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OPTIMIZATION OF EXTRACTION CONDITION OF NATURAL DYE FROM POMEGRANATE PEELS USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT

Dyes extracted from natural sources have appeared as important substitutes to synthetic dyes. The aim of the present study was to optimize the extraction conditions of natural dye from pomegranate peels using central composite design of experiment. The effect of pH of the extraction medium, extraction time and material to liquor ratio (M:L) was investigated. It was found that the pH of the extraction medium has statistically significant effect on dye extraction, whereas the effect of time and M:L, within the ranges used in this study, was not found to be significant. Optimum dye extraction was obtained in alkaline pH of the extraction medium at lower liquor ratio. Cotton fabric dyed with the optimized dye extract, showed very good washing and light fastness properties but slightly poor wet crocking fastness.

KEYWORDS: pomegranate peels, natural dye, response surface methodology, central composite design.

INTRODUCTION

Natural dyes have been a part of human life since prehistoric times. However, the introduction of man-made dyes almost completely replaced the natural dyes within a century [1]. Nevertheless, several problems have been reported on the use of man-made dyes such as the effect of their effluent on photosynthetic activity of aquatic organisms and their harmful effects on human beings, plants and animals [2,3] The increased awareness of health and environmental hazards associated with the manufacturing, processing and use of synthetic dyes, has resulted in the revival of interest in the use of natural dyes in recent years.

Natural dyes are obtained from plants and animals with minimal chemical processing. This class of dyes is recognized to be eco-friendly and less likely to be poisonous as compared to man-made dyes [4-6]. Because of being less hazardous and more eco-friendly, natural dyes are considered safer to use as compared to their man-made counterparts [7,8].

Several studies have been carried out on the extraction of coloring component from different parts of plants i.e. leaves, roots, barks, trunks or fruits [9] as well as on the identification of chemical structure of the coloring component [7]. Henna, pomegranate, madder, turmeric, eucalyptus etc. are examples of some well-known natural dyes. The coloring components in most of the plants are usually flavonoids, anthraquinones and indigo. Most common flavonoids include flavanoes, flavonlos and anthocyanins. The flavonoids give yellow, brown and green colour shades after application on textile fabrics.

Punica granatum of Punicacea family is said to be originally found in Persia but also grows in all temperate countries [10]. Its peels, when dry are brown outside, yellow inside, hard, brittle and odourless. The coloring components of pomegranate rind include tannins and flavonols. Tannins are significantly present in pomegranate peels of which about 19% is pelletierine [11,12]. Pomegranate juice consists of different types of anthocyanins including pelargonidin-3-glucoside, cyaniding-3-glucoside, 5-diglucoside and delphinidin-3-glucoside. Granatonine is the chief coloring component in the pomegranate rind which is present in the alkaloid form N-methyl granatonine [10] and its structure is composed of two condense pyridine rings in which nitrogen atom is bridge between two carbon atoms (Figure 1). Ellagitannins and gallotannins are also present in the rind of pomegranate [13]

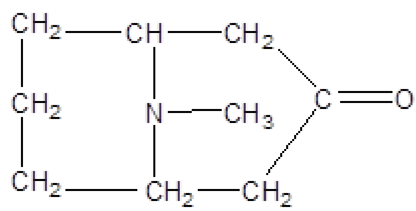

Figure 1 Structure of Granatonine

Optimization of extraction conditions is very important in order to obtain maximum coloring component in a solvent. Response surface methodology (RSM) is a well-known statistical approach for process optimization, modelling and finding the main effect and interaction of several factors concurrently with less number of experiments as compared to full-factorial approach [14]. Central composite design (CCD) is very useful for process optimization and model fitting tool of response surface methodology (RSM) [15-17]. The aim of this study was to investigate the effect of extraction pH, time and material to liquor ratio on the extraction of natural dye from pomegranate using CCD of RSM. The effect of extraction pH has not been previously reported on the extraction of colour from pomegranate peels.

MATERIALS AND METHODS

Preparation of the raw material

Pomegranate rinds were collected from the local fruit market. The rinds were washed and dried at the ambient atmosphere in the range of 37-40°C. The dried material was then ground into powder form. The powder was passed through a sieve of 25 mesh size to obtain uniform particle size of the materials. The sieved powder was then used for dye extraction at different experimental conditions.

