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ISOLATION OF CITRAL FROM LEMONGRASS OIL USING STEAM DISTILLATION: STATISTICAL OPTIMIZATION BY RESPONSE SURFACE METHODOLOGY

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ABSTRACT

In this study, citral, a major component of lemongrass oil is isolated using steam distillation. The objectives of the study were to explore the effect of the selected factors, volume ratio and time on the percentage yield for the obtained distillate (citral) and to develop a statistical relationship. The experiments were designed using response surface method (RSM) with percentage yield as the response. The results were analyzed statistically and the optimum conditions are identified as: volume ratio and time were 0.053 and 98.2126 min, respectively. Under the optimum conditions the yield is 85.1416%. A confirmation experiment under the optimum conditions showed a yield of 83.8%. This was only within experimental error range of < 5% from the predicted value. From our study, we found that specific gravity, density, flash point and refractive index of the product are 0.8904, 0.89031 g/cm^3, 91°C and 1.488, respectively, which were resembling the characteristics of standard citral.

Key words: Steam distillation, Optimization, Response surface methodology, Characterization of Citral, Central composite design.

INTRODUCTION

Lemongrass oil

Lemongrass is a perennial fast-growing aromatic grass, growing to about 1 meter (3 feet) high with long, thin leaves. Originally growing wild in India, it produces a network of roots and rootlets that rapidly exhaust the soil. In India, it is known as 'choomanapoolu' and is also referred to as 'Indian Verbena' or 'Indian Melissa oil'. In ayurvedic medicine, Lemongrass is used to help bring down fevers and treat infectious illnesses. It is a valuable ingredient in perfumes and citrus-type soaps and is also an insect deterrent.

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Lemongrass essential oil is extracted from *Cymbopogon citratus*. Lemongrass oil has a lemony, sweet smell and is dark yellow in color. The volatile oil of *Cymbopogon citratus* possesses a strong odor, with a basically lemon-like character. The lemony character is due to its high content of the aldehyde citral, which ranges from 75% to 85% of the oil's total constitution, which is isolated by steam distillation[1].

Citral

Citral, or 3,7-dimethyl-2,6-octadienal, is either of, or a mixture of, a pair of terpenoids with the molecular formula $C_{10}H_{16}O$. The two compounds are double bond isomers. The E-isomer is known as geranial or citral A. The Z-isomer is known as neral or citral B (Citral, The Merck Index, and 12th Edition). Citral is an aroma compound used in perfumery for its citrus effect. Citral is also used as a flavor and for fortifying lemon oil. It also has strong antimicrobial qualities[2], and pheromonal effects in insects[3]. Citral is used in the synthesis of vitamin A, ionone, and methyl ionone, and to mask the smell of smoke.

Citral is an example of a very large group of natural products called terpenes. The odors of camphor, menthol, lavender, rose, and hundreds of other fragrances are due to terpenes, many of which have 10 carbon atoms with double bonds or rings and aldehyde, ketone, or alcohol functional groups. In nature, these terpenes all arise from a common precursor, isopentenyl pyrophosphate. At one time, they were thought to come from the simple diene, isoprene (2-methyl-1,3-butadiene), because the skeletons of terpenes can be dissected into isoprene units, having five carbon atoms arranged as in 2-methy1butane. These isoprene units are almost always arranged in a "head-to-tail fashion."

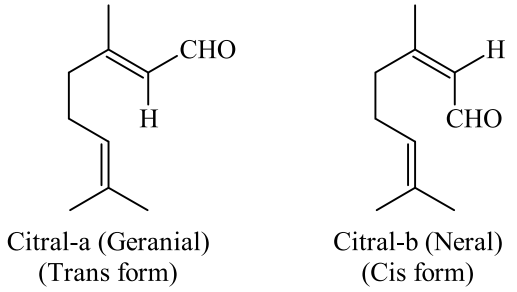

Fig. 1: Structure of citral-a and citral-b

Methods for isolation of essential oils

Fragrance extraction refers to the extraction of aromatic compounds from raw materials. There are many methods of essential oil extraction. The most popular being used is steam distillation. Other methods include Supercritical Fluid Extraction, Solvent

Extraction, and Ultra Sonication etc. The selection of appropriate extraction method will determine the quality and quantity of essential oils.

