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Study on physico-chemical properties of patchouli oil

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Abstract
Patchouli (*Pogostemon Cablin*) is an aromatic herb plant. It is a very fragrant, bushy herb with soft oval leaves and square stems. Patchouli oil is widely used in food, pharmaceutical and cosmetic industries. The shade dried samples were kept at room temperature as treated with microbial culture *Aspergillus foetidus, Penicillium citrinum* and *Trichosporon asteroides* for 2, 4, 6 and 8 days along with fresh and control samples. The patchouli oil samples extracted during the study were also analyzed for its physico-chemical quality. The extracted oil samples have shown the values of physico-chemical parameter in the standard permissible range of patchouli oil except the oil extracted after 4 days of incubation with the culture *Penicillium citrinum.* The acid value increases beyond 4.0, which is not permissible range for the patchouli oil. Higher end analysis of the oil samples with GC-MS indicated the effect biotransformation efficiency of different microorganisms on the patchouli oil component.

Keywords: Patchouli oil, fungi, density, refractive index, acid value and ester value

Introduction
Patchouli (*Pogostemon cablin* Pellet: Lamiaceae), native to South East Asia, produces oil of commercial importance, finds its extensive use in cosmetics, as a fixative and in aromatherapy. The main composition of patchouli oil is patchouli alcohol, nor-patchoulene, bulnesene and beta-patchoulene. Tenacity of odour is the virtue of patchouli oil and is one of the qualities for its versatile use (Reddy, 2012) [17].

Patchouli is an erect, branched, pubescent aromatic herb, the essential oil of which is one of the best fixatives for heavy perfumes which imparts strength, character, alluring notes and lasting qualities. In fact, it is a perfume by itself and is highly valued in perfumes, soaps, cosmetics and flavour industries. The oil is extensively used as a flavor ingredient in major food products, including alcoholic and non-alcoholic beverages, frozen dairy desserts, candy, packed foods, gelatin, meat and meat products. It blends well with the oils of sandal wood, geranium, vetiver, cedarwood, clove, lavender, bergamot and many others. The oil gives one of the finest attars when blended with sandal wood oil. Tenacity of odour is one of the great virtues of patchouli oil and is one of the reasons for its versatile use. The oil possesses antibacterial and insect repellent activity. It is also used as a masking agent for alcoholic breath. The oil is also used in aromatherapy for its antidepressant, anti-inflammatory, cytophylactic, deodorant and fungicidal properties (Joy *et al*. 2001 [6], Shukor, 2008 [19], and Ramya *et al*. 2013) [16].

Presently, the extraction of essential oil from patchouli leaves is being done with the help of age old traditional techniques. Basically, there are several methods to extract the oil. For example steam distillation, supercritical solvent extraction, ultrasonic extraction, conventional distillation method etc. (Nasharudin *et al.* 2008) [13]. The quantity along with quality of extracted oil can further be increased with the introduction of better post harvest management practices and improvement of processing technology. Though, extraction process was carried out to optimize the oil recovery by physical process, there is some biological process viz. fermentation of herbage before the extraction of oil, which can improve the recovery of oil from the herbage. Therefore, further experiments can be carried out to improve oil recovery as well as patchouli alcohol percent in patchouli oil.

Material and Method

Quality Analysis

Density

Density of an essential oil is defined as the ratio of the weight of a given volume of oil to the volume of oil at constant temperature. This is usually reported at 20 ºC. A temperature correction of approximately 0.00045 per degree may be made either by subtracting or addition to bring it to 20 ºC.

The weight of the oil can be determined with the help of a thoroughly cleaned and dried pycnometer.

$$\textbf{Density}(20^{o}\textbf{C}) = \frac{\mathbf{W_o}}{\mathbf{V_o}}$$

Where,

W_o = Weight of oil

V_o = Volume of oil

Refractive Index

This can be determined with the help of a refractometer which gives the reading directly after calibration. The reading can be observed at room temperature with the help of ATC probe.

