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ORIGINAL PAPER

Quantification and Concentration of Anthocyanidin from Indian Blackberry (Jamun) by Combination of Ultra- and Nano-filtrations

Payel Ghosh[1] · Rama Chandra Pradhan[1] · Sabyasachi Mishra[1] · P. K. Rout[2]

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Abstract

Indian blackberry (*Syzygium cumini*) is an excellent source of antioxidants and anthocyanins. The fruit juice can be concentrated by membrane filtration for use as nutritional supplements. The juice was extracted and clarified by ultrafiltration (50 kDa) and concentrated by nano-filtration (300 Da). Physicochemical properties of permeate and retentate were analysed. Protein, polyphenol and antioxidant contents were determined by spectrophotometric method. Anthocyanidins were quantified by RP-HPLC-photodiode array detector method. The nano-filtered concentrate had various anthocyanidins such as cyanidin chloride (5.9 mg/10 g), malvidin chloride (20.8 mg/10 g) and delphinidin chloride (3.6 mg/10 g). Rejection rates of protein, polyphenol and antioxidants for ultra- and nano-filtered permeates were 48%, 22.3%, 51% and 63%, 74%, 40%, respectively. The particle size distribution of the concentrated juice followed a parabolic curve justifying proper filtration. The results suggest possible use of the fruit juice concentrate in beverage and pharmaceutical industry.

Keywords Indian blackberry · Membrane filtration · Concentration · Anthocyanidins

Introduction

Indian blackberry (*Syzygium cumini*) is a tropical fruit from Myrtaceae family, traditionally known as Jamun. The fruit is known for its therapeutic value, antioxidant and nutritional properties. Jamun is rich in anthocyanins that are stable at pH 1–4 and degrade above pH 7 (Mojica et al. 2017; Li et al. 2009). Anthocyanins are in the forms of anthocyanidins glycosides and acylated anthocyanins. Anthocyanidins are generally of three types named as 3-hydroxyanthocyanidins, 3-deoxyanthocyanidins and O-methylated anthocyanidins. So it can be said that anthocyanins are in the form of glycoside while anthocyanidins are the aglycone (Khoo et al. 2017). The common anthocyanidins found in fruits are cyanidin, peonidin, delphinidin, petunidin and malvidin (Ananga et al. 2013). These compounds exhibit anti-diabetic and anti-carcinogenic properties and are also used as natural colorant by the food industry. The colour properties of the anthocyanidins are based on the number of hydroxyl groups attached to the substitute ring. It also depends on the pH and the temperature. Increase in the number of hydroxyl groups in the structure shifts the colour from orange to violet. The anthocyanidins improve the overall colour stability of the food at optimized temperature and pH range (Stintzing and Carle 2004). Various researchers have reported extraction of anthocyanins from horticultural products and their uses. Several fruits and vegetables have been used for extracting anthocyanin. Anthocyanin was obtained from purple sweet potato (Gras et al. 2017) and radish (Giusti and Wrolstad 2003) for use as colorant. Aqil et al. (2012) have reported the presence of anthocyanins (0.54%), ellagitannins (0.17%) and polyphenols in Jamun pulp. Anthocyanin obtained from grapes has been used to fortify dairy and egg products (Pineda-Vadillo et al. 2017). The regulatory authorities in European countries,

✉ Rama Chandra Pradhan
pradhanrc@nitrkl.ac.in

Payel Ghosh
payelghosh89@gmail.com

Sabyasachi Mishra
mishrasa@nitrkl.ac.in

P. K. Rout
prasantrout@gmail.com

[1] Department of Food Process Engineering, National Institute of Technology, Rourkela, Odisha 769008, India

[2] Process Chemistry & Technology Department, Central Institute of Medicinal and Aromatic Plants; PO: CIMAP, Lucknow 226015, India

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USA and China have recommended 22.3 mg of anthocyanins per day in diet (Wedick et al. 2012). Anthocyanins also have various medicinal uses. Daily intake of anthocyanins can reduce the chances of cancer, diabetics and heart diseases. Various anthocyanidins (delphinidin, cyanidin, petunidin, peonidin and malvidin) extracted from Jamun have shown anti-proliferative activity against human lung cancer (Arun et al. 2011; Aqil et al. 2012; Chung et al. 2017).

Various membrane filtration techniques such as microfiltration and ultrafiltration have been used for clarification of juices, and nano-filtration (NF) was used for concentrating bioactive molecules like phenols, anthocyanins, antioxidants and tannins. Various studies have been reported for clarification of juices through different types of microfiltration for a decade. Microfiltration was used for orange, chicory, passion fruit, pineapple, kiwi, camucamu, pomegranate, banana and melon juice clarification (Barreto et al. 2013; Cassano et al. 2006). NF is used for concentration of sugar and non-sugar compounds in beverage industry. Separation of various solutes in NF depends on pore radius and surface charge density (Chen et al. 2011; Hussain and Al-Rawajfeh 2009; Gyura et al. 2002). Sugarcane juice was fermented by *Lactobacillus plantarum* for the production and separation of unconverted sugar and lactic acid by nano-filtration (Sikder et al. 2012). Arend et al. (2017) concentrated phenolic compounds from strawberry juice using nano-filtration. Khemakhem et al. (2017) combined microfiltration (MF, 0.2 μm), ultrafiltration (UF, 5 kDa) and NF (300 Da) to extract oleuropein from the juice of olive leaves. In the present work, Jamun juice has been clarified by microfiltration and ultrafiltration and anthocyanin compounds were concentrated by nano-filtration and quantification has been made. Various physicochemical and nutritional parameters of the concentrated juice were evaluated. The main aim of the work is to concentrate the Jamun juice through nano-filter to retain the original quality of the juice as well as to obtain anthocyanidin which can be used in food and pharmaceutical industry.

