Solvent Extraction Optimization of Chlorophyll Dye from *Conocarpus lancifolius* Leaves

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Highlights:

- *Conocarpus lancifolius* Leaves were examined as a potential source of chlorophyll dye. Optimization of extraction parameters is carried out using Taguchi experimental design.
- The total chlorophyll yield is mostly dependent on extraction time, mixing ratio and temperature. There are large interactions between extraction parameters time, temperature and mixing ratio.
- The quadratic model developed for chlorophyll extraction process had R2 of 97.54 %.

Abstract. Recently, the green food industry has started using chlorophyll dye as a healthy and safe color additive for food and beverages. In this study, a conventional extraction technique was used to extract chlorophyll dye from *Conocarpus lancifolius* leaves, using 80% acetone as solvent. Taguchi's L_{25} orthogonal array was utilized to optimize the extraction parameters with total chlorophyll dye yield as the response. The optimum extraction conditions were 75 minutes of extraction time, 33.5 °C extraction temperature and a 118 ml/g solvent to raw leaves ratio. Analysis of variance using Minitab18 was conducted to identify the most significant extraction parameters and available interactions. It was found that the chlorophyll dye yield from *Conocarpus lancifolius* leaves is mostly dependent on extraction time, followed by mixing ratio and temperature. The analysis showed a large interaction between the extraction parameters studied. The experimental chlorophyll dye yield was in close agreement with the predicted model. The adequacy of the predicted model was confirmed by an experiment conducted under the optimal conditions, given a 3.9% error percentage.

Keywords: *chlorophyll dye; Conocarpus lancifolius leaves; extraction parameters; solvent extraction; Taguchi design.*

1 Introduction

In recent years, the use of synthetic dyes and food colorants as food additives has increased, despite harmful health effects. Synthetic dyes produce a hazardous residual, so the search for alternative natural formulations has become an urgent research topic. Researchers are interested in extracting safe and environmentally

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friendly dyes from plant roots [1]. The main limitations in the use of natural dyes are their high cost and low stability, which is why researchers focus on inexpensive extraction methods within normal temperatures ranges.

Chlorophylls, betalains, anthocyanins and carotenoids are four essential families of natural product dyes that have been authorized as food supplements by the Codex and Food and Drug Administration (FDA) [2]. Chlorophyll is responsible for the photosynthesis process in plants and algae. In chloroplasts, two kinds of chlorophyll are found, chlorophyll a and chlorophyll b, which differ in color gradient and structure [3]. Chlorophyll dyes a and b are used in alternative medicine, food additives, and in the industrial field. Chlorophyll a is utilized in photodynamic therapy (PDT) as a natural photosensitizer for cancer treatment [4]. The antioxidant activity of chlorophyll a supplements plays an important role against lipid oxidation and in preventing diseases. Chlorophyll dyes provide a strong green color for foods and cosmetics and improve many products, from sweets to soap [5]. In addition, it is used as a biological mordant in textile dyeing to enhance color strength [6]. Another use of chlorophyll dyes is in the fabrication of simple, environmentally friendly and low-cost dye-sensitized solar cells (DSSCs) [7].

Conocarpus lancifolius is a popular riverine tree native to Somalia. Its spread was originally limited to a small region on the southern red sea coast, but over the last decade it has spread to the south and middle of Iraq due to the ability of this species to grow under aggressive conditions (temperature $\geq$ 40˚C, salinity and water inadequacy). Conocarpus is now the most important perennial decorative tree in Iraq. It is a rapidly spreading evergreen tree that is able to create a large amount of biomass with drip irrigation [8,9].

Chlorophyll is the only natural source of green dye existing in large quantities in nature, with world production at 1.2 billion tons from marine and terrestrial origins. The instability of the middle magnesium atom in chlorophyll when extracted from chloroplasts indirectly leads to its displacement by metallic molecules such as copper to create a stable bluish green dye. Cu-chlorophyllin, Na-Cu-chlorophyllin and related structures are authorized as foods dyes in the European Union and the United States of America [10].