Density and refractive indices are very sensitive to temperature changes. The low temperatures tend to increase the values considerably while at higher temperatures the values are sufficiently lowered.

Acid Value

Most of the essential oil contains small amounts of various free acids and therefore, the content is usually reported as acid number rather than its percentage. The acid number of oil is defined as the number of mg of potassium hydroxide required to neutralize the free acids in 1 g of oil. While determining the acid number the alkalis should be quite dilute (0.1 N) as strong alkalis may hydrolyze the esters even in cold conditions, thereby giving a higher acid number. In case, large excess of phenols are present, the indicator should be changed from phenolphthalein to phenol red.

Procedure

Weight accurately about 500 mg of the oil into 100 ml saponification flask. Add 10 ml of neutral alcohol and a drop of phenolphthalein. Titrate this against standard solution of 0.1 N KOH to the end point. This titration requires only a few drops of the alkali. Keep a blank reading also.

Calculation

Volume consumed = Y– X = Z
Volume of alkali used = P – Z = R
Blank reading = P
Initial reading = X
Final reading = Y

$$\mathbf{A_v} = \frac{\mathbf{56.1} \times \boldsymbol{R} \times \boldsymbol{N}}{\boldsymbol{W_o}}$$

Where,

A_v=Acid value ;

N = Normality; and

W_o= Weight of oil (mg).

Ester value

Esters are normally calculated as ester number or value because they are usually mixtures of unknown esters. An ester number is defined as the number of mg of potassium hydroxide required to saponify the esters present in 1g of oil. For the esters of dibasic acids or dihydroxy alcohols, the ester number is divided by 2.

Procedure

Weight accurately about 500 mg of the oil into a 100 ml saponification flask. Add about 10 ml of neutral alcohol and a drop of phenolphthalein. Titrate this against standard solution of 0.1 N KOH to the end point. This titration requires only a few drops of the alkali. Keep a blank reading also.

To the above flask add 10 ml of 0.5 N alcoholic KOH, attach the air condenser and reflux the contents on the water bath for 2 hr. Cool and titrate against 0.5 N HCl.

Calculation

Volume consumed = $Y' - X' = Z'$
Amount used for saponification = $P' - Z' = R'$
Blank reading = P'
Initial reading = X'
Final reading = Y'

$$\mathbf{E_v} = \frac{\mathbf{56.1} \times \mathbf{R'} \times \mathbf{N}}{\mathbf{W_o}}$$

Where,

E_v=Acid value;

N = Normality; and

W_o = Weight of oil in mg

Results and Discussion

Physico-Chemical Quality of Patchouli Oil

The physico-chemical quality of patchouli essential oil extracted from different samples were analyzed by using standard methods as mention in the materials and methods section. Density, refractive index, acid value and ester value were analyzed for physico-chemical quality of patchouli oil extracted from different extracts.

Physico-chemical quality of patchouli oil extracted after incubation with *Aspergillus foetidus, Penicillium citrinum and Trichosporon asteroides*

The patchouli oil extracted at different intervals of days after fermentation was analyzed for density, refractive index, acid value and ester value. The data obtained are presented in Table. The given data indicates that the physico-chemical quality; density, refractive index, acid value and ester value of patchouli oil are significantly affected by the treatment of cultures with respect to days. Data indicates the increase in density however, decrease in refractive index, acid value and ester values with respect to increase in incubation time.

1. The patchouli oil extracted after treatment with *Aspergillus foetidus* were analyzed for its physico-chemical quality. Density, refractive index, acid values and ester values for oil extracted from the different sample *viz.* fresh, control 2, 4, 6 and 8 days was found 0.972, 0.974, 0.975, 0.977, 0.982 and 0.982g/ml; 1.5116, 1.5095, 1.5086, 1.5078, 1.5064 and 1.5064; 2.74, 2.81, 3.36, 3.48, 3.81 and 3.81; 9.83, 8.88, 7.83, 7.33, and 6.17 respectively.
2. The patchouli oil extracted after treatment with *Penicillium citrinum* were analyzed for its physico-chemical quality. Density, refractive index, acid values and ester values for oil extracted from the different sample *viz.* fresh, control 2, 4, 6 and 8 days was found 0.972, 0.974, 0.976, 0.981, 0.985 and 0.985 g/ml; 1.5116,

1.5095, 1.5090, 1.5068, 1.5052 and 1.5051; 2.74, 3.01, 3.36, 3.93, 4.15 and 4.17; 9.83, 6.73, 6.17, 5.61, 5.05 and 5.01 respectively.