Experimental variables

Based on the preliminary trials, three experimental variables were identified for this study, viz., the extraction pH, extraction time and material to liquor ratio (M:L). The coded and actual levels of these variables are given in Table1.

Table 1 Experimental Variable and their Levels for Dye Extraction

Variables	**Levels**				
	- alpha	- 1	0	+ 1	+ alpha
pH	2	4	7	10	12
Time (min.)	30	42	60	78	90
M:L	20	26	35	44	50

Design of Experiment

Central composite design (CCD) of response surface methodology (RSM) was used to find the optimized extraction conditions for natural dye from dried pomegranate [18,19]. The experiments were designed using Design-Expert 7.0 statistical software package. The complete experimental design given in Table 2 comprises 8 experiments at factorial points and 6 each at centre and axial points. The central point replicates reduce the error and also determine the reproducibility of the data. In this experimental design, -1 and +1 represent the low and high levels. The axial points are situated at distance of α (alpha) from central point and thus make the design rotatable [18]. The experimental factors are extraction pH, time and material to liquor ratio (M:L) and the response variable is the colour strength (K/S) of cotton fabric dyed with the dye extracts at different extraction conditions.

Dye extraction

The extraction of natural dye from pomegranate peels was done in distilled water using reflux boiling methodology. Specimens of 5g of pomegranate peels powder were added in round bottom flasks at different extraction conditions as given in Table 3. pH of the extraction liquors was adjusted using 0.1 M NaOH or 0.1 M HCl solutions for alkaline or acidic conditions respectively, with the help of a digital pH meter. Sample extracts were taken at different time intervals (30 min, 42 min, 60 min, 78 min and 90 min) and different M:L as given in Table 3.

Application of the extracted dye on cotton fabric
All the extracted solutions were used to dye bleached cotton fabric at the following dyeing condition: 60°C temperature, 60 minutes dyeing time, 1:15 material to liquor ratio and 1g/L salt concentration [12]. At the completion of dyeing, the dyed cotton samples were removed from the lab dyeing machine and washed with tap water until there was no more colour bleeding followed by air drying under the shade.

Measurement of colour strength values
Colour strength (K/S) values of dyed samples were measured with the help of Spectraflash SF 650 spectrophotometer by Datacolor International. These values were calculated by using Kubelka-Munk equation. This equation describes the colour absorbing & scattering properties of the dyed material. K/S value is the ratio of the coefficient of absorption (K) to that of scattering (S) [20].

$$(K/S)_\lambda = (1-R_\lambda)^2 / 2R_\lambda$$

Quality assurance tests of the dyed fabric
Most dyes are organic compounds and therefore, some natural destructive agencies e.g., weather, oxygen, light and other atmospheric gases can fade and destroy certain dyes. In addition to natural agencies, there are many chemicals used in finishing treatments or in home laundering detergents that may also affect the fastness properties of dyed fabrics.

For the evaluation of fastness properties, washing fastness, rubbing fastness and light fastness tests were performed. Colour fastness to washing was determined using launder-o-meter according to ISO 105-CO2 method. Colour fastness to rubbing was determined using crockmeter according to ISO 105-X12 method and colour fastness to light was determined using Fade-o-meter according to ISO-B-02.

Statistical Analysis
The analysis of variance (ANOVA) was used to check the statistical significance of the regression coefficients and adequacy of the developed model. Response surface plots were drawn to study the interaction among the different independent process factors and their effect on the colour yield. A surface plot is a graphical demonstration of a three dimensional response surface which gives interaction between two independent variables while maintaining the third variable at fixed level [21].

RESULTS AND DISCUSSIONS

Extraction of dye from pomegranate rind by conventional method is a time consuming process because in conventional method a large number of experiments are required to be performed in order to check the interaction among different. In such situations response surface (RSM) proves very helpful to study and analyze the effects of multiple factors which influence the responses through variation in the factor combinations simultaneously. The colour strength (K/S) values of cotton fabric samples dyed with the dye extracted from pomegranate peels at different extraction conditions, are given in Table 2. A higher value of K/S indicates higher colour strength, thus better dye extraction efficiency.

