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INFLUENCE OF ULTRASOUND-ASSISTED OSMOTIC DEHYDRATION PRE-TREATMENT ON TOTAL PHENOLIC CONTENT, ANTIOXIDANT CAPACITY AND P-CYMENE CONTENT OF *EUCALYPTUS DEGLUPTA*

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Convective drying is widely used in the drying of medicinal plants, however, long duration and high temperature often compromise the quality through degradation or loss of bioactive antioxidants. This problem can be overcome by incorporating pre-treatment such as ultrasound-assisted osmotic dehydration (UAOD) to efficiently and effectively remove excess moisture. Therefore, the main objective of the current research is to optimise the incorporation of UAOD pre-treatment before convective drying of *Eucalyptus deglupta* leaves for enhanced retention of phenolic compounds, p-cymene yield and antioxidant activity. This was achieved by evaluating the effect of four independent variables: UAOD temperature (20–60 °C), UAOD duration (60–100 min), ultrasound intensity (198–330 W) and UAOD sucrose concentration (30–50% w v^{-1}). Optimal UAOD conditions were achieved at a temperature of 58.18 °C, duration of 60 min, ultrasound intensity of 330 W and sucrose concentration of 36.02%. The optimum UAOD pre-treatment produced higher total phenolic content, antioxidant activity and p-cymene yield compared to convective drying on its own by two-fold. Lewis model predicted the drying kinetics with the highest accuracy based on R^2 (0.9889) and RMSE (6.52×10^{-8}). The results justify the inclusion of UAOD pre-treatment before convective drying for improved phenolic compound extraction with high p-cymene yield and antioxidant activity from *Eucalyptus deglupta*.

Keywords: Hybrid drying, pre-dehydration, sucrose solution, optimisation, kinetic, rainbow *Eucalyptus*

INTRODUCTION

The genus *Eucalyptus* has been identified as a potential source of natural antioxidants in traditional and complementary medicine *via* literature (Salehi et al. 2019). *Eucalyptus deglupta*, belonging to the same family, often gets overlooked. Although *E. deglupta* (rainbow *Eucalyptus*) is categorised under the *M*yrtaceae family that originates from Australia, this particular species is more native to the Asian regions of Indonesia and the Philippines (Cornelius et al. 1995). Past findings have shown that *E. deglupta* possesses a high quantity of volatile bioactive compounds in its rich essential oil (Chaverri & Ciccio 2018). The potent antioxidant activity in *E. deglupta* is due to the presence of phenolic compounds, particularly p-cymene (Chaves et al. 2018). Despite such promising potential, literature highlighted a major gap regarding the impact of processing technologies of this plant, one of them being drying or dehydration.

Drying is an essential post-harvest process before the phytochemical extraction process, as high moisture significantly affects the quality of natural extracts. Commercial drying processes, namely conventional hot air drying, solar drying and freeze-drying, have major concerns (Chua et al. 2019). The above-mentioned methods have high energy expenditure and costs since they involve tediously long processes. In fact, the drying process, reportedly, consumes 10–20% of the total energy requirements of all food industries in developed countries (Klemes et al. 2008). Moreover, they have major implications for economic and environmental aspects. Reduced product quality and nutritional value are additional drawbacks of conventional hot air drying, commonly referred to as convective

drying. The loss of potent compounds in the natural source is linked to the loss of bioactivity and its quality. Thus, a preliminary step is introduced before complete convective drying to reduce the overall drying period and retain higher quality.

Osmotic dehydration is a common preliminary treatment implemented in the industry. This method is based on the permeation of water from a low solute concentration to a high solute concentration through a semi-permeable membrane to achieve equilibrium. Osmotic dehydration is a simple process, however, its characteristic slow diffusion process cannot remove the moisture content to an acceptable level. Thus, it is paired with convective drying for complete moisture removal (Yadav & Singh 2014). Besides, the introduction of ultrasound-assisted drying can increase the pore sizes on the leaf surface for more efficient mass transfer of water (Bozkir et al. 2019). According to previous studies, ultrasonic waves can improve the drying rate of numerous fruits and vegetables without affecting the final product quality (Kroehnke et al. 2018). Thereby, the pairing of ultrasound as a preliminary pre-treatment step to the convective drying sounds promising.

To take a step forward, recent research has moved onto a more advanced technique by integrating the two pre-treatments in a single stage for enhanced moisture removal. The hybrid form of this method, namely ultrasound-assisted osmotic dehydration, reportedly performed better than the solo methods in terms of product quality and compound preservation/retention (Bozkir et al. 2019, Rahaman et al. 2019). Thereby, the present research investigated the ultrasound-assisted osmotic dehydration of *E. deglupta* leaves to enhance its antioxidant activity.

The current study focused on the ultrasound-assisted osmotic dehydration. The application of this technique on *E. deglupta* leaves, before convective drying, will be the first attempt based on literature survey. The primary objective is the optimisation of multiple UAOD conditions (temperature, ultrasound intensity, drying time and sucrose concentration) using response surface methodology (RSM) optmisation in retaining and maximising the total phenolic content, antioxidant capacity and p-cymene content of *E. deglupta*. The drying kinetics of the optimised ultrasound-assisted osmotic dehydration was analysed by best-fitting the data to well-known thin-layer drying models.

