ULTRASOUND-ASSISTED THREE PHASE PARTITIONING OF PHYCOCYANIN FROM *SPIRULINA PLATENSIS*

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ABSTRACT

In this study ,conventional three phase partitioning (TPP) and ultrasound assisted three phase partitioning (UATPP) were explored for the extraction and purification of phycocyanin from *Spirulina platensis.* and the process were optimized. rhizomes. Factors affecting partitioning efficiency such as ammonium sulfate concentration, crude extract to t-butanol ratio, time and pH on phycocyanin partitioning were optimized for conventional TPP. Except the similar parameters were also optimized for UATPP. Irradiation time at different frequencies, duty cycle and rated power were also studied for UATPP. Optimal purification parameters for conventional TPP were 0-50%(w/v)ammonium sulfate concentration with 1:1(v/v)ratio of crude extract:t-butanol at pH 7.0, which gave 4.13 purification factor with 77.3% recovery of phycocyanin after 20 min of conventional stirring. The optimized parameters for UATPP yielding maximum purity of 6.69 purification factor of phycocyanin with 94.3% recovery comprised of 0-50%(w/v)ammonium sulfate concentration, crude extract to t-butanol ratio 1:1 (v/v), pH 7, at 25 kHz frequency and 150W ultrasonication power with 50% duty cycle for 5min irradiation time. SDS PAGE analysis of partitioned phycocyanin shows two bands α and β in the range of 18 and 20 KDa. UATPP was found to be an attractive technique for the extraction and purification of phycocyanin from *Spirulina platensis.*

Keywords: ultrasound assisted three phase partitioning (UATPP), phycocyanin, *Spirulina platensi*.

INTRODUCTION

Spirulina platensis, one of the blue-green algae among microalgae, is composed of polysaccharides[1], fat [2], β-carotene[3],vitamins [4], and phycobiliproteins [5]. Phycobiliproteins, which are brightly colored pigments, function as a receiver of light for driving photosynthesis in the *Spirulina* microalga [6]. Microalgal phycobiliproteins include phycoerythrin, allophycocyanin, and phycocyanin [6]. The predominant pigment in the phycobiliprotein family is phycocyanin [7]. Phycocyanin, a blue natural pigment, is purported to be of high value [6]. as it has been reported to have anti-aging, antioxidant, and anti-inflammatory activities as well as to suppress cancer metastases[8-10]. For this reason, a simple and efficient process of separation and purification of phycocyanin is important and indispensable.

Up to now, several methods for the separation of phycocyanin have been reported. Aqueous two-phase systems extraction[11-12] (ATPE) was shown to be effective for obtention of

phycocyanin from *Spirulina platensis*, However, the majority of phycocyanin was gathered in the PEG phase. The viscosity of the PEG was increased with the increasing of the molecular weight of the PEG. There was a hindrance to the selective removal of the PEG. Although phycocyanin could be obtained by ammonium sulphate precipitation[13], anion exchange chromatography[14], and hydrophobic interaction chromatography[15].these methods are time consuming and laborious, lead to solvent wastage and lower recoveries and thus they are limited in industrial use. High-speed counter-current chromatography (HSCCC) was shown to be effective for obtention of phycocyanin from *Spirulina platensis* [16], resulting in a purity of 4.25 based on the absorbance ratio of A(620) /A(280). However, HSCCC is a particularly demanding technique that results in long separation times and high solvent consumption, and consequently to high process costs.

Three-phase partitioning (TPP) is a simple and efficient method for the separation and enrichment of protein compounds such as enzymes from complex mixtures[17-19]. It consists in the sequential addition of a sufficient amount of salt (typically ammonium sulphate) and an organic solvent (mainly t-butanol, The boiling point of t-butanol is high and less flammable compared to ethanol and methanol[20].) in the crude extract and after agitation and decantation[21], the mixture separates into three distinct phases: the upper t-butanol phase contains non-polar compounds which are separated from the lower aqueous phase (containing polar compounds) by an interfacial protein precipitate. The desired proteins are selectively partitioned to one phase and other contaminant proteins to the other phase. This causes partial purification and concentration of the protein[22]. The extraction process is an amalgamation of kosmotropic, salting out, isotonic cosolvent and osmolytic precipitation of proteins[23].

TPP has also been employed as an extraction and purification technique for active ingredients such as proteins[17],Oil[20],polysaccharide[23], and enzyme[24]. In the TPP system enzyme partitioning occurs through mass transfer phenomenon and an increase in mass transfer augment the partitioning and purification fold of the target enzyme and protein,but TPP has a drawback at it still requires more time for the extraction because of its mass transfer limitations of inefficient mixing[25]. This mass transfer can be improved using ultrasound. Ultrasound assisted extraction (UAE) has been reported for intensifying the extraction efficiency of various natural products by improving the mass transfer. In UAE when an ultrasonic pressure wave propagates through liquid medium, it induces a cavitational force that facilitates greater penetration of solvent into cellular material thereby leading to a significant increase in mass transfer[21]. There are reports available where, UAE has been coupled with different extraction or separation techniques such as ATPE[26], ionic liquid[27], and TPP for increasing recovery and purification fold of targeted protein. Hence, TPP has been coupled with ultrasound in order to enhance purification fold and increase in mass transfer.

