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HIGH HYDROSTATIC PRESSURE EXTRACTION OF ANTIOXIDANTS FROM MORINDA CITRIFOLIA FRUIT – PROCESS PARAMETERS OPTIMIZATION

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Abstract

A modified version of high hydrostatic pressure extraction has been performed for extraction of antioxidants from M. citrifolia fruit at 5, 15, 25 bar and temperature 30˚ to 70˚C for time duration 1, 2, 4 and 6 hours. The antioxidant activity of the extracts was determined by di-phenylpicrylhydrazyl radical scavenging method. The process parameters were optimized for antioxidant activity by central composite design method of response surface methodology using the statistical package, design expert. The results are expressed as 3D surface graphs. The optimum antioxidant activity was achieved at 58˚C and 5 hours for 25bar. The optimal result achieved was within the region of response surface methodology. The statistical results were compared with the experimental result at 25bar, 2hour and 30˚ to 70˚C and were found to be in proximate. The antioxidant activities of the extracts were found to increase with increase in pressure. It was also found that the response surface methodology works effectively for shorter range of parameters considered.

Keywords: Statistical experimental design, Phytochemical extraction, Response surface methodology, Diphenylpicrylhydrazyl method

1. Introduction

Phytochemicals are the chemicals derived from plants. Medicines from herbal and natural products have been used for centuries in every culture throughout the world.

Nomenclatures

P	Pressure
T	Temperature
t	Extraction time
HHPE	High hydrostatic pressure extraction
DPPH	2,2-DiPhenylPicrylHydrazyl (Chemical)
RSM	Response surface methodology
CCD	Central Composite Design

The true health benefits of these remedies without any side effects have shown increased interest in this field from scientists and medical professionals. Morinda citrifolia or mengkudu is one of the traditional folk medicinal plants that has been used for over 2000 years in Polynesia [1]. It has been reported to have a broad range of health benefits and for treatment of cancer, infection, arthritis, diabetes, asthma, hypertension, and pain [2]. A number of phytochemical antioxidants have been identified in the Noni plant. Scopoletin, vitamin C, terpenoids, alkaloids, anthraquinones (such as nordamnacanthal, morindone, rubiadin, and rubiadin- ı-methyl ether, anthraquinone glycoside), β-sitosterol, carotene, vitamin A, flavone glycosides, rutin, and a putative proxeronine are some of the major and rare antioxidants present [3]. Mohd. Zin et. al. [4] showed that the antioxidants present in the fruit of *Morinda citrifolia* were of non-polar nature and a non-polar solvent for extraction leads to higher antioxidant activity of the extracts [4].

There are various extraction processes for the extraction of required phytochemicals from plants. Microwave assisted extraction (MAE), ultrasonic extraction, supercritical fluid extraction, subcritical water extraction, pressurized liquid extraction etc. are some of the available extraction methods. The disadvantage with these processes are either the denaturation of the phytochemicals present in the plant due to the methodology involved in extraction such as high temperature of the solvent as in the case of pressurized liquid extraction and microwave assisted extraction or the cost incurred as in supercritical fluid extraction [5]. A modified version of high hydrostatic pressure extraction (HHPE) has been developed to extract the phytochemicals without much effect on their properties. In this process, parameters such as pressure, temperature and time duration can be varied according to the biology of the plant in order to obtain maximum yield. By HHPE, single component of high purity can be easily obtained. Strong, weak, and non polar compounds can all be extracted by using different solvents. Saving energy and safety are also advantages of HHPE. HHPE can be used as a tool for drug discovery, allowing chemical reactions to occur under more suitable conditions. HHPE was found to give higher yield of essential components when compared with ultrasonic and heat reflux extractions [6].

Previous work on HHPE for the extraction of flavonoids from propolis have showed that the pressure increases the yield in shortest extraction time [6, 7].

