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Enzyme-assisted turmeric oil extraction from turmeric rhizomes

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An enzyme-assisted modified steam distillation process was adopted to extract turmeric oil from *Curcuma longa* L. rhizomes. The diastase, xylose, cellulase, pectinase, and lipase enzymes were used for the pre-treatment of fresh turmeric rhizome to obtain a higher yield by rapturing biological cells. The quantitative and qualitative analysis of turmeric oil was performed by GC–MS. The various influencing parameters for the extraction of turmeric oil such as an enzyme, incubation/pre-treatment time, distillation time have been studied in the present work. The obtained turmeric oil by enzymatic pre-treatment process is richer in bioactive/medicinal components than in the other traditional methods. The maximum yield was obtained with cellulase enzyme, which is 25–27% higher than the yield obtained by the traditional hydro distillation process. The detailed qualitative and quantitative analyses are also presented. The present method can be considered energy-efficient, effective, economical, and eco-friendly.

Keywords: turmeric oil, enzyme assisted, diastase, cellulase, pectinase

1. INTRODUCTION

Turmeric (*Curcuma longa* L.) is one of the prominent herbs/spices used in food preparation and preservation, traditional/herbal/Ayurvedic medicines, cosmetics, dye, etc. (Laokuldilok et al., 2015; Singh et al., 2010). Its pretended uses include the treatment of jaundice, malaria, skin diseases, gastric ulcer, diabetes, rheumatoid arthritis, cold-flu, hypertension, convulsions, and various emotional disorders (Ayati et al., 2019; Shosan et al., 2014). Turmeric is rich in curcuminoids and is usually consumed as fresh rhizome,

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dried rhizome, powdered, in the form of essential oil, and/or oleoresins. It shows anti-inflammatory, antioxidant, antispasmodic, and antimicrobial properties (Priyadarsini, 2014). According to Chang et al. (2006), turmeric oil consists of different sesquiterpenes and α–atlantone along with curcuminoids. The major component found in turmeric rhizome consists of turmerone, which protects cells from damage, enhances wound healing capacities, inhibits replication of HIV, controls type 2 diabetes symptoms, conceals tumour formation, protects the damage of the liver, etc. (Chassagnez-Mendez et al, 2000; Dosoky and Setzer, 2018; Shirsath et al., 2017).

The shelf life of turmeric rhizomes is limited due to bacterial and fungal growth. The dried herbs usually have an unacceptable hay-like aroma, unpleasant smell, dusty and difficult to handle in bulk. The extraction of essential oil and production of oleoresin are the ways to handle these problems, including efficient use, ease of transport, etc. The chemical composition of *Curcuma longa* L. was studied by Lee (2006), and almost 235 compounds were identified (Dosoky and Setzer, 2018; Ferreira et al., 2013). The antifungal, anti-arthritic activity of turmeric oil was observed by Funk et al. (2010). Priya et al. (2012) observed the antioxidant potential of turmeric leaf essential oil. Further, Kumar et al. (2016) confirmed the potential application of turmeric oil in the food industry to control a fungal infestation. Due to high bioactive content and aggressive bioactivity, turmeric has excellent value in traditional medicine and is widely used across the globe. Now, it is crucial to find out the optimum process for turmeric oil with increased yield (qualitative and quantitative). Beside steam distillation (Manzan et al., 2003), essential oil extraction obtained by solvent extraction is another widely used process (Ibáñez and Blázquez, 2021; Jayaprakasha et al., 2001; Negi et al., 1999) to extract essential oil and curcumin oleoresin. An increased yield of turmeric oil from turmeric leaves and rhizomes have been obtained by Chandra et al. (2016) using continuous water circulation distillation process (a modified steam distillation). The essential oil yield of the turmeric varies from 2 to 5.5% (Contesini et al., 2018; Srivastava et al., 2001).

Alternatively, these isolates can be derived synthetically from petroleum origins, which is not economical and energy intensive and costly. We should consider these factors for the production/isolation of bioactive compounds from natural resources. The enzyme based process provides the energy-efficient natural route to obtain an increased yield of turmeric oil. In this work, we are using an eco-friendly method to isolate turmeric oil with enzyme-assisted pre-treatment. The enzymes interact with the substrate (raw material) in various ways and lyse the cell wall, which facilitates the extraction of different cell components. The pre-treated raw material undergoes the modified steam distillation process to obtain turmeric oil. The obtained turmeric oil has been analysed by gas chromatography hyphenated with the mass spectrometer.