The extraction of bioactive components from plant biomass by mixing with a selective solvent is a crucial step in the synthesis of phytochemical extracts. Solvent extraction is an efficient and inexpensive technique to produce bioactive solute extract for applications such as food colorants and nutraceutical additives [11]. Mixing and well stirring of the solvent/biomass matrix are the main steps of the traditional extraction of phytochemical compounds. In addition, the solvent must be selected carefully to reduce interference of the product. To achieve significant extraction of the desired biomolecules, the experimental parameters (pH, time, temperature, biomass to solvent ratio and stirring speed) should be optimized. The extraction process has a wide industrial application in the food industry and an important research trend in this area is the use of non-toxic and cheap solvents [12,13]. Recent studies have recommended using acetone diluted in water or methanol to extract chlorophyll dye because these solvents have high extraction efficiency, create a large dielectric constant, and are environmentally friendly, safe and cheap [14-18].

There are many kinds of factorial experimental design methods that have been used to optimize the different extraction processes, for example Taguchi design and response surface. When the number of studied parameter levels are high, Taguchi design is a powerful factorial design that links potential combinations between levels and factors and reduces the number of experiments [19-20]. By choosing the best orthogonal array (OA) and parameter levels suitable for Taguchi design, optimal conditions for each level of the extraction process and the interactions between the optimized parameters can be established [11]. Previous researches did not use Taguchi statistical design to verify the effect of the process parameters on chlorophyll dye extraction. In this study, the solvent extraction method was used to extract chlorophyll dye from *Conocarpus lancifolius* fresh leaves by using 80% acetone as solvent. Taguchi's technique was applied to study the influence of three extraction parameters: time, temperature, and mixing ratio. From reviewing the literature, the use of *Conocarpus lancifolius* as a source of chlorophyll dye has not yet been investigated. Hence, the present study attempted to develop a new empirical model to investigate the optimum levels and interaction effects of the studied parameters, i.e. time, temperature and mixing ratio.

2 Experimentation

2.1 Raw Material and Chemicals

Conocarpus lancifolius leaves were collected from trees near the research lab in the middle part of the campus of Southern Technical University, Basra Province, Iraq. The *Conocarpus lancifolius* leaves were cleaned and cut to mixed particle sizes of approximately 2 to 5 mm. The leaves were rinsed in deionized water several times and then dried in the dark at about 10 to 13 °C for 24 hours. Analytical grade acetone (ROMIL) was purchased from the local market in Basra.

2.2 Solvent Extraction and Determination of Total Chlorophyll

The acetone and the deionized water were used as solvent in the chlorophyll dye extraction process at an 80:20 ratio. The solvent was added to a 250-mL

Erlenmeyer flask with a known quantity of *Conocarpus lancifolius* leave particles. The flask was firmly covered by a polyethylene cover to save the solvent from escaping by evaporation. The flask was kept in a water bath shaker (Polyscience) connected to a chiller (DHC) to control the temperature. The mixing speed was fixed at 190 r.p.m. The parameters studied were: solvent to dry leaves mixing ratio, extraction temperature, and extraction time, with five levels for each parameter, as shown Table 1. The chlorophyll dye solution extracted from the *Conocarpus lancifolius* leaves was identified by UV-Vis spectrophotometer (Apel PD-303 UV) and the absorbance was read at 400-700 nm. It was observed that the chlorophyll had maximum absorbance at 645 nm and chlorophyll b at 663 nm. The quantification of the total chlorophyll concentration in 80% acetone solvent was determined using the formula by Arnon [3,21]:

$$\textit{Total Chlorophyll Concentration}\ \text{(mg/l)} = 20.2\ A_{645} + 8.02 A_{663} \tag{1}$$

where A_{645} is the absorbance at 645 nm and A_{663} is the absorbance at 663 nm. The total chlorophyll dye extraction yield was calculated using the following equation:

$$\textit{Total Chlorophyll Yield}\ \% = \left(\frac{S}{W}\right) 100 \tag{2}$$

where S is the mass of total chlorophyll dye extracted (mg), and W is the mass of *Conocarpus lancifolius* feed leaves (g) [22].

Table 1 Extraction parameters and their levels.

Parameters	Code	Unit	Levels				
			1	**2**	**3**	**4**	**5**
Time	t	minutes	15	30	45	60	75
Temperature	T	°C	15	20	25	30	35
Mixing ratio	M	ml/g	10	20	40	80	120

2.3 Taguchi Method

Taguchi's L_{25} orthogonal array was utilized to define the optimum levels and parameter interactions for the chlorophyll dye extraction process. Graphical and statistical analyses were performed using the statistical program Minitab 18. The three parameters (extraction time, mixing ratio and extraction temperature) were investigated at five levels. In total, twenty-five experiments were conducted, as displayed in Table 1. The total chlorophyll yield was selected as the response of the dye extraction process. General Linear Model (GLM) statistical analysis was performed to analyze the variance and regression of the investigated response variable. The theoretical and experimental values were compared in order to confirm the studied model.

