Standardization of conditions for effective clarification and concentration of green tea extract by membrane filtration

S Ramarethinam, G R Anitha and K Latha*
The United Nilgiri Tea Estates Co Ltd., Race Course, Coimbatore 641 018

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Studies on clarification of tea extracts with membrane filters was carried out, as a mode of concentration, of the soluble components of tea, prior to drying to reduce creaming in tea based products. The reduction of flux rate was high in reverse osmosis membranes microfiltration compared to ultrafiltration, and prefiltration enabled removal of mainly particulate components of the tea. Catechin content was high in the retentate under 1000 Kpa pressures in all types of filtration process as compared to the original content in the fresh green tea leaves. Alkali and enzyme treatments of the precipitate for maximum recovery of catechins that developed on chilling after filtration improved the solubility of the extract. A partial reduction in the pectin content in the retentates with a concomitant increase in carbohydrates, protein, turbidity, catechins was noted with ultrafiltered and microfiltered extracts. Ultrafiltration caused a rejection of 24% pectin, 23.40% catechins in permeate, and 18.11% caffeine in the green tea extract.

Keywords: Catechins, Clarification, Green tea extracts, Membrane filters

Introduction

Tea components, soluble polyphenols (20-25%) as flavanols and their derivatives, contribute to flavour, colour and mouth feel of tea. The preparation of polyphenols extracts, essentially comprises of extraction of water soluble components and involves the use of tea waste, with pretreatment of tannase followed by hot water extraction and concentration. During these operations, a large part of the volatile substances responsible for flavour are lost and since concentration of the extract thermally damages the quality of tea, an alternate mode of concentration through membrane filtration was studied. Several technical applications utilise synthetic polymer membranes for separation and isolation of components as it retains the quality of the product in addition to being a process of low energy consumption. Reverse osmosis is an effective alternative to evaporative techniques and membrane separations energy efficient process for concentration of fractionated liquid products. Clarification is an important step in the production of packaged green and oolong tea drinks, and membrane processes have the advantage of being non-thermal and reducing the sediments after packaging.

Present study relates to the clarifications and concentration of polyphenols of tea (Camellia sinensis Linn.) with prefilter, microfilter, ultrafilter, and reverse osmosis membranes. The extracts were estimated for important constituents after each step of filtration and the precipitates from filtration process were subjected to alkali and enzyme treatment to reduce loss of polyphenols and analysed. These extracts have potential use singly or in combination with other active principles in the food, pharmaceutical, and cosmetic industry.

Materials and Methods

Green tea as BOP grade was obtained from Chamraj Tea Estates, Nilgiris. Extraction was carried out with tea (1 kg) in water (10 l) at 90-100°C for 10 min. and filtered in 140-mesh sieve. Extract was rapidly cooled (27°C) and used for experiments as green tea crude extract. Operating pressure applied was 1000 Kpa. The change in components concentration after filtration was assessed and analysed in terms of polyphenols, catechins, carbohydrate, protein, caffeine and pectin. Pre-filters (membrane area, 0.10 m²) were used for initial clarification after filtration through a nylon membrane cloth of 140-mesh sieve without, operating pressure to remove large insoluble particles. This extract was then forced through the membrane separation...
systems under pressure resulting in a water phase, containing a small quantity of dissolved extract and the concentrated extract. Ultrafiltration membranes were made of polyethylen sulphone material (normal pore size, 0.014µ). Membrane area of the ultra filtration unit was 0.20 m² and sediment dry weight was estimated after centrifugation (REMI Cooling Centrifuge) at 5000 g, for 20 min twice and dried in vacuum at 50°C. Feed volume (10 l) was reduced to approx 8.5 l after the prefiltration and further reduced to 7.8 l after ultrafiltration. The volume of permeate was very low in microfiltration and ultrafiltration compared to the reverse osmosis membrane.