Materials and Methods

Sample Preparation

Fresh and ripe Jamun (variety: Ram Jamun) was procured from local market (Rourkela, Odisha, India). It was cleaned with $CaCl_2$ solution. The fruits were deseeded, and pulp was extracted manually for juice preparation. The pulp was treated with pectinase (*Aspergillus aculeatus*) enzyme (Activity 3800 units/ml, Pectinex Ultra SPL, Sigma-Aldrich, India) for 80 min at 44 °C with a concentration of 0.05% (*w*/*w*) (Ghosh et al. 2016b).

List of Materials

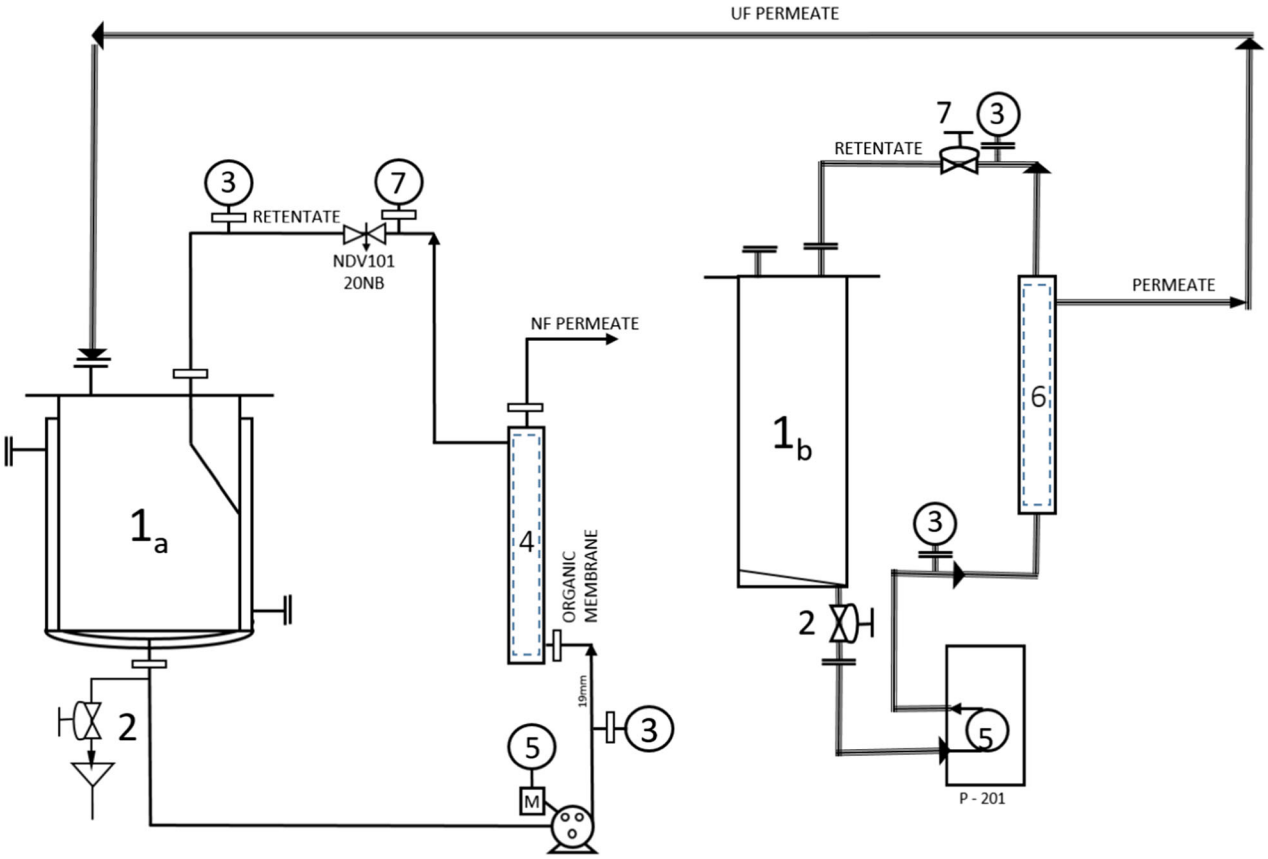
Potassium sodium tartrate, hydrazine sulphate and Hexamine LR grade were procured from HiMedia Laboratories Pvt. Ltd. (Mumbai, India) and used for enzymatic treatment. Pectinase (Commercial Name: Pectinex Ultra SPL; activity 3800 units/ml) from *Aspergillus aculeatus*, and Folin–Ciocalteu reagent for protein estimation were purchased from Sigma-Aldrich (Bengaluru, India). Anthocyanidin standards (cyanidin chloride, malvidin chloride, delphinidin chloride) were procured from Sigma-Aldrich (Bengaluru, India). Anhydrous sodium carbonate procured from Sisco Research Pvt. Ltd. (Mumbai, India). Copper(II) sulphate pentahydrate was purchased from Merck Specialists Pvt. Ltd. (Mumbai, India). Ethyl alcohol was purchased from Merck KGaA, Germany. Bovine serum albumin (BSA) was used for calibration for protein estimation, and it was procured from Otto Chemie Pvt. Ltd. (Mumbai, India). Gallic acid standard was obtained from Otto Chemie Pvt. Ltd. (Mumbai, India). All the chemicals listed above were of analytical grade.