3. The patchouli oil extracted after treatment with *Trichosporon asteroides* were analyzed for its physico-chemical quality. Density, refractive index, acid values and ester values for oil extracted from the different sample *viz* .fresh, control 2, 4, 6 and 8 days was found 0.972, 0.974, 0.980, 0.982, 0.988 and 0.99 g/ml; 1.5116, 1.5095, 1.5093, 1.5074, 1.5048 and 1.5046; 2.74, 2.81, 2.94, 3.37, 4.05 and 4.09; 9.83, 8.88, 7.83, 7.30, 6.73 and 5.61 respectively.

It is observed that the density of patchouli oil increases from 0.972 to 0.982 g/ml. The density of patchouli oil extracted from various incubated and control samples are 0.972, 0.974, 0.975, 0.977 and 0.982 g/ml for fresh control, 2, 4 and 6 days treatment of microorganism. The oil extracted from fresh and control patchouli has lower value of density 0.972 and 0.974 g/ml, respectively. The oil extracted from 6 days *Aspergillus foetidus* incubated sample gives highest value 0.982 g/ml compared to other samples.

It may be inferred From Table 4.7 and Fig. 4.4 that refractive index of patchouli oil decreases as interval of day increases. Refractive index of different extracts decreases from 1.5116 to 1.5064. Refractive indexes of patchouli oil were found 1.5116, 1.5095, 1.5086, 1.5078, and 1.5064 for fresh, control & 2, 4 and 6 days *Aspergillus foetidus* extracts, respectively. The oil extracted from fresh patchouli gives highest refractive index 1.5116 and the oil extracted from 6 days *Aspergillus foetidus* sample gives least value 1.5064 compared to other samples on essential oil from patchouli.

Acid value of oil obtained after different treated and untreated samples increases from 2.74 to 3.81. The oil extracted from fresh and control sample gives lower acid value 2.74 and 2.81 respectively, whereas oil from the samples treated with the culture gives the acid value from 2.74 to 3.81. Further increase in acid value will lead to no market for the oil. The acid value higher than 4.0 indicates the poor quality of oil and is not acceptable in the market.

Ester value of different extracts decreases gradually from 9.83 to 6.17. When the oil extracted from fresh patchouli gives highest ester value 9.83 and the oil extracted from 6 days AS treated sample gives least value 6.17 as compared to other treatment.

Table1: Physico-chemical quality of patchouli oil extracted after incubation with *Aspergillus foetidus*

Treatment	Density (g/ml)	Refractive Index	Acid Value	Ester Value
Fresh	0.972	1.5116	2.74	9.83
Control	0.974	1.5095	2.81	8.88
Day 2	0.975	1.5086	3.36	7.83
Day 4	0.977	1.5078	3.48	7.33
Day 6	0.982	1.5064	3.81	6.17
Day 8	0.982	1.5064	3.81	6.17