Microfiltration unit was a laboratory model (Alwin Pumps & Systems Aro model MF – Mini Lab) that allowed a continuous filtration of liquid, and solids being rejected (>1µ). These systems used polystyrene membranes (area 0.02 m²) housed in polyurethane in a cross flow configuration. The extract was passed around the hollow fibers with sediments accumulating on the outside of the fibers, while the filtered extract was passed through membranes into the center, which is permeate filter. Unfiltered part of the extract was retained. A lab model reverse osmosis unit was used with a plate and frame type membrane (pore size, 0.02µ) at operating pressure of 200-3000 Kpa. Clarification levels, as turbidity was measured at 660 nm by using UV-VIS spectrophotometer (Chemito 2100). Caffeine, catechins, polyphenols, carbohydrate, protein and pectin were estimated in the ultra and microfiltered extracts at different pressures (500, 1000, 1500 Kpa) and the flux with time was calculated in terms of l/m²/h. Retentate after filtration were chilled overnight and centrifuged at 5000 g for 20 min. Precipitate was subjected to enzyme and alkali treatment for further solubilization and estimated of polyphenols and catechins. The extract after passing through the reverse osmosis membranes was subjected to analysis in the first retentate, which was then returned and passed through the reverse osmosis membranes for the second time, to get the second retentate and also in the water that was used to wash after completion to test for any loss, while processing. The membrane function was restored by cleaning with sodium hypochlorite (10 ppm) solution, and flushing.

Results and Discussion

Clarification levels, rejections of the components with microfilters, ultrafilters and reverse osmosis membranes, were assessed in this study. Pre-filter treatment was effective (wound cartridge) in increasing flux rate, and the flow was stable, as the pores were not completely blocked. The extract was passed through 140-mesh sieve prior to pre-filtration. In aqueous tea extracts, substances of varying solubility’s are encountered, with one fraction of the extracted solids, being readily soluble in hot water, but essentially insoluble in cold water. Chilling (10-18°C) and separating the precipitated tannins from the aqueous supernatant by centrifugation removes the cold-water insoluble polyphenol fraction, constituting about 10% of the total tea solids. Water-soluble portion is concentrated, and the insoluble fraction generally discarded. To avoid substantial loss of the tea components other possible alternative tried out was by treating the precipitates of the cold-water insoluble tannin fraction of a chilled aqueous tea extract. The precipitate that was subjected to oxidation under highly basic condition or by tannase treatment adequately improved its solubility. Thereafter, insoluble fraction is acidified to return to its normal pH, and recombined with the other fraction of the original extract before spray drying to minimize loss of the desired components. Treatment of fresh green leaves with an aqueous suspension of tannase in presence of water in the temperature range, in which tannase was active, improved the characteristics of tea extracts and substantially reduced the amount of tea cream, during polyphenol extraction. The addition of alkali reagents for solubilization of the polyphenols complex was not required, when high concentration of the enzyme was used for the dissolution of the insoluble polyphenols, but can be expensive.

Dry-weight at various levels of filtration showed a removal of 66% of the insoluble solid tea components in the prefiltration stage, and 19% removal at the ultrafiltration stage (Fig. 1). The pore size of the prefilter was 5µ. As the pore size of the microfilter membranes and ultrafilters, reduced, dry weight decreased gradually. The optical transmission increased at the ultrafiltration stage in the retentate (0.24%) compared to the permeate (0.18%).

Prefiltration step rejected particles larger than 5µ, and there was a partial removal of macromolecules in the ultrafilters (0.01 µm), and in microfilters (1 µm). Rejection of solutes by ultrafilters depends on the number and pore size (0.008-0.014 µ). Since the macromolecules in permeate are larger than the pore size of ultrafilters, rejected solids are concentrated on the membrane surface, as filtration is by molecular
diffusion under pressure. Effects of membrane pore size, on dry weight of the insoluble solids in the retentate, shows that the dry weight decreased with increased pressure and reduced at 1500 Kpa pressure (Fig. 1). Turbidity levels decreased in the prefilter extract by 24.45%, in ultra-filter retentate by 3.05%, at 500 Kpa pressure, an increase of 3.78% at 1000 Kpa pressure, with a decrease of 2.62% at 1500 Kpa pressure, and a decrease in the permeate at 18.77% (500 Kpa pressure) compared to the fresh green tea extract, 22.27% (1000 Kpa pressure) and 24.89% (1500 Kpa pressure).

Brix of the fresh green tea extract was 4-4.5° and there was no change after microfiltration, since soluble components passed through the microfilter membranes. The extract forms the first permeate after filtering through membrane (pore size, 10 µ). In the crude extract, prior to filtration, dry weight and optical transmission were 660 g and 0.21 absorbance units respectively. After prefiltration, 60-70% insoluble components removed and the optical transmission increased up to 0.27 absorbance unit.

