



Effect of Solid-State Fermentation on the Essential Oil Yield of *Curcuma longa* Residues

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Abstract

The solid waste of *Curcuma longa* rhizomes generated after its cold juice process making is mostly unused and discarded even though they can contain essential oil. Conventional techniques such as hydrodistillation can be used to extract essential oil, but this generally results in low essential oil yield and inefficient extraction time. Solid-state fermentation as a pretreatment of distillation could improve the yield of essential oil. In this study, we evaluated the effect of solid state fermentation on the yield of extraction of *Curcuma longa* solid wastes essential oil. The solid-state fermentation was carried out naturally without any addition of inoculum and the extraction was performed by hydrodistillation. Under experimental conditions at room temperature (25°C) with a moisture content of 44% and anaerobically in the dark, the treatment of 7 days of solid state fermentation followed by 2 h of hydrodistillation provided the highest yield of 1.21% as compared to non-fermented of 0.35% and of 0.96% relative to the raw plant material representing an increase of 71% and 21% respectively. A set of experiments was then carried out by a Doehlert matrix to optimize the yield of extraction. Two independent variables, namely the distillation time and the fermentation time, were studied. Under optimal experimental conditions of 10 days and 4 h, a yield of 1.96% was obtained validating the statistical model. The solid state fermentation applied before the hydrodistillation step has been successful and proves its potential to improve the efficiency of essential oil extraction.

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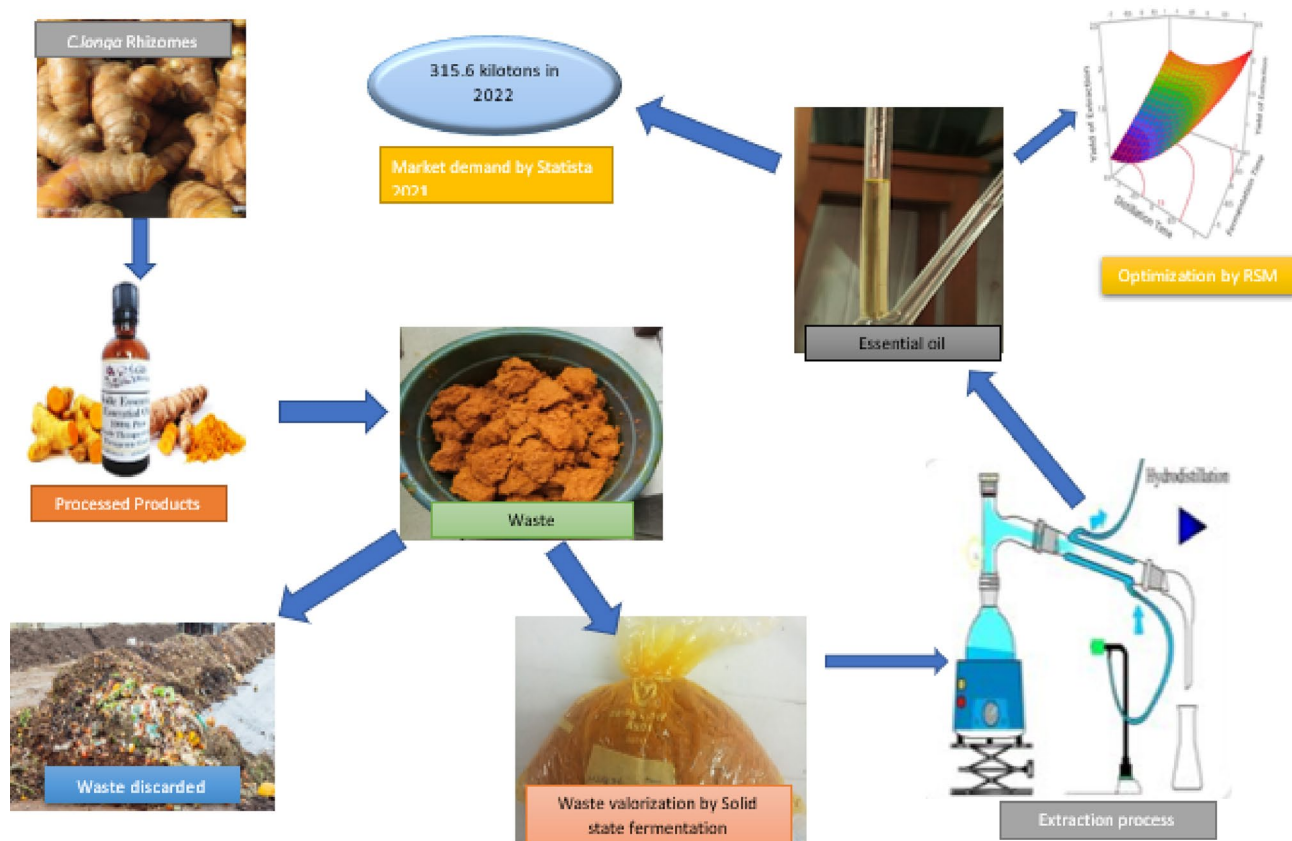
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Graphical Abstract



