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Evaluation of various techniques for extraction of natural colorants from pomegranate rind— Ultrasound and enzyme assisted extraction

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Ultrasound, enzyme and enzyme-mediated ultrasound assisted extraction processes have been used for the extraction of dye from pomegranate rind. Optimum parameters for dye extraction are found to be *p*H 10, extraction time 40 min and temperature 50 °C. The yields for ultrasound, enzyme, enzyme-mediated ultrasound assisted and solvent extraction are found to be 29.2%, 26.5%, 35.6% and 8.8% respectively. The dye obtained has been used for dyeing wool and cotton, keeping the optimum dye bath concentration as 10% and 8% (w/v) respectively. The fastness properties of wool are found to be very good while that of cotton is only satisfactory.

Keywords: Cotton, Enzyme assisted extraction, Enzyme-mediated ultrasound assisted extraction, Pomegranate dye, Pomegranate rind, Ultrasound assisted extraction, Wool

1 Introduction

The use of natural colours for dyeing fabrics has been in practice for ancient times. Indigo is known to be the oldest natural colorant in India. The advent of synthetic dye during 1856-1900 jeopardized the market of natural colorants, as synthetic dyes were cheaper and gave excellent fastness and reproducible colour shades. The growing awareness of environmental problems coupled with the toxicity associated with synthetic dyes[1] brings promising prospects to investigate the cheaper extraction technology of colorants from natural, renewable resources. The colorants (from plant origin) used in dyeing various fabrics are mainly flavonoids[2], along with anthraquinones and indigoids. Most commonly found flavonoids are flavonols, flavonones and anthocyanins. These flavonoids give variety of yellow, brown and green shades. The principal colouring components from pomegranate (*Punica granatum*) rind are tannins and flavonols.

Many investigations have been carried out for extraction of colorants from different parts of the plants[3] and their chemical identification[4-6]. Various studies are reported, where ultrasonic assisted extraction and enzyme assisted extraction have been used to improve the yield[7-12].

Pomegranate juice contains various anthocyanins mainly cyanidin-3-glucoside, delphinidin 3-glucoside, pelargonidin-3,5-diglucoside, cyanidin-3,5-diglucoside, delphinidin-3,5-diglucoside and pelargonidin-3-glucoside[13]. Outer covering of pomegranate rind has been known to be very rich in ellagitannins and gallotannins[14]. Though the pomegranate peels have been used since antiquity in the Middle East as colorant for textiles because of their high tannin and phenolic contents, the literature is rare[15]. The present investigation has therefore been made to explore the suitability of ultrasonic assisted extraction (UAE), enzyme assisted extraction (EAE) and enzyme – mediated ultrasonic assisted extraction (EUAE) techniques for the extraction of natural colorants from pomegranate rind. The dyeing characteristics of extracted dye on cotton and wool have also been studied.

2 Materials and Methods

2.1 Materials

Pomegranate rind was collected from local fruit market, washed with water to remove dirt and dried in tray drier at 50 °C. The dried rind was then crushed in a ball mill and passed through a standard test sieve (BSS-14). Enzymes pectinase and cellulase were procured from Varuna Bio-Cell, Industrial Area, Ramnagar, Varanasi, India and stored as per standard norms.

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2.2 Extraction of Dye

2.2.1 Ultrasound Assisted Extraction (UAE)

Standard extraction protocol was used using a 500 mL beaker containing 25 g of powdered pomegranate rind using water (250 mL) as solvent. The different *p*H (7-10) was maintained using different amounts of Na_2CO_3. For maintaining *p*H 11, few drops of 2% aqueous NaOH was added. The beaker was then immersed into the ultrasonic bath with working frequency of 27-30 MHz at 160 V under a controlled water level at about 2-3 cm from the bottom of the bath. The mixture in the beaker was ultra-sonicated for 10-60 min at different temperatures, viz. 30, 40, 50, 60 and 70 °C, which were maintained by using hot water and ice cubes.

To estimate the yield at different time intervals, during the course of extraction 1.0 mL liquid was pipetted out and then diluted to make 10.0 mL volume. This solution was centrifuged at 2000 rpm to remove suspension. Finally, the concentration (% w/w) of the diluted solution was measured spectrophotometrically at 460 nm.