EXPERIMENTAL

Materials and methods

Lemongrass oil of 1 liter quantity is purchased from Susheel Aromatics (essential and aromatic oils), Visakhapatnam. It is stored in plastic bottles at 25°C. Diethyl ether is taken from our chemistry lab, which was stored in ambient colored bottle at room temperature.

Experimental procedure

The quantity of lemongrass oil and water are taken in the distillation flask according to the volume ratio (oil : water). 600 mL of distilled water was taken in the steam generation flask for generating steam. In presence of steam, the mixture of lemongrass oil and water was volatilized at a temperature close to 100°C at atmospheric pressure. The mixtures of hot vapors were allowed to pass through a condenser to form a liquid in which the oil and water comprise two distinct layers. The top layer is oil and the bottom layer was water, which was separated by separating funnel. Some amount of water may be present in separated oil layer that was removed by adding diethyl ether.

Then, we need to extract the oil layer from the co-distilled Water. We took advantage of the fact that the oil was insoluble in water and is soluble in non-polar solvents like Diethyl ether ($CH_3CH_2OCH_2CH_3$). Ether was added to the Oil-Water mixture in a separating funnel. This funnel allows the two solvents to layer and subsequently, we can drain one solvent layer away from the other. After a few moments of shaking, the oil will partition into the ether layer. Draining the water layer from the ether removes the oil from the water. The partitioning is almost never complete, so an extraction is usually carried-out multiple times. The resulting ether layers were collected and combined. (Caution must be observed when shaking the system. First, the system must be vented continuously because of vapor build-up that occurs within the flask. Second, the shaking cannot be too vigorous or else the system will emulsify. If emulsification occurs, separation of the two layers will become very difficult). It may seem as though we have traded one problem for another; we now have a mixture of oil and ether that must be separated. However, this is not a major problem. The ether boils at a low enough temperature that it can simply be boiled off from the oil layer. After the ether is stripped off, our isolation of the citral oil is complete.

Experimental design and optimization of parameters

The independent variables (k = 2) were volume ratio, ml of oil/ml of water (X_1) and time, minutes (X_2) while the percentage yield (Y, %) was chosen as the response variable. The experimental range and levels of independent process variables are given in Table 1. A 2k full-factorial experimental design with 5 replicates (n_0) at the center-point, and thus a total of 13 (2k + 2k + no) experiments were performed in this study. The center-point was repeated 5 times, to give 4 degrees of freedom, to verify any change in the estimation procedure and as a measure of precision property[4]. The experimental design illustrated in Table 2 shows the coded and uncoded values of the individual variables and the corresponding experimental and model predicted values for the Y, the yield. The variables were coded according to Equation (1).

$$X_i = \frac{(x_i - x_o)}{\Delta x} \quad \ldots(1)$$

where, X_i is the coded value of variable i, x_i is the dimensionless un-coded (actual) value of X_i, x_o is the value of X_i at the center point and Δx is the step change between levels -1 and 0.

The results were fitted using the response surface regression procedure, using the following second order polynomial equation (Equation 2), and by analyzing the response surface and contour plots.

$$Y = \beta_o + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i} \sum_{j} \beta_{ij} X_i X_j \quad \ldots(2)$$

where, Y is the predicted response, *k* is the number of variables, β_o is the offset term, β_i is the i^th^ coefficient of linear effect, β_{ii} is the i^th^ coefficient of squared effect, β_{ij} is the ij^th^ coefficient of interaction effect, and X_i and X_j are the coded values of independent variables i and j, respectively. Analysis of variance (ANOVA) was applied to estimate the main (linear) effects of independent variables and their potential interaction effects on the Y. The ANOVA table provides information on the following terms: DF (degrees of freedom); Seq SS (sequential sum of squares); Adj SS (adjusted sum of squares); Adj MS (adjusted mean squares); *F* (Fischer's variance ratio); *P* (probability value). The goodness of fit of the regression model and the significance of parameters estimates were determined through appropriate statistical methods. The results of this experimental design were analyzed and interpreted by MINITAB 16 (PA, USA) statistical software. For optimizing Y, the

``Response Optimizer´´ function in the MINITAB software that adopts the desirability function method to optimize response was used.