Keywords *Curcuma longa* · Fermentation · Hydrodistillation · Doehlert matrix · Oil percentage

Statement of Novelty

The management of waste from plant materials is becoming an increasingly growing concern. From a chemical point of view, it is a real loss when we know that many molecules of interest and high value-added products have been found through the extraction residues. Instead of pouring them into the wild, they can be re-exploited into essential oil by hydrodistillation. Objective which integrates the fact that the residues are not exhausted in phytochemical compounds, but also takes into consideration the limits in yield and in time of extraction by hydrodistillation. Studies in the literature have reviewed different methods of valorization of extraction residues in essential oil while taking into account the improvement of the yield by solid-state fermentation as a pretreatment of hydrodistillation. However, this pretreatment of the material includes the addition of microorganisms and the sterilization of the medium during experimental conditions. This study thus explores the process of solid-state fermentation as a

pretreatment for hydrodistillation without adding inoculum and without sterilization in order to allow the endophytes of the material to produce specific enzymes to the plant material to extract the essential oils with an appreciable yield compared to no pretreatment.

Introduction

Medicinal and aromatic plant species have been broadly exploited as food flavourings, medicinal agents, preservatives and ornaments, as well as beauty and personal delight products, becoming natural alternatives over synthetic products that offer reliability, safety and sustainability to the populations [1]. Amongst them, the popular natural products that gain much attention are those derived from aromatic plants namely: essential oils. Essential oils are volatile oils extracted from aromatic plants that capture the aromatic plant's essence and offer many health-related benefits through their interesting biological activities that include: the antibacterial, antioxidant, antiviral, insecticidal,

activities etc. [2–4]. As a result, the worldwide essential oil market demand was 226.8 kilotons in 2018 and is expected to expand to 404.2 in 2025 [3]. In the midst of all the popular worldwide essential oil that can exist (lavender, citrus, eucalyptus, lemongrass, tea tree etc.), *Curcuma longa* essential oil is especially popular because of the specific interest that resides in the exploitation of its rhizomes as a traditional medicine treatment (for malaria, jaundice, gastric ulcer, skin diseases, emotional disorders and convulsions) [5, 6], as well as in its numerous pharmacological activities such as: antioxidant, anticancer, anti-inflammatory, neuro- and dermatoprotective, antiasthmatic, as a flavouring agent, and as a potential contributor against the life-threatening viral disease COVID-19 by inhibiting the main spike protease enzyme [1]. Moreover, the countless beneficial health effects attributed to its rhizome essential oil are: cardiovascular protection, antihyperlipidemic, antiglycaemic, antioxidant, antiplatelet, anti-inflammatory, antioxidant, antiarthritic, antimicrobial etc. [1]. This rising concern of consumers about essential oils encourages researchers to develop new techniques to satisfy the demand.