After extraction, the contents of beaker were filtered through standard test sieve (BSS-36) to remove solid material. The filtered dark brown liquid was vacuum evaporated in a rotary vacuum evaporator to about half of the original volume. This concentrated dye extract was spray dried in Spraymate (Jay Instruments and Systems Pvt. Ltd. TTC Industrial Area, Turbhe, Navi Mumbai). The inlet and outlet temperatures of spray drier were maintained at 140 °C and 80 °C respectively and aspiration speed was 1200 rpm, while flow rate was 35 mL/min. The dried powder was collected, stored in a desiccator and used as dye without further purification. All experiments were carried out in triplicate and the gravimetric yields (%w/w) were reported as an average of three values. The spectrophotometric yields were found to be higher (1-2%) than that of gravimetric yields owing to the loss of dye in the chamber, cyclone and vacuum chamber cloth of the spray drier.

2.2.2 Enzyme Assisted Extraction (EAE)

A 2% solution of pectinase:cellulase (2:1) was sprayed on pomegranate rind (25g) for better soaking and contact and then left overnight. The enzyme treated material was washed with little amount of distilled water. This material was then transferred in to 500 ml conical flask and 250 mL aqueous Na_2CO_3 (*p*H 10) was poured into it. The contents of the conical flask were shaken in orbital shaker at 150 rpm for 20-80 min at desired temperature. The filtration and drying were similar as for ultrasonic assisted extraction.

2.2.3 Enzyme – mediated Ultrasound Assisted Extraction (EUAE)

Enzyme treated pomegranate rind (section **2.2.2**) was placed in beaker, ultrasonicated and extraction was carried out as mentioned in section **2.2.1.**

2.3 Analysis

2.3.1 Spectrophotometric Analysis

The ultraviolet-visible (UV-VIS) adsorption spectra was recorded on PC based double beam spectrophotometer (Systronics 2202) over the range of 200-800 nm. The concentration of dye solution was determined with calibration curve of the pomegranate dye at 460 nm.

2.3.2 Fastness Studies

Fastness properties of the dyed yarns were evaluated using the tests ISO 105-X12 for colour fastness to rubbing; ISO 105-C02 for colour fastness to washing; and ISO 105-B02 for colour fastness to light.

2.4 Dyeing of Fibres

The wool and cotton yarns, generally used in textiles and carpet industry, were selected for dyeing. The scoured and bleached cotton and wool fibres were procured from Carpet Weavers (India). The fabrics were washed with 1% non-ionic soap (Labolene) at 50 °C for 15 min, maintaining the material-to-liquor ratio (M:L) at 1:50. Finally, the material was washed with plenty of water and dried at room temperature.

Dye solutions (2, 4, 6, 8, 10 and 12% w/v) were made by dissolving the dye obtained from pomegranate rind in water at different *p*H (6 and 8). The *p*H of the dye solution was maintained by using aqueous Na_2CO_3 and dilute acetic acid.

Cotton yarn was dyed at *p*H 8 and M:L ratio 1:15. A bunch of 5.0 g pre-treated cotton yarn was dipped into 75 mL of dye solution in 250 mL beaker and heated (80-90 °C) for 1.0 h. The dyed yarn was boiled in 75 mL water for half an hour. The washings were mixed in dye bath and the volume was made up to 150 mL. The dye bath solution before and after dyeing was further suitably diluted to get the absorbency below 4.0. Wool yarn was similarly dyed at *p*H 6.

2.4.1 Estimation of Quantity of Dye Uptake

The dye bath solution, with certain dilution, was scanned over 200-800 nm range before and after dyeing; peaks were obtained at 424 and 460 nm and compared with standard plot. The quantity of dye uptake was estimated using the following equation[16]:

$$Q=\frac{(C_o - C_f)}{W}$$

where Q is the quantity of dye uptake (mg/g); C_o and C_f , the initial and final concentrations of dye in solution (mg/L) respectively; V, the volume of dye bath (L); and W, *the* weight of fibre (g). The concentrations of the dye solutions before and after dyeing were determined using calibration curve of the dye at 460 nm.