2. MATERIAL AND METHOD

Fresh turmeric rhizomes were purchased from the markets of northern India. The washed and surface dried rhizomes were chopped into small pieces (of ~4–5 mm size) to increase the exposed surface area for enzymatic pre-treatment. The enzyme was used to facilitate the rapture of the biological cells through the digestion/degradation of turmeric rhizomes. The cellulose (~0.8 units/mg) pectinase (~400 units/g), lipase (~160 units/mg), diastase (~100 units/mg), and xylose (~350 units/g) were chosen for the pre-treatment of turmeric rhizomes. These enzymes were purchased from the authorized Merck dealer/institute vendor.

2.1. Pre-treatment of turmeric rhizomes

The enzyme concentration was optimized as 50 mg/l (~25 °C) for the optimum process and the deviation from its optimum value will lead to over/ under digestion of cell. 10 g of chopped turmeric rhizomes were taken into a petri dish with the enzymatic solution and kept in the constant temperature water bath for

180 minutes at 40 °C. The pH of the turmeric rhizome pre-treatment mixture was observed between 4.5 and 6. It has been observed that all enzymes worked well at low pH (acidic condition) except lipase, as it works well in an alkaline medium (Contesini et al., 2018).

2.2. Experimental setup and procedure

The steam was continuously produced in flask A through electrical heating and passed through the turmeric fixed-bed column containing random packing of longitudinal slices of turmeric rhizomes. Collector B was added to collect transient condensate of the steam passing through the turmeric bed. Initially, as the steam approached the turmeric bed, it got condensed, and the condensate would be collected in collector B, and the bed temperature increased slowly. During this initial heating of the bed, vapours did not pass through the bed, although they got condensed in the bed itself. After some time, as the system approached the equilibrium, the steam passed through the turmeric bed, the steam along with the volatile contents (vapours) rose from the column C and entered the cold-water condenser D. In the condenser, the vapours were condensed and collected in collector E. Being a lighter component, the turmeric oil was collected as a top product in the collector, whereas the condensed water layer remained as the bottom layer. The turmeric oil was separated and obtained as the top product through tap F. In this continuous operation, the loss of water in the boiling flask A due to continuous boiling and steam generation was being compensated by the condensed water from column E as reflux. This operation continued for 4.5 hours (300 min). An additional vent may provided for the periodical release of uncondensed gases and excessive pressure. The process schematic is shown in Figure 1.

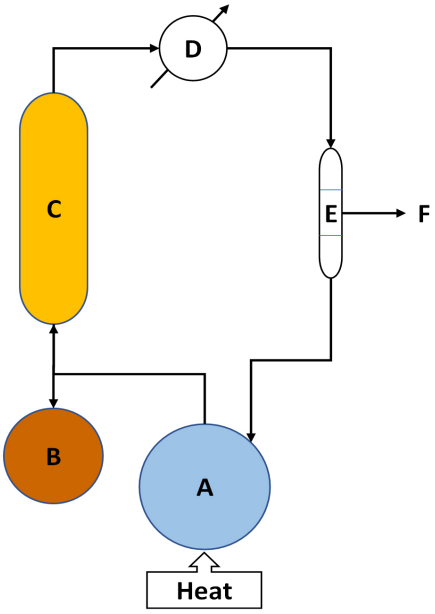

Fig. 1. Modified steam distillation process

2.3. Gas chromatography – mass spectrometry analysis of the turmeric oil

The obtained extract was separated by filtration through the membrane (0.22 μm), and a 1μ L-1 aliquot was injected in GC apparatus (Perkin Elmer model number Clarus 500) connected with a mass spectrometer (quadrupole) using a capillary column (30 m × 0.25 mm Φ, Phenomenex). The temperature was programmed at 40 °C for 2 min and ramped up to 200 °C (5 m) and 300 °C at 11 °C/min. The pure helium gas (> 99.999% pure) was used as the carrier gas at a constant flow velocity of 35.9 cm/s. The electron energy of 70 eV was set for a full scan of mass range of 50–550 m/z. The real-time data was collected

on the attached PC. The GC–MS analysis was performed to identify the eluted compounds by comparing the retention indices and mass spectra with the literature values and National Institute of Standards and Technology (NIST), Wiley.

