Study on Extracting Natural Plant Dyestuff by Enzyme-ultrasonic Method and its Dyeing Ability

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***Abstract*:** The natural colorant was extracted from cortex phellodendri and rubia using biological enzyme and ultrasonic technology together, and the effect of biological enzyme and ultrasonic technology on natural plant dyes extract yield was discussed through absorbance values of extracted solution in this work. Also, the dyeing solution (cortex phellodendri and rubia) extracted by enzyme-ultrasonic method was found to give the highest color difference (ΔE) among dyed fabrics. It was proved through the experiment that, natural plant dyes extract yield can be increased by using biological enzyme and ultrasonic technology together, with high efficiency, energy saving and pollution dipping features. The optimized condition for dye extraction was to extract the dry cortex phellodendri and drubia at 50℃ for 60 mins. with pH 4.5.

***Keywords*:** Enzyme, ultrasonic, natural dyes, extracts, cortex phellodendri, rubia.

1. Introduction

The coloring of textile, wood, leather and other natural commodities with dyes from plants and other natural products is receiving increasing attention. What attracts people to textile colored with natural plant dyes may be due to one or a combination of factors. Natural plant dyes are based on nature and have small impact on environment during dyeing process. Many natural dyes are from Chinese medicine resources, have high officinal value and beneficial to human body such as cortex phellodendri and rubia used in this work. Therefore, it is an inevitable trend that natural dye will replace chemical dye, there are many reports about studies on natural dyes [1-4]. Generally, the natural dye should be extracted before dyeing process. Currently the extract technology includes water extract, drying method, heating drying, ultrasonic, enzymatic, etc. The traditional extractions, such as water extract, heating drying method causes a certain environmental pollution and numerous energy consumption due to the organic solvent used in separation and purification for extraction process, so the clean extract technologies such as biological enzyme and ultrasonic method are most popular. Biological enzyme functions at the plant's material surface, destructs cell wall, reduces the resistance of extracting colorant from the cells, so as to improve the extract yield, with low temperature, high purity and less pollution character. Ultrasonic method is a broken method. Ultrasonic wave stays in plant cell longer and deeper than what an electromagnetic wave can penetrate, and the effective components of the plant can be extracted easily [5]. In recent years, there are many reports on extracting natural dyes by enzyme or ultrasonic method. Zhao YH extracted anthocyanin in indigo slag with cellulase and pectase by enzyme hydrolysis extraction method [6], it was found that the extract yield by adopting composite double enzyme hydrolysis was 3.05 and 1.53 times higher than cellulase and pectase used by itself respectively. Cai J researched the application of enzyme in lycopene extraction process [7], the result was that, extract time can be reduced by adding pectase and cellulose simultaneously. Qiu B, et al. compared water extract and pectase by adding in the extract process of gardenia yellow [8], the conclusion was, the extract yield of gardenia yellow with the presence of pectase was higher than that of traditional extraction. Zhao CL extracted lycopene from fresh tomato by auxiliary function of ultrasonic extraction [9], the content of lycopene in the extracted solution was 55.72mg/100g under the optimum process by ultrasonic method. Wang B, et al. extracted capsicum red by ultrasonic method [10], it was found that the extract yield of capsicum red reached 10.68%, with less pollution, short time, high yield and purity. Jiang SJ et al. studied the process of extracted orange peel dyestuff by ultrasonic technology [11], finally it was confirmed that, ultrasonic extract method was much more excellent than traditional process. According to the researches above we can see, both enzyme and ultrasonic technology are able to increase the extract

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JFBI Vol. 2 No. 1 2009 doi:10.3993/jfbi06200904

yield in the extracting process of natural plant dyestuff, but there is litter report on extracting natural dye by enzyme/ultrasonic technology. In order to increase the extract yield, we chose enzyme – ultrasonic method to extract cortex phellodendri and rubia and studied various factors in the extract process, which improves the extract efficiency and saves energy at the same time.

2. Experiment

2.1 Materials and Agents

Samples of knitted fabric made of 100% cotton (32s, single) supplied by Dalian Puqi Knitted Co., Ltd (China) were used. Dried cortex phellodendri and rubia (China) were chosen. Cellulase was provided by Zhaodong Richeng enzyme agent Co., Ltd (China). Citric acid and sodium citrate (China) were grade of purity.

