fat was estimated by using soxhlet apparatus as method described by Ranganna. (2005) [8].

$$\text{Crude Fat (\%)} = \frac{\text{Weight of ether soluble material}}{\text{Weight of sample}} \times 100$$

**3) Protein:** Protein content was estimated by using microkjeldhal technique standardized by Ranganna (2005) [8].

$$\text{Nitrogen (\%)} = \frac{(\text{Sample titre} - \text{Blank titre}) \times \text{Normality of HCL} \times 14}{\text{Weight of sample} \times 1000} \times 100$$

$$\text{Protein content (\%)} = \text{Nitrogen (\%)} \times 6.25$$

**4) Total Carbohydrates:** Total carbohydrate content was estimated by using Anthrone method standardized by Ranganna, (2005) [8].

**5) Crude fibre:** Crude fibre was estimated by using method standardized by Ranganna (2005) [8]. Crude fibre was determined by following formula.

$$\text{Crude Fibre (\%)} = \frac{\text{Loss in weight noted}}{\text{Weight of sample taken}} \times 100$$

**6) Total ash and acid insoluble ash:** Total ash and acid insoluble ash content of turmeric powder was estimated by standard methods proposed by using Ranganna, 2005) [8].

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

### 3. Extraction of curcumin from turmeric

Good quality dried rhizomes of selected three cultivars (Salem, Rajapuri and local) were cut in small pieces, powdered by food grinder. 2 g of sample were taken into a

thimble and placed in a soxtron apparatus. 90 ml of methanol solvent was added and extracted according to their boiling point for seven hours. After completion of extraction the dark brown extract was cooled, concentrated using water bath. The absorbance is measured using spectrophotometer at 420 nm. Curcumin content is calculated using following formula

$$\text{Curcumin (g/100g)} = \frac{0.0025 \times \text{Absorbance at 425 nm} \times \text{volume made up} \times \text{Dilution factor}}{0.42 \times \text{weight of sample} \times 1000} \times 100$$

#### 4. Separation of curcumin by thin layer chromatography

Curcumin cultivar extracts were run by using thin layer chromatography. The mobile phase was prepared using

chloroform and methanol in the ratio of 95:5 respectively. This experimental setup was left as it is until mobile phase reaches  $\frac{3}{4}$  th of the TLC sheet. The plates were spread with 1% KOH solution. The three curcuminoids were separated into 3 different single bands of each cultivar. RF value is calculated by using following formula.

$$\text{RF} = \frac{\text{Distance travelled by spot}}{\text{Distance travelled by solvent}}$$

### Result and Discussion

#### 1. Physical parameters of turmeric rhizome

**Table 1:** Physical parameters of turmeric rhizome

S. No.	Properties	Cultivars		
		Salem	Rajapuri	Local
1	Colour	Yellowish	Yellowish orange	Yellowish brown
2	Shape	Irregular	Irregular	Irregular
3	Weight(g)	11.63	10.54	9.85
4	Length(mm)	83.33	66.07	62.01
5	Width(mm)	14.24	12.91	10.87
6	Thickness(mm)	12.87	10.87	9.30
7	Bulk volume(mm <sup>3</sup> )	13.8 x 10 <sup>3</sup>	9.27 x 10 <sup>3</sup>	6.27 x 10 <sup>3</sup>

\*Each observation is an average of three determinations.

The data on physical parameters justifiable for techno-economical feasibility status of turmeric rhizome for quality powder depicted in table no.1 reflects on overall physico-chemical development.

The turmeric rhizome colour of various cultivars observed to be yellowish to yellowish brown. The average rhizome weight was recorded in the range of 9.85 to 11.63g (10.74g Avg). The length and width of rhizomes was observed to be in the range of 62.01 to 83.33 mm(72.67mm Avg) and 10.87 to 14.24 mm (12.55mm Avg) respectively. The thickness as a bulk determining one of the parameters was also observed in the

range of 9.30 to 12.87mm (11.08mm Avg). Bulk volume was also recorded an appeared to be highest in Salem cultivar (13.8 x 10<sup>3</sup> mm<sup>3</sup>). Bulk volume parameter may act as an option to correlate curcumin percent yield extraction ability and accessible technology. The powdered turmeric percent yield determining parameters as length, width and thickness are justifiable for techno economical commercial feasibility development.