Clarification by Microfiltration and Ultrafiltration

Before concentration, the juice was passed through microfiltration and ultrafiltration system. A laboratory scale filtration system (CleanSep, Mumbai, India) attached with hollow fibre membrane (Explorer 12, polysulphone material, effective area 0.005 m^2, inner diameter 0.013 m with a height of 0.312 m) was used for microfiltration and ultrafiltration of Jamun juice. The sample feed volume was 2 l for both the filtration system. Schematic diagram of the filtration process is given in Fig. 1. Membranes with pore sizes of 0.45 μm and 50 kDa were used for micro and ultrafiltration, respectively at a pressure of 0.137 mPa.

Concentration by Nano-filtration

Spiral wound membrane (PS MINI hydracore, polysulphone material, effective area 0.3m^2, inner diameter 0.0457 m with a height of 0.3048 m) was used to concentrate the clarified juice supplied by (CleanSep, Mumbai, India) (Fig. 1). The sample feed (3 l) was supplied to the nano-filtration membrane through a pump (0.5 hp). The inlet (P1) and outlet (P2) pressures were monitored through the attached digital LED display. The trans-membrane pressure was calculated as the average of inlet and outlet pressures. A membrane with pore size of 300 Da and a pressure of 2.5 mPa were used for concentrating the juice. A pressure valve was used to maintain the back pressure throughout the experiment. The filtrate was collected in a measuring cylinder. The time required



1-Feed tank (a) nano filtration (b) ultra and micro filtration; 2-Outlet valve; 3-Pressure gauge; 4- Nano filtration membrane; 5- Peristaltic pump; 6- Micro and ultrafiltration filtration membrane; 7- Pressure control valve

Fig. 1 Schematic diagram of the filtration system

to collect 50 ml of juice was recorded, and the permeate flux (J) was calculated. Volumetric reduction ratio (VRR) was also calculated with the ratio of initial feed volume to retentate volume.

$$\mathrm{VRR} = V_o/V_R$$

where V_o is the initial feed volume and V_R is the retentate volume.

It was useful to determine the nature of the fluid flow and used to calculate the fouling nature. The retentate was recirculated to the feed tank to maintain the concentration of the sample juice. Various physicochemical properties of both filtrate and retentate were analysed.

Physicochemical Analysis

The pH of the samples was measured using a digital pH meter (Elico).Total soluble sugar (TSS, °Bx) was measured by an Abbe-type digital refractometer. Turbidity (Nephelometric Turbidity Units, NTU) was measured by digital turbidity meter (Model 335, Deluxe Company, India) following standard protocols (Sin et al. 2006). Various colour parameters such as brightness/darkness (L^*), redness/greenness (a^*) and yellowness/blueness (b^*) were analysed using a colorimeter (Colour Flex EZ, HunterLab, USA). Clarity of the juice was evaluated by transmittance (%T) at 660 nm in a UV–Vis spectrophotometer (Model: AU 2701, Systronics India Ltd) as described by Ghosh et al. (2016a). Spectrophotometric method was used for measuring both protein and polyphenol concentrations. Protein concentration was determined by modified Lowry method with bovine serum albumin (BSA) (concentration range of 10–100 μg/ml) as standard (Hartree 1972) with a R^2 value of 0.99. Sample volume was 0.1 ml. The absorbance of the sample was measured at 660 nm using UV–Vis Double Beam Spectrophotometer (Systronics India Limited, Ahmedabad, India). Folin–Ciocalteu method with Gallic acid standard was followed to measure the polyphenol concentration (Ghosh et al. 2016a). Folin–Ciocalteu reagent was added to the juice. After 3 min, Na_2CO_3 solution (7.5% *w*/*v*) was added to it and was mixed well. The solutions were kept at room temperature for 1 h and then analysed by UV–Vis

Double Beam Spectrophotometer (Systronics India Limited, Ahmedabad, India) at 750 nm. Five different concentrations were used to plot the standard curve. The standard R^2 value for Gallic acid was 1.