MATERIALS AND METHODS

Experimental setup

Eucalyptus deglupta leaves (2 kg) were purchased from SJH Nursery, Muar, Johor. The 2,2-diphenylpicrylhydrazyl (DPPH), gallic acid, Folin-Ciocalteu reagent, absolute ethanol (HPLC grade), sodium carbonate and p-cymene standard were purchased from Sigma-Aldrich. Sucrose crystals (multi-compendial grade) were procured from J.T. Baker.

Experimental design of response surface methodology

The effects of pre-treatment temperature (T_p, 20–60 °C), duration (T_{pd}, 60–100 min), the intensity of the ultrasound (I_p, 198–330 W) and sucrose solution concentration (O_p, 30–50% (w v^{-1})) on *E. deglupta* leaves were investigated in the current study. The selection of the above-mentioned variables and their corresponding ranges were identified from previous studies (Amami et al. 2017). A four-factor, three-level central composite experimental design with a total of 27 combinations of ultrasound-assisted osmotic dehydration pre-treatment conditions were implemented for optimisation using Design expert software 8.0.6. Experimental responses of TPC (Y_1), antioxidant activity (Y_2), and p-cymene yield (Y_3) were fitted separately into three second-order polynomial equations.

Pre-treatment of *Eucalyptus deglupta* leaves (ultrasound-assisted osmotic dehydration)

Leaf samples with a respective sucrose solution (30–50% w v^{-1}) in Schott bottles were immersed into the ultrasonic bath and sonicated for a drying duration of 60–100 min at 20–60 °C, respectively. The ultrasound intensity varied from 198–330 W while the frequency was kept constant at 80 kHz. All pre-treatments were carried out in the sequence of design matrix obtained from Design expert software 8.0.6. Once the pre-treatment was completed, the samples were removed from the sucrose solution and wiped with tissue paper before proceeding with complete moisture removal using convective drying.

Complete moisture removal with convective drying

The moisture of the pre-treated samples from ultrasound-assisted osmotic dehydration was completely removed in the second stage with convective drying at 60 °C using a convective oven. Convective drying at 60 °C is recommended by researchers as optimal drying temperature for natural sources, in general (Chua et al. 2019). *Eucalyptus deglupta* leaves without any ultrasound-assisted osmotic dehydration pre-treatment were also completely dried in the same manner as above which served as a control in the study. The drying processes were accomplished when the constant weight was obtained. Fully dried and ground leaves were sieved through a 500 µm mesh size sieve and stored in air-tight Schott bottles before extraction of the essential oil.

Extraction of essential oil from *Eucalyptus deglupta* leaves

The essential oil was extracted from *E. deglupta* leaves based on previous studies (Gullón et al. 2017). A total of 1 g of dried powder was added to ethanol (HPLC Grade) in constant speed of 120 rpm at 25 °C for 30 min. The extracted sample was centrifuged at 10000 rpm to collect the supernatant.

Quantification of total phenolic content (TPC)

Total phenolic contant (TPC) was determined with Folin-Ciocalteu as described in the literature (Bobo-García et al. 2015). A total of 25 µL of the extract samples were mixed with 25 µL of diluted Folin-Ciocalteu reagent. Then, 75 µL of ultrapure water was added and homogenised for 1 min with an orbital shaker. After a settling period of 5 min, 100 µL of 75 g L^{-1} of a sodium carbonate solution was added and allowed to react for 90 min. The absorbance of each mixture was read at 760 nm using a microplate reader. All experiments were performed in a dark room. The TPC was calculated in terms of mg of gallic acid equivalents (GAE) per 0.1 g of the dried leaf (DL) using a calibration curve for the standard marker, $R^2 = 0.999$.

Determination of antioxidant capacity

The DPPH radical scavenging assay was carried out according to the method described in literature with slight modifications (equation I) (Bobo-García et al. 2015). Briefly, 100 µL of extract samples and 100 µL of 0.2 mM DPPH reagent in methanol were mixed and allowed to react for 30 min in the dark. The absorbance of the mixtures was read at 517 nm using a microplate reader.

$$\text{DPPH radical scavenging activity (\%)} = \frac{R_C - R_C}{R_C} \times 100\% \quad \text{(I)}$$

where R_C represents the absorbance of DPPH reagent and R_S represents the absorbance of DPPH reagent with *E. deglupta* extract.

Identification and quantification of p-cymene

Identification of the p-cymene in the extract samples was performed in a gas chromatography-mass spectrometer interfaced with an HP-5 MS capillary column (30 m × 0.25 mm, coated with 5% phenylmethyl silicone, 95% dimethylpolysiloxane, 0.25 mm film thickness; HP). The column temperature was programmed to rise from 50–240 °C at a rate of 5 °C min^{-1}. Helium was used as the carrier gas with a flow rate of 1.2 mL min^{-1} and a split ratio of 1:60. Scan time and mass ranges were 1 s and 40–300 m z^{-1}, respectively (Salem et al. 2018). Gas chromatography/mass spectrometry (GC/MS) was also performed for the standard marker of p-cymene with concentrations ranging from 0–10 ppm (diluted with HPLC grade ethanol) to construct a calibration curve for p-cymene quantification.