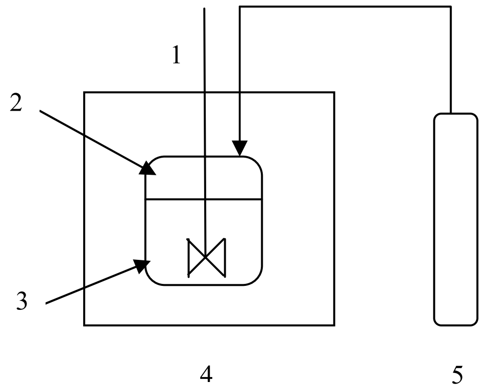
Experiments such as HHPE lead to a voluminous data for analysis and optimization. A number of statistical techniques are available to perform these operations [8]. Response surface methodology (RSM) is one such statistical method that uses quantitative data from appropriate experiments to determine thereby reducing the number of experiments necessary to be performed and simultaneously solve multivarient equations [9]. Generally speaking, Response surface methodology attempts to fit a polynomial of appropriate degree to the response of the system of interest. The goal of the system of interest is termed the response. This response is normally measured on a continuous scale and is a variable which likely represents the most important function of the system, though this does not rule out the possibility of investigating more than one system function, i.e., more than one response. Also contained in the system are input variables that affect the response and are subject to control [10]. The response surface procedures involve experimental strategy, mathematical methods, and statistical inference which, when combined, enable users to make an efficient empirical exploration of the system in which they are interested [11]. The experimental strategy enables the analyst to explore the response surface with equal precision, in any direction. The experimental design initially limits the region under investigation. Subsequent to the initial investigation, the experimental design enables the analyst to explore the response surface in a systematic manner in the direction that offers the most promise for improvement [12].

RSM can be applied to any system that has the following key elements: (1) a criterion of effectiveness, which is measurable on a continuous scale (extraction time), and (2) quantifiable independent variables (both controllable and uncontrollable) that affect the system's performance (such as temperature, pressure, particle size etc.). Given these conditions, RSM offers techniques for finding the optimum response of the system in an efficient manner [13]. The major advantage of RSM is the amount of data needed for evaluation, analysis and optimisation. It significantly reduces the number of experiments required.

2. Methods and Materials

High hydrostatic pressure extraction on dried and powdered (10-100µm) *Morinda citrifolia* fruit was performed in an modified parr 4842 series high pressure extractor (figure. 1) at varying pressures of 5, 15, 25bar and at various temperatures of 30, 40 ,50, 60 and 70˚ C for an extraction time of 1, 2, 4 and 6 hours. Radical scavenging activity of the extracts was determined by Di-phenylpicrylhydrazyl (DPPH) radical scavenging method [14]. The results were optimized by response surface methodology using the software package, Design expert 6.0.10. The statistical experimental design was suggested by the central composite design (CCD) method of this software as shown in Table 1. The data was numerically optimized and represented as 3D surface graphs in terms of activity. Activity in the graph is expressed till unity. However, it corresponds to the DPPH radical scavenging activity of the extracts in terms of percentage as shown in Table.1.

Many designed experiments involve more than one response. In some processes such as the current suggested high hydrostatic pressure extraction, 3 to 4 variables are used. Nowadays, many software packages on response surface methodology are available. Their common approach in multiple response optimizations is they model each response individually and then graphically superimpose contour plots. This is due to the fact that rarely does one operating condition produce the optimum for all responses simultaneously [15].



1. Stirrer; 2. N_2 Gas; 3. M. citrifolia powder in ethyl acetate; 4. High pressure reactor; 5. N_2 Cylinder.

Fig. 1.High hydrostatic pressure extractor

3. Results and discussion

ANOVA Analysis for response surface quadratic model gives the following equation. Pressure, time and temperature are indicated as P, t and T.

$$\text{DPPH radical scavenging activity} = 25.77529 - 0.082917 \times P + 5.32530 \times t + 0.45034 \times T - 4.90661 \times 10^{-5} \times P^2 - 0.71999 \times t^2 - 4.58592 \times 10^{-3} \times T^2 + 0.023128 \times P \times t + 1.50028 \times 10^{-3} \times P \times T + 8.87405 \times 10^{-3} \times t \times T \quad (1)$$

The equation (1) was checked for its proximity by substituting 25 bar, 2 hours and temperature 30˚ C to 70˚ C. These results were compared with the experimental results as shown in Fig. 2.

Figure 3 shows the results of activity for the varying temperature and extraction time for a fixed pressure of 5bar. The maximum activity was found to be 0.679. The response, DPPH radical scavenging activity was found to increase with the rise in temperature from 30˚ C and reach its peak at 57˚ C.