2.2 Instruments

Ultrasonic instrument (KQ2200E, 50Hz, 220V) was made by Kunshan ultrasonic Instrument Co., Ltd (China). Color difference meter (ADIC-60-C) (China) was provided by Beijing Chentaike Co., Ltd (China). UV spectrophotometer instrument was provided by The American PerkinElmer Co., Ltd.

2.3 Extract Process

2.3.1 Extraction Process of Cortex Phellodendri and Rubia by Enzyme Method

Citric acid (2.1g) and sodium citrate (2.9g) were added into water (100g) and a citric acid-sodium citrate buffer solution was prepared, the pH range was adjusted to 3.5-4.5. Cortex phellodendri and rubia were washed and dried, then cortex phellodendri/ rubia was added into water in material-liquor ratio 1:50, and then the mixed solution was stirred for 60min at 50℃.

2.3.2 Extraction Process of Cortex Phellodendri and Rubia by Ultrasonic Method

Cortex phellodendri/rubia was added in water with a material-liquor ratio of 1:50.Then the solution was heated and extracted from an ultrasonic instrument for 60min at 50 ℃. The extracted solution was centrifugally separated and clear solution at the top of the liquor was used.

2.3.3 Extraction Process of Cortex Phellodendri and Rubia by Enzyme-Ultrasonic Method

Cortex phellodendri/rubia was added in water with a material-liquor ratio of 1:50, then cellulose (0.04g) was added. Then the mixed solution was heated and extracted from an ultrasonic instrument for 60min at 50 ℃. The extracted solution was centrifugally separated and clear solution at the top of the liquor was used.

2.3.4 Extraction Process of Cortex Phellodendri and Rubia by Water Extract Method

The process was the same as 2.3.3, besides not applying ultrasonic technology.

2.4 Measurement of Extracting Yield

Ultraviolet spectrum instrument was taken to measure the absorbance of extracted solution and evaluate the extracting effect of different methods.

2.5 Optimized Extraction Process of Cortex Phellodendri and Rubia

The effect of temperature, time and pH value on extraction of cortex phellodendri and rubia were determined by evaluating the absorbance values of the extracted solution.

2.6 Evaluation of Dyeing Properties

The cotton fabrics were dyed with the solution extracted by water extract, enzyme, ultrasonic and enzyme-ultrasonic method, respectively. The color difference between dyed samples reflected the extraction yield, indirectly. Color difference ΔE was measured by color difference meter ADIC-60-C under illuminant D65, with a 10° standard observer. The corresponding CIE L*, a*, b* and ΔE were valued and compared with the blank sample.

3. Results and Discussion

3.1 Extraction Yield of Cortex Phellodendri and Rubia by Four Different Methods

In order to compare the extraction yield of four different methods, cortex phellodendri and rubia were extracted in that order in this experiment. It can be found from Figure 1 and 2 that, enzyme-ultrasonic

extraction yield is the largest among the four methods used, followed by ultrasonic method, enzyme method, and traditional water extract method. As it is well known that, for cellulase, the colorant extract rate is improved through the degradation of walls and stroma of hemicelluloses as well as cellulose; for ultrasonic, the coloring matter in plant is easy to diffuse in water and extraction yield is increased through ultrasonic cavitations effect. Both biological enzyme and ultrasonic technology have good extraction effect for natural plant dyes, moreover, they have no mutual influence when two methods are used in conjunction, finally the synergistic effect realized.

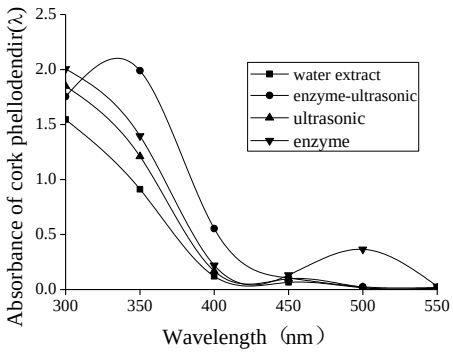

Figure 1 Absorbance of cortex phellodendri in water.

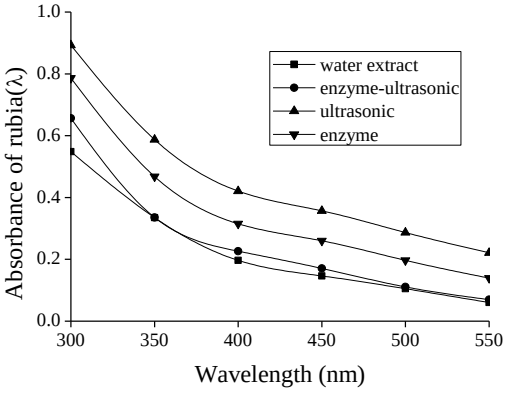

Figure 2 Absorbance of rubia in water.

