Optimization of Colorants Extraction from Gromwell (*Lithospermum erythrorhizon*)

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1. INTRODUCTION

The roots of gromwell (*Lithospermum erythrorhizon)* in the Orient have been used for many centuries as natural red dyes and as crude drugs with magic property of accelerating wound healing. Its main component is shikonin, a kind of naphthoquinones, and it has a high value both for its medicinal and coloring properties, especially in Japan [1]. In Korea, it has been a precious purple dye and its color on dyed fabrics varied depending on extraction process and dyeing method. In this work, colorants extraction process from gromwell was studied for making powder form of colorants. In order to do that, sugar extracted together with colorants must be removed. For sugar decomposition, gromwell roots were pretreated with various enzyme solutions. Accordingly, the effects of enzyme and pretreatment on sugar decomposition were investigated to find appropriate enzyme and conditions.

2. EXPERIMENTAL

Materials

Dried gromwell roots (originated from China) were obtained commercially from an oriental medicine shop. Enzymes (Sigma Co.) including amylase, hemicellulase, and pectinase were used for pretreatment. Reagents used were of first grades. For dyeing test, wool and silk fabrics were obtained commercially.

Method

Table I shows conditions in each step for preparing gromwell colorants powder.

For determining the sugar concentration in pretreated solutions, phenol-sulfuric acid method was used [2]. Absorbance of colorants extraction was measured using a UV-Vis spectrophotometer (Agilant 845, Agilant Technologies, Waldbronm, Germany).

Table 1. Extraction of Gromwell Colorants

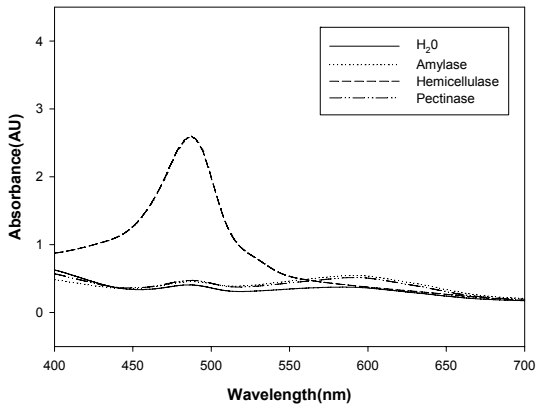
Step	Instrument	Condition
Pretreatment	Shaker	Solvents: H_20 Enzyme conc.: 500~3000unit/g 30~60℃/ 3hr
Dry	Dry oven	40~50℃/ 24hr
Colorants extraction	Shaker	Solvent: methanol Ratio 1:20, 40℃/ 3hr Stirring, Filtering
Concentration	Evaporator	Volume ratio 30%
Shock freezing	Deep freezer	-80℃, 1 day
Freeze-dry	Freezing dryer	-50℃, 5 days

Using colorant extracts, dyeing was carried out 50°C for 30min at a liquor ratio of 1:50 using an infra-red automatic dyeing machine (Ahiba Nuance, Data Color International, USA), and rinsed for further evaluation. Dye uptake was assessed by measuring K/S value at the maximum adsorption wavelength (λmax; 400nm) using a Macbeth Coloreye 3100 spectrophotometer. CIELab coordinates (Illuminant D_{65}/10°Observer) was measured with a Macbeth Coloreye 3100 spectrophotometer at 640 nm. H V/C values were obtained from $L^*a^*b^*$ data using CIE Munsell conversion program.

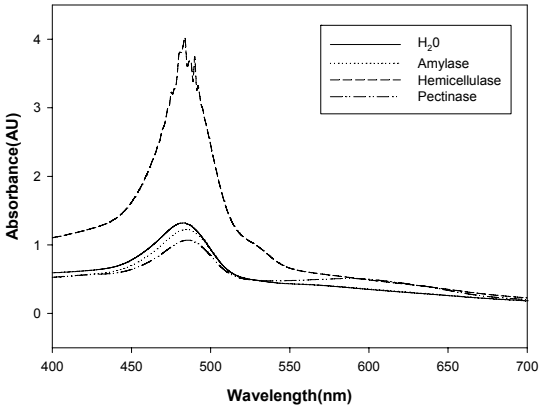
3. RESULTS AND DISCUSSION

Shikonin is not soluble in water and so, When gromwell colorants were extracted in methanol, sugar component was also extracted with colorants. The sugar component made extracts viscous during concentration using an evaporator and prevented from making colorant powder. To remove sugar component, gromwell roots were pretreated with various enzyme solutions to decompose sugar. Fig. 1 shows absorption spectra of sugar extracts pretreated with different enzyme solutions. Among enzymes used, hemi-cellulase was the most effective to

remove sugar. Colorants were extracted during pretreatment longer than 3hrs. Water was also possible solvent for extracting sugar.



(a)



(b)

Fig. 1. Absorption spectra of pretreated enzyme solutions; (a) 10 min dipping and (b) 3hrs dipping.

The gromwell roots pretreated enzyme solutions were used for extracting colorants in methanol. Dyeing was carried out using methanol extracts/ water (50:50) mixture. The results are presented in Table 1.

Table 1. Effect of enzyme pretreatment on the color of dyed fabrics

Enzyme	Fabric	K/S	H V /C	L*	a*	b*
No	Silk	2.7	6.8RP 4.8/4.8	49.2	19.36	-0.38
Amylase		2.5	6.6RP 5.0/5.2	50.8	20.66	-0.75
Hemicellulase		2.3	6.4RP 5.1/4.8	51.9	18.99	-0.80
Pectinase		3.0	7.2RP 4.8/5.6	49.0	22.97	-0.06
No	Wool	5.5	3.9RP 3.5/2.4	35.8	9.95	-2.83
Amylase		6.0	3.7RP 3.4/2.4	34.5	9.91	-3.03
Hemicellulase		5.2	3.4RP 3.6/2.4	36.7	9.65	-3.18
Pectinase		6.0	4.4RP 3.2/2.6	32.8	11.19	-2.79

Wool had better affinity than silk. Irrespective of pretreatment enzyme type, silk fabrics showed more purplish color indicating 6.4-7.2RP, while wool fabrics showed more reddish color indicating 3.4-4.4 RP.

Some colorants were extracted in hemicellulase and pectinase solutions during pretreatment. The pretreated gromwell roots were used for colorants extraction and it had no sticky problem experienced during freeze drying to obtain powdery colorants.

4. ACKNOWLEDGEMENTS

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (**NRF**) funded by the Ministry of Education, Science and Technology (No. 20090091276).

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