3 Results and Discussion

The Taguchi method analyzes the influence of the studied parameters on the response and proposes the optimum parameter values with the lowest number of experiments. In this study, the controllable factors time, temperature and mixing ratio were considered at five selected levels, as shown in Table 2. The total chlorophyll yield (Eq. (2)) was taken as the response variable in the extraction experiment. The Taguchi design uses the signal to noise ratio (S/N) to analyze the results. In the literature, three defined S/N ratio analyses are available: Higher is Better, Lower is Better, and Nominal is Best [23,24]. In the current study, the destination value of the total chlorophyll yield was to achieve the greatest possible dye extraction, so the equation for the Higher is Better S/N ratio was selected. The S/N ratio for each level was calculated using Minitab 18 according to the following equation:

$$S/N = \sum_{i=1}^{n} \frac{1}{y_i^2} - 10 \ \log\left[\frac{1}{n}\right] \tag{3}$$

where n is the number of repeats and y_i is the measured variable value [25].

3.1 Main Effect Plot

The experimental results of total chlorophyll yield and S/N ratio for different Taguchi level runs are reported in Table 2. Each level condition was repeated three times and the average value was recorded. It can be observed that the highest yield was 62.09%, achieved at experimental run number 20 with temperature 30 °C, extraction time 75 min, and mixing ratio 120. The main effect curves for the individual parameters against the S/N ratio and mean are given in Figures 1 and 2, which show a pictorial plot for each parameter.

The extraction time of chlorophyll dye was varied from 15 to 75 min. It is clear from Figure 1 that the chlorophyll extraction yield was highly affected by the extraction time. It can be seen that the chlorophyll yield increased rapidly with the increase of the extraction time in the initial stages and increased slightly in the final stages. This phenomenon can be attributed to the high mass transfer rate at the beginning of the extraction process. The mass transfer rate increases with an increasing concentration gradient of dye in solution, which enhances the dye transfer to the solvent. The selected optimum extraction time was 75 min. The same time behavior has been observed in a previous study [26].

Tests were conducted to investigate the influence of temperature (varied at 15, 20, 25, 30 and 35 °C) on the chlorophyll dye yield. From the results on the effect of temperature it was observed that the chlorophyll yield increased by increasing the temperature. When the temperature rises, the kinetic energy of the chlorophyll molecules increases and leads to enhanced dye solubility in the solvent [27]. The total yield increased with increasing temperature until the optimum value of 33.5 °C was reached. A further increase in temperature over the optimum value was insignificant. The same observation was recorded in Reference [28].

Table 2 Results of total chlorophyll yield response and S/N ratio.

Run	Temperature	Time	Mixing Ratio	Total Chlorophyll Yield	SN Ratio
1	15	15	40	9.80	19.82
2	15	30	80	25.91	28.27
3	15	45	120	33.64	30.54
4	15	60	20	19.01	25.58
5	15	75	10	27.91	28.92
6	20	15	80	19.17	25.65
7	20	30	120	27.74	28.86
8	20	45	20	24.89	27.92
9	20	60	10	29.36	29.36
10	20	75	40	33.34	30.46
11	25	15	120	17.43	24.83
12	25	30	20	23.60	27.46
13	25	45	10	28.21	29.01
14	25	60	40	42.20	32.51
15	25	75	80	50.42	34.05
16	30	15	20	22.26	26.95
17	30	30	10	22.23	26.94
18	30	45	40	36.00	31.13
19	30	60	80	46.52	33.35
20	30	75	120	62.10	35.86
21	35	15	10	20.14	26.08
22	35	30	40	34.71	30.81
23	35	45	80	57.96	35.26
24	35	60	120	49.77	33.94
25	35	75	20	38.85	31.79

The last parameter investigated was the mixing ratio. It can be observed from Figures 1 and 2 that the extracting yield increased with increasing mixing ratio until the optimum value of 118 ml/g was reached and then became insignificant. When the solvent quantity increased, the solubility of chlorophyll in the solvent was increased. In addition, the concentration gradient developed and the chance of chlorophyll molecules to contact the solvent was increased.