3 Results and Discussion

3.1 Effect of Extraction Time and *p*H on Yield

Figure 1 shows the effect of extraction time on yield of dye at different *p*H maintaining the temperature at 50 °C. It is clear that at every *p*H the maximum yield is obtained at 40 min extraction time. Thereafter, notable increment on yield is not observed, and hence *p*H 10 and 40 min ultrasonication time are found to be optimum for UAE. It is noteworthy that the increase in the rate of extraction of dye is significantly higher initially, i.e. up to 20 min (Fig. 1). This may be attributed to the higher concentration gradient initially. Moreover, the collapse of cavitations into a micro-jet can easily strip off the dye initially from the surface of the rind. Afterwards the extraction rate of dye decreases because the solvent is gradually more loaded with the dye and results in the lower concentration gradient and also the remaining colorants are located in the inner part of the pomegranate rind.

The yield of dye increases with the increase in *p*H in UAE. A maximum of 31.2% yield of colorants is found at *p*H 10. The dye yield in EAE is found to be 26.5% under similar conditions, though the extraction time is 80 min. The yield increases up to 35.6% in EUAE. These yields are significantly higher than that of control experiment (8.8%) under the same conditions and 80 min shaking time.

The higher yield of colorants is ascribed to the collapse of acoustic cavitations, which are generated by ultrasound waves through liquid. The collapse of acoustic cavitations on the surface of the material momentarily produces high temperature (about 1000 °C) and high pressure (about 100 atm)[17] which facilitates release of colorant from inner core. The collapse of acoustic cavitations takes the shape of micro-jet on the surface, which ruptures the cell walls of pomegranate rind, thereby facilitating an easy percolation of solvent through the interior of the cell walls. This enhances the extraction of the colorants from the interior of the cell walls taking less extraction time for greater degree of extraction of colorants.

A measurably higher yield in EUAE may be attributed to dissolution of cell walls by enzymes and efficient stripping off colorants by ultrasound from deep inside the cell.

3.2 Effect of Temperature

Increase in temperature of extraction increases the yield of the dye in control experiment and EAE up to 80 °C (Fig. 2), but no significant increase is observed in case of EAE above 60 °C. On the other hand, the yield is even higher in EUAE than that in UAE. The temperature may change many physical properties of the solvent, like viscosity, diffusivity and surface tension, but the micro-jet generated by ultrasound cavitaional bubble collapse is most significant effect for the efficient extraction of the dye. Had it not been the main reason, the yield would have increased even at temperature higher than 50 °C. The number of cavitations bubbles increase with the increase in

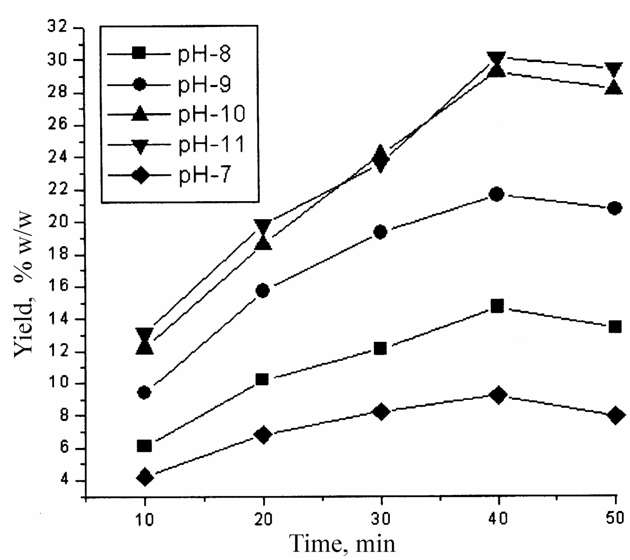

Fig. 1—Effect of time on ultrasound assisted extraction at different *p*H (temperature 50 °C)