By this method, the desired enzymes or proteins are selectively partitioned to one phase while contaminants such as lipids and inhibitors to the other one. It does not only purify proteins but also concentrate them into one of the phases. In this short survey no mention on the use of this technique for precipitation of phycocyanin has been reported.

The present study describes for the first time, the purification and recovery of phycocyanin from *Spirulina platensis* using the TPP system and UATPP. Hence, the main focus of the present study was to achieve a maximum purity and yield of phycocyanin using this one-step purification approach. The influence of various parameters such as $(NH_4)_2SO_4$ saturation ,

crude extract to t butanol ratio, agitation time, pH, ultrasonic power ,extraction time, and duty cycle on purification of phycocyanin has been studied.

MATERIALS AND METHODS
Materials and chemicals

The lyophilized *S. platensis* was provided by research center of gansu province microalgae engineering. (Gansu, China). The dried microalgae were ground to reduce the average particle size to less than 25 μm before examining the extraction process.

t-butanol, ammonium sulfate, acrylamide, bisacrylamide, sodium dodecyl sulfate (SDS), dithiothreitol (DTT),ammonium persulfate, N,N,N,N-tetramethylethylenediamine (TEMED), were of pure grade and were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Molecular weight standards (Cat.# 3450) and Bradford Reagent were purchased from TaKaRa (Shiga, Japan). Unless otherwise stated, all other chemicals and reagents were of the highest available purity and used as purchased. All solutions were prepared using deionized water and stored at the appropriate temperatures.

Extraction and purification of phycocyanin
Extraction

Phycocyanin was extracted by repeated freezing (-20℃) and thawing at room temperature until the blue color becomes in acetate buffer. Cell debris was removed by centrifugation at 5,000 rpm for 10 min and the extract thus obtained was termed as crude extract.

Three phase partitioning (TPP) of phycocyanin

Different $(NH_4)_2SO_4$ concentrations such as 0-30, 0-40, 0-50, 0-60,and 0-70% (w/v) were added into 10 mL crude extract of phycocyanin in an ice bath at 4℃ and vortexed gently to dissolve the salt. After complete dissolution of different concentration of salts, t-butanol was added at ratio of 1:1 (v/v). Further these mixtures were stirred on magnetic stirrer with magnetic needle of dimension (206mm) at 400 rpm for 30 min. The mixtures were then centrifuged at 8000× g for 20 min to facilitate the separation of phases. The upper t-butanol layer was removed carefully with the help of pipette, and the separated bottom phase was analyzed for phycocyanin purity as well as the efficiency of the purification process.

After ammonium salt concentration different parameters such as effect of crude extract to t-butanol from range 1:0.5 to 1:2.5(v/v), pH (5-8), on phycocyanin partitioning were studied. The highest phycocyanin recovery and purity was chosen for further study and all the experiments were run in triplicate.

Ultrasound assisted three phase partitioning (UATPP)

An ultrasonic bath having dimensions of 0.3×0.15×0.15m (Model SCQ-3201E, shengyan,Shanghai), maximum rated power of 200 W with dual frequencies 25 and 40 kHz was used for performing UATPP of phycocyanin. The temperature of the ultrasound bath was varied and controlled with the recirculation water. UATTP was carried out in a glass vessel of 250 mL capacity kept 0.2 mm above from bottom of ultrasonic bath. To perform experiment, 10mL of crude extract long with 0-50% ammonium sulfate concentration and 10mL of t-butanol at pH 7 was added in a glass vessel. Ultrasound was irradiated for 3 min at 25 kHz

ultrasonic frequency with rated power 50 W and 50% duty cycle. Irradiated mixture was centrifuged at 8,000g for 10 min to facilitate the separation of phases and the bottom phase was analyzed for phycocyanin recovery and purity. Further, different operating parameter such as crude extract to t-butanol ratio, irradiation time with frequency, pH, duty cycle, rated power and temperature were studied by using one factor at-a-time method. The experiment was carried out in triplicate and results are presented with the standard deviation.