A further increase of solvent to solid above 118 did not affect the extraction yield due to equilibrium being reached. On the other hand, increasing the solid quantity against the solvent quantity may increase the quantity of chlorophyll extracted, but no significant effect on the yield was observed. The same observation has been reported in Ref. [29,30].

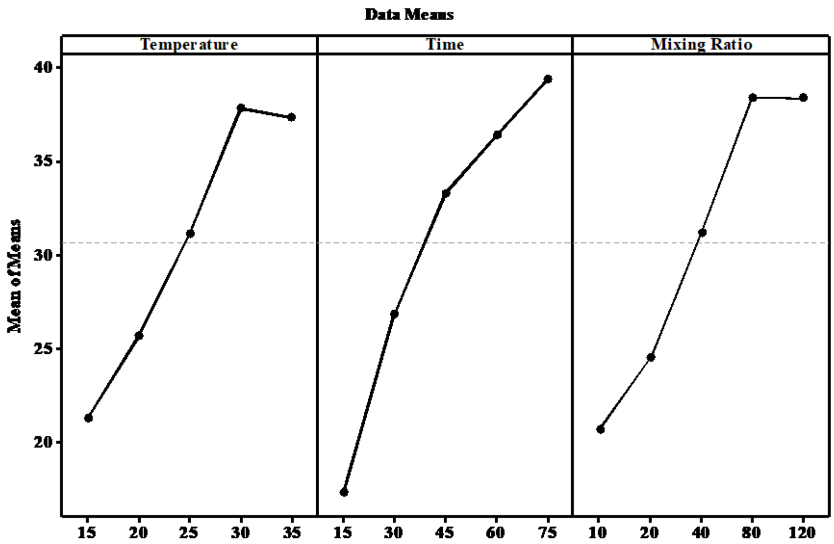


Figure 1 Effect of extraction parameters on mean response (total chlorophyll yield mg/g).

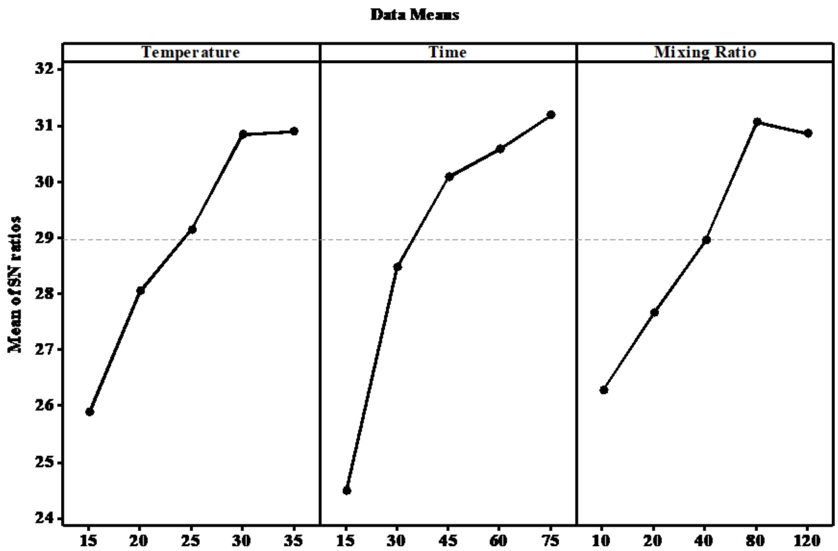


Figure 2 Effect of the extraction parameters on the Higher is Better S/N ratio.

The S/N ratios (Higher is Better) for each parameter at five levels, ranks and Delta values are given in Table 3. The Delta value for each studied parameter is defined as the variance between the highest and the lowest S/N ratio, and indicates the relative impact of the effect.

The higher the Delta value, the more intense the impact, which helps in identifying the parameters with the largest effect [31]. It is understood from the Delta values that the extraction time is the parameter with the largest effect on the chlorophyll extraction yield and S/N ratio, followed by temperature and mixing ratio, respectively.

Table 3 Response table for higher is better S/N ratio.