Antioxidant activity of the juice sample was determined as the measure of free radical scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Bermúdez-Soto and Tomás-Barberán 2004). The standard curve has been plotted with DPPH concentration of 10–100 μg/ml. The R^2 value for the standard curve was 0.99. Juice was mixed with DPPH solution (0.002% *w/v* in ethanol), and the mixture was kept in dark for 30 min. Sample volume was 0.01 ml. Its absorbance was measured against the blank sample at 516 nm using UV–Vis Double Beam Spectrophotometer (Systronics India Limited, Ahmedabad, India). The result was expressed as percentage reduction in the DPPH• radicals.

$$\text{DPPH free radical scavenging activity } (\%) = (Y-X) \times 100/Y$$

where X = absorbance of sample and Y = absorbance of the blank sample.

Particle Size Analysis

Particle sizes of the clarified Jamun juice from micro- and ultrafiltration and that of concentrated juice from nanofiltration were measured by Zetasizer (Zetasizernano ZS90, Malvern Instruments, Worcestershire, UK) with a refractive index of 1.36. The average pore size of the membrane was calculated using the following empirical correlation given by Singh et al. (1998).

$$r_{\text{avg}} = 16.73 \times 10^{-10}\ (\text{MWCO})^{0.557}$$

where r_{avg} is the average pore radius (cm) and MWCO is the molecular weight cut-off (Dalton).

Quantification of Anthocyanidins by HPLC Method

A RP-HPLC-photodiode array detector (PDA) system was used for the separation and characterization of Anthocyanidins in Jamun juice samples. A HPLC system (Water, Milford MA, USA) equipped with binary pump, manual injector, PDA (model 996) and run by Empower Pro software (Waters, USA) was used for the analysis. A RP Column (Sunfire C18, 4.6 × 250 mm, 5 μm coating; Waters, USA) was used for the separation and quantification of the above compounds.

The feed as well as concentrated extracts were acid hydrolysed before HPLC analysis as per the method reported by Aqil et al. (2012) with little modification. The extracts (10 ml) were refluxed with 1 ml of 1 N HCl for an hour. Then, after the reaction, mixture was cooled immediately in an ice bath to stop the hydrolysis process. The reaction mixture was purified and concentrated in a column packed with Amberlite XAD-7 resin for adsorption of the organics as per our reported procedure (Rout et al. 2015). Further organics were desorbed from the resin using acidified (10 mM HCl) ethanol as eluent. The enriched extracts were dried in a rotary evaporator (Buchi Rotavapor R-210) at 150 mbar pressure.

The stock solution of each anthocyanidin standard was dissolved in methanol (*v/v*) at a concentration of 1 mg/ml. Sample analysis was carried out at 30 °C using gradient solvent system. The mobile phases for gradient elution comprised of solvents A (3.5% aqueous phosphoric acid) and B (methanol, 100%), mixed according to the following profile: 5% A (0–10 min), then 5% A to 10% A (10–20 min), then 10% A to 13% A (20–30 min), then 13% A to 18% A (30–35 min), 18% A to 25% A (35–40 min), 25% A to 60% A (40–41 min), 60% A (41–61 min) and 60% A to 5% A (61–65 min). The injection volume of standards and extracts was 20 μl with 0.75 ml/min flow rate. Signal detection at 268 nm was used for the quantification of anthocyanidins (Aqil et al. 2012).

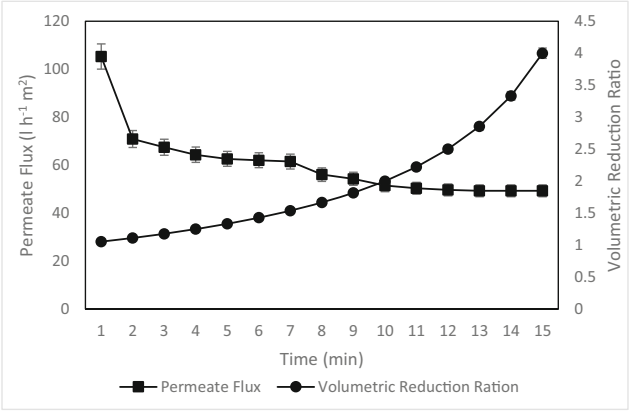
Statistical Analysis

All the experiments for filtration and physicochemical properties were determined in random order in triplicate. All results obtained were expressed as mean ± SD of three independent evaluations and were analysed by statistical program (SPSS21.0) with the help of analysis of variance (ANOVA). Least significance difference test (LSD) was performed to detect any statistically significant difference ($p < 0.05$).

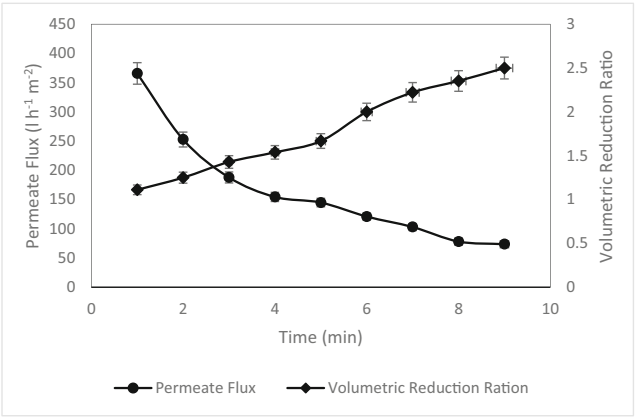
Results and Discussions

Permeate Flux of UF and NF Processes

Effect of flux behaviour for UF and NF was evaluated with time given in Fig. 2a, b. In case of ultrafiltration, permeate flux was 105 l/hm^2 with volumetric reduction ratio (VRR) of 4 at a pressure of 0.137 mPa. There is a sudden change in permeate flux in the first minute, then the rate of change of permeate flux decrease and became constant after 9 min. Permeate flux was high (366 l/hm^2) at a higher pressure (2.5 mPa) during nano-filtration. It decreased with time (Fig. 2b). At the initial stage, permeate flux decreased significantly and stabilised after 8 min. This initial decrease is because of membrane fouling. Rapid change in permeate flux is due to polarization of