Analytical method
Estimation of phycocyanin

According to Bennett and Bogorad[28], the phycocyanin concentration(PC)was defined as:

$$PC = \frac{A_{615} - 0.474A_{652}}{5.34}$$

where PC is the phycocyanin concentration (mg/mL), A_{615} is the optical density of the sample at 615 nm and A_{652} is the optical density of the sample at 652 nm.
Phycocyanin purity

The phycocyanin purity (EP) was calculated spectrophotometrically using the following relationship[14].

$$EP = \frac{A_{620}}{A_{280}}$$

where A_{620} is the optical density of the sample at 620 nm and A_{280} is the optical density of the sample at 280 nm. This relationship is indicative of the phycocyanin extract purity with respect to most forms of contaminating protein. The absorbance at 620 nm indicates the phycocyanin concentration, while that at 280 nm is due to the total concentration of proteins in the solution.
Phycocyanin purification fold
    The purification factor was defined as:

$$PF = \frac{EP_p}{EP_c}$$

where PF is the purification factor, EP_p is the extract purity after the purification process and EP_c is the purity of the crude extract.
Phycocyanin recovery
    The extract recovery (%) was defined as:

$$\%Recovery = \frac{PC_b \times V_b}{PC_{crude-ext} \times V_{in}} \times 100$$

where PC_b is the phycocyanin concentration in the bottom phase considered (mg/mL), PC_{crude_ext} is the phycocyanin concentration in crude extract (mg/mL), V_b is the volume of the bottom phase considered (mL) and V_{in} is the initial volume of the extract added (mL).

SDS-PAGE

Sodium dodecyl sulfate polyacrylamide gel electrophoresis(SDS-PAGE) was used for analysis of TPP and UATPP bottom phase samples. DYCZ-24DN electrophoresis apparatus(Six One Instrument Factory, Beijing, China) was used for the SDS-PAGE. Experiment was performed with 12% gel for determination of molecular weight and

homogeneity of phycocyanin sample. Standard molecular weight marker was used and gel was stained overnight at room temperature with Coomassie blue solution (in 10% acetic acid) and then de-stained with 10% acetic acid for 2-3h.

Statistical analysis

Statistical analysis is very important to summarize and interpret the obtained data. All experimental results were performed in triplicate and the data are expressed as means±SD. The statistical significances of process parameters were evaluated by analysis of variance (ANOVA) using Microsoft Excel.

RESULTS AND DISCUSSION
Effect of ammonium sulfate concentration on phycocyanin partitioning

Ammonium sulfate saturation has very important role in TPP and UATPP. This is due to salting out phenomenon. Salting out can be used to separate proteins based on their solubility in the presence of high salt concentration [29].Additionally, NH_4^+ and SO_4^- are at the ends of their respective hofmeister series and have been shown to stabilize protein structure. $(NH_4)_2SO_4$ saturation must be optimized to get the maximum recovery. The efficiency of the salting out will first depend on $(NH_4)_2SO_4$ and second on the net charge. Herein, the experiment was carried out by keeping the experimental parameters as follows: ammonium sulfate concentration was studied by varying it from 0–30 to 0–70% (w/v) at constant crude extract to t-butanol ratio 1:0.5 (v/v), room temperature , pH 7 and agitation time 15 min for conventional TPP. In the case of UATPP along with above mentioned constant parameters of TPP except agitation time, additional ultrasonic parameters such as duty cycle 50%, ultrasonic frequency 25 kHz, irradiation time 3 min and rated power 50W were used. Figure 1 showed that increased $(NH_4)_2SO_4$ concentration enhanced the purification factor and recovery due to salting out effect. Maximum phycocyanin recovery of 78.4% along with 3.28 purification factor in bottom phase was obtained at $(NH_4)_2SO_4$ 0-50% (w/v) concentration for conventional TPP.

On the other hand in UATPP, acoustic cavitation effect was utilized as a mode of mass transfer enhancement that gave higher mass transfer between electrolyte ions and hydrated layer around protein. Thus, the protein surface loses its electric charge and hydrated layer gets neutralized which consequently enables it to become flocculated and deposited. However, the extraction rate of protein may increase and showed maximum recovery of 88.2% and purification factor 5.01 of phycocyanin in bottom phase of system. On further increasing the $(NH_4)_2SO_4$ concentration up to 0-70% (w/v) it tends to reduce selective partitioning of phycocyanin with decreased purify. This may be because of precipitation of phycocyanin and its irreversible denaturation at higher $(NH_4)_2SO_4$ saturation.However,0-50% (w/v) $(NH_4)_2SO_4$ saturation was found to be most favorable as it showed highest recovery and purity. Hence, it was selected for further experimentation.

Effect of crude extract to t-butanol ratio on phycocyanin partitioning

Conventional TPP and UATPP of phycocyanin were studied by using t-butanol as co-solvent owing to its greater advantage over other solvents reported in literature. t-Butanol has higher molecular size and branched chain structure so it does not permeate inside the folded protein molecule and it prevents denaturation of protein because of its higher molecular size and branched chain structure. Therefore, crude extract to t-butanol ratio was varied from 1:0.5 to

1:2.5(v/v) in order to study effect on TPP and UATTP of phycocyanin and all the other parameters were kept constant. As shown in Figure 2, in both systems purification fold and recovery are increased up to crude extract to t-butanol(1:1)ratio then decreased from ratio (1:1 to 1:2.5). If small amounts of t-butanol were used, then t-butanol is unable to adequately synergize with $(NH_4)_2SO_4$, whereas higher amounts of t-butanol would lead to protein denaturation. Therefore, an appropriate amount of crude extract to t-butanol ratio 1:1(v/v) investigates the best results with high purification fold and recovery in the TPP without ultrasound (Figure 2).