Table 1: Experimental range and levels of independent process variables

Independent variable	Range and level				
	-1.414	-1.000	0.000	+1.000	+1.414
Volume ratio (X_1)	0.052969	0.0667	0.09985	0.133	0.146731
Time (mins) (X_2)	29.645	40	65	90	100.355

Table 2: Full factorial central composite design matrix

S. No.	Volume ratio (X_1)	Time (min) (X_2)	Experimental	Predicted
			Y	Y
1	–1 (0.0667)	–1 (40)	35.00	40.38
2	+1 (0.133)	–1 (40)	35.00	35.225
3	–1 (0.0667)	+1 (90)	80.00	84.626
4	+1 (0.133)	+1 (90)	70.00	69.462
5	–α (0.052969)	0 (65)	75.00	71.903
6	+α (0.146731)	0 (65)	53.33	57.53
7	0 (0.09985)	–α (29.645)	25.00	22.389
8	0 (0.09985)	+α (100.355)	73.33	77.879
9	0 (0.09985)	0 (65)	65.00	66.384
10	0 (0.09985)	0 (65)	65.00	66.384
11	0 (0.09985)	0 (65)	65.00	66.384
12	0 (0.09985)	0 (65)	65.00	66.384
13	0 (0.09985)	0 (65)	65.00	66.384

RESULTS AND DISCUSSION

Statistical analysis

The data from steam distillation experiments in our study collectively displayed the successful effects in increasing the yield. These effects depended on the range of volume ratio and the time taken. These results produced main (linear), squared and interaction effects, which are statistically interpreted in this paper.

Analysis of variance & regression in model equation

The experimental results were analyzed in the form of analysis of variance (ANOVA) by using yield (Y_1) as the response variable (Table 3). ANOVA is a statistical technique that subdivides the total variation in a set of data into component parts associated with specific sources of variation for the purpose of testing hypotheses on the different parameters of the model. The statistical significance of the ratio of mean square due to regression and mean square due to residual error was tested using ANOVA. The *P* values were used to judge whether *F* is large enough to indicate statistical significance[5]. Thus, high *F* (statistics) and low *P* values (< 0.05) indicate that the effect is significant at the 95% confidence level. The *P* values of the linear and squared (quadratic) were < 0.05 for yield, while interaction effect found to be statistically insignificant. The regression values indicate that the second order polynomial model would produce some errors while representing the relationship between the process parameters and the response variable. The residual error in Table 3 indicates the amount of variation in the response data left unexplained by the model.

Furthermore, Students ´*t*´- test was used to determine the significance of the regression coefficients of the parameters. The corresponding *P* values were used as a tool to check the significance of each of the interactions among the variables, which in turn may indicate the patterns of interactions between the variables. The regression coefficient, t and P values for all the linear, squared, and interaction effects of the parameter are given in Table 4. The regression model equation for the yield is given in Equation (3).

$$Y_1 = -51.389 + 194.325X_1 + 2.776X_2 - 758.698X_1^2 - 0.013X_2^2 - 3.017X_1 \times X_2 \quad \ldots(3)$$

The positive terms of the coefficients given in Table 4 indicate a direct effect on the yield (%) values. The linear effect of volume ratio with $P > 0.05$ and the linear effect of time with $P = 0.000$ showed the direct effect whereas all the other effects such as squared effect of volume ratio, squared effect of time and the interaction effect of time and volume ratio showed an indirect effect. The coefficient of linear effect of time 2.776 with *P* value 0.000 showed both the direct effect and significant probability. The experimental and predicted values were shown in the Table 2. The experimental values and the predicted values were not s interaction effect of probability with 0.172 in ANOVA table showed that the 82.8% of variations in the yield was not statistically explained by the model equation, reflecting ratio and time were 0.053 and 98.2126 significantly different from each other. The interaction effect of probability with 0.172 in ANOVA table showed that the 82.8% of variations in the yield was not statistically explained by the model equation, reflecting the goodness of fit of the regression model to analyze trends in the responses.

Table 3: ANOVA for yield as response

Source	DF	Seq SS	Adj MS	*F*	*P*
	Y	Y	Y	Y	Y
Linear	2	2957.44	422.759	39.04	0.000
Square	2	483.59	241.797	22.33	0.001
Interaction	1	25	25	2.31	0.172
Residual error	7	75.8	10.828		
Total	12	3541.83			

Table 4: Estimated regression coefficients & corresponding t and P values

Term	Coefficient	SE Coefficient	*t*	*P*
	Y	Y	Y	Y
Constant	-51.389	19.48	-2.638	0.034
X_1	194.325	263.22	0.738	0.484
X_2	2.776	0.33	8.417	0.000
X_1^2	-758.698	1135.29	-0.668	0.525
X_2^2	-0.013	0.00	-6.680	0.000
$X_1 \times X_2$	-3.017	1.99	-1.519	0.172