(a)



(b)

Fig. 2 Effect of time on VRR and permeate flux during ultrafiltration (**a**) and nano-filtration (**b**) of Jamun juice

solute concentration. VRR ranged between 2.5 and 1.1 which stabilised at the later stage. This value signifies that the concentration of the filtrate is decreasing. The trend of permeate flux was due to presence of macromolecules. It reduces the flow rate which is responsible for decreasing permeate flux after a certain time period (Bacchin et al. 2002; Nabetani et al. 2009; Tanaka et al. 1998). Similar results have been reported by Khemakhem et al. (2017). In case of UF, deposition of macromolecular particles was more than that of NF. Deposition of polyphenols at the membrane barrier reduces the mass transfer of the permeate resulting in high pressure (Dammak et al. 2015). Higher operating pressure caused lower recovery and membrane fouling (Cai et al. 2017). Maximum recovery rate for permeate flux was at 2.5 mPa. Membrane fouling could be avoided at this pressure.

Physicochemical Properties

Colour is one of the major quality parameters in the beverage industry. Colour can also suggest the presence of anthocyanins and flavonoids (Arend et al. 2017). The colour properties of the samples are given in Table 1. For permeate, the lightness of the juice (L^* value) increased after ultrafiltration process. The redness (a^* value) and blueness (b^* value) decreased because of deposition of anthocyanins at the membrane module and the purple colour of the retentate became darker. The overall purple colour of the juice was preserved until the end of the process.

The pH of the juice remained constant even after passing through nano-filtration membrane. The TSS of the permeate and the retentate were 10.17 and 19.53°Bx, respectively. Due to less suspended particles and other micromolecules, clarity of the permeate increased up to 95%T and the turbidity decreased to 0.03NTU. Clarity and turbidity of the retentate were 70.43%T and 1.43 NTU, respectively. Similar type of observation has been reported for strawberry juice (Crecente-Campo et al. 2012).

The protein, polyphenol content and antioxidant properties of the samples are given in Table 2. In case of ultrafiltration protein, polyphenols cannot pass through the membrane module due to high molecular weight cut-off of membrane. Maximum concentration was found in the ultrafiltered retentate. During nano-filtration, protein (55%), polyphenol (75%) and antioxidants (70%) could be recovered in retentate. In this case, rejection rate is more than that of ultrafiltration. This high recovery of

Table 1 Physicochemical properties of ultrafiltered and nano-filtered feed, permeate and retentate

	L^*	a^*	b^*	pH	TSS (°B)	Turbidity (NTU)	Clarity (%T)
UF feed	0.80a ± 0.02	0.45a ± 0.01	0.13a ± 0.02	3.50a ± 0.05	17.2a ± 0.001	8.56a ± 0.001	83.5a ± 0.001
UF permeate	0.17b ± 0.01	0.47b ± 0.01	0.26b ± 0.01	3.6b ± 0.23	16.07b ± 0.34	0.03b ± 0.001	89.33b ± 0.41
UF retentate	0.127c ± 0.006	0.463b ± 0.01	0.21c ± 0.01	3.69c ± 0.05	16.61c ± 0.02	7.24c ± 0.006	70.23c ± 0.05
NF feed	0.17a ± 0.01	0.47a ± 0.01	0.26a ± 0.01	3.6a ± 0.23	16.07a ± 0.34	0.03a ± 0.001	89.33a ± 0.41
NF permeate	0.44b ± 0.01	0.98b ± 0.02	0.62b ± 0.01	3.52a ± 0.11	14.32b ± 0.21	0.03a ± 0.001	94.4b ± 0.57
NF retentate	1.15c ± 0.02	0.91b ± 0.02	0.42c ± 0.01	3.56a ± 0.16	19.36c ± 0.32	0.17b ± 0.011	70.43c ± 0.37

Different letters in the same column indicate significant differences ($p < 0.05$) between fractions of the same membrane

Table 2 Presence of protein, polyphenol and antioxidant in feed, permeate and retentate of both ultrafiltered and nano-filtered feed, permeate and retentate