Level	Temperature	Time	Mixing Ratio
1	25.87	24.47	26.27
2	28.04	28.47	27.66
3	29.14	30.08	28.95
4	30.85	30.58	31.06
5	30.88	31.18	30.85
Delta	5.01	6.71	4.79
Rank	2	1	3

3.2 Regression Analysis and Model Fitting

The response yield of total chlorophyll in the observed experimental results was subjected to a regression analysis to take out the coefficients and estimate the fit of the model. A general linear model for the tested data was achieved using the Minitab18 software. In the present study, three parameters were investigated at five levels each.

The predicted model for the total chlorophyll extraction is represented by Eq. (4). The number of terms within the predicted model builds on the degree of freedom of the major influence terms and the related effective interactions.

$$\begin{aligned} &\mathit{Total\ Chlorophyll\ Yield}\ \% = -38.2 + 0.766t + 0.387M + 2.23T - \\ &0.00531t^2 - 0.0023M^2 - 0.0252T^2 + 0.0028tM - 0.00588Tt - 0.0032MT \end{aligned} \tag{4}$$

where t is the extraction time (min), T is the extraction temperature (°C) and M is the mixing ratio (ml/g). The results of the variance analysis are tabulated in Table 4.

The effects of time (t), temperature (T) and mixing ratio (M), the interaction effect and the square effect can clearly be seen from Table 4. The significance of the predicted model equation was estimated by the corresponding p-values ($p < 0.05$)

and F-values. The variance analysis proved that the prediction of the total chlorophyll extraction yield model was highly significant (p = 000 < 0.05).

The results show a significant effect of time (p = 0.019 < 0.05) and temperature (p = 0.025 < 0.05). A high significant square effect of mixing ratio (p = 000 < 0.05) and time (p = 0.003 < 0.05) was observed. For the total chlorophyll extraction yield parameters mentioned in Eq. (4) it was observed that R^2 = 97.54%. Thus, it can be concluded that the estimated model has good predictability. The R^2 achieved in this study is significant and more accurate than regressions in other studies using expensive extraction techniques and solvents [32-33].

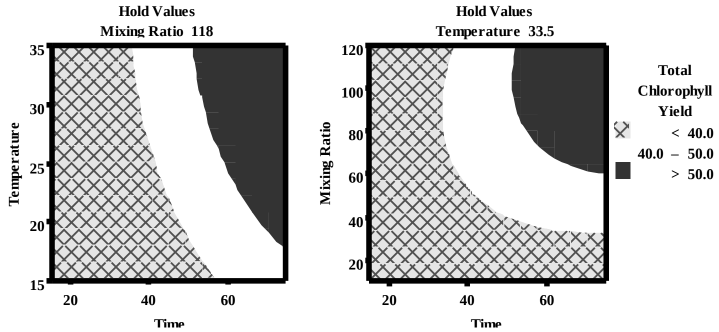
Table 4 Analysis of variance of the total chlorophyll yield for the studied parameters (S = 2.71909, R2 = 97.54%, R2 (adjusted) = 95.78%, and R2 (predicted) = 91.71%).

Source	Degree of freedom	Sum of square	Mean square	F-Value	P-Value
Regression	10	4096.81	409.681	55.41	0.000
Time	1	52.12	52.116	7.05	0.019
Mixing Ratio	1	11.57	11.574	1.57	0.231
Temperature	1	46.24	46.244	6.25	0.025
Time× Time	1	92.27	92.268	12.48	0.003
Mixing Ratio× Mixing Ratio	1	171.15	171.151	23.15	0.000
Temperature× Temperature	1	25.63	25.627	3.47	0.084
Time× Mixing Ratio	1	1.91	1.914	0.26	0.619
Time× Temperature	1	4.18	4.183	0.57	0.464
Mixing Ratio× Temperature	1	0.69	0.685	0.09	0.765
Time× Mixing Ratio× Temperature	1	0.45	0.455	0.06	0.808
Error	14	103.51	7.393		
Total	24	4200.31			

3.3 Analysis of Contour Plots

Contour charts can specify the desired total chlorophyll yield values related to the parameter conditions. In these charts, the total chlorophyll yield is illustrated on a two-dimensional plane, where all points with the same value are linked to make contour lines showing the fixed chlorophyll dye yield.

The contour plots illustrated in Figures 3 and 4 show that the highest total chlorophyll extraction yields were obtained at the upper end of the parameters time, temperature and mixing ratio.