According to the absorption peaks described in Figs. 1 and 2, it was found that, for cortex phellodendri, the absorbance peak appears at the region of 425nm wavelength and for rubia at 328nm. No matter use what method(consider using whichever method was used), maximum absorption peaks of cortex phellodendri and rubia were unchanged, as well as the absorption wavelengths. These facts indicate that the molecular structure of cortex phellodendri and rubia were not affected by extraction methods when enzyme and ultrasonic methods exitsimultaneously. Because, enzyme as a catalyst, has the function of substrate specificity and selectivity only for cellulose , but won't destroy the cellular structure of the colorant; ultrasonic method makes the whole process of plant cell walls broken momentarily, it does not only accelerateplant's effective components, but also helps to retain the active ingredients. Moreover, during the extraction process, cellulose wall and mesenchymal in cellulose were destroyed by enzyme and ultrasonic methods, some material and plants impurities inside the cells were transferred to the extract solution, which lead to a turbid solution. So, the extracted solution was centrifugally separated and clear solution from the top of the liquor was used as the dyeing solution.

3.2 Optimized Extraction Process of Cortex phellodendir and Rubia

3.2.1 Effect of Temperature on Extracting of Cortex phellodendir and Rubia

Cortex phellodendir and rubia were extracted by four different methods under different temperatures, respectively. The optimum extracting temperature was determined by evaluating the absorbance values of the extracted solution. The results are shown in Figures. 3 and 4.

It was indicated that, as the temperature increased, absorbance of four extraction methods increased in the initial phase but declined at different degrees above 50℃ under enzyme and enzyme-ultrasonic method.

For water extract, absorbance reached to a high value at 50℃ but did not rise significantly after that temperature. This may be because energy movement for dye molecules was not enough before 50 ℃, when temperature rose, Brownian motion was accelerated then dyestuff diffused in water and the absorbance increased.

For enzyme method, absorbance value increased with the increasing temperature and reached to maximum value at 50℃. That is because temperature is a very important factor in the enzymatic hydrolysis reaction. It affects not only the reaction speed, but also the enzyme activity. On one hand, when the temperature rose, the reaction speeded up and enzymatic hydrolysis reacted; on the other hand, protein degenerated gradually and reaction speed decreased when temperature increased further. Therefore, there is an optimum temperature for enzyme [12]. When the temperature T=50℃, large ratio of cortex phellodendri and rubia was extracted, this temperature is best for cellulase to act on plant material.

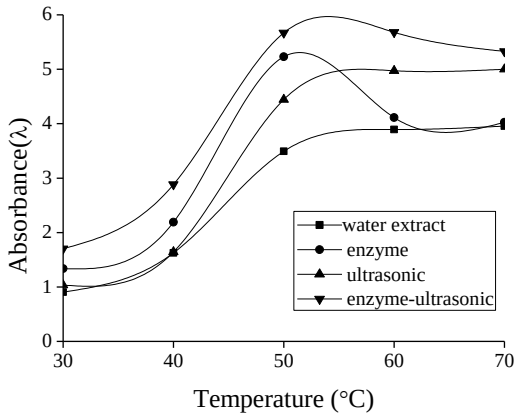

Figure 3 Effect of temperature on extraction of cortex phellodendri.

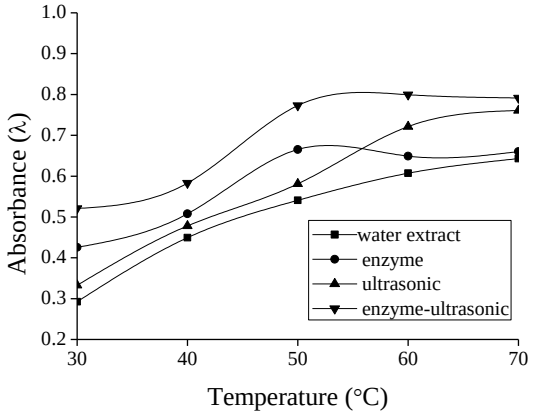

Figure 4 Effect of temperature on extraction of rubia.