Various techniques are used in the extraction process of *Curcuma longa* essential oil, but hydrodistillation is the most commonly chosen one due to its low cost and easy implementation. Unfortunately, adoption of this technique to satisfy the growing demand for essential oil production is insufficient because it's hindered by longer extraction times: 7 h according to Variyana et al. [7] and Widayat et al. [8] and low yield of extraction [9, 10] : 0.6–5.5% [1, 11–14] over 18% as prescribed by Sankara et al. [15] for aromatic plants. Point is, the recalcitrant and endogenous oil body structure of the aromatic plant tissue that inhibits the migration of the extractant and the release of intracellular metabolites [2, 4, 9, 10, 16]. Inevitably, it follows back a large quantity of residues that could still contain essential oil in addition of being abundant in phenolic compounds, oligomers and flavonoid glycosides with various biologically active principles [17–20]. Unfortunately, those residues remain unused, undervalued, and mostly are discarded or used as compost. Following this direction, the production of essential oils from negligible agro wastes as raw material such as *Curcuma longa* residues, is an interesting approach. *Curcuma longa* solid residues are the solid wastes generated during the essential oil extraction and during the traditional beverage making process. To avoid difficulties of low productivity and inefficient extraction time faced, adequate techniques and operating conditions for cell destruction are the key to obtain the maximum amount of essential oil during the extraction process. Since the plant cell wall consists mainly of cross-linked cellulose, hemicellulose and lignin, immersed in a matrix of pectic substances and reinforced by structural proteins and aromatic compounds which are difficult to destroy because they are bound together, and

therefore reduces the extraction efficiency of conventional techniques [10].

Referring to hydrodistillation, the plant material can be first subjected to a process of drying to both impede the growth of microorganisms and diminish the moisture content to facilitate essential oil extraction with a recommended time of 3 to 7 days [10] and a volatile oil content of 5–7.5% [21, 22]. In the instance of *Curcuma longa*, according to different studies related by [21, 22], *C. longa* essential oil yield in dry rhizomes varied from 1.5 to 5%. Those records, in comparison with the recommended range suggest that the drying process could result in the loss of volatile oil content up to 25% by evaporation and by volatilization, and in the destruction of some of the light-sensitive oil constituents [1, 10, 22].

Another technique to favor the essential oil release without alteration of the qualitative traits of the product to be extracted can be a microbial development process through enzymatic action. The use of solid-state fermentation (SSF) in this instance as a means to improve high productivity rates and higher product stability is recommended [23, 24]. Solid-State Fermentation is a process carried out in a solid matrix with sufficient moisture content for microbial growth and metabolism requirements but almost no free water in the system [9, 24, 25]. It appears as a method of fractionating cell walls where growing conditions are similar to those from the natural habitat using enzymes produced by microorganisms [26–28] thrives on solid, moist substrates that act as sources of nutrients and support microbial growth.

The plant cell wall is composed of a rigid complex of carbohydrates (cellulose, hemicellulose, xylan, pectin and lignin) which requires the joint action of enzymes with different functions in order to be broken down [29]. Therefore, microorganisms are used here as bioconversion agents, using lignocellulosic biomass as an energy source and excreting various metabolites including enzymes (biological catalysts) which are intended to cause ruptures at the cellular level of the storage glands of essential oils by speeding up reactions and hydrolyzing glycosidic bonds in plants (mainly cellulases, hemicellulases, and pectinases), to thus facilitate their diffusion during extraction [4, 10, 28, 30].

Wei-Qian et al. [31] improved with the use of enzymes by solid-state fermentation the extraction rate of essential oil from goyava leaves by 6.53 times that of the unfermented group, while Costa et al. [2] for *Croton argyrophyllus* leaves got an improve in the essential oil yield from fresh leaves subjected to enzymatic pre-treatment by solid state fermentation of 9.35% and that for dry leaves by 6.77%. Nurhandianty et al. [32] too, got an improve in the yield and quality characteristics of kefir lemon oil of 20% compared to unfermented leaves by solid fermentation pre-treatment using tempe yeast. Smigielski et al. [4] got an increase of 48% on the yield of extraction of essential oil

from waste carrot seed by solid-state fermentation using commercial enzymes: Lipex® and two lipases produced by *Rhizomucor miehei* and *Aspergillus niger*, pectate lyase (XPect®), amylase (Stainzyme®), cellulase (Celluclean®), and serine protease (Esperase®) and a noncommercial lipase, (specially prepared mycelium of *Mucor circinelloides*).