#### 2. Proximate composition of turmeric rhizome

**Table 2:** Proximate composition of turmeric cultivars

S. No.	Parameters	Salem	Rajapuri	Local
1	Moisture (%)	7.6	8.1	8.9
2	Carbohydrate (%)	68.1	64	62.4
3	Protein (%)	7.1	7.5	6.3
4	Fat (%)	5.2	4.4	4.07
5	Crude Fiber (%)	4.6	4.3	5.6
6	Ash (%)	6.03	5.5	5.9
7	Acid Insoluble Ash (%)	1.6	1.4	1.7

\*Each observation is an average of three determinations.

The data on turmeric rhizome composition illustrated in table no.2, reflects on cultivar diversity of major and minor nutrition base components to justify the overall quality. Salem cultivar contains highest carbohydrate (68.1%) followed by Rajapuri (64%) and local cultivar (62.4%). The protein content found highest in Rajapuri (7.5%) than Salem (7.1) and local cultivar (6.3%). Fat content as a curcumin carrier based on its solubility was observed in the range of 4.4% to 5.2%.

Crude fiber content as digestive track cleanser was recorded highest in local cultivar (5.6%) as compared to Salem (4.6) and Rajapuri (4.3). Ash and acid insoluble ash content were noted in range of 5.5% to 6.03% and 1.4% to 1.7% respectively.

#### 3. Extraction of curcumin

**Table 3:** Curcumin profile of turmeric cultivars

S. No.	Cultivars	Curcumin (%)
1	Salem	3.68
2	Rajapuri	2.73
3	Local	2.15

\*Each observation is an average of three determinations.

The data on curcumin as a targeted component justifiable for functional efficacy status of turmeric cultivars is depicted in table no.3. Curcumin was recorded to be highest in Salem cultivar (3.68%) compared to Rajapuri (2.73 %) and local variety (2.15 %). These observations support the Monitor able

extraction base technology development for commercial exploration. It also provides administered guidelines for fractionation base efficacy for its functionality.

#### 4. Curcumin molecular characterization

**Table 4:** Curcumin fraction profile of turmeric cultivars

S. No.	Cultivars	RF values			DMC/BDMC Quotient
		C	DMC	BDMC	
1	Salem	0.71	0.62	0.32	1.94
2	Rajapuri	0.66	0.61	0.29	2.10
3	Local variety	0.79	0.50	0.18	2.70
4	Standard curcumin (RML)	0.75	0.55	0.27	2.03

\*Each observation is an average of three determinations.

**C**= Curcumin

**DMC** = Demethoxycurcumin

**BDMC**= Bisdemethoxycurcumin

Cultivar diversity data on curcumin fractionation (Curcumin, DMC, BDMC) by thin layer chromatography is presented in Table 4. The RF values of fractions of various cultivars recorded in range leading to 0.66 to 0.79, 0.50- 0.61, 0.18 to 0.32 for curcumin, demethoxycurcumin and bisdemethoxycurcumin respectively observed to be at par with curcumin standard values. DMC/BDMC quotient values of various cultivars depicted in same table also helped in designing a curcumin functional efficacy hypothesis to justify its nutraceutical value. Higher the quotient value more is the functional efficacy and vice versa. Salem as a yield base (3.68%) techno economically feasible curcumin cultivar represents lowest DMC/BDMC quotient admissible for highest functional efficacy against rest of the cultivars. Highest quotient of local cultivar may be associated with traditional crop development superimposed by advanced agricultural crop development either by tissue culture or genetical plant breeding.

#### Conclusion

Molecular fractionation base curcumin characterization of turmeric with reference to DMC/BDMC quotient is an important aspect to justify nutraceutical potential reflecting on human health claim under the hypothesis entitled as 'let food be a medicine and medicine be a food'. DMC/BDMC quotient is emerged out as a specialty feature of curcumin for recognition of its value as an ingredient empowered with functional efficacy. Higher the quotient value more is the functional efficacy and vice versa. It has also provided a lucrative option to food technologists to standardize curcumin demethoxylation process exclusively in the interest of functional efficacy as an innovative technology for production of health claim base food products.

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