Mathematical modelling of drying kinetics of ultrasound-assisted osmotic dehydration pre-treatment

The drying kinetics of optimised ultrasound-assisted osmotic dehydration pre-treatment was studied by mathematical modelling of the moisture loss from *E. deglupta* leaves using Microsoft excel. The recorded weight loss data of the leaves was determined using equation II at

a time interval of every 30 min, until a constant value was obtained. The moisture loss data was used to calculate the dimensionless moisture ratio (MR) by applying equation III. Drying kinetics were modeled using five thin-layer drying models which are Page, Henderson & Pabis, Lewis, Logarithmic and Avhad & Marchetti (Liu et al. 2017). The selected thin layer models were chosen to represent a wide variation of semi-theoretical models with diverse theories.

$$\text{MC (dry basis)} = \frac{w_i - w_{db}}{w_i} \quad \text{(II)}$$

$$\text{MR} = \frac{M_t - M_e}{M_0 - M_e} \quad \text{(III)}$$

where M_0 is the initial moisture content, M_t is moisture content at time t, M_e is equilibrium moisture content of the samples, w_i and w_{bd} are the initial weight and weight on a dry basis, respectively, M_e was experimentally obtained by drying the samples in the oven until no change in weight was noted for three successive weight measurements for every 30 min time interval.

Statistical analysis

Each experimental run was conducted in triplicates and the average value was utilised for statistical analysis. All the experiments were carried out in a randomised error to minimise the effects of unexplained variability in response to extraneous factors. Regression and statistical analysis were performed using Design expert software 8.0.6. Analysis of variance (ANOVA) was used to evaluate the ultrasound-assisted osmotic dehydration pre-treatment for each response, $\rho < 0.05$. The accuracy of thin-layer drying models was checked based on coefficient of determination (R^2) and root mean square error (RMSE).

Validation of response surface methodology of optimised conditions

Using the predictive model developed by the response surface methodology, the optimised ultrasound-assisted osmotic dehydration pre-treatment conditions were applied to pre-treat the leaves. Three confirmation experiments were performed to obtain a new set of responses which were compared with predicted response to validate the model's adequacy.

RESULTS AND DISCUSSION

The current study investigated the combined impact of both processes (osmotic dehydration and ultrasound) in the form of hybrid pre-treatment as a part of the two-stage drying process, to improve the quality of the final product. Four process variables of UAOD conditions (temperature, duration, ultrasound intensity and sucrose concentration), identified from the literature with the most significant impact, were studied and discussed in the subsections. The optimised conditions were evaluated based on the highest retention of bioactive compound (p-cymene), TPC and antioxidant activity. Lastly, the drying kinetics were also studied for further evaluation of the mass transfer process.

The impact of temperature on TPC, DPPH radical scavenging activity and p-cymene

The experimental responses (Table 1) shows the yields of TPC and DPPH radical scavenging activity, in the range of 0.305–0.527 mg GAE g^{-1} DL and 6.29–49.21% activity, respectively. The yields of TPC were drastically reduced with increasing temperature as the lowest value was obtained at 60 °C (0.305 mg GAE g^{-1} DL). The decline in TPC is associated with the loss of essential oil at a temperature beyond 40 °C, which aligns with previous findings (Samani et al. 2017). Reportedly, the epithelial cells in the leaves can collapse and split open at ambient temperatures and above, which aid in the escape of highly volatile essential oil to the surroundings (Díaz-Maroto et al. 2003). The biological structures in medicinal and aromatic plants can also be affected by ultrasound treatment. Sonication-induced cavitation can cause the plant cell's structure to breakdown, thus, producing a more porous structure with open and expanded microscopic channels (Sledz et al. 2017). The resulting structural change can also cause the essential oil to diffuse from the cells.

Contrary to TPC results, DPPH radical scavenging activity was observed to increase at higher temperatures with maximum activity at 60 °C (49.21%). The increase in antioxidant activity may be attributed to the formation of melanoidins. Melanoidin is a compound that forms under the presence of low water and high sugar activity (Balzarini et al. 2018). The presence of sugar in the sucrose solution

facilitates the formation of melanoidin which is known to exhibit antioxidant activities (Langner & Rzeski 2013). The TPC exhibited an inversely proportional relationship to antioxidant activity in the current research; some phenolic compounds possess strong antioxidant activity (Maran et al. 2017). As for p-cymene, the yield was in the range of 1.442–8.991 ppm. An increasing trend was noted at the beginning until the temperature reached approximately 40 °C, around which the yield began to decline. Previous studies have reported the conversion of unstable volatile compounds to p-cymene that was supported by light and temperature through oxidation and other chemical reactions (Turek & Stintzing 2012). The gradual decrease in the yield beyond 40 °C could be caused by the possible degradation of bioactive compounds (Shakthi-Deve et al. 2014).