	Feed	Permeate	%R	Retentate
Ultrafiltration				
Protein (mg/g)	209.4a ± 1.63	109.43b ± 0.87	47.74	216.17c ± 1.66
Polyphenol (mg GAE/g)	161.9a ± 0.94	125.74b ± 0.98	22.33	257.65c ± 1.68
Antioxidant (% DPPH)	69.31a ± 0.73	34.22b ± 0.12	50.62	55.27c ± 0.77
Nano-filtration				
Protein (mg/g)	109.43a ± 0.87	40.63b ± 0.34	62.87	120.42c ± 0.96
Polyphenol (mg GAE/g)	125.74a ± 0.98	32.27b ± 0.26	74.33	90.71c ± 0.67
Antioxidant (% DPPH)	34.22a ± 0.12	3.61b ± 0.02	39.61	89.45c ± 0.53

Different letters in the same row indicate significant differences ($p < 0.05$) between fractions of the same membrane. %R referred as retention of the particular compound in the permeate

macromolecules could be because of lower molecular weight cut-off (MWCO) and reversible fouling at the membrane. Concentration of phenolic compounds and antioxidants by nanofiltration has been mentioned by several researchers (Cai et al. 2017; Machado et al. 2013; Vivekanand et al. 2012). Khemakhem et al. (2017) have observed similar trends in result for concentration of oleuropein from the extract of olive leaves.

Particle Size Distribution

The particles size distributions of feed, nano-filtered permeate and retentate are given in Fig. 3. The NF feed had a maximum size distribution intensity of 25% for 1–3 nm. The NF filtrate had a size distribution of 0.19–0.45 nm with a maximum intensity of 30%. However, retentate had a significantly low intensity (13%) with a wide size distribution range. NF feed passed through 50 kDa membrane which is equivalent to a pore size of 6.93 nm. NF filtrate passed through 300 Da membrane which is equivalent to a pore size of 0.43 nm. According to the particle size distribution, the presence of macromolecules (sugars, protein and polyphenols in the form of anthocyanin) is maximum in the NF retentate. In case of NF filtrate, micromolecules (salt, sugar in the form of monomers, protein in the form of amino acids) are present. These data are in line with the theoretical and graphical values. All the three distributions have single peaks suggesting proper filtration.

Anthocyanidin

The anthocyanidins (cyanidin chloride, malvidin chloride and delphinidin chloride) in the ultrafiltration samples (feed, permeate and retentate) and nano-filtration samples (permeate and retentate) were quantified using HPLC (Fig. 4). The