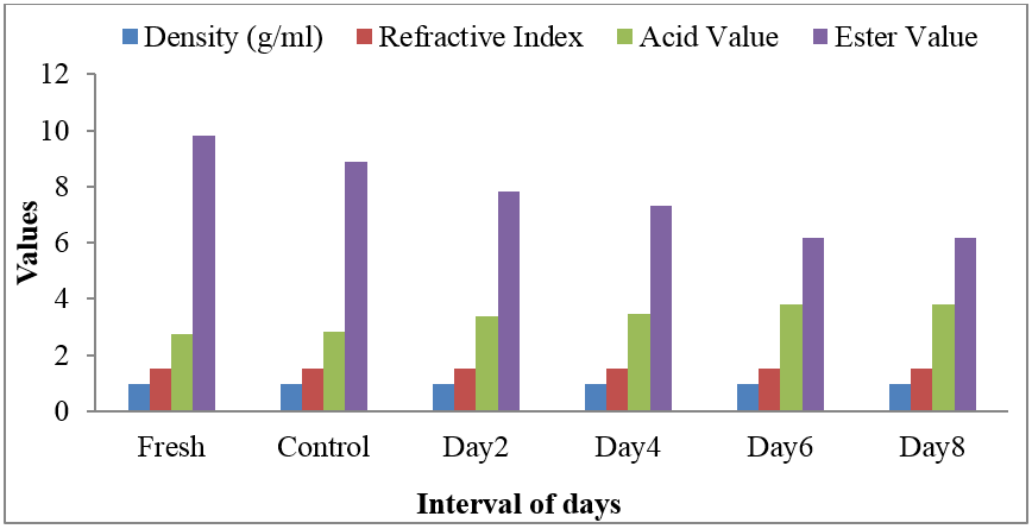

Fig 1: Physico-chemical quality of patchouli oil extracted after incubation with *Aspergillus foetidus*

Physico-chemical quality of patchouli oil extracted after incubation with *Penicillium citrinum*

The patchouli oil extracted at different intervals of days after fermentation was analyzed for density, refractive index, acid value and ester value. The data obtained are presented in Table. The given data indicates that the physico-chemical quality; density, refractive index, acid value and ester value of patchouli oil are significantly affected by the treatment of cultures with respect to days. Data indicates the increase in density however, decrease in refractive index, acid value and ester values with respect to increase in incubation time.

It is observed that the density of patchouli oil increases from 0.972 to 0.985 g/ml. The density of patchouli oil extracted from various incubated and control samples determined was 0.972, 0.974, 0.976, 0.981, 0.985 and 0.985 g/ml for fresh control, 2, 4, 6 and 8 days treatment. The oil extracted from fresh and control patchouli gives least value of density 0.972 and 0.974 g/ml. The oil extracted from *Penicillium citrinum* incubated sample after 6 days gives highest density 0.985 g/ml. compared to other samples.

Table 2: Physico-chemical quality of patchouli oil extracted after incubation with *Penicillium citrinum*

Treatment	Density (g/ml)	Refractive Index	Acid Value	Ester Value
Fresh	0.972	1.5116	2.74	9.83
Control	0.974	1.5095	3.01	6.73
Day 2	0.976	1.5090	3.36	6.17
Day 4	0.981	1.5068	3.93	5.61
Day 6	0.985	1.5052	4.15	5.05
Day 8	0.985	1.5051	4.17	5.01

It may be inferred From Table 4.8 and Fig. 4.5 that refractive index of patchouli oil decreases as interval of day increases. Refractive index of different extracts decreases from 1.5116 to 1.5051. Refractive index of patchouli oil obtained was 1.5116, 1.5095, 1.5090, 1.5068, 1.5052 and 1.5051 for fresh, control, 2, 4, 6 and 8 days sample incubate with *Penicillium citrinum* extracts. The oil extracted from fresh patchouli gives highest value of refractive index 1.5116 and the oil extracted from sample treated with *Penicillium citrinum* after 8 days sample gives least value 1.5051 compared to other samples.