The impact of duration on TPC, DPPH radical scavenging activity and p-cymene

The duration had a significant impact on all responses (TPC yield, DPPH radical scavenging activity, and p-cymene yield). With increasing duration, the yields of TPC shows an almost linear decreasing trend with a maximum value of 0.433 mg GAE g^{-1} DL obtained at 60 min. On the other hand, the maximum DPPH radical scavenging activity of 35.55 % was noted at 80 min, although it began to decline beyond this stage. The long duration in UAOD is advantageous for the overall drying process. Since the majority of the moisture content is removed by sucrose solution, less time is needed for consequent convective drying. At the same time, long-duration allows for enhanced mass transfer of the phenolic and other bioactive compounds to the sucrose solution (Yadav & Singh 2014). Additionally, the action of cavitation on the pores and structure of the leaves accentuates the mass transfer process (Sledz et al. 2017). Therefore, this leads to the loss of valuable antioxidant compounds. The results indicate that osmotic treatment for a long duration may be unfavourable. As for the yield of p-cymene, a decreasing trend was noted at the beginning before showing an exponential increase after approximately 80 min. It can be speculated that the compounds other than p-cymene diffused out of the leaves, thereby, increasing the compound yield.

The impact of ultrasound intensity on TPC, DPPH radical scavenging activity and p-cymene

It was noted that ultrasound intensity had no impact on TPC results (Table 1), regardless of the different powers applied. On the other hand, DPPH radical scavenging activity increased after an applied power of 264 W. This outcome was unexpected, as according to reported literature, high-intensity ultrasound is proportional to the high ultrasound amplitude that is applied on the product, which results in intense cavitational bubble collapse (Chemat et al. 2017). As a result, the physio-mechanical effects such as cracked or damaged cell walls increase, leading to increased solute diffusion, interfacial turbulence and local energy dissipation (Chemat et al. 2017). A similar result was expected with decreasing compound yield and antioxidant activity, although that was not the case for DPPH radical scavenging activity. However, the decreasing yields of p-cymene could be explained by the above phenomenon, since its yield began to subside after 264 W. Further insight is needed to determine the true impact of ultrasound intensity on the pre-treatment of *E. deglupta* regarding the TPC yield.

The impact of sucrose solution on TPC, DPPH radical scavenging activity and p-cymene

Similar to duration, the yields of TPC (Table 1) shows a linearly decreasing trend with the highest value of 0.437 mg GAE g^{-1} DL obtained at 20% concentration. In contrast to this, the maximum DPPH radical scavenging activity of approximately 35% was obtained at 50% concentration. High osmotic pressure (sucrose solution) enhances moisture loss, however, it also forces the migration of phenolic compounds out of the plant leaves into the sucrose solution (Bozkir et al. 2019). With the aid of ultrasonic action, the dual mass transfer of water and phenolic compounds is accelerated (Rahaman et al. 2019). This phenomenon could also explain the decreasing yield of p-cymene after 40% sucrose concentration. The results also indicated the increase of antioxidant activity at higher concentrations.

The physical attributes of sucrose solution, such as viscosity and fluidity, may have influenced the outcome of the antioxidant activity. The high viscosity of the solution possibly acted as a

Table 1 Central composite design matrix with experimental and predicted responses of TPC, DPPH antioxidant activity and p-cymene yield

Std	Run	Temperature (°C)	Duration (min)	Intensity (%)	Osmotic concentration (%)	TPC, Y_1 (mg GAE/0.1 g dried leaf)		Yield of DPPH radical scavenging activity Y_2 (%)		Yield of p-cymene	
						Experimental	Predicted	Experimental	Predicted	Experimental	Predicted
18	1	20	60	60	30	0.414	0.408	6.290	4.044	1.442	1.531
23	2	20	60	60	50	0.383	0.381	6.980	8.400	2.197	2.611
2	3	20	60	100	30	0.513	0.496	9.290	14.320	8.698	8.016
9	4	20	60	100	50	0.492	0.502	25.830	20.565	3.532	3.790
16	5	20	100	60	30	0.472	0.478	15.630	15.542	8.060	7.885
7	6	20	100	60	50	0.527	0.518	12.530	12.472	4.925	4.889
3	7	20	100	100	50	0.393	0.408	22.330	27.653	4.344	4.261
13	8	20	100	100	50	0.415	0.408	31.390	27.653	3.882	4.261
17	9	60	60	60	30	0.471	0.462	43.090	43.765	3.183	2.910
6	10	60	60	60	50	0.305	0.305	57.400	56.124	8.756	8.944
15	11	60	60	100	30	0.521	0.531	43.420	37.857	6.982	7.393
24	12	60	60	100	50	0.489	0.472	43.890	48.103	5.721	5.646
25	13	60	100	60	30	0.482	0.473	39.210	41.282	6.232	6.349
8	14	60	100	60	50	0.442	0.448	49.210	47.115	5.399	5.831
4	15	60	100	100	30	0.345	0.337	32.260	32.499	8.591	8.114
12	16	60	100	100	50	0.347	0.345	39.210	39.957	2.278	2.291
5	17	40	80	80	40	0.402	0.432	35.210	35.073	6.543	6.424
27	18	40	80	80	40	0.428	0.432	34.760	35.073	6.530	7.004
20	19	40	80	80	40	0.434	0.452	35.460	33.925	7.123	7.004
21	20	20	80	80	40	0.454	0.441	15.920	15.465	6.432	6.524
11	21	60	80	80	40	0.405	0.423	39.290	43.020	6.592	6.684
26	22	40	60	80	40	0.432	0.446	30.070	33.083	7.652	7.322
14	23	40	100	80	40	0.421	0.418	38.520	33.975	8.991	8.821
19	24	40	80	60	40	0.438	0.423	39.780	38.845	5.992	5.236
1	25	40	80	100	40	0.434	0.428	40.830	40.224	6.452	6.708
10	26	40	80	80	30	0.427	0.437	29.580	30.010	6.334	7.324
27	27	40	80	80	50	0.443	0.427	37.690	35.728	6.442	4.952