Table 1: Statistical experimental design suggested by CCD method of RSM by Design-Expert software

Std	**Run**	**Block**	**Factor 1** A: Pressure bar	**Factor 2** B:time hour	**Factor 3** C:Temp. deg C	**Response** Activity %
11	1	Block 1	5.00	4.00	5.00	48.18
1	2	Block 1	25.00	2.00	70.00	45.34
5	3	Block 1	25.00	4.00	50.00	51.51
12	4	Block 1	15.00	5.00	50.00	48.41
4	5	Block 1	5.00	2.00	70.00	44.64
2	6	Block 1	25.00	2.00	30.00	43.76
14	7	Block 1	25.00	6.00	30.00	44.69
6	8	Block 1	15.00	2.00	50.00	46.61
3	9	Block 1	25.00	6.00	70.00	44.33
7	10	Block 1	15.00	4.00	70.00	45.46
8	11	Block 1	5.00	2.00	30.00	43.31
9	12	Block 1	5.00	6.00	70.00	45.35
18	13	Block 1	5.00	2.00	30.00	43.31
16	14	Block 1	25.00	6.00	70.00	44.33
17	15	Block 1	5.00	6.00	70.00	45.35
13	16	Block 1	5.00	6.00	30.00	44.04
15	17	Block 1	15.00	4.00	70.00	45.46
10	18	Block 1	15.00	6.00	30.00	44.39

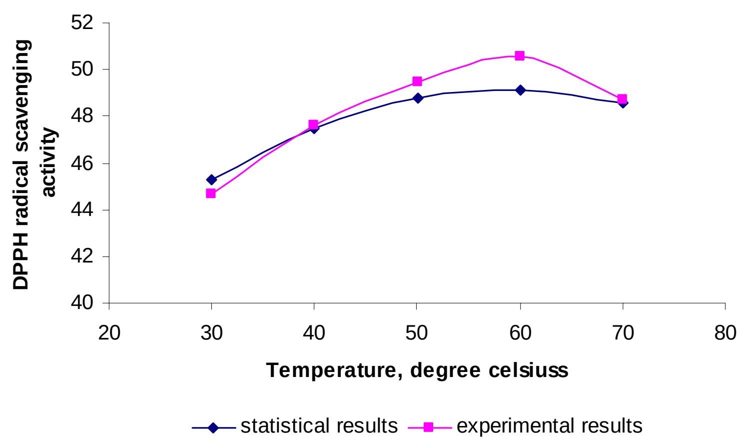


Fig. 2. Comparison between experimental and statistical results at 25 bar and 2 hour

There was a drop in the activity with further rise in temperature to 70˚C. There was a significant increase in the radical scavenging activity of the extracts with increase in extraction time. It reached optimum at 5 hours showing less significant difference in radical scavenging activity thereafter. For the pressure of 5 bar, the optimum temperature and extraction time as predicted by RSM was 57˚ C and 5 hours. The values for activity were found to be higher for 15 bar comparatively with 5 bar as represented graphically using RSM as shown in Fig. 4. Activity of 0.795 was achieved. The trend of the 3D mesh generated was found to be similar to that of the 5 bar. So, the increase in radical scavenging activity should be due to the increase in the pressure for extraction. According to the mass transfer theory, the rate of mass transfer = pressure/resistance, i.e. pressurized cells show increased permeability [7]. The higher the hydrostatic pressure is, the more solvent can enter into the cell and the more compounds can permeate the cell membrane thereby extracting the phytochemicals such as antioxidants present in their matrices.

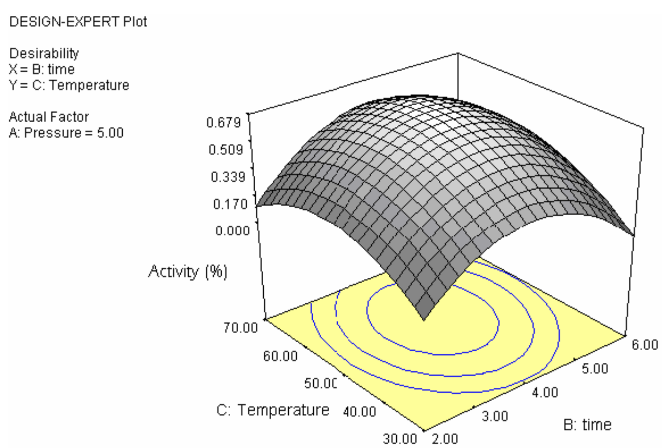


Fig. 3. DPPH radical scavenging activity at a fixed pressure of 5 bar

Activity was achieved a maximum of 0.918 at a pressure of 25 bar as shown in Fig. 5. The trend of the mesh obtained was same as the former graphs. There was an increase in radical scavenging activity with the increase in pressure. Radical scavenging activity is directly proportional to the concentration of extract. According to the phase behaviour theory, the solubility of the sample to be extracted is greater as the pressure increases. The increased solubility may have lead to the increase in the concentration of the antioxidants in the extracts from HHPE.