For ultrasonic method, absorbance variation curve was similar to water extract method but absorbance value was higher. In addition to the reason mentioned above, higher temperature may cause decreasing cavitations effect. Beginning with the difficulty of producing cavitations bubble, when temperature increased, liquid surface tension coefficient and viscous coefficient decreased, which lead to the decreasing cavitations threshold and made it easier to form cavitations bubble [13].

For enzyme-ultrasonic method, absorbance value is higher than the other three methods at any temperature. Enzyme worked together with ultrasonic technology below 50℃ and extraction yield was improved greatly but declined obviously at 60℃. That may be due to the activity loosing of enzyme at 50℃, as a result, the action speed slowed down significantly and the increasing extraction yield was realized mainly by ultrasonic function. So 50 ℃ was the optimum temperature for extraction.

3.2.2 Effect of Time on Extraction of Cortex phellodenron and Rubia

Extractions were carried out at 50℃ varying the time from 30-70 min, respectively. The optimized extracting time was determined by evaluating the absorbance values of dye solution. The results are shown in Figure 5 and 6.

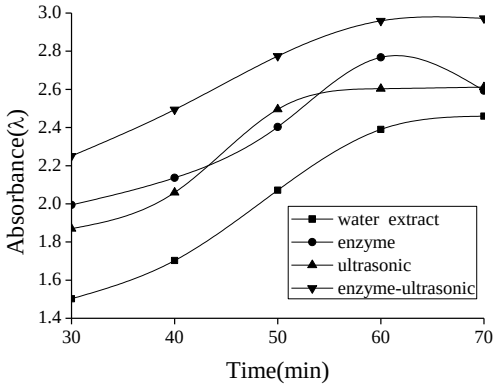

Figure 5 Effect of time on extraction of cortex phellodendri.

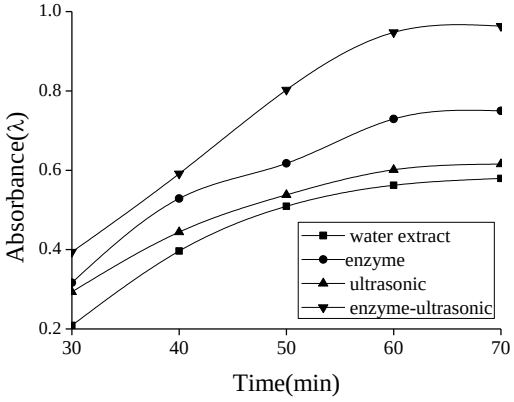

Figure 6 Effect of time on extraction of rubia.

It can be seen from Figure 5 and 6 that when time was between 30 and 60 mins., absorbance value was increasing but lost velocity at a steady rate when time was past 60 mins. because extracting quantity would not increase a lot after the maximum value by increasing time due to which colorant extracted was limited.

For ultrasonic method, concussion enhanced the activity of molecular chain in amorphous area as well as the space inside the fiber. As a result, movement speed of dye molecules increased, these may be the reasons why extraction was accelerated [13]. So the optimum time was 60 min. Extraction rate approached saturation at this temperature.

3.2.3 Effect of pH on Extraction of Cortex phellodenron and Rubia

As an attempt to investigate the influence of the pH of extraction (buffer solution) on extract yield of cortex phellodendri and rubia, cortex phellodendir/rubia was added into citric acid-sodium citrate buffer solution with a liquor ratio of 1:50. The optimum pH for extraction was determined by evaluating the absorbance values of dye solution. The results are shown in Figure 7 and 8.

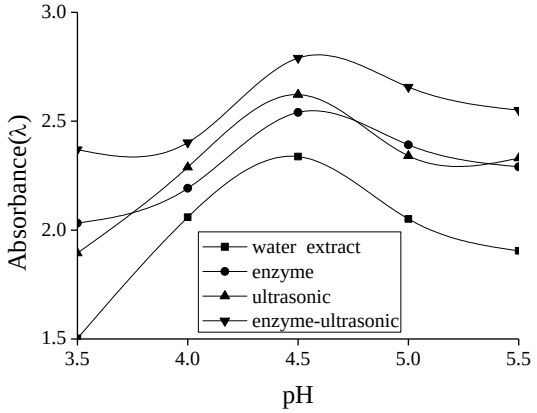

Figure7 Effect of pH on extraction of cortex phellodendri.