Table 2 Colour strength (K/S) at different extraction conditions

No	pH	Time(min)	M:L (ml)	K/S
1	4	42	26	5.5
2	12	60	35	9.2
3	10	42	26	8.9
4	10	42	44	8.5
5	7	60	35	7.0
6	7	60	35	7.1

7	4	78	44	5.4
8	7	60	35	6.9
9	7	90	35	7.8
10	2	60	35	4.5
11	7	60	50	6.9
12	7	30	35	6.5
13	7	60	35	7.0
14	10	78	26	8.3
15	7	60	35	7.1
16	10	78	44	8.5
17	7	60	35	6.9
18	4	78	26	5.6
19	7	60	20	6.7
20	4	42	44	5.2

Analysis of Variance (ANOVA)

The analysis of variance results for colour strength values are given in Table 3. It is clear from the results that the model is significant with p-value < 0.0001. The effect of pH on colour strength is also statistically significant (p-value <0.0001). Thus it may concluded that the change in pH has significant effect on the extraction of color component from plant material and have subsequent effect on the color strength values of fabric dyed with the extract. However, the extraction time and the material to liquor ratio do not significantly affect the colour strength of the extracted dye, within the range of values experimented in this study.

Table 3 ANOVA for colour strength at different dye extraction variables

Source	Sum of Squares	DF	Mean Square	F-value	p-value	Remarks
Model	31.08	9	3.45	43.18	< 0.0001	Significant
A-pH	30.49	1	30.49	381.19	< 0.0001	Significant
B-Time	0.26	1	0.26	3.26	0.1012	
C-M:L	9.683E-003	1	9.683E-003	0.12	0.7351	
AB	0.10	1	0.10	1.27	0.2868	
AC	0.011	1	0.011	0.14	0.7155	
BC	0.061	1	0.061	0.77	0.4020	
A2	0.024	1	0.024	0.30	0.5964	
B2	0.061	1	0.061	0.77	0.4012	
C2	0.049	1	0.049	0.62	0.4510	
Residual	0.80	10	0.080			
Lack of Fit	0.80	5	0.16			
Pure Error	0.000	5	0.000			
Total	31.88	19				

Regression equation for colour strength

The second order quardatic equation for colour strength is given as follows:

$$\text{Colour Strength (K/S)} = 7.00 + 1.49\,A + 0.14B - 0.027\,C - 0.11AB + 0.038AC + 0.088BC - 0.041\,A^2 + 0.065B^2 - 0.05C^2 \quad (1)$$

Where A, B and C are the coded values of the process parameters pH, time and material to liquor ratio. The positive sign with each term indicate the synergistic effect while the negative sign indicates the antagonistic effect. The R^2

value for the regression equation is 0.97, which means that 97% change in colour strength can be explained by the terms included in the equation.

Effect experimental variables on the extraction of dye
Figure 2 shows the effect of pH and extraction time on colour strength of the dyed cotton fabric. It is clear that the colour yield of the extracted dye increases with increase in extraction pH and time. The effect of pH is much more pronounced as compared to that of time. ANOVA results showed that the effect of pH was statistically significant. As the coloring component in the rind of pomegranate belongs to the class of tannins which are acidic in nature thus increased pH enhance its leaching process in to the extraction medium. Therefore alkalinity in the solution withdraws more coloring component during extraction and causes neutralization of tannins. Also the salt in the dyeing medium help to neutralize the negative charged on the fabric which facilitates the transfer of dye moieties toward fabric and form hydrogen boding with cellulosic fabric resulting in increased colour strength values. However, there was no significant increase in colour yield when the extraction time was increased from 30 minutes to 90 minutes. Within the first 30 minutes of extraction time, there was sufficient extraction of the colour. As pH and extraction time of the extraction medium is increased, the process of dye extraction is enhanced by breaking up of the plant cell membranes leading to improve transport of chromophoric constituent of biomass in the solvent medium. However, further increasing extraction time possibly led to the degradation of coloring component resulting decrease uptake by the fabric and lowering K/S values. The prolong heating during extraction for longer times also solubilised the impurities which caused unevenness in dyeing [12].