3. RESULTS AND DISCUSSION

3.1. Estimation of moisture content

The moisture contents of the turmeric rhizomes play an imperative role in the extraction process. The moisture contents of the turmeric rhizome were estimated by keeping the measured amount of turmeric at 105 °C for 4 hours. The loss of weight gives the % moisture content, and the moisture contents of the turmeric rhizome are calculated using the following expression:

$$\text{moisture content (\%)} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \cdot 100 \tag{1}$$

In our case, the average moisture content of the turmeric was estimated as 76%.

3.2. Optimization of incubation time

The incubation time was defined as the time at which biomass is exposed to the enzyme activity for pre-treatment. The incubation time was optimized by conducting various experiments for the time range of 60 to 300 minutes (1–5 hours) at 60 minute time intervals (Table 1). The enzyme concentration was taken as 50 mg/l for 100 g turmeric sample. The sample S1, S2, S3, S4, S5 was incubated for 60, 120, 180, 240, and 300 minutes (min), respectively. The yield became constant after 180 min of incubation. Hence, 180 minutes has been chosen as the optimum incubation time.

Table 1. Effect of incubation period on the yield of turmeric oil

Sample number	S1	S2	S3	S4	S5
Incubation time, min.	60	120	180	240	300
% oil yield, ml/g	0.84	0.97	1.36	1.37	1.38

3.3. Optimization of process time

After optimization of incubation time for pre-treatment, we have optimized the time of the processing. The pre-treated turmeric rhizomes were fed to the packed column C for further processing to obtain turmeric oil. Several experiments were conducted at 30 minutes of processing time intervals, and the results are reported in Table 2. The results of Table 2 show that the oil yield became constant after 270 minutes of operation. Hence, 270 minutes is considered as the optimum time for the processing of the pre-treated turmeric rhizomes.

Table 2. Effect of process time on turmeric oil yield

Sample number	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15
Time, min.	30	60	90	120	150	180	210	240	270	300
% Oil yield, ml/g	0.33	0.53	0.91	1.37	1.54	1.89	2.06	2.47	2.76	2.77

3.4. Turmeric oil yield

The obtained turmeric oil in the form of % yield (ml/g) for all enzymes is tabulated in Table 3. The experiments were repeatedly performed several times to check the repeatability of the results, and the average values are reported in Table 3. The essential oil yield from turmeric rhizomes was estimated using the following expression.

$$\text{Oil yield (\%)} = \frac{\text{Essential oil obtained}}{\left[\text{Fresh feed weight} \cdot \left\{1 - \left(\text{moisture content}/100\right)\right\}\right]} \cdot 100 \tag{2}$$

The observation of Table 3 shows that the maximum yield came out to be 3.75% for the cellulase enzyme. In the case of lipase pre-treatment, the turmeric oil yield was lowest among these, with 2.08%. Since lipase shows high activity at pH > 7 whereas turmeric shows acidic behaviour, hence the reaction (conversion) goes down due to the reduced activity of the lipase enzyme in acidic media (Contesini et al., 2018). In a previous study by Srivastava et al. (2001), the turmeric oil content was about 2.2% (v/wt). The essential oil yield increased with the enzymatic pre-treatment of the turmeric rhizome. This was due to the fact that the major component of the turmeric rhizome is carbohydrates (69.4%) which is made up of cellulosic material that is degraded by an enzyme.

