

# Optimization of the Various Conditions of Extraction for the Phenolic Compounds from The *Moringa Oleifera* Leaves

M. Selvarathi, S.Elizabeth Amudhini Stephen, Veninstine Vivik J. Abel Kooran Varghese

**Abstract:** The main objective of this particular study is in optimizing the various methods for extracting the phenolic compounds present in the *Moringa Oleifera* leaves by using a method called response surface methodology (RSM). In this study, a user-defined design was used to determine the effect of the time taken for extraction, the temperature during the extraction and then the ethanol concentration (percentage) on total phenolic content (TPC). The extraction process is done on three different types of *Moringa Oleifera* leaves. They differ in the method in which these leaves are dried (oven dried, sun-dried and ambient air-dried). The RSM method was used to attain conditions by which the extraction can be maximized i.e. Maximum extraction of TPC of the *Moringa Oleifera* leaves. The conditions that gives maximum extraction of phenolic compounds were the extraction time 73 min; the extraction temperature, 44°C and the percentage of methanol 97.55. The TPC that were extracted under these conditions were 12.28, 12.65 and 12.43 MG GAE/gm DW for the samples that were dried by using different methods. The difference between the three drying methods were found ( $p < 0.05$ ).

**Keywords:** Optimization *Moringa Oleifera*, phenolic compounds, response surface methodology, the drying methods.

## I. INTRODUCTION

*Moringa Oleifera* leaves are a very important plant due to its various usages in fields like nutrition, agriculture, pharmacological and industrial (Kasolo, 2010). The extract that we obtain from the *Moringa Oleifera* leaves are having antioxidant potential, ample amount of protein, vitamin C, calcium, potassium and natural antioxidants such as ascorbic acid phenolic are present in the *Moringa Oleifera* leaves. Due to this reason, they are used for increasing the shelf life of foods especially the foods that contain fat (Anwar 2007).

The availability of these phenolic compounds in the *Moringa Oleifera* leaves such as the antioxidant source are ensured. A high extraction efficiency is necessary for an industry when

considering it as economically feasible. There are many factors that influence the efficiency of extraction, such as the method of extraction, particle size, solvent type, the concentration of the solvent, solvent-to-solid ratio, the temperature of extraction, the extraction time and pH (Banik Pandey, 2007; Silva et al., 2007).

The current study is conducted with the objective of primarily using the Response surface methodology (RSM) for the optimizing of the extraction parameters including extraction time, extraction temperature and the percentage of methanol for the extraction of TPC from the *Moringa Oleifera* leaves. Secondly, to compare the effect of various drying methods on the TPC that is extracted from the *Moringa Oleifera* leaves.

## II. MATERIALS AND METHODS

### Materials from plant

Leaves of *Moringa Oleifera* leaves also commonly known as Sohanjna were collected from a plant. Sunlight, oven and ambient air are the various drying methods used for drying the leaves.

### Extraction of solvent

The leaves that were dried in the above methods were finely ground into powder. Approximately 10 g of the dried sample was weighed and extracted with 100 ml by using the Soxhlet apparatus. Extracts were concentrated at a reduced pressure at 30-45°C on a rotary evaporator. The concentrated samples were preserved in labeled sample bottles in a refrigerator (-4°C) for further analysis.

### Determination of phenolic content

Parejo's method was used to determine the total phenolic content by using the Folin-ciocalteu reagent. The solvent extract (0.5 ml), 0.5 ml Folin-ciocalteu reagent and about 25 ml of deionized water was added as a final volume. After an hour, the absorbance of the sample was measured at 745 nm using a spectrophotometer.

### Design of the experiment

The effect of the three factors, extraction time (time), extraction temperature (temp) and % of methanol on the TPC from the *Moringa Oleifera* leaves and were studied using the response surface methodology. By using this design we can test the three variables by three different levels; extraction time at 30, 60 and 90 min.; extraction temperature 30, 60, 90°C; percentage of methanol 50, 75 and 100.

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The Table 1 shows the experimental design, with the experimental variables of the independent variable and accompanied with the result that was obtained from the response analyzed variables for each of the drying methods.