Despite the fact that solid-state fermentation with addition of enzymes procedure has been shown to achieve high extraction yields for essential oil of the aforementioned species, there are no reports on its application of this procedure for *Curcuma longa* residues, especially for those produced after its traditional juice making process. Bearing this in mind and taking into account the context of undervalued and unused residues and the increased market demand of essential oil, the present contribution aim for the first time at evaluating the effect of solid-state fermentation of *Curcuma longa* residues on the yield of their essential oil.

Material

Collection of Plant Material and Sample Preparation

The fresh rhizomes of *Curcuma* (*C. longa*) used in this study were obtained at a locality of Santchou in the west region of Cameroon in August 2021 and were stored in an open polyethylene bag and kept under room temperature (25°C). The fresh rhizomes of *Curcuma longa* (8 kg) were cleaned, washed, crushed using a blender. The ground curcuma rhizomes were then added to distilled water and the homogeneous mixture were deposited for 22 h maceration. A total of 3190 g of solid residues representing 40% of a fresh material were obtained after maceration and separated from *Curcuma longa* water by filtration and were also squeezed. *Curcuma longa* juice was then stored and kept as beverages while the waste were used for natural Solid-State fermentation.

Methods

Solid-State Fermentation

Solid-State fermentation (SSF) was based on the flowchart of valorization of organic waste to produce valuable bio-products by Abu et al. [25]. The fermentation were carried out with an agricultural organic waste (grinded solid residues) without pretreatment and addition of inoculum in polyethylene bags and kept anaerobically in the dark for seven days at room temperature (25°C) with a moisture content of 44%. A total of 290 g of residues were used (Fig. 1).

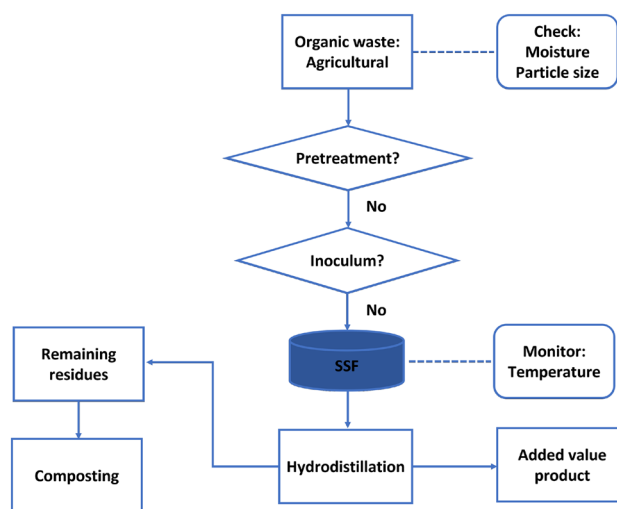


Fig. 1 Flowchart of the method used for essential oil production by solid-state fermentation of *Curcuma longa* residues

Distillation Process

The essential oil from *Curcuma longa* residues prepared according to step 2.1 was extracted by hydrodistillation using a Clevenger type apparatus of 12 L with 290 g of residue for 2900 g of distilled water (1:10 w/w) for 2 h. The oil was separated and dried with anhydrous sodium sulfate, and stored at 4°C in an opaque closed container. The yield of extraction of the essential oil was determined by the ratio of essential oil mass per 290 g of plant material following Eq. (1)

$$\text{Yield of Essential Oil}(Y_{EO}) = 100 * \frac{\text{Mass of the essential oil (g)}}{\text{Mass of plant material (g)}} \quad (1)$$

Experimental Design

The Response Surface Methodology (RSM) is a group of methods that used to evaluate of the variables and the practices involved in develop to optimize for extraction process [7]. The fermentation and the extraction of *Curcuma longa* residues were conducted by manipulating two parameters namely distillation time (hours, X_1) and fermentation time (days, X_2). Optimization of these variables were analyzed using a Doehlert Matrix based on Response Surface Methodology. A total of ten experiments was computed with three center points of replicates. The range and the designed levels of process variables are given in Table 1. The performance of the optimization process was evaluated in terms of yield of extraction (Y).