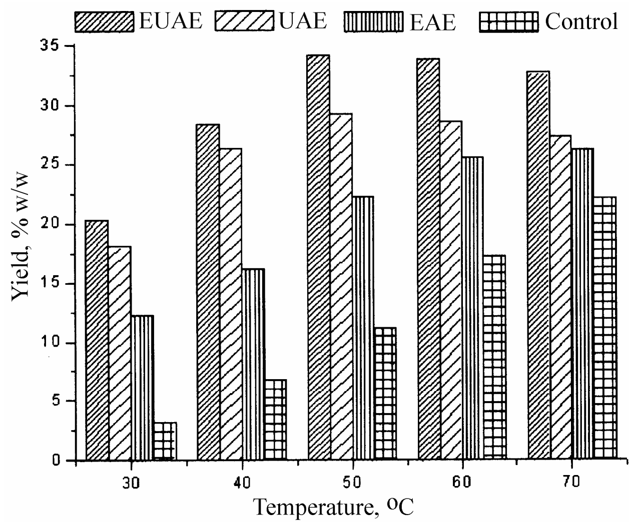

Fig. 2 — Effect of temperature on gravimetric yield of dye (extraction time: 40 min for UAE & EUAE and 80 min for control and EAE)

temperature but their collapse is powerful enough to render easy extraction from 40 °C to 50 °C. The collapse of micro-bubbles is less efficient at 60 °C and onwards albeit the number of bubbles is higher, as it has been known that at higher vapour pressure more bubbles are formed but collapse with lower intensity owing to smaller internal and external pressure difference[18]. Interestingly, the yield started decreasing in case of UAE and EUAE after 50 °C (Fig. 2). Ultrasound is known to generate OH free radicals in water, which are highly energetic species and capable of causing cleavage in the dye molecules, resulting in the decrease in yield.

3.3 Effect of Dye Bath Concentration on Dyeability of Cotton and Wool

It is found that the dyeability of cotton is lesser than that of wool as indicated by quantity of dye uptake (Fig. 3). The dye uptake increases gradually in cotton with the increase in concentration of dye bath up to 8% (w/v); at this the dye uptake is 61.3 mg/g. On the other hand, the quantity of dye uptake increases in wool till the concentration of dye bath reaches at 10% (w/v); at this the dye uptake is found to be 76.1 mg/g. The more dye uptake in wool is attributed to the adherence with >CO and -NH_2 groups of protein molecules of wool due to hydrogen bonding with polyphenolics present in the dye.

The light, wash and rubbing fastness are found to be very good in case of wool (light fastness: 4, wash fastness: 4-5 and rub fastness: 4-5) while these are only in satisfactory range for cotton fibres (light fastness: 3, wash fastness: 3-4 and rub fastness: 3-4). The pomegranate rind dye gives brown colour on wool and yellow colour on cotton fabric.

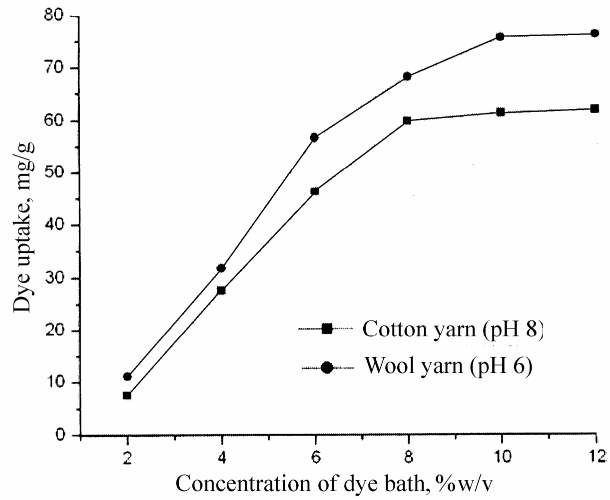

Fig. 3— Quantity of dye absorbed in cotton and wool yarns

4 Conclusions

The assistance of ultrasound and enzyme both increases the efficiency of extraction processes. Highest yield is obtained by EUAE followed by UAE, EAE and controlled conditions. Optimum conditions for extraction of dye from pomegranate rind are temperature 50 °C, *p*H 10 and extraction time 40 min for UAE and EUAE, while extraction time 80 min and temperature 60 °C for EAE and 80 °C for controlled conditions at the same *p*H are found optimal.

Better dyeability is observed on wool followed by cotton, whereas the dye cannot be used for dyeing silk yarn. Use of other mordants may also be considered for improving fastness of dye on the yarn.

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