### III. STATISTICAL ANALYSIS:

The results that were obtained from the experiment were analyzed using the SPSS software. The Design experiment statistical software was employed to analyze the experiment data in RSM and to optimize the conditions.

### IV. RESULTS

In the research work that was conducted, three drying methods were used. The phenols obtained from the oven-dried, sun-dried and ambient air-dried samples of *Moringa Oleifera* leaves are given in the table 1. The type of the file version used, the study type and all other information regarding the software are given in table 2. The factors used also i.e. that variables etc. are also mentioned in the table 1. The point prediction result is mentioned in table 3. The confirmation table (table 4) gives the predicted mean and predicted median. This model demonstrated the significance difference between the average TPC obtained from oven-dried, sun-dried and the ambient air-dried samples of *Moringa Oleifera* leaves.

$$Y1 = 9.11873 + 0.20921X_1 + 0.7005X_2 + 0.04025X_3 + 0.42X_1X_2 - 2.01869X_1X_3 + 0.55X_2X_3 - 0.266X_1^2 - 0.374X_2^2 - 0.514X_3^2$$

$$Y2 = 13.4876 - 0.165X_1 + 0.36X_2 + 0.5214X_3 - 1.14X_1X_2 + 0.37X_1X_3 - 0.055X_2X_3 + 1.074X_2^2 - 0.726X_3^2 - 0.021X_3^2$$

$$Y3 = 11.074 - 0.13375X_1 + 0.4X_2 + 0.8593X_3 - 0.6425X_1X_2 + 0.585X_1X_3 + 0.1975X_2X_3 - 0.062X_1^2 + 0.4905X_2^2 - 0.417X_3^2$$

Where  $X_1$  extraction time,  $X_2$  extraction temperature,  $X_3$  percentage of methonal,  $Y_1$  oven dried sample,  $Y_2$  sun dried sample,  $Y_3$  ambient air dried sample

Run	Factor 1 Extraction time min	Factor 2 Extraction temp. degree celcius	Factor 3 Concentration %	Response 1 oven dried sam. g	Response 2 sunlight dried s. percent air dried	Response 3
1	15	30	90	75	52	11.88
2	15	30	60	75	11.77	9.43
3	15	30	30	75	12.15	10.72
4	15	60	90	100	9.91	12.11
5	15	60	60	75	10.78	11.45
6	15	60	30	100	12.28	9.47
7	15	90	90	100	11.65	12.44
8	15	90	60	75	9.97	11.33
9	15	90	30	100	10.81	10.07
10	30	30	90	75	11.34	12.45
11	30	30	60	75	9.56	11.79
12	30	30	30	75	9.97	10.37
13	30	60	90	75	10.36	10.32
14	30	60	60	75	9.81	11.87
15	30	60	30	75	10.46	9.76
16	45	30	90	75	12.12	11.21

Table 1: phenolic content and the values obtained from the three drying methods.

### DISCUSSION

In this current study the phenolic content of the oven-dried, sun-dried and the ambient air-dried samples of the *Moringa Oleifera* leaves were determined. The obtained values are shown in the table 1.

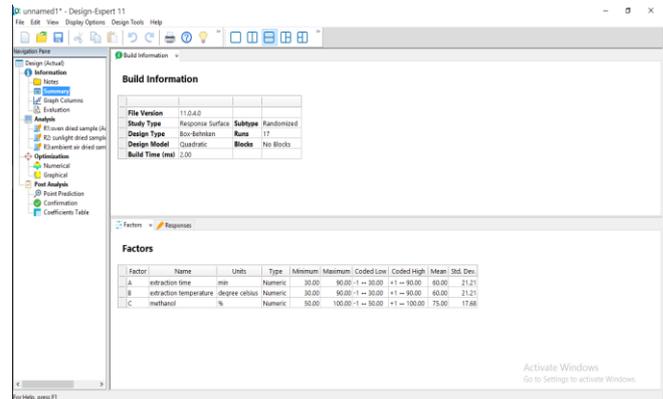


Table 2: information regarding the build or the software used.

### V. ANALYSIS OF THE RESPONSE SURFACE

In this the 2D RSM method are used for the visually predicting future responses, and for the determination of the factor values that optimize the response function.

#### Effect of the extraction time on ambient air-dried, sun-dried and the oven-dried samples.

This also a comparison between all the different drying methods.