Table 1 Doehlert Matrix used in the treatment of *Curcuma longa* residues for the optimization of essential oil extraction)

Experiments	Factors		Response: Yield (%)	
	X ₁ (hours)	X ₂ (days)	Observed values	Predicted values
1	3 (0)	6 (0)	1.32	1.4
2	3 (0)	10 (1)	1.69	1.713
3	4 (0.866)	8 (0.5)	1.86	1.84
4	4 (0.866)	4 (- 0.5)	1.67	1.69
5	3 (0)	2 (- 1)	1.19	1.17
6	2 (- 0.866)	4 (- 0.5)	1.07	1.09
7	2 (- 0.866)	8 (0.5)	1.52	1.4
8	3 (0)	6 (0)	1.45	1.4
9	3 (0)	6 (0)	1.41	1.4
10	3 (0)	6 (0)	1.42	1.4

X₁ : Distillation Time; X₂: Fermentation Time

JMP PRO 16 Software was used for data and graphical analysis. A full second order model with linear, quadratic and interaction term was used (Eq. 2) in order to obtain the optimum combinations of the different factors level. This second order model develop by Giovanni and Maria [33] is written as follows:

$$Y = \beta_0 + \sum_{j=1}^k \beta_j x_j + \sum_{j=1}^k \beta_{jj} x_j^2 + \sum_{i < j} \sum_{i < j} \beta_{ij} x_i x_j + \epsilon \tag{2}$$

- Y : The response
- β₀ : The constant
- β_j; β_{jj}; β_{ij} : Model coefficients
- x_j : Independant variable
- x_i : Independant variable
- ε : The error

Meanwhile, analysis of variance (ANOVA) was used to statistically analyse the main and interaction effects of model term, the statistical significance level of the constructed model at 5% significance (p < 0.05), the regression coefficients evaluated from the standardized effects based on t-Student test (p < 0.05) . The quality of fit for the second order polynomial model equation was evaluated using the coefficient of determination (R²), the adjusted coefficient of determination (Radj²) as well as the lack of fit test. The obtained polynomial equation was then expressed in the form of three dimensional surface plot as to illustrate the relationship between the response and the experimental levels of each of the two variables investigated in the study.

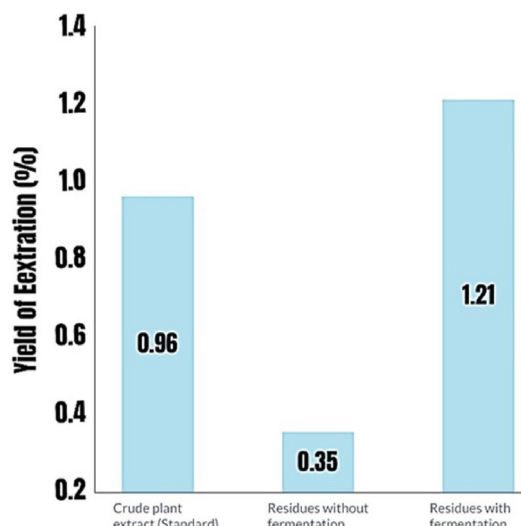


Fig. 2 Effect of fermentation on the yield of *Curcuma longa* waste

Validation of Response Surface Methodology Prediction Point

The optimization predicted point was tested for confirmation run in order to validate the prediction point. The confirmation run was conducted in one trial.

Results and Discussion

Yield of Extraction

As shown in Fig. 2, the highest yield of extraction of essential oil was obtained from the fermented residues (1.21%) compared to the crude plant extract (0.96%) and the control which is the residues without fermentation (0.35%). The release of essential oil by enzymatic action through the fermentation process of the residues was 3.46 times greater than the control (0.35% vs 1.21%) and 1.26 times greater than the crude material (0.96% vs 1.21%). This increase of essential oil yield can be explained by the disruption of the structure and integrity of the cell wall, where the highest oil extracted simply represent the one with the lowest cellulose content according to Indira et al. [9] and Hosni et al. [34].