Optimization and confirmation experiment

The numerical point prediction tool of Minitab Release 16 was used to find the optimum values of the test variables to maximize the yield (> 80%) for production of citral. The optimum values of the test variables were obtained when the volume ratio and time were 0.053 and 98.2126 mins, respectively. Under optimal conditions the model predicted the yield of 85.1416%. In order to confirm the validity of the statistical experimental strategy; steam distillation experiment was performed under optimal conditions in duplicate. The calculated optima are in close agreement with the observed value of yield of 84.1% with deviations less than 5% attributed to experimental error. The composite desirability value (D) of the predicted yield at optimized levels of variables was found to be close to 1. This result showed that the regression model developed in this study resulted in good agreement between the actual and predicted responses.

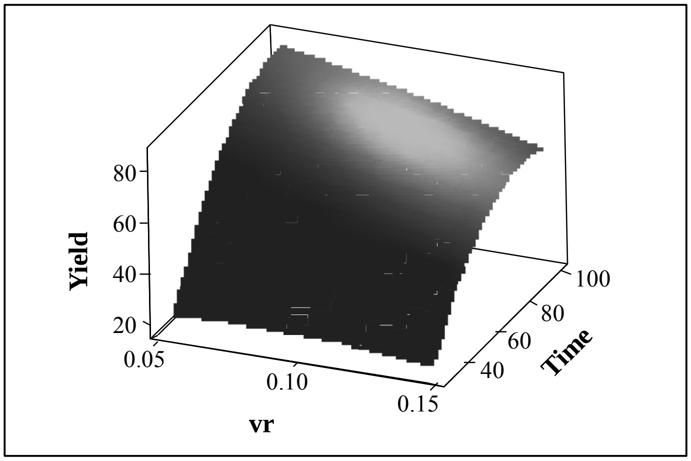

Fig. 2: Response surface plot showing the effect of volume ratio and time on the yield

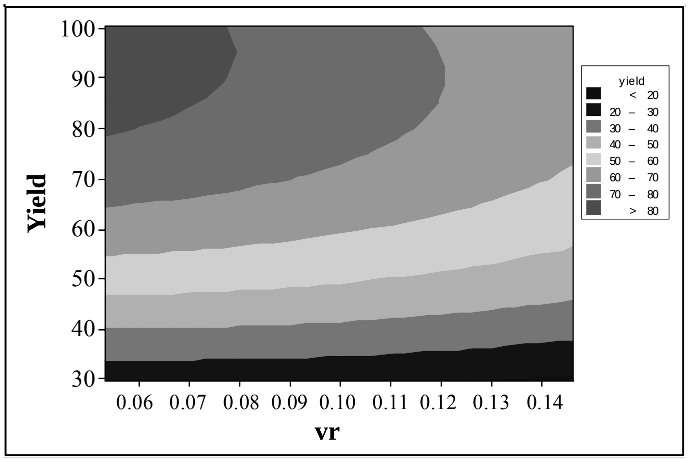

Fig. 3: Response contour plot showing the effect of volume ratio and time on the yield

CONCLUSION

The present work was focused on the optimization of the key process parameters for improvement in the increase in the yield of citral using statistical methodology.

Experimental results observed using RSM showed that among the linear effects, the volume ratio had the greatest influence (t = 0.738, P = 0.484) on the yield % and the time (t = 8.417, P = 0.000) showed the greatest effect on the yield%.

The ANOVA of the regression models for the yield% (F = 22.33, P = 0.001) demonstrated that the model is highly significant at 100% confidence intervals. Accurate prediction of the maximum value of the experimental responses indicated that the quadratic models had been adequately selected to describe the response surface within the experimental region. The RSM results indicated that the optimum conditions for maximizing the yield were volume ratio 0.053 and time 98.2126 min for which the predicted yield% is 85.1416 and the experimental yield % obtained is 84.1%. From the confirmation experiment, it is clear, that predicted yield % and experimental yield % are very to each other.

Isolation of essential oils using steam distillation can be used on industrial scale to make various finished products, which includes body oils, cosmetic lotions, baths, hair rinses, soaps, perfumes and room sprays. From our study, we found that specific gravity, density and flash point are 0.8904, 0.89031 and 91^{o}C, respectively.

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