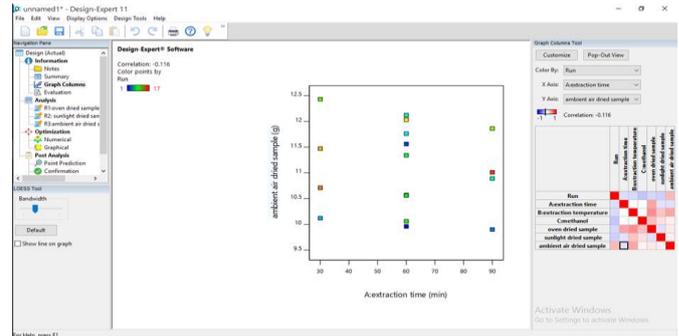


Fig. 1: The effect of extracting between the extraction time and the ambient air-dried sample.

From the Fig. 1 we can see that that the extraction time of 90 min the extraction of TPC from and ambient air-dried sample is the highest. In addition, when comparing the figures 2 and 3 we can see that the extraction time for the sun dried sample is also 90 min and whereas for the oven dried sample the extraction time is 30 min. so the maximum time and the minimum time taken is 90 min. and 30 min.

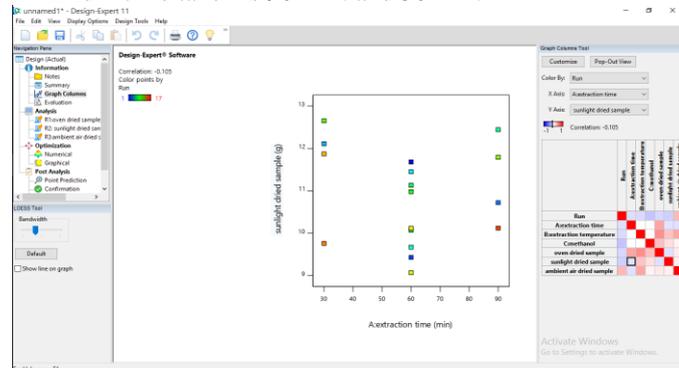


Fig. 2: The effect of extracting between the extraction time and the sun dried sample.



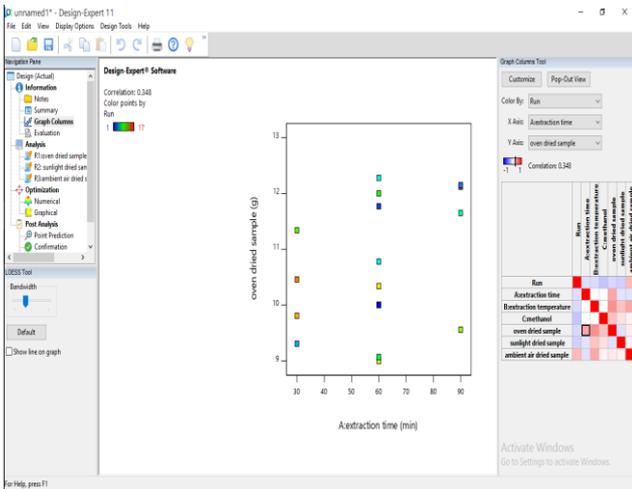


Fig.3: The effect of extracting between the extraction time and the oven dried sample.

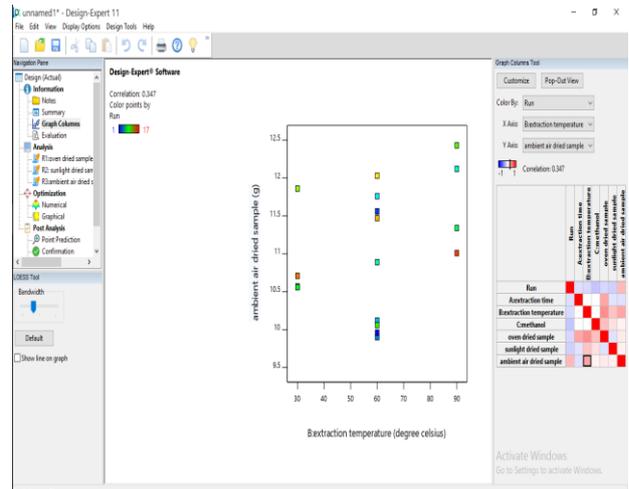


Fig.6: The effect of extracting between the extraction time and the ambient air-dried sample.