Actually, the synergistic action of the natural enzyme-complex synthetized in the fermented substrate was effective in destroying its cell wall by cutting off the intersugar linkages and glycosidic bonds, promoting the release of free and bound aromatic compounds of essential oils [?], since the plant cell wall is composed of a rigid complex of carbohydrates (cellulose, hemicellulose, xylan, pectin and lignin) which are arranged and connected in a well-organized manner, forming a material with a rigid and highly

resistant structure. Therefore, it can require the joint action of enzymes with different functions in order to be broken down [2, 10]. Those enzymes could be: cellulases for hydrolyzing cellulose and generating monomer units making it possible both to localize and to destructure the substrate and thus facilitating the enzyme-substrate interaction; hemicellulases and accessory enzymes (unplugging glycoside hydrolases and carbohydrate esterases) for facilitating the attachment of enzymes to insoluble polysaccharides in particular that of the starch present in the plant cell wall which will promote the release of essential oils in the storage glands by hydrolysis; possible enzymes degrading or modifying lignin which is responsible for rigidity and impermeability since it is a large group of complex aromatic polymers, representing between 10 and 30% of the dry matter of plant cell walls [10, 35]. Hence, Fig. 2 revealed and confirm that efficient essential oil yield extraction was recovered during hydrodistillation after fermentation.

In addition, those results revealed that natural solid-state fermentation of *C. longa* residues result in an increase of about 21% compared to the crude plant extract. While a sharp increase was obtained to about 71% compared to the non fermented residues. Our result, is therefore higher than the one obtained by Nurhadianty et al. [32] from Keffir lemon oil with tempeh yeast (71% vs 20% of increase) and the one of carrot seeds oil using commercial enzymes (71% vs 48%) by Smigielski et al. [4].

The obtained results by solid fermentation are in contrary to those of the drying step which rather reduces the essential oil content due to the volatilization or degradation of the chemical constituents [10]. But also due to the physiological activity (enzymatic reaction) which occurs within the plant matrix resulting in strong dehydration of the dry plant material which stops all enzymatic activity by causing the death of the cells which contain the essential oils [36]. Thus,

the essential oil yields of *Curcuma longa* obtained by [8] after 1 to 4 days of drying for 3 to 7 h of steam distillation (0.2–0.8% and 0.2–0.75% respectively) remain much more lower than our findings.

Optimization of the Yield of Extraction

Ten (10) trials for optimization was computed based on the Doehlert Matrix as shown in Table 1. The Yield of extraction started to increase from day 2 of fermentation (1.19%) to day 8 (1.86%) before decreasing at day 10 of fermentation (1.69%). Referring to Table 2, the highest yield was obtained when the experimental conditions were set to 4 h of distillation after 8 days of fermentation, while the lowest yield was obtained at 4 days of fermentation with 2 h of distillation. Fermentation time between 2 and 8 days is then the best condition to increase yield of extraction with a distillation time of minimum 3 h. The ANOVA was performed and tabulated in Table 3. Generally, statistically speaking, significant model terms are those whose "Prob > F" values are less than 0.05 and inversely values greater than 0.05 indicate that the model terms are insignificant.

In the present study, the ANOVA results obtained demonstrate that the model presented statistical significance with an F value of 31.37 ($p < 0.05$). The R^2 value (98%) demonstrates that there was a good correlation between the experimental results and the predicted values with the value of R^2_{adj} highlighting the significance of the model. The lack of fit was insignificant with an F-value of 1.0426 and a p-value of 0.3824 which was good and implied that all data obtained from the conducted experiments were sufficient as well as the good predictability of the model. The model is then valid for construction of the response surface and prediction of the desired regions and was described by Eq. (3) as follows:

Table 2 ANOVA analysis of statistical significance of input factors for solid-state fermentation pretreatment of *Curcuma longa* residues according to the Doehlert method