Table 3. Percentage (%) turmeric oil yields for different enzymes

Enzymes	Pectinase	Diastase	Lipase	Cellulase	Xylose
% yield, ml/g	2.9	2.5	2.1	3.75	2.29

For the qualitative analysis, the GC–MS of the obtained turmeric oils was performed and various compounds, such as monoterpenoid, sesquiterpenoid, bisabolane, caryophellane, sesquisabinane, bergamotane, cedrane, salinane, santalane, etc. were identified. Dosoky et al. (2018) and Angel et al. (2014) reported that the species are known for producing assembles of volatile components such as sesquiterpenes, monoterpenes, etc. and other aromatic components (Dosoky and Setzer, 2018; Angel et al., 2014). The major components of turmeric oil were p–cymen–8–ol (1.5–2.0%), zingiberene (1.6–3.01%), α–santalol (13.5–15.6%), ar–termerone (10.30–20.7%), termerone (0.9–1.4%), caryophyllene (2.1–2.9%), α–turpinene (9.7–10.7%), cineole (1.2–16.5%), α–turpinolene (10.5–15.8%) and others mentioned in Table 4 are still unidentified. The unidentified volume consists of approximately 68 compounds.

The turmeric oil obtained by the enzymatic pre-treatment process is found to be rich as it has more constituents as compared to the oil obtained without pre-treatment (Table 4). It may be possible that the rich turmeric oil obtained by the enzymatic process will have more medicinal properties than the turmeric oil obtained by other traditional essential oil extraction methods. The maximum number of compounds was obtained in the case of cellulose pre-treatment, whereas lipase is the next one. Table 4 also shows that the minimum number of compounds was found in the case of pectinase.

Table 4. Composition of turmeric oil with different enzymes

S. No.	Compound	Type	Without enzymes	Cellulase	Lipase	Xylose	Pectinase	Diastase
1	α–pinene	monoterpenoid	×	✓	✓	✓	✓	✓
2	β–pinene	monoterpenoid	×	×	×	×	✓	✓
3	δ–carene	monoterpenoid	✓	✓	✓	✓	✓	✓
4	β–myrcene	monoterpenoid	×	✓	✓	✓	✓	✓

Continued on next page

Table 4 [cont.]

S. No.	Compound	Type	Without enzymes	Cellulase	Lipase	Xylose	Pectinase	Diastase
5	geraniol	monoterpenoid	×	✓	✓	✓	✓	✓
7	α–phellandrene	monoterpenoid	✓	✓	✓	✓	✓	✓
8	nerol	monoterpenoid	×	✓	×	×	×	×
9	linalool	monoterpenoid	×	✓	×	×	×	×
10	α–thujene	monoterpenoid	✓	✓	✓	✓	✓	✓
11	γ–terpinene	monoterpenoid	✓	✓	✓	✓	✓	✓
12	α–humulene	sesquiterpene	×	✓	✓	✓	✓	✓
13	α–terpinene	monoterpenoid	×	✓	✓	✓	✓	✓
14	α–terpinolene	monoterpenoid	✓	✓	✓	✓	✓	✓
15	cineole	monoterpenoid	✓	✓	✓	✓	✓	✓
16	xanthorrhizol	bisabolane	×	×	×	×	×	✓
17	tans–sesqui sabinene hydrat	sesquisabinane	×	×	✓	✓	×	×
18	carene	monoterpenoid	×	✓	✓	×	✓	×
19	p–cymen–8–ol	monoterpenoid	✓	×	✓	✓	✓	✓
20	caryophyllene	sesquiterpene	✓	✓	✓	✓	×	✓
21	di–epi–α–cedrene	cedrane	×	×	×	✓	×	×
22	zingebrene	bisabolane	✓	✓	✓	✓	×	✓
23	β–sesqui phellenderen	bisabolane	✓	✓	✓	✓	✓	✓
24	β–bisabolene	bisabolane	×	×	✓	✓	×	✓
25	α–bergamotene	bergamotane	✓	✓	✓	✓	×	✓
26	ar-turmerone	bisabolane	✓	✓	✓	✓	✓	✓
27	α–turmerone	bisabolane	✓	×	✓	×	×	✓
28	β–turmerone	bisabolane	×	×	✓	✓	×	×
29	α–atlantone	bisabolane	×	×	✓	✓	✓	✓
30	β–atlantone	bisabolane	×	✓	✓	✓	✓	✓
31	α–santalol	santalane	×	×	✓	✓	×	×
32	ascaridole	monoterpenoid	×	×	✓	✓	✓	✓
33	α–cubebene	sesquiterpene	×	×	×	✓	✓	×
34	geranyl acetate	monoterpenoid	×	×	×	×	×	✓
35	β–cedrene	cadrane	✓	×	×	×	×	×
36	himachalene	himachalene	✓	×	×	×	×	×