**Effect of extraction between the extraction temperature and the differently dried samples.**

The Figures 4,5and6 shows the extraction of TPC from the three different types of dried samples against the extraction temperature.

**Effect of extraction between the percentage of methanol and the different dried samples.**

In this, the three different dried samples are analyzed against the percentage of methanol. With this graphical study, we can find out how much percentage of methanol is required for the maximum extraction of TPC from the three different samples.

The figures 7, 8 and 9 shows the graphical representation between the percentage of methanol and the differently dried samples.

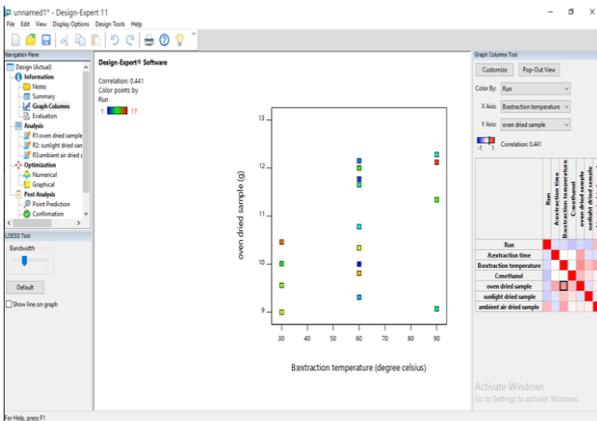


Fig.4: The effect of extracting between the extraction temperature and the oven dried sample.

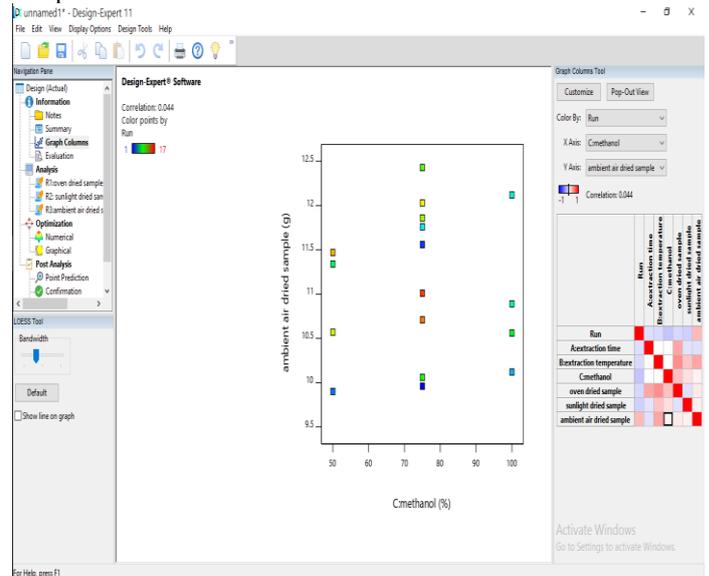


Fig.7: The effect of extracting between the percentage of methanol and the ambient air-dried sample.

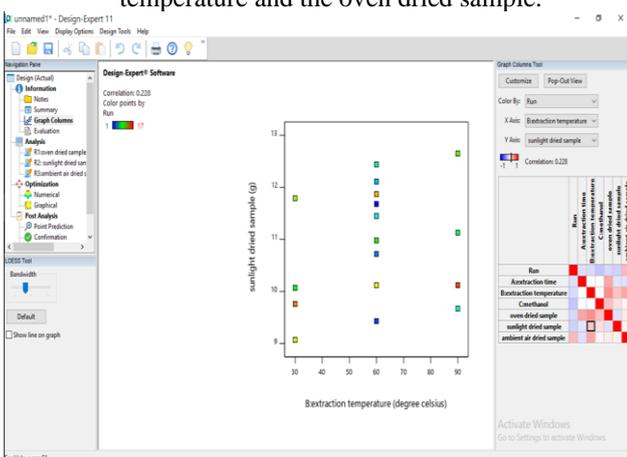


Fig.5: The effect of extracting between the extraction time and the sundried sample.