Dry weight decreased gradually and optical transmission increased. Optical transmission (95%) of the permeate was similar to that of ultra filter membrane (Fig. 1). On the other hand, brix value decreased approx to 3° after ultrafiltration treatment as 20-25% of pectin was reduced (Table 1). The rejection of catechins in the permeate (190-290 mol wt cut off) with microfilters was more compared to ultrafilters. Though caffeine and catechins are smaller, rejection was lesser as they are generally found as complexes with polyphenols, carbohydrates etc.

Protein content increased in the concentrate by 58.8% in the ultrafilter retentate at 500 Kpa pressure, 105.8% increase at 10 kg pressure and 11.76% decrease in the retentate at 1500 Kpa pressure (Table 1). Ultrafiltration thus showed an increase in protein content at 1000 Kpa, and a reduction at 1500 Kpa pressure. Increase in carbohydrate content in the prefilter extract, and after passing through ultrafilters at 500 Kpa pressure and a decrease of 29.7% at 1000 Kpa pressure, 27.02% at 1500 Kpa pressure in retentate and also a decrease in the permeate at 500 Kpa pressure. The permeate showed reduction in concentration of polyphenols at the rate of 23.47% at 500 Kpa, 42.55% at 1000 Kpa and 61.70% at 1500 Kpa pressure. Ultrafilter treatment removed 23.5% of pectin at 500 Kpa pressure retentate, compared to high-pressure treatment that aids in retardation of sedimentation in tea.

Entrapment of particles inside the pores and the layer formation on the microfilters were observed high compared to the ultrafilters. The yield after filtration in the ultrafilters, with an initial feed rate of 10 l of the extract, was 7.8 l of retentate and 0.1 l

Fig.1—Effect of filtration pressure on insoluble solids and optical transmission

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<th>Table— Effect of filtration pressure on tea components</th>
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<td>Increase (+) or decrease (-) compared to fresh green tea extract</td>
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<tr>
<td>Pre filter retentate</td>
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<td>Micro filter retentate</td>
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<td>Micro filter permeate</td>
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<td>Reverse osmosis retentate</td>
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permeate. In the retentate, catechin content was concentrated from 4.7 to 5.8% and caffeine content reduced from 1.27 to 1.05%. Significant changes in the caffeine content were not observed in the various treatments given both in the microfilter and ultrafiltration studies.

Concentration of catechin was high, after treating the precipitate with alkali, more so in the treatment with sodium bicarbonate than enzyme and sodium hydroxide. Total polyphenolic content was high in precipitate solubilised with enzyme than alkali treatment with an increase of 13.5% (Fig. 2). Concentration of catechins was recovered by either of the alkali or enzyme treatment as the precipitate showed only 18% of the catechin present in the retentate.

Study indicates that the prefilter treatment is effective in increasing the flow rate, and the flux reached the levels of 3600 l/m²/h. With ultrafilter membranes, though the flux rate was initially low, it was stablised later till completion, as the pores were not totally blocked. Ultrafiltration system (membrane size, 0.2 m²; flow rate 1 l/min) showed that an initial feed volume of 10 l was reduced to 7.8 l.

Clarification was observed better with ultrafilters than the microfilters and the molecular cut off need to be still smaller for effective clarification, and for concentration, reverse osmosis membranes were suitable as the concentration of polyphenols was significantly increased (33 %) in the second concentrate. Solubility limit of the extract was increased with enzyme and alkali pretreatment of the precipitate after chilling, thereby enhancing concentration of the components. Additionally, since tea extracts contain important constituents that are susceptible to reaction or loss during processing, it is advantageous to use membrane separation to minimize the loss of desired components, when compared to thermal concentration. Efficiency of the membranes in terms of the tea components showed that a combination of reverse osmosis membranes with ultrafilters was more efficient than with the microfiltration system.

Conclusions

Clarification performance, flux and rejection of components with different membranes was studied in cross flow filtration mode. The particle size cut off was reduced considerably even with a larger pore size, due to blockage of the particles, thereby restricting the small size particles. Optimum conditions to maximize concentration of the soluble components in tea were a pre-filter (10 µ), with an ultrafilter (0.014 µ) at a trans-membrane pressure of 1000 Kpa with a flow rate 1.2 l/min. It can be concluded that combination of ultrafiltration and reverse osmosis treatment was suitable for clarification and concentration of green tea extracts. The effects of various molecular weight cutoffs in ultrafiltration membrane need to be studied in greater detail, as the study was restricted to one pore size filter. The process of extraction, concentration and recovery of these vital components, in conjunction with the manufacture of the value added tea and tea based products is of paramount importance in future studies.

References