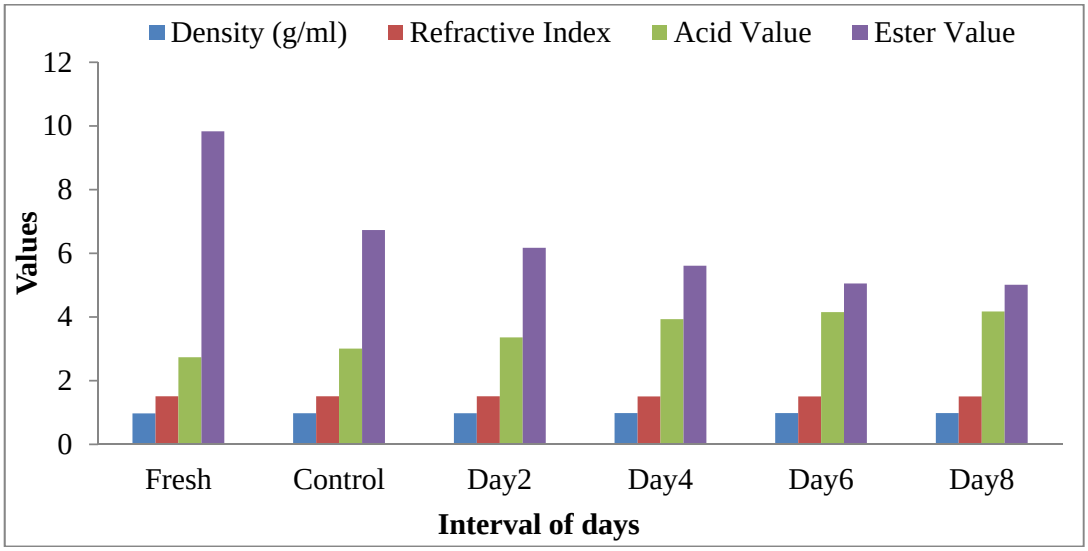

Fig 2: Physico-chemical quality of patchouli oil extracted after incubation with *Penicillium citrinum*

Acid value of oil obtained after different treated and untreated samples increases from 2.74 to 3.85. The oil extracted from fresh and control sample gives lower acid value 2.74 and 2.85, respectively, whereas oil sample from the samples treated with the culture gives the acid value from 2.74 to 3.85. Further increase in acid value will lead to no market for the oil. The acid value higher than 4.0 indicates the poor quality of oil and is not acceptable in the market.

Ester value of different extracts decreases gradually from 9.83 to 5.01, when the oil extracted from fresh patchouli gives highest ester value 9.83 and the oil extracted from 6 days *Penicillium citrinum* treated sample gives least value 5.05 as compared to other treatment.

Physico-chemical quality of patchouli oil extracted after incubation with *Trichosporon asteroides*

The patchouli oil extracted at different intervals of days after fermentation was analyzed for density, refractive index, acid value and ester value. The data obtained are presented in Table 4.9. The given data indicates that the physico-chemical quality; density, refractive index, acid value and ester value of patchouli oil are significantly affected by the treatment of cultures with respect to days. Data indicates the increase in density however, decrease in refractive index, acid value and ester values with respect to increase in incubation time.

It is observed that the density of patchouli oil increases from 0.972 to 0.991 g/ml. The density of patchouli oil extracted from various incubated and control samples are 0.972, 0.974, 0.980, 0.982, 0.988 and 0.991 g/ml for fresh control, 2, 4, 6 and 8 days treatment. The oil extracted from fresh and control patchouli gives least value of density 0.972 and 0.974 g/ml. The oil extracted from *Trichosporon asteroides* incubated sample after 8 days gives highest density 0.991 g/ml. compared to other samples on patchouli oil.

Table 3: Physico-chemical quality of patchouli oil after incubation with *Trichosporon asteroides*

Treatment	Density (g/ml)	Refractive Index	Acid Value	Ester Value
Fresh	0.972	1.5116	2.74	9.83
Control	0.974	1.5095	2.81	8.88
Day 2	0.980	1.5093	2.94	7.83
Day 4	0.982	1.5074	3.37	7.30
Day 6	0.988	1.5048	4.05	6.73
Day 8	0.991	1.5046	4.09	5.61

It may be inferred From Table 4.9 and Fig. 4.6 that refractive index of patchouli oil decreases as interval of day increases. Refractive index of different extracts decreases from 1.5116 to 1.5046. Refractive index of patchouli oil obtained was 1.5116, 1.5095, 1.5093, 1.5074, 1.5048 and 1.5046 for fresh, control, 2, 4, 6 and 8 days sample incubated with *Trichosporon asteroides* extracts. The oil extracted from fresh patchouli gives highest value of refractive index 1.5116 and the oil extracted from sample treated with *Trichosporon asteroides* after 8 days sample gives least value 1.5046 compared to other samples.