From the figures 7, 8, 9 we can see that as the percentage of methanol increases there is reduction in TPC. A mixture of alcohols and a little water is more efficient to extract phenolic compounds from the sample (Spigno et al., 2007). Polarity of the solvent increases with the addition of water to methanol. (Zhang et al., 2007).

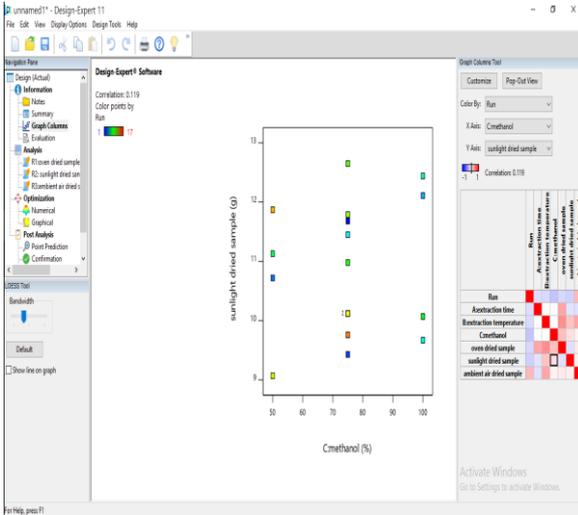


Fig.8: The effect of extracting between the percentage of methanol and the sundried sample.

During sundrying the climatic factors play a vital role as it is mentioned and proved by Muller- Harvey (2001). Generally drying process would reduce the naturally occurring antioxidants due to intense or prolonged drying (Tomaino *et al.*, 2001). Since most of these compounds are comparatively unstable, its natural antioxidants are destroyed (Nicoli *et al.*, 1999).

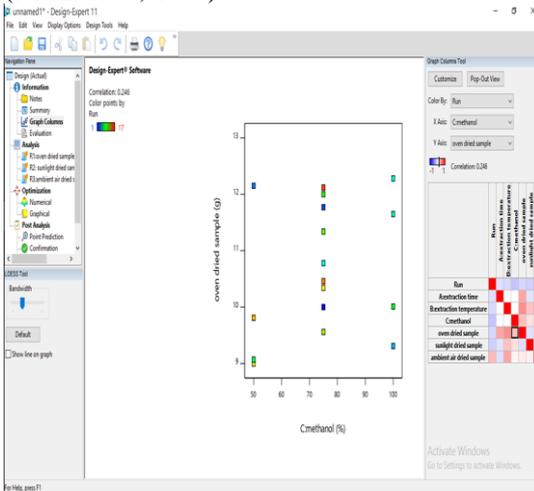


Fig.9: The effect of extracting between the percentage of methanol and the oven-dried sample.

In the Fig.10, we can see the Numerical analysis of the threefactors, extraction time, extraction temperature and the percentage of methanol. Along with it is also the numerical analysis of the three methods of drying.

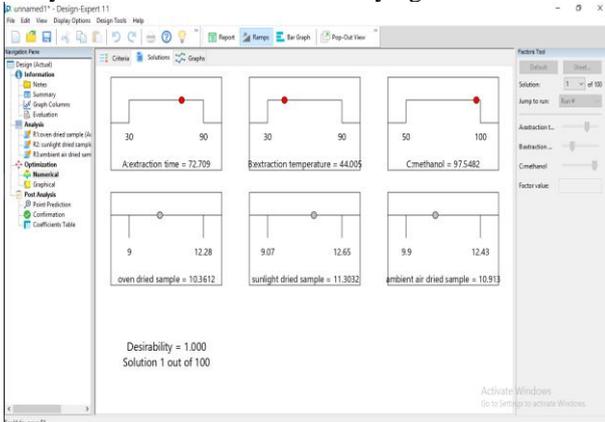


Fig.10: Numerical analysis of the independent variables and the dependent variables.

The next Figure (Fig. 11) shows the graphical representation of the desirability of the drying methods used based on the comparison of the extraction temperature and the extraction time.