In the above UATTP associated effect, t-butanol was combined with the cavitation phenomena and thus recovery and purity are higher than conventional TPP. Lower amount of t-butanol tends to increase the surface tension and vapor pressure of the system. At 1:2.5 (v/v) crude extract to t-butanol ratio, high t-butanol quantity and ultrasonic cavitation combination would affect the protein structure leading to phycocyanin denaturation. Therefore less cavitation and prevention of phycocyanin denaturation limits the minimum utilization of extract to t-butanol ratio 1:1(v/v) for maximum partitioning with 5.11 purification factor of phycocyanin from UATPP.

Effect of pH on phycocyanin partitioning

Effect of pH on TPP and UATPP system for partitioning of phycocyanin was studied at different pH between 5 and 8 and keeping other parameters constant such as ammonium sulfate 0-50% (w/v) concentration with crude extract to t-butanol ratio1:1 (v/v), agitation time 15 min, room temperature , duty 50% cycle, rated power 50 W, frequency 25 kHz, irradiation and time 3 min. As shown in Figure 3, when the pH increases from 5 to 7, the % recovery and purity increase in the bottom phase. In both TPP and UATPP the maximum purification factor and recovery was found at pH 7. Partitioning of target protein mainly depends on its isoelectric point (pI). Variation in pH of the system affects the ionizable groups of a protein and changes its surface charges. When pH values are above the pI, protein will acquire net negative charge and will be propelled to bottom aqueous phase. On the other hand, if system pH is below pI of target protein, it will be precipitated or accumulated at the interphase of three phase system[24]. Rito-Palomares[30] et al. have reported a pI of 5.8 for c-phycocyanin from *Spirulina*. num tuberosum). Figure 5 shows that in pH 5, which is below pI 5.8, results in damaging some amount of the proteins due to acidic conditions and precipitate at interphase. Therefore, the phycocyanin partitioned to bottom phase was less along with lesser % yield. In both TPP and UATPP the maximum purity and recovery was found at pH 7. As the pI of phycocyanin is 5.8, at pH of 7 negative charges on the protein surface were enhanced which resulted in increased partitioning of phycocyanin to the bottom phase. Impurities and contaminated proteins were partitioned at the upper t-butanol phase or precipitated or accumulated at the interfacial phase. For this reason the purity of phycocyanin was increased. Mohammed[31] et al. reported similar finding by performing TPP of a milk-clotting enzyme, in which the enzyme partioned into the bottom phase at pH 7, because the pH was above its pI value of 4.8. Hence, pH 7.0 was found to be optimum in this study for partitioning of phycocyanin.

Effect of time on TPP of phycocyanin

From the industrial scale up point of view, agitation time optimization is an important factor and efforts are made to maximize recovery and purity in the smallest duration of time. Therefore, the agitation time was varied from 5, 10, 15, 20, and 25 min keeping a constant

agitation speed of 400 rpm, $(NH_4)_2SO_4$ 0-50% (w/v) concentration, crude extract to t-butanol ratio of 1:1 (v/v), pH 7 and room temperature to study the effect on the partitioning of phycocyanin. Agitation resulted in the formation of large turbulence in solvent phase, which leads to increased mass transfer. As shown in Figure 4, the result demonstrated that an agitation time of 20 min gave the highest partition and 4.13 purification factor of phycocyanin. After 20 min no significant increase in purity and yield was observed as three phase partitioning is an equilibrium process and adequate time must be allowed for the system to reach equilibrium for partitioning of phycocyanin between the phases. It was found that the equilibrium was achieved at 20 min of contact time and a further increase in the agitation time had no influence on the recovery and purity of phycocyanin. Hence, for conventional TPP an optimized agitation time of 20 min was used for further experimentation.

Effect of frequency and irradiation time on UATPP of phycocyanin

Experiments were performed at two ultrasonic frequencies (25 and 40 kHz) by keeping other parameters constant such as 50% duty cycle, ammonium sulfate saturation 0-50% (w/v), crude extract to t-butanol ratio 1:1 (v/v), pH 7, room temperature and power 50 W. Experiments were carried out separately for different extraction time between 1 and 9 min for each frequency. Figure 5 shows the results of the frequency effect based on purity and yield. It shows that 25 kHz gave maximum 6.45 purification factor and 90.5% recovery as compared to 40 kHz at irradiation time of 5min. This may be due to different power dissipation at both frequencies. The power dissipations were determined by calorimetric method at a power of 50 W and they were found to be 42 W and 24 W for 25 kHz and 40 kHz, respectively. Thus, higher dissipation of power at 25 kHz would favor higher purity of phycocyanin.