barrier by providing external resistance to the mass transfer of compounds (Yadav & Singh 2014). Previous findings also support these results where the best antioxidant activity was observed at higher concentrations (Rahman et al. 2018). Reported literature also shows that osmotic stress can increase the enzymatic activity, which in turn promotes key chemical reactions such as esterification (Escriche et al. 2006). The enhanced enzyme activity encourages the synthesis of secondary metabolites with better antioxidant activity.

Optimisation and validation of quadratic polynomial model

Optimisation of the ultrasound-assisted osmotic pre-treatment was based on the maximised yield of TPC, DPPH radical scavenging activity and p-cymene yield. The statistical analysis performed by ANOVA (Table 2, Table 3 and Table 4) indicated that temperature and duration imposed significant linear influence on the response of TPC (Y_1) and DPPH radical scavenging activity (Y_2). Meanwhile, ultrasound intensity and sucrose concentration were the significant linear influencers for the response of p-cymene yield (Y_3). The results also indicated that the only significant quadratic influence was imposed by temperature on DPPH radical scavenging activity and p-cymene yield. In terms of interaction effects, the combined influence of UAOD conditions (temperature, duration and ultrasound intensity) was significant for all responses. Moreover, it was noted that all pre-treatment parameters had a significant combined impact on p-cymene yield except for temperature and sucrose concentration. All developed second-order regression models (equations IV, V and VI) were found to be significant with an F-value of 20.542, 16.588 and 9.075 for TPC, DPPH radical scavenging activity and p-cymene yield, respectively. All three developed models, excluding the non-significant terms, are shown below:

Numerical optimisation revealed that the best pre-treatment conditions for maximum TPC, DPPH radical scavenging activity and p-cymene yield was at a temperature of 58.18 °C with a

Table 2 ANOVA analysis for quadratic model of TPC

Source	Sum of squares	DF	Mean square	F - value	p-value
Model	0.070783	14	0.005056	20.54195	< 0.0001
Temperature - T_p	0.001601	1	0.001601	6.503713	0.0255
Duration - T_{pd}	0.001951	1	0.001951	7.926619	0.0156
Intensity - I_p	5.67E-05	1	5.67E-05	0.230234	0.6400
Osmotic - O_p concentration	0.000308	1	0.000308	1.251767	0.2851
T_pT_{pd}	0.000281	1	0.000281	1.14249	0.3062
T_pI_p	0.000207	1	0.000207	0.841633	0.3770
T_pO_p	0.007278	1	0.007278	29.57044	0.0002
$T_{pd}I_p$	0.0338	1	0.0338	137.3273	< 0.0001
$T_{pd}O_p$	0.00386	1	0.00386	15.68286	0.0019
I_pO_p	0.00098	1	0.00098	3.983374	0.0692
T_p^2	0.000136	1	0.000136	0.550595	0.4723
T_{pd}^2	6.38E-05	1	6.38E-05	0.259131	0.6199
I_p^2	4.56E-05	1	4.56E-05	0.185115	0.6746
O_p^2	2.56E-05	1	2.56E-05	0.103982	0.7527
Residual	0.002954	12	0.000246		
Lack of fit	0.00214	9	0.000238	0.87616	0.6162
Pure error	0.000814	3	0.000271		
Standard deviation	0.016				
Mean	0.43				
Coefficient variation (%)	3.61				
Press	0.018				
R^2	0.9599				
Adjusted-R^2	0.9132				

Table 3 ANOVA analysis for quadratic model of DPPH radical scavenging activity

Source	Sum of squares	DF	Mean square	F - value	p-value
Model	4358.328	14	311.3091	16.58839	< 0.0001
Temperature - T_p	2460.536	1	2460.536	131.1119	< 0.0001
Duration - T_{pd}	2.45048	1	2.45048	0.130576	0.7241
Intensity - I_p	5.778894	1	5.778894	0.307934	0.5891
Osmotic - O_p concentration	120.5062	1	120.5062	6.421286	0.0262
T_pT_{pd}	152.8496	1	152.8496	8.144733	0.0145
T_pI_p	242.0697	1	242.0697	12.89891	0.0037
T_pO_p	27.72625	1	27.72625	1.477419	0.2476
$T_{pd}I_p$	0.001689	1	0.001689	9E-05	0.9926
$T_{pd}O_p$	8.509084	1	8.509084	0.453414	0.5135
I_pO_p	3.027887	1	3.027887	0.161344	0.6950
T_p^2	321.8854	1	321.8854	17.15196	0.0014
T_{pd}^2	6.188335	1	6.188335	0.329751	0.5764
I_p^2	51.71788	1	51.71788	2.755835	0.1228
O_p^2	12.60656	1	12.60656	0.671752	0.4284
Residual	225.2002	12	18.76668		
Lack of fit	183.9067	9	20.43408	1.484551	0.4104
Pure error	41.29347	3	13.76449		
Standard deviation	4.33				
Mean	31.67				
Coefficient variation (%)	13.68				
Press	1777.35				
R^2	0.9509				
Adjusted-R^2	0.8935				