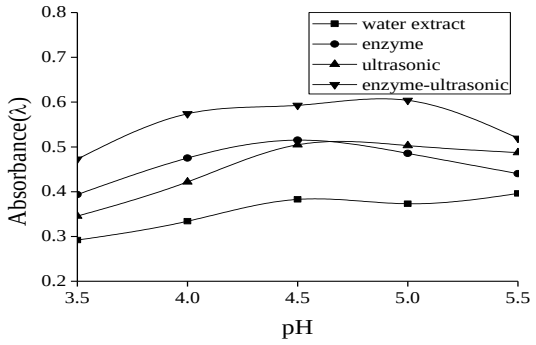

Figure 8 Effect of pH on extraction of rubia.

From Figure 7, it can be found that for cortex phellodendri, no matter using what kind of extraction method (consider using whichever extraction method is used), absorbance value increased prior to pH=4.5 while decreased after that. Because cortex phellodendri has strong resistance toward alkaline condition [14-17], acid condition would effect the extraction. In addition, most of the enzyme activities are influenced by pH. Enzyme gets maximum speed under a certain pH value; the action speed will rise above or dip below that value. The change of pH can damage space conformation as well as disintegration of catalytic groups of enzyme, then lead to energy losses. The regularity of the four methods mentioned above can also be explained by the mechanism.

Rubia has strong resistance towards both acid and alkaline condition. This experiment used the same kind of cellulose enzyme to extract cortex phellodendri and rubia. In order to make the experiment comparable, we chose the same pH range as cortex phellodendri. From Figure 8, for rubia, with the pH value increasing, absorbance values obtained from four extract methods had no obvious changes in regularity and fluctuation, which indicated that pH value, has little impact on rubia. In conclusion, 4.5 is the optimum pH value for extraction.

3.3 Dyeing Properties

In order to investigate the effect of extraction yield by extraction method, cotton fabrics were dyed with the solution extracted under the optimum extraction process by four different methods, respectively. The extraction yield was evaluated through color difference among the dyed samples indirectly. The results are tabulated in Tables 1 and 2.

From the above two tables, it is evident that, color differences among the dyed samples under enzyme-ultrasonic method are higher than the other three extract methods.

For the samples dyed with cortex phellodendir, its maximum ΔE is 25.39, which is 2.45 higher than water extract; for the samples dyed with rubia, its maximum ΔE is 33.77, which is 4.02 higher than water extract. So it can be concluded from these results that, extraction yield of cortex phellodendir and rubia by enzyme – ultrasonic method is better than water extract, enzyme and ultrasonic method.

Table 1 Color difference of samples dyed with cortex phellodendri

Samples	L*	S.D.	a*	S.D.	b*	S.D.	Δ E	S.D.
Water extract	89.81	0.548	-1.57	0.0792	19.87	0.2112	22.94	0.4056
Enzyme	90.77	0.4544	-2.79	0.2552	21.43	0.188	24.53	0.3384
Ultrasonic	90.05	0.6104	-2.18	0.1576	20.88	0.1544	24.71	0.4608
Enzyme-ultrasonic	92.32	0.4024	-2.9	0.1368	23.57	0.3768	25.39	0.3704

Table 2 Color difference of samples dyed with rubia

Samples	L*	S.D.	a*	S.D.	b*	S.D.	ΔE	S.D.
Water extract	76.40	0.4171	8.91	0.1928	20.40	0.1704	29.75	0.2456
Enzyme	74.32	0.3672	10.43	0.3408	21.98	0.6496	32.59	0.2568
Ultrasonic	74.68	0.3544	11.06	0.1136	20.87	0.2776	31.77	0.348
Enzyme-ultrasonic	74.44	0.4224	11.82	0.2224	22.97	0.1896	33.77	0.2904

4. Conclusion

The purpose of this study is to investigate the effect of extraction yield by enzyme – ultrasonic method. In this paper, cortex phellodendri and rubia were extracted by water extract, enzyme, ultrasonic and enzyme – ultrasonic method, and the optimized extraction method and process were determined by evaluating the absorbance values of different extracted solutions. Extraction yield of naturural plants dyestuff using biological enzyme and ultrasonic technology together in extract process was higher than enzyme, ultrasonic and water extraction method. Meanwhile, the pollution can be reduced and energy also can be saved. The optimized process parameters for extracting dyestuff from cortex phellodendir and rubia is: time at 60 min, temperature at 50℃, pH of 4.5.

Acknowledgement

This research was supported by 2008T012 from science and technology innovation program of Liaoning education department.

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