It observed that for both frequencies the purity and recovery increased with time until 5 min and decreased afterward. This may possible due to excessive utilization of irradiation time that causes change in protein structural conformation leading to degradation of phycocyanin. Our results were in agreement with previous finding of Mangesh[21] et al., where UATPP of peroxidase from waste orange peels were performed. Moreover its purification factor and recovery were increased at 25 kHz up to 5 min of irradiation time; a further increase in irradiation time exhibited a decline in purification fold.

Effect of power on UATPP of phycocyanin

Ultrasonic power is an important factor because it affects the cost of the process, especially at the industrial scale. As ultrasonic power increases it generates a large amplitude ultrasonic wave, which passes through the medium and causes the production of more cavitation bubbles. These bubbles collapse violently due to strong shock wave which leads to improved mass transfer. Hence

UATPP was carried at different rated powers from 50 to 200W at ammonium sulfate saturation 0-50% (w/v), pH 7, room temperature and crude extract to t-butanol ratio 1:1(v/v) with 50% duty cycle at 25 kHz frequency for 5 min. Dissipated power was also estimated by calorimetric method for 5min at different rated powers such as 50, 100, 150, and 200W for 25 kHz and it was found to be 42, 46.2, 61.6, and 72.8W, respectively. It can be observed from Figure 6 that with an increase in the rated power, the purification fold and recovery were increased. This may be because as the rated power increased, it induced mechanical shear to

the three phase system, which enhanced the mass transfer and recovery. At 150W rated power (power dissipated 61.6W), a maximum purification factor of 6.67 and 93.1% recovery were obtained and a further increase in rated power until 200 W may cause protein degradation.

Effect of duty cycle on UATPP of phycocyanin

Ultrasound can be operated in either a continuous mode or a pulse mode. The continuous mode of ultrasonic irradiation may cause erosion of transducers and also utilize higher energy. Additionally the continuous mode generates excessive heat that may cause degradation of heat sensitive biomolecules. It has been reported by Dey and Rathod [33]that using the pulse mode helps to intensify the recovery and prevent excessive heat and energy loss while also increasing the life span of the transducer. There are reports available which supported the use of pulse mode ultrasonication for intensification of several bioprocesses[31-32]. Therefore, the effect of the duty cycle (changing ON-OFF time of ultrasound) on the partitioning of phycocyanin was carried out at 30, 50, 70, and 90% duty cycles with constant rated power of 150 W at a frequency of 25 kHz for 5 min. Purity of phycocyanin was increased up to 50% duty cycle (30s ON and 30s OFF) and decreased thereafter till 90% duty cycle (54sON and 6sOFF) as shown in Fig.7.At higher duty cycle, extensive cavitation was occurred which caused degradation of protein structure due to imparted mechanical shear along with elevated temperature. In addition to this, higher duty cycle causes temperature elevation which is also one of the factors of protein degradation. Swapnil et al. had extracted serratiopeptidase from Serratia marcescens NRRL B 23112 at different duty cycle and reported similar trend of variation in yield of serratiopeptidase[32] .

Comparison of conventional TPP and UATPP

TPP displays selective partitioning of phycocyanin in the bottom phase and other contaminant proteins to the upper and intermediate phase. Results of UATPP are compared with TPP at optimized conditions as shown below. At optimum conditions, i.e. 0-50% (w/v)ammonium sulfate concentration, pH 7, and crude extract to t-butanol ratio 1:1(v/v), TPP has shown 4.13 fold purity with 77.3% recovery of phycocyanin after 20 min of conventional stirring. However, UATPP has shown increase in fold purity of phycocyanin up to 6.69 at optimized conditions of irradiation viz; 25 kHz frequency, 50% duty cycle and 150W rated power for 5 min. Thus, ultrasound irradiation not only decreases the time of operation of TPP, but also increases the fold purity of phycocyanin. Thus, UATPP would be an attractive alternative to purification of active components.

SDS-PAGE analysis

SDS-PAGE analysis phycocyanin samples from crude, TPP and UATPP were analyzed using SDS-PAGE as shown in Figure 8. In crude phycocyanin (lane1) and TPP (lane 3) many other protein fraction bands are observed whereas UATPP (lane 4) showed fewer bands. It indicated that the purity of phycocyanin is significantly increased in the UTPP system compared to TPP. The phycocyanin shows two bands α and β in the range of 18 and 20KDa. This demonstrated that UATPP can be used to obtain a high purification factor than convectional TPP.

CONCLUSION

Purification of phycocyanin from *spirulina platensis* using UATPP has been successfully optimized. Enhanced mass transfer in UATPP results in higher % recovery of phycocyanin as compared to TPP. Higher values of irradiation time, rated power and duty cycle had reported unfavorable effects on phycocyanin partitioning. Optimized parameters of UATPP increase fold purity and % recovery of phycocyanin, reducing time of operation from 20 min to 5 min as compared to conventional TPP. Hence, enhanced purity with reduced time of operation symbolizes UATPP as an attractive contention for a future integrated bioseparation protocol. The combination of ultrasound and TPP enhances the benefits of the both processes and makes this integrated approach an interesting alternative to the downstream purification step.