Further, the composition of turmeric oil in terms of major components is shown in Table 5. Again, the maximum composition of each compound was found in the case of cellulase pre-treatment, whereas lipase is the next one. The composition of turmeric oil was analysed by GC and GC–MS, and it revealed the remarkable enrichment in the turmeric oil composition derived from enzyme based pre-treated process. The main enrich component were oxygenated monoterpenes, which leads to better antioxidant activity. Furthermore, the enzyme base pre-treatment significantly enhances the release of phenolic compounds also and consequently increases antioxidant activity. These obtained results suggest that enzyme base pre-treatment could be a useful process for extracting bioactive components, and it holds good potential for future use in the food, cosmetic as well as in pharmaceutical industries.

Table 5. Identified compounds with their composition

S. No.	Compound Name	Compounds present in percentage (%)					
		Without enzymes	Cellulase	Lipase	Xylose	Pectinase	Diastase
1	ar–turmerone	7.5	20.7	10.25	10.56	10.54	10.3
2	alpha–santalol	10.9	15.6	14.5	15.4	13.5	14.7
3	turmerone	0.8	1.4	1.3	1.3	1.4	0.9
4	zingiberene	1.6	3.01	2.5	2.9	2.7	1.6
5	caryophyllene	1.9	2.3	2.9	2.7	2.1	2.5
6	p–cymen–8–ol	1.0	2.0	1.6	1.8	1.5	1.8
7	alpha–turpinene	8.5	10.7	10.4	10.5	9.7	10.5
8	cineole	1.5	16.5	1.2	9.6	1.8	16.8
9	alpha–phellandrene	7.6	15.8	14.8	10.5	10.5	14.5

The major component found in our present study is turmerone, which possesses potent anti-inflammatory anti-oxidative and antiplatelet properties (Park et al. 2012; Lee 2006). The obtained compounds are listed in Table 5. The spectrum of α–turmerone has shown the peak at m/z 216 with the loss of methyl ion at m/z 201, α–cleavage to the aromatic ring at m/z 119, and α–cleavage to the carbonyl at m/z 83 (Al-Reza et al., 2010; Rao et al., 1989; Kuroda et al., 2005). Two odd electron ions at m/z 132 and m/z 98 showed the Mc-Lafferty rearrangements. These turmerones have analogous chemical structures, similar physical properties, and almost equal molecular weights, but the tastes are different. The literature shows that the ar-turmerone is the best medicine for the treatment of edema, hemorrhage, and focal necrosis (Kuroda et al., 2005). Moreover, ar-turmerone shows antiplatelet activity and is found to be a more potent platelet inhibitor against platelet aggregation than aspirin. Additionally, turmerone is also found effective in improving insulin resistance and ameliorating type 2 diabetes (Kuroda et al., 2005). The quality of turmeric oil can be identified by the turmerone content, and it may be an active marker for the quality assessment.

4. CONCLUSIONS

The presented work was aimed to explore the possibility of enzymatic degradation as pre-treatment to obtain enhanced turmeric oil yield over the conventional process such as hydro-distillation, steam distillation, cold pressing, etc. The present process is the combination of enzymatic degradation of pre-treatment followed by modified steam distillation to obtain turmeric oil from fresh turmeric rhizome. The proposed enzyme-

assisted method shows an efficient way to obtain essential oil from turmeric rhizomes. Consequently, this process can be considered as a promising alternative for getting turmeric oil. The cellulase enzyme was found to be the best for the pre-treatment of turmeric rhizome over other enzymes investigated in the present work. The obtained turmeric oil by enzymatic pre-treatment process was found rich in terms of more constituents (medicinal components) than the other traditional methods with increased yield. The quality of turmeric oil (essential oil) is usually estimated with the chemical composition of the obtained essential oil. In the case of enzymatic pre-treatment with cellulase, the obtained oil is rich in terms of composition and yield. Furthermore, qualitative and quantitative analysis has been done, and the current process can be concluded as less energy-intensive, efficient, economical, and eco-friendly.

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