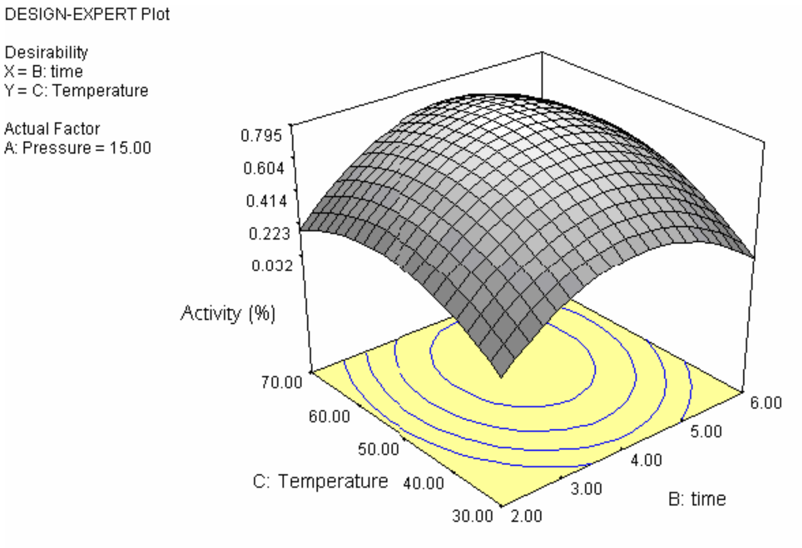


Fig. 4. DPPH radical scavenging activity at a fixed pressure of 15 bar.

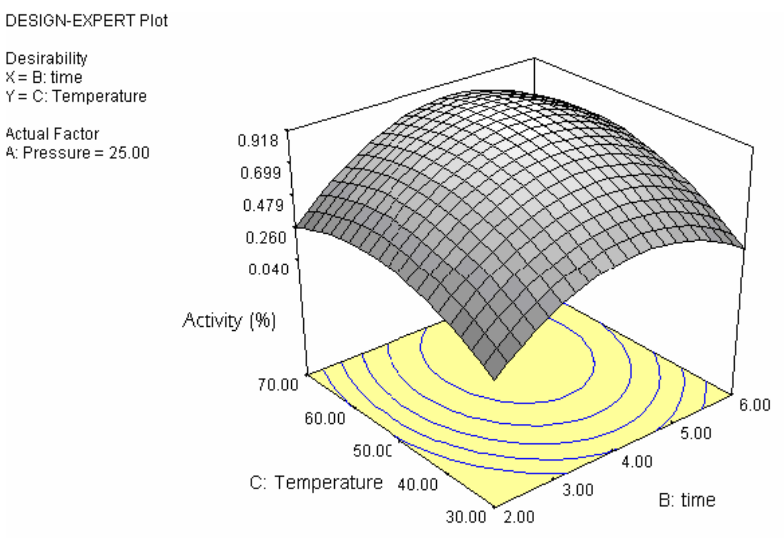


Fig. 5. DPPH radical scavenging activity at a fixed pressure of 25 bar

3. Conclusion

With the advent of statistical techniques such as response surface methodology, especially as software packages for problem solving such as optimization of the processes, products, design etc. have reduced the time required to perform these operations not only by the speed of the computer but also by reducing the quantity of experimental data required for optimization analysis. They have lead to better understanding of the process through results represented in the form of graphs such as 3D surface, contour etc. From the RSM applied to the data acquired from high hydrostatic pressure extraction of antioxidants from *Morinda citrifolia* fruit it can be deciphered that there is an increase in the antioxidant activity of the extracts as the pressure increases within the range of temperature 30˚ to 70˚C and extraction time 1 to 6 hours. The optimum temperature and extraction time determined experimentally at 25 bar were 60˚C and 6 hours. These results are in proximate with the RSM results of 58˚C and 5 hours at 25bar. It was found that the response surface methodology works effectively for shorter range of parameters considered. Thus, the results achieved lead us to the conclusion that further research in the high hydrostatic pressure extraction for higher antioxidant activity is possible at larger pressures.

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