Source	Sum of square	DF	Mean square	F value	Prob > F	
Model	0.4967	5	0.099	31.37	< 0.026*	Significant
X_1	0.220	1	0.220	69.75	0.0011**	Very significant
X_2	0.224	1	0.224	70.77	0.0011**	Very significant
X_1^2	0.034	1	0.034	10.77	0.0304*	Significant
X_2^2	0.002	1	0.002	0.67	0.4579	Not significant
X_1X_2	0.016	1	0.016	5.33	0.0820	Not significant
Error	0.0126	4	0.0031			
Total	0.5034	9	1.0484			
Lack of fit	0.0032	1	0.0032	1.0426	0.3824	Not significant
Pure Error	0.0094	3	0.0031			
Total error	0.0126	4				
R^2	0.98					
R^2 adjusted	0.9440					

X_1 : Distillation Time; X_2 : Fermentation Time

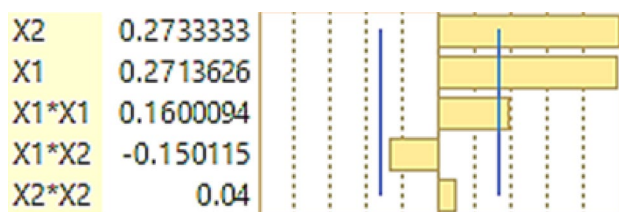


Fig. 3 Histogram of the factors influence and their contribution on the yield

$$Y = 1,4 + 0,271X_1 + 0,273X_2 + 0,16X_1^2 + 0,04X_2^2 - 0,15X_1X_2 \tag{3}$$

In the chart showing the effect and the contribution of the variables on the yield of extraction (Fig. 3) each factor were plotted in comparison with a minimum significant factor effect of 95% reliability (p=0.05) represented by the vertical line. When combine with the ANOVA analysis (Table 2), model reduction was then applied to improve the model by considering the significant terms.

In present study, it can be noted that all the linear terms namely : Fermentation time and Distillation time; and one quadratic term namely : Distillation time*Distillation time term were statistically significant. With Fermentation time exercising the greatest influence on the Yield of extraction, followed by Distillation time (Fig. 3) suggesting that for an increase in essential oil yield through solid-state fermentation, those factors are important.

Therefore, Eq. (4) represents the reduced model, adjusted based on the statistically significance of the model.

$$Y = 1,4 + 0,271X_1 + 0,273X_2 + 0,16X_1^2 \tag{4}$$

The response surface graph (Fig. 4), constructed from Eq. (4) using the response surface methodology for optimization of experimental parameters by the Doelhart matrix illustrate the effect of the variables and their interactions. It demonstrates the 3D response surface plot for the interaction effects of distillation and fermentation time versus yield of extraction as response. Both of the interaction effects were distillation and fermentation time (varying from 2 to 4 h and 2–10 days, respectively) on the yield of extraction. It was noticed that the highest yield (1.94%) could be attained on 10 days of fermentation with 4 h of distillation. This finding suggested that it might be take a certain time for the endophytes to grow and enable the synthesis of specific enzymes to break the glycosidic bonds that unite the polysaccharides to the plant cell wall and thus, causing ruptures in the oil storage glands which will facilitate extraction [2].

At the start of fermentation, there is no growth of microbes and this phase is known as lag phase, which is the phase where microbes were stil adapting with the environmental conditions as well as the occurrence of enzyme

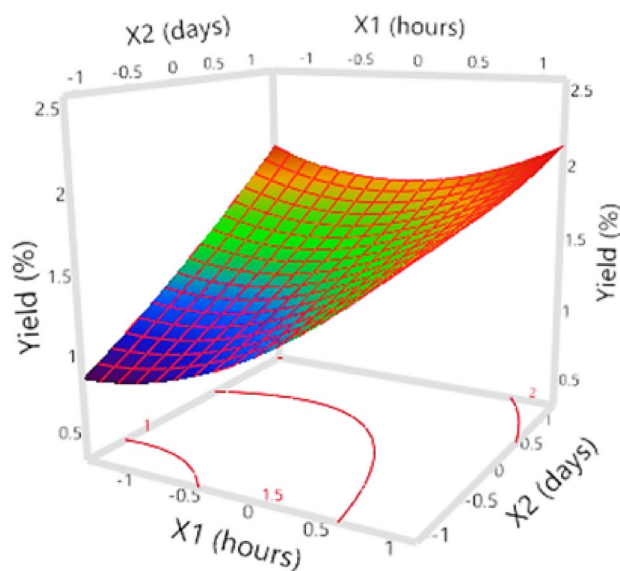


Fig. 4 Response surface graphs constructed based on the yield model representing the interactions between the distillation time and fermentation time, having the yield of extracted *Curcuma longa* residues essential oil as response