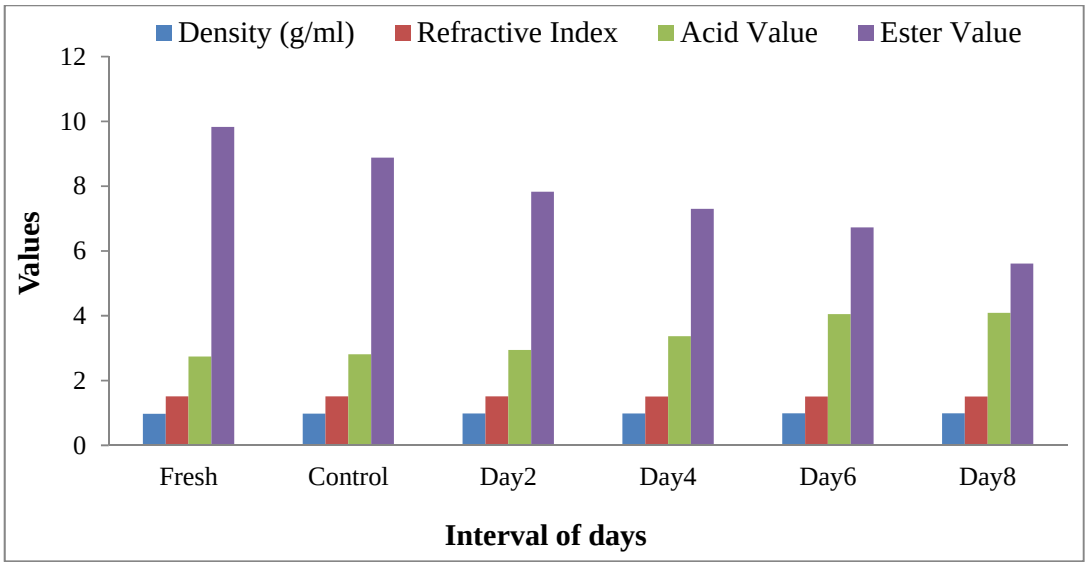

Fig 3: Physico-chemical quality of patchouli oil extracted after incubation with *Trichosporon asteroides*

Acid value of oil obtained after different treated and untreated samples increases from 2.74 to 4.09. The oil extracted from fresh and control sample gives lower acid value 2.74 and 2.85, respectively, whereas oil obtained from the samples treated with the culture gives the acid value from 2.74 to 4.09. Further increase in acid value will lead to no market for the oil. The acid value higher than 4.0 indicates the poor quality of oil and is not acceptable in the market.

Ester value of different extracts decreases gradually from 9.83 to 5.61. When the oil extracted from fresh patchouli gives highest ester value 9.83 and the oil extracted from 6 days *Trichosporon asteroides* treated sample gives least value 6.73 as compared to other treatment.

Conclusions

Biotransformation method can be utilized to improve oil recovery along with the oil quality. The source of raw materials and distillation method used (vapor versus water and vapor, condenser in a stagnant water bath or inside flowing water) will affect the yield, refractive index, patchouli alcohol content, acid number, and the clarity of the patchouli oil produced. Patchouli oil produced in general has yield range from 2.85% to 4.5%, refractive index of 1.5010 to 1.5056 and acid number of 2.40 to 4.17 g/ml. The longer the time used for the distillation process is, the higher the yield of patchouli oil, the higher the specific gravity (reach up to 0.955), and the higher the patchouli alcohol concentration is (34.03%). Quality of oil deteriorates, especially acid value increase with the increase in incubation period.

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