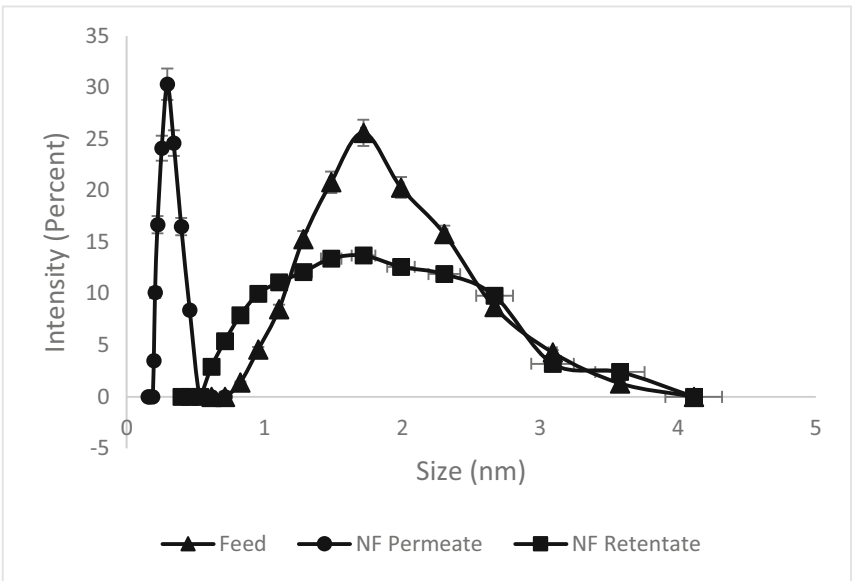


Fig. 3 Intensity vs. particle size distribution for nano-filtration

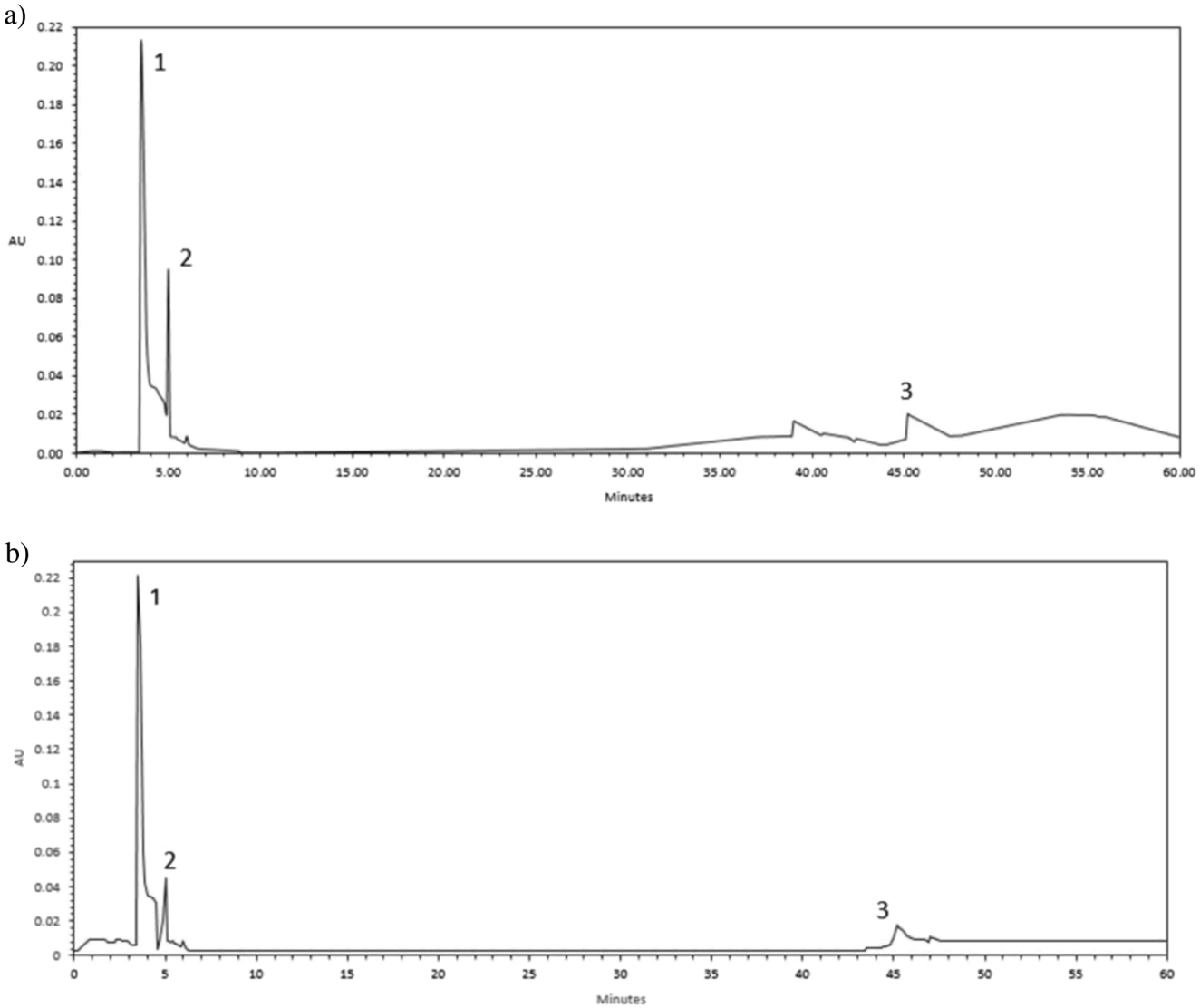


Fig. 4 HPLC chromatograms of anthocyanidins [1—cyanidin chloride (3.5 min); 2—malvidin chloride (5 min); 3—delphinidin chloride (45.2 min)] in original juice (**a**), UF filtrate (**b**), UF retentate (**c**) and NF retentate (**d**)

molecular weights of the above-mentioned anthocyanidins were 322.67 g/mol, 366.75 g/mol and 338.69 g/mol, respectively. These molecules could not pass through 300 Da membrane pore size and remained in the retentate of NF. Most of the anthocyanidins passed through the ultrafiltration membrane along with permeate. This could be because of larger membrane pore size than the molecular weight of the materials. The anthocyanidin retention was in the range of 15–17% in ultrafiltration. Remaining 83–85% anthocyanidins passed to permeate of the ultrafiltration and became the feed for nanofiltration. Table 3 suggests that there was no anthocyanidin in permeate of nano-filtration. Most of the anthocyanidins (5.9 mg/10 g of cyanidin chloride, 20.8 mg/10 g of malvidin chloride and 3.6 mg/10 g of delphinidin chloride) were found in the concentrated juice (Fig. 4d). The enrichment of cyanidin chloride, malvidin chloride and delphinidin chloride is 1.2-, 2.47- and 1.05-fold respectively. Total amount of anthocyanin present in the initial feed was 1.64 mg/ml, whereas in the NF retentate, the amount was 3.03 mg/ml. Hence, the concentration became double. Similar observations have been reported by various researchers for other fruits (Arend et al. 2017; Khemakhem et al. 2017; Li et al. 2017; Mojica et al. 2017).

Conclusion

Jamun is known as wonder fruit due to its medicinal properties. The fruit is unexploited and available only in the monsoon month (June to September) due to its unavailability throughout the year. The juice concentrate can be used to obtain high amount of anthocyanidin. The combination of ultra- and nano-filtrations could be used to concentrate