Table 4 ANOVA analysis for quadratic model of p-cymene yield

Source	Sum of squares	DF	Mean square	F - value	p-value
Model	67.4148	14	4.815345	9.07464	0.0002
Temperature - T_p	1.61803	1	1.618039	3.049236	0.1063
Duration - T_{pd}	1.36705	1	1.367057	2.576254	0.1345
Intensity - I_p	5.28400	1	5.284002	9.957836	0.0083
Osmotic - O_p concentration	16.0486	1	16.04816	30.24316	0.0001
T_pT_{pd}	3.32013	1	3.320133	6.256875	0.0278
T_pI_p	3.01852	1	3.01852	5.688478	0.0344
T_pO_p	0.12913	1	0.129137	0.243361	0.6307
$T_{pd}I_p$	2.85576	1	2.855765	5.381762	0.0388
$T_{pd}O_p$	4.46859	1	4.468595	8.421182	0.0133
I_pO_p	13.5974	1	13.59741	25.62467	0.0003
T_p^2	2.35270	1	2.352706	4.433734	0.0570
T_{pd}^2	0.75936	1	0.759367	1.431047	0.2547
I_p^2	1.13640	1	1.136407	2.141588	0.1690
O_p^2	0.45626	1	0.456268	0.859848	0.3721
Residual	6.36765	12	0.530638		
Lack of fit	6.03234	9	0.670261	5.996871	0.0839
Pure error	0.33530	3	0.111768		
Standard deviation	0.73				
Mean	5.69				
Coefficient variation (%)	12.79				
Press	58.85				
R^2	0.9137				
Adjusted-R^2	0.8130				

duration of 60 min, ultrasound intensity of 330 W and sucrose concentration of 36.02%. The maximum predicted responses obtained were a TPC yield of 0.510 mg GAE g^{-1} DL, DPPH radical scavenging activity of 42.661% and 7.66 ppm of p-cymene. The experimental responses with the optimised conditions yielded a TPC of 0.489 mg GAE g^{-1} DL, 44.57% DPPH radical scavenging activity and 7.8233 ppm of p-cymene. The deviation from the predicted responses was 4.06%, 4.47% and 2.13% for TPC yield, DPPH radical scavenging activity and p-cymene yield. Further validation implemented through comparison with the control method (Table 5) shows a remarkable increase in the three responses by two-fold. Therefore, the result shows the success of integrating ultrasound-assisted osmotic dehydration pre-treatment method in improving the final quality of the extract.

Modelling of drying kinetics

According to the moisture ratio curves, an exponential decrease was observed throughout the drying process, confirming that the diffusion process was governed by internal mass transfer (Shittu & Raji 2011). The constant moisture ratio at the altered phase after 23.5 hrs was the indication that further moisture removal was not possible. The drying kinetics was assessed by fitting to five thin-layer drying models which were Page, Henderson & Pabis, Lewi, Logarithmic and Avhad & Marchetti model (Table 6). According to statistical criteria, the fitness of models was in the following order: Lewis > Henderson & Pabis > Avhad & Machetti > Page > Logarithmic model. Lewis model, a semi-theoretical newton model satisfied the statistical evaluation of the highest R^2 and lowest RMSE, revealing that it is suitable for future predictions of drying kinetics in *E. deglupta*. On the other hand, Page and logarithmic are modified versions of Lewis and Handerson & Pabis model, respectively, with additional constants to minimise the errors (Onwude et al. 2016). However, it seems that it could be the reason behind the low accuracy leading to low R^2 values.

CONCLUSION

The present research studied the potential and impact of applying the ultrasound-assisted osmotic dehydration as pre-treatment for effective moisture removal from *E. deglupta*. Four crucial variables of UAOD conditions (temperature (40–60 °C), duration (60–100 min), ultrasound intensity (198–330 W), and sucrose concentration (30–50%)) were investigated. The optimum

Table 5 Validation of optimisation with control method

Assays	Results			Percentage difference between responses (%)	Percentage increase from control (%)
	Control	Predicted response	Experimental response		
TPC	0.309 mg GAE/g DL	0.510 mg GAE/g DL	0.489 mg GAE/g DL	4.06	36.84
DPPH radical scavenging activity	12.293%	42.661%	44.57%	4.47	70.41
p-cymene yield	3.563 ppm	7.660 ppm	7.823 ppm	2.13	54.45