(a) (b)

Figure 3 Contour plots of the combined effects of (a) total chlorophyll yield vs temperature, time (b) total chlorophyll yield vs mixing ratio, time.

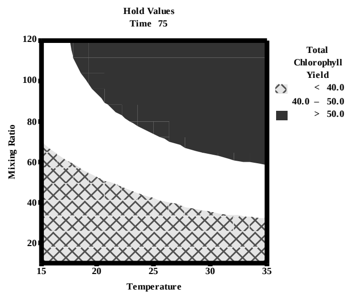


Figure 4 Contour plot of total chlorophyll yield vs mixing ratio, temperature.

3.4 Analysis of the Interaction Plots

Interaction plots were used to explain the relationships between the studied parameters and the total chlorophyll yield. Interaction occurs when the change in response value (total chlorophyll yield) from the lowest level to the highest level of one parameter does not resemble the second parameter under the same response values. Based on the interaction plots in Figure 5, it seems that the studied parameters are highly dependent and that there are large interactions between them (temperature, mixing ratio and time).

3.5 Confirmation Test

To get the validity of the studied regression model, a confirmation test was conducted with the parameters at the optimum levels. Table 5 shows the levels of the parameters selected for the confirmation test and compares them with the values predicted by the model. The error between the model values and the experimental values was very low (3.9%). The developed regression model provides a feasible and effective way to predict the total chlorophyll extraction yield from *Conocarpus lancifolius*.

Table 5 Model validity confirmation test.

Parameters /unit	Values	Experimental yield value (mg/g)	Model yield value (mg/g)
Extraction time (min)	75		
Temperature (°C)	33.5	62.1	59.64
Mixing ratio (ml/g)	118		

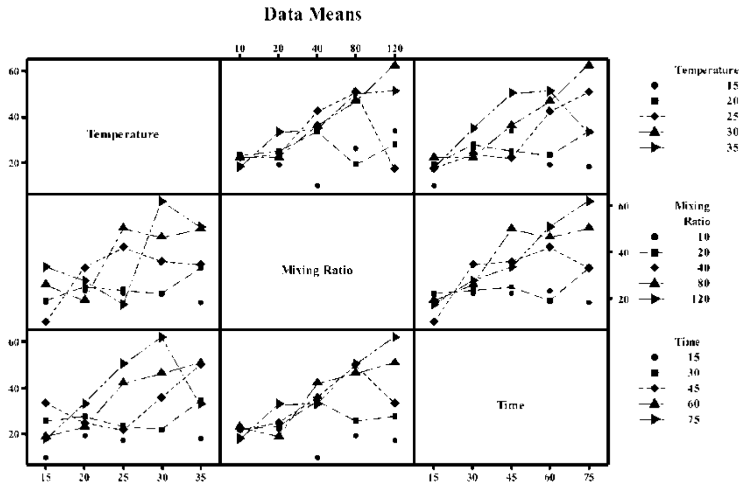


Figure 5 Interaction plot between temperature, time and mixing ratio.

4 Conclusions

In this study, solvent extraction of chlorophyll dye by 80% acetone was tested using a Taguchi experimental design to optimize the extraction parameters. The conventional method for extracting chlorophyll dye from *Conocarpus lancifolius* leaves for further use as a food supplement was found to be feasible and achievable within the temperature range of 25 to 35 °C. The experiments showed that the studied extraction parameters (time, temperature and mixing ratio) had a significant effect on the total chlorophyll dye yield.

The levels of importance of the extraction parameters were determined using analysis of variance. Moreover, a mathematical model was established to represent the relationship between the extraction parameters and the total chlorophyll extraction yield. The theoretical results demonstrated excellent agreement with the experimental results. There were large interactions between the studied parameters within the range of selected levels. The proposed experimental setup was very effective in extracting chlorophyll dye from *Conocarpus lancifolius* leaves.

References

[1] Nayak, C.A., Chethana, S., Rastogi, N.K. & Raghavarao, K.S.M.S., E*nhanced Mass Transfer During Solid-Liquid Extraction of Gamma-Irradiated Red Beetroot*, Radiation Physics and Chemistry, **75**(1), pp. 173-178, 2006.

[2] Ngamwonglumlert, L., Devahastin, S. & Chiewchan, N., *Natural Colorants: Pigment Stability and Extraction Yield Enhancement via Utilization of Appropriate Pretreatment and Extraction Methods*, Critical Reviews in Food Science and Nutrition, **57**(15), pp. 3243-3259, 2017.