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Caption to Figures

Fig. 1. Effect of ammonium sulfate concentration(w/v%) on TPP and UATPP of phycocyanin (crude extract to t-butanol ratio 1:0.5 (v/v); pH 7 and agitation time 15 min; duty cycle 50%;ultrasonic frequency 25 kHz; irradiation time 3 min and rated power 50W).
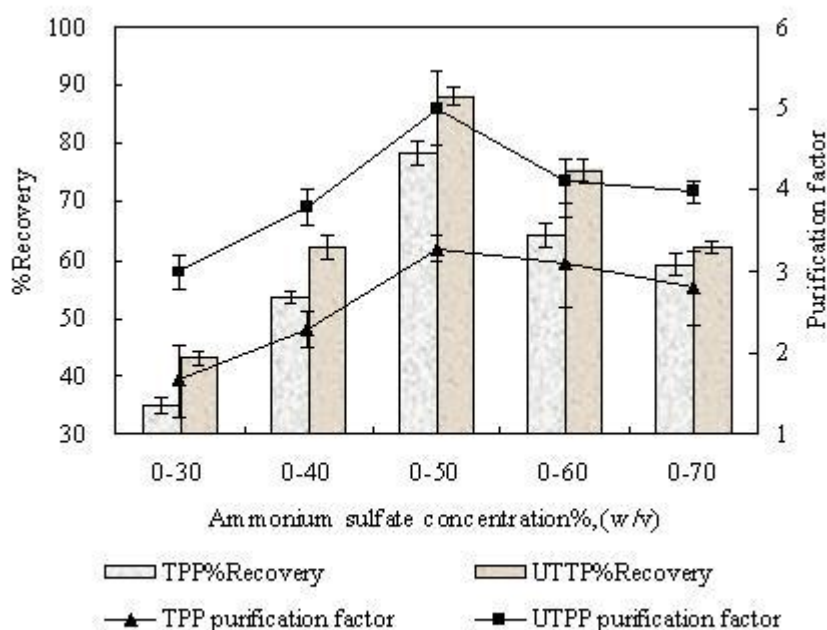
Fig. 2. Effect of crude extract to t-butanol ratio on TPP and UATPP of phycocyanin (ammonium sulfate concentration 0-50%(w/v%); pH 7 and agitation time 15 min; duty cycle 50%; ultrasonic frequency 25 kHz; irradiation time 3 min and rated power 50W).
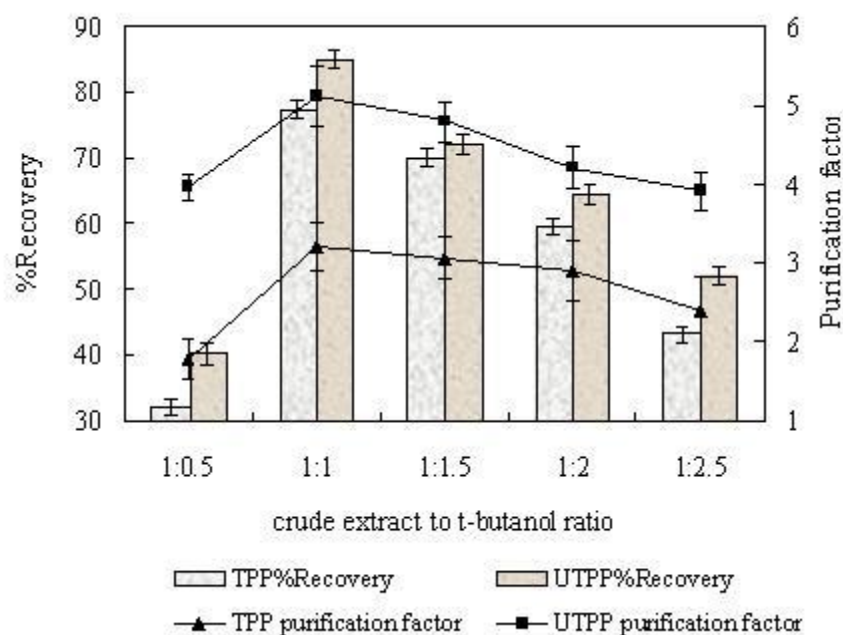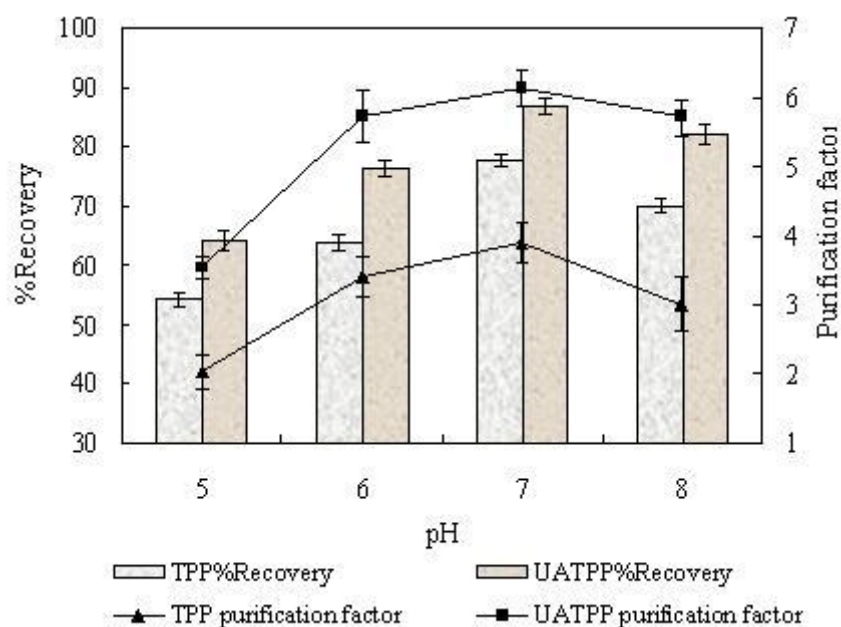