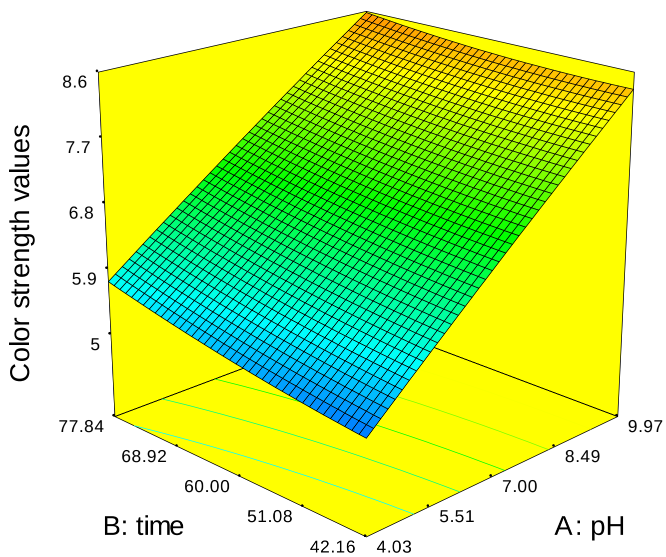

Figure 2 Effect of extraction pH and time on colour strength (K/S)

Figure 3 depicts the effect of pH and M:L on the colour yield. Under alkaline condition dye particles evenly sorbed on to the fabric, resulting good colour strength values while at acidic pH other components like gallic acid might also sorbed onto the fabric which cause unevenness of dye onto the fabric [22]. ANOVA results revealed that there was no significant increase in colour strength values by increasing the M:L from 42 to 78.

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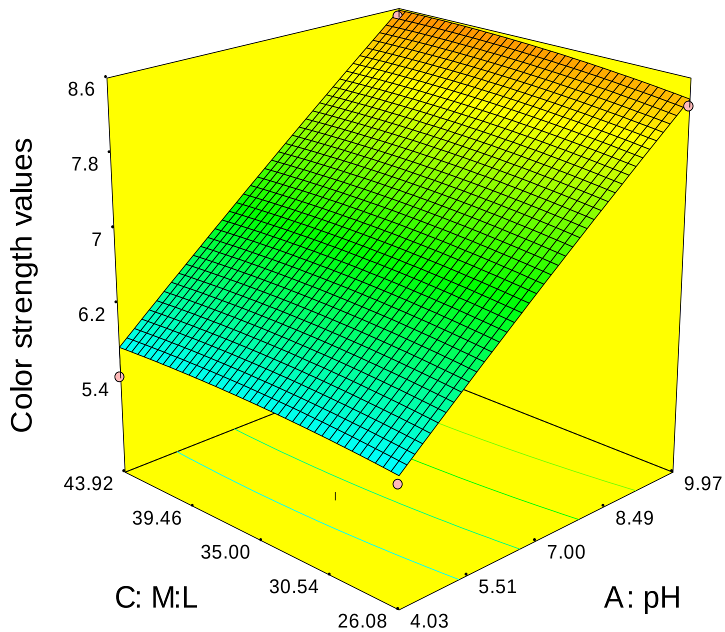

Figure 3 Effect of extraction pH and M:L ratio on colour strength (K/S)

The optimum extraction conditions

The optimum extraction pH for maximum colour strength was found to be 12, i.e. alkaline. The fabric dyed with the optimized extract showed 4-5 rating for color fastness to washing, which was quite good. Color fastness to light was also good with 5 rating on the blue wool scale. Dry crocking fastness was also good with a rating of 4-5 but wet crocking fastness was slightly poor with 3-4 rating on the grey scale.

CONCLUSION

It could be concluded from the study that the pH of the extraction liquor has highly significant effect on the colour yield of pomegranate peels. The colour yield is very poor in the acidic medium, moderate in the neutral pH and significantly better in the alkaline pH of 12. The colour yield only slightly increased by increasing the extraction time from 30 minutes to 90 minutes and the effect of increase in time was not statistically significant. Similarly, the colour yield showed a slight decrease with increase in M:L from 1:20 to 1:50 and the effect of M:L was also not statistically significant

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