[3] Manolopoulou, E., Varzakas, T. & Petsalaki, A., *Chlorophyll Determination in Green Pepper Using Two Different Extraction Methods*, Current Research in Nutrition and Food Science Journal, **4**(1), pp. 52-60, 2016.

[4] Semeraro, P., Chimienti, G., Altamura, E., Fini, P., Rizzi, V. & Cosma, P., *Chlorophyll A in Cyclodextrin Supramolecular Complexes as a Natural Photosensitizer for Photodynamic Therapy (PDT) Applications*, Materials Science and Engineering C, **85**, pp. 47-56, 2018.

[5] Hung, S.M., Hsu, B.D. & Lee, S., *Modelling of Isothermal Chlorophyll Extraction from Herbaceous Plants*, Journal of Food Engineering, **128**, pp. 17-23, 2014.

[6] Guesmi, A., Ladhari, N., Ben Hamadi, N., Msaddek, M. & Sakli, F., *First Application of Chlorophyll-A as Biomordant: Sonicator Dyeing of Wool With Betanin Dye*, Journal of Cleaner Production, **39**, pp. 97-104, 2013.

[7] Panda, B.B., Mahapatra, P.K. & Ghosh, M.K., *Application of Chlorophyll as Sensitizer for ZnS Photoanode in a Dye-Sensitized Solar Cell (DSSC)*, Journal of Electronic Materials, **47**(7), pp. 3657-3665, 2018.

[8] Suleman, P., Redha, A., Afzal, M. & Al-Hasan, R., *Temperature-induced Changes of Malondialdehyde, Heat-Shock Proteins in Relation to Chlorophyll Fluorescence and Photosynthesis in Conocarpus lancifolius (Engl.)*, Acta Physiologiae Plantarum, **35**(4), pp. 1223-1231, 2013.

[9] Ali, B.A.A., *Resistance of Conocarpus lancifolius Engl. to Different Levels of Salinity and Water Supply*, ARPN Journal of Agricultural and Biological Science, **9**(6), pp. 211-215, 2014.

[10] Wrolstad, R.E., *Symposium 12: Interaction of Natural Colors with Anthocyanin Pigments - Bioactivity and Coloring Properties*, Journal of Food Science, **69**(5), pp. 419-421, 2004.

[11] Pompeu, D.R., Silva, E.M. & Rogez, H., *Optimisation of the Solvent Extraction of Phenolic Antioxidants from Fruits of Euterpe Oleracea*

Using Response Surface Methodology, Bioresource Technology, **100**(23), pp. 6076-6082, 2009.

[12] Luthria, D.L., *Influence of Experimental Conditions on The Extraction of Phenolic Compounds from Parsley (Petroselinum Crispum) Flakes Using a Pressurized Liquid Extractor*, Food Chemistry, **107**(2), pp. 745-752, 2008.

[13] Baiano, A., *Recovery of Biomolecules from Food Wastes – A Review*, Molecules, **19**(9), pp. 14821-14842, 2014.

[14] Su, S., Zhou, Y., Qin, J.G., Yao, W. & Z. Ma, *Optimization of the Method for Chlorophyll Extraction in Aquatic Plants*, Journal of Freshwater Ecology, **25**(4), pp. 531-538, 2010.

[15] Costache, M.A., Campeanu, G. & Neata, G., *Studies Concerning the Extraction of Chlorophyll and Total Carotenoids from Vegetables*, Romanian Biotechnological Letters, **17**(5), pp. 7702-7708, 2012.

[16] Thambavani, D.S. & Sabitha, M.A., *The Spectral Determination of Chlorophylls a, b and Total Carotenoids Using Various Solvents for Tree Species Growing Near Sugar Mill*, The Asian Journal of Experimental Chemistry, **7**(1), pp. 5-9, 2012.

[17] Sumanta, N., Haque, C.I., Nishika, J. & Suprakash, R., *Spectro-photometric Analysis of Chlorophylls and Carotenoids from Commonly Grown Fern Species by Using Various Extracting Solvents*, Research Journal of Chemical Sciences Res. J. Chem. Sci, **4**(9), pp. 2231-606, 2014.