Fig. 3. Effect of pH on TPP and UATPP of phycocyanin (ammonium sulfate concentration 0-50%(w/v%); crude extract to t-butanol ratio 1:1 (v/v); agitation time 15 min; duty cycle 50%; ultrasonic frequency 25 kHz; irradiation time 3 min and rated power 50W).

Fig. 4. Effect of time on TPP of phycocyanin (ammonium sulfate concentration 0-50%(w/v%); crude extract to t-butanol ratio 1:1 (v/v); pH 7).
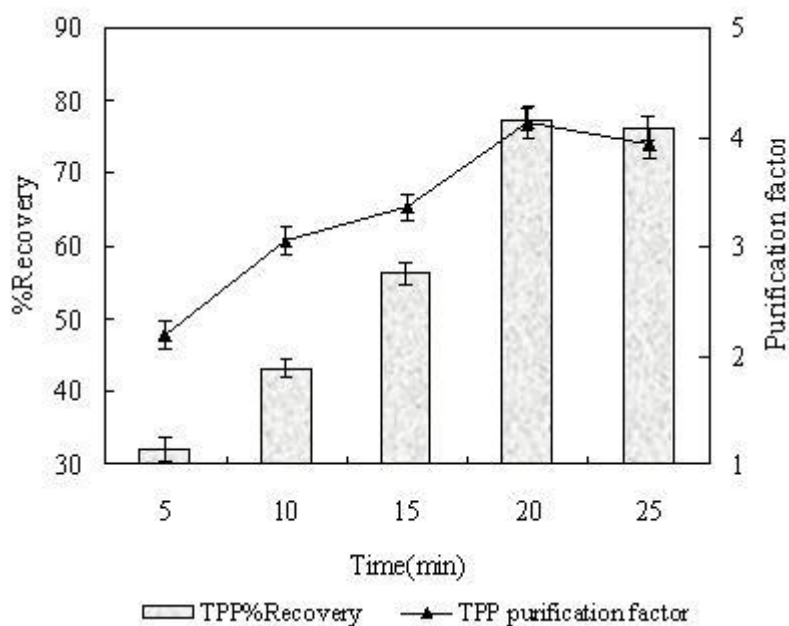