GAE = gallic acid equivalents, DL = dried leaf

Table 6 The fitness of different models at optimum UOAD pre-treatment

No	Model name	Coefficients and constants	R^2	RMSE
1	Lewis	k = 0.18139 min^{-1}	0.9889	6.52E-8
2	Henderson & Pabis	a = 1.13591, k = 0.22839 min^{-1}	0.9783	7.16E-9
3	Page	k = 0.99098, n = 0.00986	0.2444	3.29E-9
4	Logarithmic	a = 4.44, k = 20.0025, c = 0.18944	0.2255	0.0107
5	Avhad & Machetti	a = 2.90515, k = 0.01995, n = 30	0.5445	3.20E-11

R^2 = coefficient of determination, RMSE = root mean square error

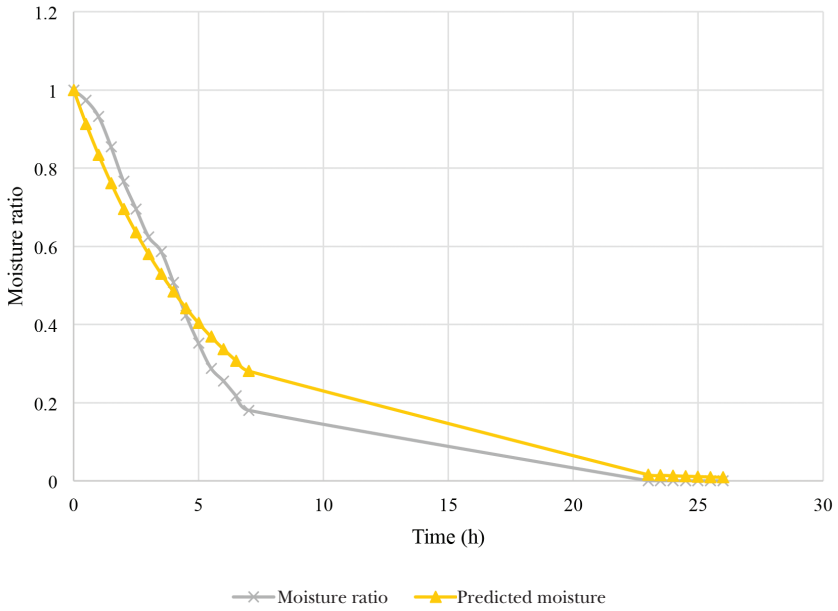

Figure 1 Moisture kinetic data for the Lewis thin layer drying model

pre-treatment conditions were identified from three-level, four-factor composite experimental design experiments as 58.18 °C, 60 min, 330 W and 36.02%, respectively. The experimental responses at the optimised condition yielded a TPC of 0.489 mg GAE g^{-1} DL, 44.57% DPPH radical scavenging activity and 7.8233 ppm of p-cymene. The deviation between the predicted and experimental responses at optimised conditions was 4.06%, 4.47% and 2.13% for TPC yield, DPPH radical scavenging activity and p-cymene yield. Under the optimised conditions, the three second-order polynomial regression models developed were deemed adequate. The incorporation of optimum ultrasound-assisted osmotic dehydration as pre-treatment yielded higher TPC, antioxidant activity and p-cymene compared to convective drying on its own by two-fold. Kinetic studies revealed that the proposed Lewis model is appropriate in predicting the moisture removal process. Overall, these results justify the benefits of incorporating ultrasound-assisted osmotic dehydration pre-treatment prior to convective drying for obtaining a final product with higher quality attributes.

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REFERENCES

Amami E, Khezami W, Mezrigui S, Badwaik LS, Bejar AK, Perez CT & Kechaou N. 2017. Effect of ultrasound-assisted osmotic dehydration pretreatment on the convective drying of strawberry. *Ultrasonics Sonochemistry* 36: 286–300.

Balzarini MF, Reinheimer MA, Ciappini MC & Scenna NJ. 2018. Comparative study of hot air and vacuum drying on the drying kinetics and physicochemical properties of chicory roots. *Journal of Food Science and Technology* 55: 4067–4078.

Bobo-Garcia G, Davidov-Pardo G, Arroquic, Virseda P, Marin-Arroyo MR & Navarro M. 2015. Intra-laboratory validation of microplate methods for total phenolic content and antioxidant activity on polyphenolic extracts, and comparison with conventional spectrophotometric methods. *Journal of the Science of Food and Agriculture* 95: 204–209.

Bozkir H, Ergun AR, Serdar E, Metin G & Baysal T. 2019. Influence of ultrasound and osmotic dehydration pretreatments on drying and quality properties of persimmon fruit. *Ultrasonics Sonochemistry* 54: 135–141.

Chaverri C & Ciccio JF. 2018. Essential oils from Eucalyptus deglupta (Myrtaceae) cultivated in Costa Rica. *American Journal of Essential Oils and Natural Products* 6: 1–6.

Chaves TP, Pinheiro REE, Melo ES et al. 2018. Essential oil of *Eucalyptus camaldulensis* Dehn potentiates β-lactam activity against *Staphylococcus aureus* and *Escherichia coli* resistant strains. *Industrial Crops and Products* 112: 70–74.

Chemat F, Rombaut N, Sicaire A-G, Meullemiestre A, Fabiano-Tixier A-S & Abert-Vian M. 2017. Ultrasound assisted extraction of food and natural products.

Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrasonics Sonochemistry* 34: 540–560.

Chua LYW, Chua BL, Figiel A, Chong CH, Wojdylo A, Szumny A & Łyczko J. 2019. Drying of Phyla nodiflora leaves: antioxidant activity,volatile and phytosterol content, energy consumption and quality studies. *Processes* 7: 210.

Cornelius JP, Corea EA & Mesen JF. 1995. Genetic variation in height growth and leaf colour of *Eucalyptus deglupta* Blume at ages up to 16 months in Costa Rica. *Forest Ecology and Management* 75: 49–59.

Diaz-Maroto MC, Perez-Coello MS, Vinas MAG & Cabezudo MD. 2003. Influence of drying on the flavor quality of spearmint (*Mentha spicata* L.). *Journal of Agricultural Food and Chemistry* 51: 1265–1269.

Escriche I, Chiralta, Moreno J & Serra JA. 2006. Influence of blanching-osmotic dehydration treatments on volatile fraction of strawberries. *Journal of Food Science 65*: 1107–1111.

Gullon B, Gullon P, Lu-Chau TA, Moreira MT, Lema JM & Eibes G. 2017. Optimization of solvent extraction of antioxidants from Eucalyptus globulus leaves by response surface methodology: Characterization and assessment of their bioactive properties. *Industrial Crops and Products* 108: 649–659.

Klemes J, Smith R & Kim, J.-K. 2008. *Handbook of Water and Energy Management in Food Processing*. Woodhead Publishing, Cambridge.

Kroehnke J, Szadzinskazadzińska J, Stasiak M, Radziejewska-Kubzdela E, Bieganska-Marecik R & Musielak G. 2018. Ultrasound- and microwave-assisted convective drying of carrots – Process kinetics and product's quality analysis. *Ultrasonics Sonochemistry* 48: 249–258.

Langner E & Rzeski W. 2013. Biological properties of melanoidins: A review. *Internationa Journal of Food Properties* 17: 344–353.

Liu J, Li L, Zhou L, Li B & Xu Z. 2017. Effect of ultrasound treatment conditions on Saccharomyces cerevisiae by response surface methodology. *Microbial Pathogenesis* 111: 497–502.

Maran JP, Manikandan S, Nivetha CV & Dinesh R. 2017. Ultrasound assisted extraction of bioactive compounds from Nephelium lappaceum L. fruit peel using central composite face centered response surface design. *Arabian Journal of Chemistry* 10: S1145–S1157.

Onwude DI, Hashim N, Janius RB, Nawi MN, Abdan K. 2016. Modelling the thin- layer drying of fruits and vegetables: A review. *Comprehensive Reviews in Food Science and Food Safety* 15: 599–618.

Rahaman A, Zeng X-A, Kumari A, Rafiq M, Siddeeg A, Manzoor MF, Baloch Z & Ahmed Z. 2019. Influence of ultrasound-assisted osmotic dehydration on texture, bioactive compounds and metabolites analysis of plum. *Ultrasonics Sonochemistry* 58: 104643.

Rahman N, Xin TB, Kamilah H & Ariffin F. 2018. Effects of osmotic dehydration treatment on volatile compound (Myristicin) content and antioxidants property of nutmeg (Myristica fragrans) pericarp. *Journal of Food Science and Technology* 55: 183–189.

Salehi B, Sharifi-Rad J, Quispe C et al. 2019. Insights into Eucalyptus genus chemical constituents, biological activities and health-promoting effects. *Trends in Food Science & Technology* 91: 609–624.

Salem N, Kefi S, Tabben O et al. 2018. Variation in chemical composition of Eucalyptus globulus essential oil under phenological stages and evidence synergism with antimicrobial standards. *Industrial Crops and Products* 124: 115–125.

Samani BH, Lorigooini Z & Zareiforoush H. 2017. Effect of ultrasound and infrared drying methods on quantitative and qualitative characteristics of Satureja bachtiarica essential oil. *Journal of Essential Oil Bearing Plants* 25: 1196–1208.

Shakthi-Deve A, Sathish Kumar T, Kumaresan K & Rapheal VS. 2014. The effect of extraction solvents, temperature and time on the composition and mass fraction of polyphenols in Dalmation Wild Sage (*Salvia officinalis* L.) extracts. *Journal of Diabetes and Metabolic Disorders* 13: 84–91.

Shittu TA & Raji AO. 2011. Thin layer drying of african breadfruit (*Treculia africana*) seeds: modelling and rehydration capacity. *Food and Bioprocess Technology* 4: 224–231.

Sledz M, Wiktor A, Nowacka M & Witrowa-Rajchert D. 2017. Drying kinetics, microstructure and antioxidant properties of basil treated by ultrasound. *Journal of Food Process Engineering* 40: 1–13.

Turek C & Stintzing FC. 2012. Impact of different storage conditions on the quality of selected essential oils. *Food Research International* 46: 341–353.

Yadav AK & Singh SV. 2014. Osmotic dehydration of fruits and vegetables: a review. *Journal of Food Science and Technology* 51: 1654–1673.