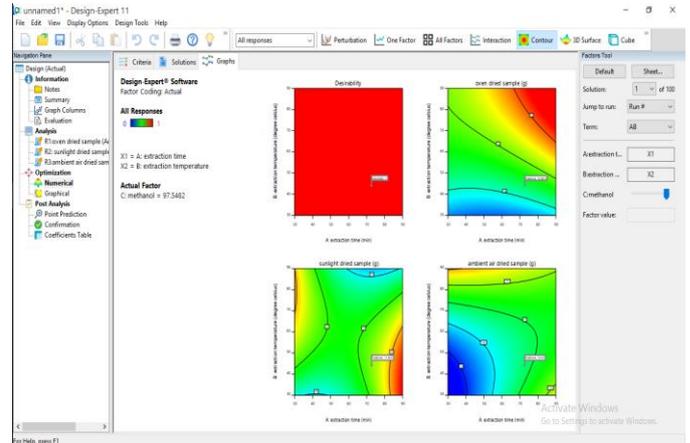


Fig. 11: Desirability comparing the three drying methods for the extraction of the TPC.

Factor	Name	Level	Low Level	High Level	Std. Dev.	Coding
A	extraction time	72.71	30.00	90.00	0.0000	Actual
B	extraction temperature	44.00	30.00	90.00	0.0000	Actual
C	methanol	97.50	50.00	100.00	0.0000	Actual

Response	Predicted Mean	Predicted Median	Observed	Std Dev	SE Mean	95% CI low	95% CI high	95% TI low	95% TI high
oven dried sample	10.3612	10.3612	1.18569	0.722866	8.65188	12.0706	3.59552	17.1269	
sunlight dried sample	11.3032	11.3032	0.780587	0.488962	10.1519	12.4544	6.74647	15.8398	
ambient air dried sample	10.9113	10.9113	0.780407	0.488921	9.75894	12.0692	6.3371	15.489	

Table 3: Point prediction of the different drying methods.

Factor	Name	Level	Low Level	High Level	Std. Dev.	Coding
A	extraction time	72.71	30.00	90.00	0.0000	Actual
B	extraction temperature	44.00	30.00	90.00	0.0000	Actual
C	methanol	97.50	50.00	100.00	0.0000	Actual

Response	Predicted Mean	Predicted Median	Observed	Std Dev	SE Pred	95% PI low	Data Mean	95% PI high
oven dried sample	10.3612	10.3612	1.18569	1.13754	1.11762	13.6046		
sunlight dried sample	11.3032	11.3032	0.780587	1.032374	9.11873	13.4876		
ambient air dried sample	10.9113	10.9113	0.780407	1.032721	8.71939	13.1087		

Table 4: Confirmation chart of the various drying methods.

	Intercept	A	B	C	AB	AC	BC	AC <sup>2</sup>	B <sup>2</sup>	C <sup>2</sup>
oven dried sample	16.978	0.571	0.7225	0.4025	0.42	-0.018696141	0.55	0.266	0.174	-0.114
p-values	0.0002	0.0290	0.0013	0.0045	0.0001	0.3760	0.0030	0.0010	0.0010	0.0001
sunlight dried sample	16.762	-0.163	-0.36	0.1875	0.14	0.27	-0.13	0.074	-0.206	-0.021
p-values	0.0016	0.0096	0.0010	0.0029	0.0010	0.0010	0.0010	0.0010	0.0010	0.0010
ambient air dried sample	11.074	-0.13373	0.4	0.00125	-0.0425	0.003	0.0015	0.002	0.002	-0.417
p-values	0.0480	0.1944	0.0001	0.1472	0.1814	0.0017	0.0014	0.0020	0.0010	0.0010

Table 5: The coefficient table of the various oven dried samples and their p values.

### VI. CONCLUSION

The current study that was conducted indicated that the advantages of RSM while comparing with the old methods in optimization of the extraction conditions for the extraction of phenolic antioxidants from the *Moringa Oleifera* leaves. The optimum condition that maximizes the extraction of TPC where the extraction time taken 73 min, extraction temperature required 44°C and the percentage of the methanol required 97.55. The Maximum TPC that were extracted from the samples that were dried by different drying methods are 12.28, 12.65 and 12.43. The sample that was dried by sun-dried method provided the best results.

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