Fig. 5. Effect of ultrasound irradiation time on UATPP of phycocyanin at ultrasonic frequency 25 and 40 kHz (pH7;ammonium sulfate concentration 0-50%(w/v%); crude extract to t-butanol ratio 1:1 (v/v); duty cycle 50%; rated power 50W).
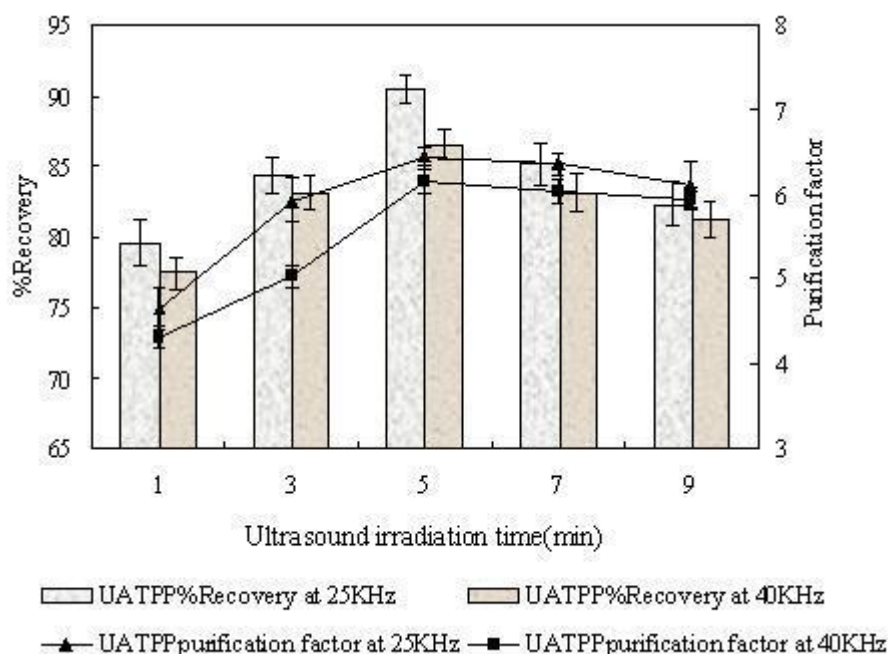
Fig. 6. Effect of ultrasound power on UATPP of phycocyanin (pH7;ammonium sulfate concentration 0-50%(w/v%); crude extract to t-butanol ratio 1:1 (v/v); duty cycle 50%; irradiation time 5 min; ultrasonic frequency 25 kHz).
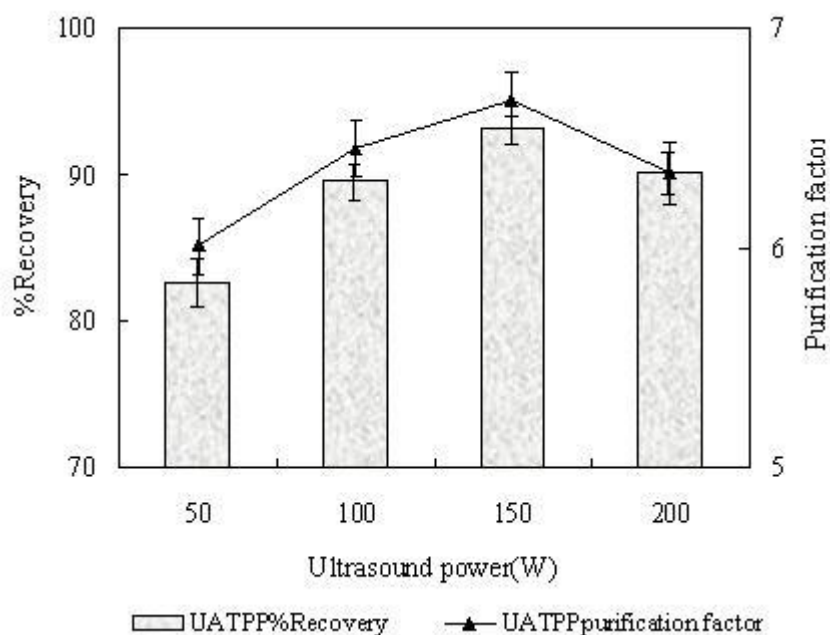

Fig. 7. Effect of duty cycle on UATPP of phycocyanin (pH7;ammonium sulfate concentration 0-50%(w/v%); crude extract to t-butanol ratio 1:1 (v/v); irradiation time 5 min; ultrasonic frequency 25 kHz; rated power 50W).
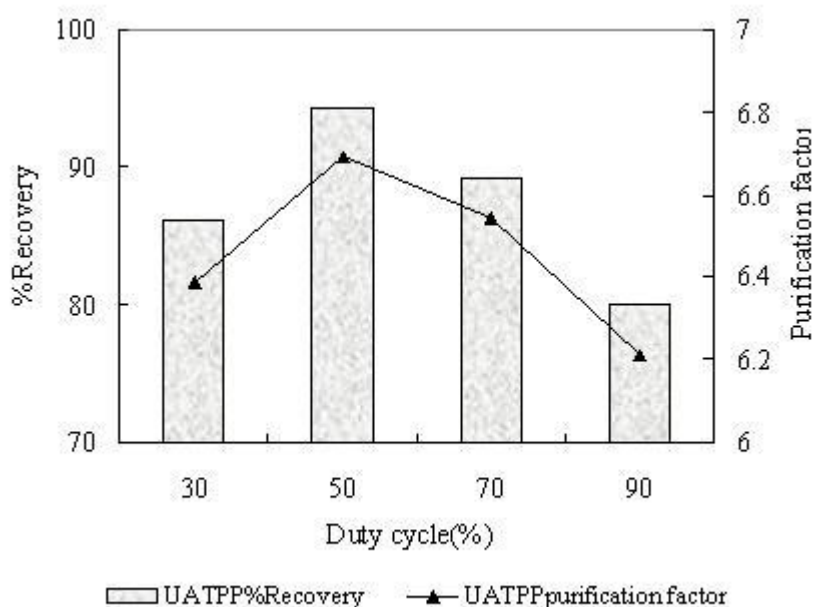
Fig. 8. SDS-PAGE analysis of TPP and UATPP partitioning of phycocyanin (Lane 1: crude phycocyanin, Lane 2: Molecular weight marker, Lane 3: conventional TPP, Lane4:UATPP)