[18] Derrien, M., Badr, A., Gosselin, A., Desjardins, Y. & Angers, P., *Optimization of a Green Process for The Extraction of Lutein and Chlorophyll from Spinach By-products Using Response Surface Methodology (RSM)*, LWT – Food Science and Technology, **79**, pp. 170-177, 2017.

[19] Hinkelmann, K., Kempthorne, O. & Hinkelmann, K., *Design and Analysis of Experiments*, Ed. 2, Wiley-Interscience, 2008.

[20] Souissi, M., Guesmi, A. & Moussa, A., *Valorization of Natural Dye Extracted from Date Palm Pits (Phoenix Dactylifera) for Dyeing of Cotton Fabric, Part 1: Optimization of Extraction Process Using Taguchi Design*, Journal of Cleaner Production, **202**, pp. 1045-1055, 2018.

[21] Arnon, D.I., *Copper Enzymes in Isolated Chloroplasts. Polyphenoloxidase in Beta Vulgaris*., Plant physiology, **24**(1), pp. 1-15, Jan. 1949.

[22] Gala, S., Sumarno, S. & Mahfud, M., *Microwave-assisted Extraction of Natural Dyes from Coleus Atropurpureus Leaves: The Effect of Solvent*, MATEC Web of Conferences, **156**, p. 06011, 2018.

[23] Shahavi, M.H., Hosseini, M., Jahanshahi, M., Meyer, R.L. & Darzi, G. N., *Clove Oil Nanoemulsion as an Effective Antibacterial Agent: Taguchi Optimization Method*, Desalination and Water Treatment, **57**(39), pp. 18379-18390, 2016.

[24] Fernández-López, J.A., Angosto, J.M., Roca, M.J. & Doval Miñarro, M., *Taguchi Design-based Enhancement of Heavy Metals Bioremoval by Agroindustrial Waste Biomass from Artichoke*, Science of the Total Environment, **653**, pp. 55-63, 2019.

[25] Demirci, M.T., Samanc, A., Tarakçıoğlu, N. & Asiltürk, I., *Optimization of Fatigue Life Parameters with Taguchi Method*, 6^{th} International Advanced Technologies Symposium, pp. 16-18, 2011.

[26] Zainal Abidin, Z., *Optimization of Chlorophyll Extraction from Gynura Procumbens*, Malaysian Journal of Analytical Science, **20**(6), pp. 1421-1428, 2016.

[27] Prakash Maran, J. & Manikandan, S., *Response Surface Modeling and Optimization of Process Parameters for Aqueous Extraction of Pigments from Prickly Pear (Opuntia Ficus-Indica) Fruit*, Dyes and Pigments, **95**(3), pp. 465-472, 2012.

[28] Jinasena, M., Amarasinghe, A., Amarasinghe, B. & Prashantha, M., *Extraction and Degradation of Chlorophyll a and b from Alternanthera Sessilis*, Journal of the National Science Foundation of Sri Lanka, **44**(1), pp. 11-21, 2016.

[29] Kamal, I., *Extraction of Chlorophyll from Alfalfa Plant*, Al-Khwarizmi Engineering Journal, **2**(1), pp. 85-97, 2006.

[30] Tavanandi, H.A. & Raghavarao, K.S.M.S., *Recovery of Chlorophylls from Spent Biomass of Arthrospira Platensis Obtained after Extraction of Phycobiliproteins*, Bioresource Technology, **271**, pp. 391-401, 2019.

[31] Fernández-López, J.A., Angosto, J.M., Roca, M.J. & Doval Miñarro, M., *Taguchi Design-based Enhancement of Heavy Metals Bioremoval by Agroindustrial Waste Biomass from Artichoke*, Science of The Total Environment, **653**, pp. 55-63, 2019.

[32] Macías-Sánchez, M.D., Mantell, C., Rodríguez, Martínez de la Ossa, M. E., Lubián, L.M. & Montero, O., S*upercritical Fluid Extraction of Carotenoids and Chlorophyll a from Synechococcus Sp*., Journal of Supercritical Fluids, **39**(3), pp. 323-329, 2007.

[33] Park, H.S., Im, N.G. & Kim, K.H., *Extraction Behaviors of Caffeine and Chlorophylls in Supercritical Decaffeination of Green Tea Leaves*, LWT – Food Science and Technology, **45**(1), pp. 73-78, 2012.