synthesis [32]. Cellulose and lignin that compose the solid and insoluble crystal structure of cellulose are considered to be essential oil release inhibitors (made with 75 % of polysaccharides where the monosaccharide units are linked to each other by glycosidic bonds) which need to be sufficiently hydrolyzed through a consequent fractionning [30, 37].

Besides that, Wei-Qian et al.[?] have reported that, in comparison of fermented and non-fermented samples, fermented guava leaves has produced higher essential oil at 10 days of fermentation; Hamidi et al. [30] too have reported that the maximum volume of patchouli oil increased with increasing fermentation time from 2 to 8 days. Then, whatever the duration of extraction, if the glycosidic bonds that unite the polysaccharides to the plant cell wall are not completely or mainly broken, the water vapor during its passage will be unable to release the maximum volatile oil content present in the matrix.

Therefore, we had a good agreement between the results obtained of the constinuously increased yield of extraction over fermentation and distillation time.

In agro-industry, residues after fermentation can constitute a rich source of biomolecules (secondary metabolites) that can be used for the bioprotection of plants against harmful pathogens and other environmental parasites. Also, we could find antibiotic molecules useful in dermocosmetics and/ or phytopharmacy to fight against microorganisms harmful to human health (yeasts, bacteria, dermatophytes etc ...). According to Abu Yazid et al. [25] after the recovery of products, a solid waste with different level of biodegradability still remains once solid-state fermentation

processing is finished. It has a potential of being reuse as compost and anaerobic digestion. Moreover, it has been observed that solid residues remained after production of citric acid in solid-state fermentation can be reutilized in a sequential extraction process to produce fungal chitosan as an eco-friendly alternative to the chitosan derived from marine shells; and also, can be served as animal feed like Babassu fermented cake as example which was sold after enzyme extraction done by solid-state fermentation for animal feed [25].

Prediction and Verification of Optimization Condition

The desirability option for the response surface plot has provided a solution that will be used to predict the optimal condition to obtain the maximum yield of extraction of essential oil from solid state fermentation of *Curcuma longa* residues. The high desirability value is proposed to attain a yield of 1.94% when the experiment conditions are conducted based on Table 3.

Therefore in order to validate this, a validation run was performed as to verify the prediction.

According to Table 3, it can be seen that the maximum essential oil yield predicted by the model was 1.94%, obtained at 10 days of fermentation with 4 h of hydrodistillation whereas the experimental value in the same extraction condition was 1.96%. The experimental and the predicted values are in close agreement to each other with a difference of 0.02 but corroborates the good fit within the experimental domain.

Conclusion

The use of Solid-State Fermentation as a pre-treatment to enhance the yield of extraction of *Curcuma longa* solid waste essential oil has proved to be effective. Even without addition of inoculum, its application was effective by increasing the essential oil yield from 0.35 to 1.21% which represents an increase of 71% compared to the standard; and from 0.96 to 1.21% which represents an increase of 21% compare to the crude plant material. Following this good result, the yield of extraction through solid-state fermentation was optimized through the Doehlert Matrix and the results obtained demonstrated an evident improvement in the degradation of the cell wall during fermentation days which consequently has facilitated the release of essential oil by hydrodistillation and ameliorated the yield of extraction.

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Table 3 Optimum conditions suggested for the response

Parameter	Coded value	Real value	Predicted response (%)	Experimental response (%)
X_1 (hours)	0.86	4	1.94%	1.96%
X_2 (days)	1	10		

X_1 : Distillation Time; X_2 : Fermentation Time

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Data Availability Enquiries about data availability should be directed to the authors.

Declarations

Conflict of interest The authors declare that they have no conflict of interest

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