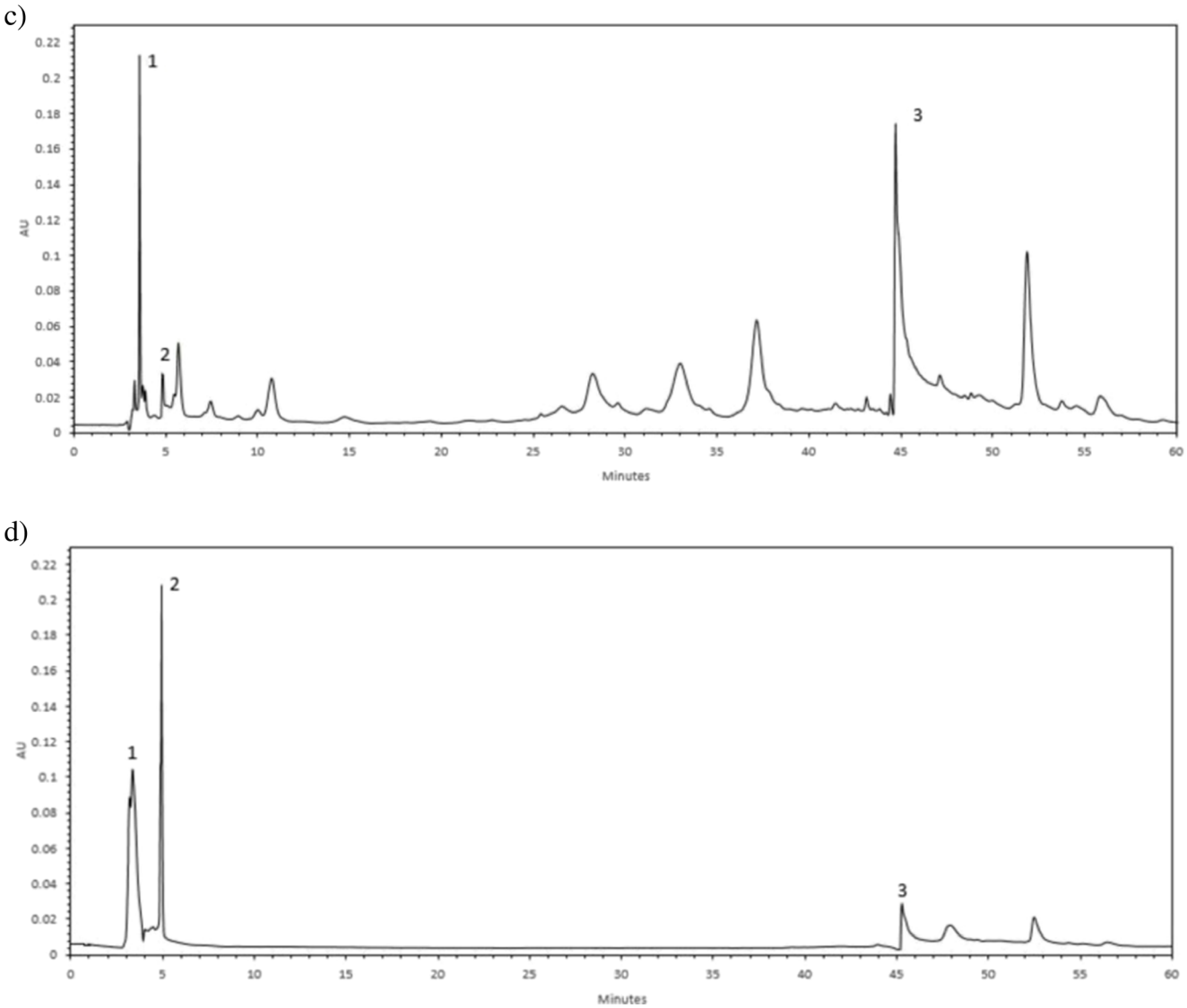


Fig. 4 continued.

Jamun juice. The concentrated product was rich is in protein (120.42 mg/g), polyphenols (90.71 mg GAE/g) and antioxidants (89.45% DPPH). Maximum amount of anthocyanidins (0.59 mg/g of cyanidin chloride, 2.08 mg/g of malvidin chloride and 0.36 mg/g of delphinidin chloride) could be recovered in the product by nano-filtration from *Jamun* juice using

Table 3 Quantification of the anthocyanidins present in the ultrafiltration and nano-filtration process

		Cyanidin chloride (mg/10 g)	%R	Malvidin chloride (mg/10 g)	%R	Delphinidin chloride (mg/10 g)	%R
Ultrafiltration	Feed	4.6a ± 0.01		8.4a ± 0.02		3.4a ± 0.01	
	UF permeate	3.8b ± 0.01	17.39	7.1a ± 0.01	15.47	2.8b ± 0.01	17.64
	UF retentate	1.2c ± 0.01		1.6b ± 0.02		0.9c ± 0.01	
Nano-filtration	Feed	3.8a ± 0.01		7.1a ± 0.01		2.8a ± 0.01	
	NF permeate	0	100	0	100	0	100
	NF retentate	5.9b ± 0.02		20.8b ± 0.02		3.6b ± 0.01	

Different letters in the same column indicate significant differences ($p < 0.05$) between fractions of the same membrane. %R referred as retention of the particular compound in the permeate

a membrane of 300 Da at a pressure of 2.5 mPa. The particle size distribution was in the range of 0.19–0.45 nm with an average NF membrane pore radius of 0.43 nm. The filtration method for concentrating the juice has a benefit as it runs under low temperature. The concentrated product can be used in the pharmaceutical and beverage industry.

Compliance with Ethical Standards

Conflict of Interest The authors declare that there is no conflict of interest.

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