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R. Subramanian [a], Chandini S. Kumar [a] & Pankaj Sharma [a]
[a] Department of Food Engineering, Central Food Technological Research Institute, Council of Scientific and Industrial Research, Mysore, 570020, India
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Membrane clarification of tea extracts

R. SUBRAMANIAN*, CHANDINI S. KUMAR and PANKAJ SHARMA[1]

Department of Food Engineering, Central Food Technological Research Institute, Council of Scientific and Industrial Research, Mysore-570020, India

*Corresponding author

Tel: +91 821 251 3910; Fax: +91 821 251 7233

E-mail: subbu@cftri.res.in (R. Subramanian)

[1]Present address: Department of Food Science, University of Otago, Dunedin, New Zealand

The RTD (ready-to-drink) tea beverages are becoming increasingly popular owing to the health benefits associated with tea polyphenols, but instability due to development of haze and formation of tea cream is a common problem encountered in the product. Membrane technology provides a scope to produce natural-additive free RTD teas while overcoming the major disadvantages associated with the conventional decreaming methods. Approaches employing membranes for the clarification of extracts from black and green tea have been discussed along with their relative advantages and limitations. The article also outlines the concerns to be addressed in the future attempts employing membrane technology.

Key words Black tea, green tea, membrane technology, polyphenols, RTD teas, tea cream

INTRODUCTION

Tea is the one of the most widely consumed beverage worldwide, made from the processed leaves of *Camellia sinensis.* It is characterized by its high polyphenolic compounds, tea flavanoids and their associated antioxidant properties which offer several health benefits (Wiseman et al., 1997). Traditionally, tea produced is classified into black (fully fermented),

oolong (partially fermented) and green (unfermented) tea based on the period of fermentation, the leaves and buds have undergone during processing. This process is not a true fermentation but an enzymatic oxidation, herein simple polyphenols undergo an enzymatic polymerization by tea polyphenol oxidase leading to formation of complex condensation compounds. The amount of polyphenols in fresh leaf, green and black teas are in the range 30-35%, 10-25% and 8-21%, respectively (Lunder, 1992). The polyphenolic composition of green, oolong and black tea leaves is mainly responsible for the taste, colour, astringency and delightful aroma of their infusion.

India is the second largest producer (21%) after China (32.8%) and one of the largest consumers of tea in the world (FAO, 2008). Tea processing has undergone many changes over the last 100 years, from loose to blended tea, tea packets, tea bags, instant teas, and finally ready-to-drink (RTD) teas. USA, China and Japan are the important markets for RTD teas, which are catching up with other countries as well. Traditionally, cold tea is prepared by infusion of tea leaves and then taste enhancers like sugar or lemon juice are added, which is then cooled before consumption. Industrially, it is generally prepared by using tea extracts or reconstituted tea powder with addition of sugar, lemon/peach juice, citric acid and colorants to modify its flavour, taste and colour. Besides, various additives are used as stabilising agents. Such type of cold teas, available in the market, does not ideally meet the consumers' demands who are looking for additive-free foods of high nutritional value (Todisco et al., 2002). One of the most relevant problems encountered in the production of natural and additive-free RTD cold tea is its instability due to development of haze and formation of tea cream, especially so when the expected shelf life of RTD teas is commonly 6–12 months.

Cold-water insolubles are known as 'tea cream' in the art. Tea catechins and their oxidation products when interact with caffeine, protein, pectins and metal ions in the extract form larger complexes that eventually precipitate out (Ekanayake et al., 2001). Tea cream formation is governed by various types of interactions, including polyphenol–caffeine (with or without lipid) and polyphenol–protein interactions (Jobstl et al., 2005). Factors affecting cream formation are the concentration, composition, pH and temperature-time history of the infusion (Tolstoguzov, 2002). Studies on the influence of extraction conditions in black tea extracts revealed that the conditions standardized for greater polyphenols content in extracted solids, also co-extracted other tea components (proteins, calcium and pectin) responsible for various interactions leading to tea cream formation (Chandini et al., 2011). Liang et al. (2002) reported that caffeine, gallocatechin (GC) and epigallocatechin gallate (EGCG) were the predominant compounds in green tea cream while thearubigins (TRs), caffeine and theaflavins (TFs) in black tea cream. Gallated compounds offer more hydroxyl groups for hydrogen bonding for tea cream formation. Also oxidation products of catechins have a stronger creaming capacity than the unoxidized catechins. Hence, the cream formation is lesser in green tea compared to oolong and black tea. However, average size of cream particles was bigger in green than black tea.

Decreaming is an important step in the process to meet the cold stability requirements of RTD teas. Conventional decreaming methods (removal of the precipitating complexes) include clarification by centrifugation/filtration after adjustments in temperature, enhancing the solubility by employing chemicals and enzymes (Chandini et al., 2011), and other equivalent techniques or combination of these methods. Cream composition is similar to tea, therefore decreaming employing any separation technique results in loss of flavor, color, and taste including health

enhancing polyphenols. Also chemicals and enzymes are not preferred in the production of additive-free natural products. Membrane technology could overcome some of these disadvantages.

During enzymatic polymerization, ~10% of the catechins are converted to TFs, bisflavanols and other oligomers with molecular weights of 500-3000 Da and 75% of them are converted to TRs in black tea. The size of TRs is reported to be in the range of 700-40000 Da (0.002-0.04 μm) (Todisco et al., 2002). Green tea extracts initially contain high levels of unoxidized flavanols, especially monomeric catechins such as EC, ECG, EGC and EGCG that impart a desired taste (astringency) to the beverage. Unfortunately these catechins remaining in the extract will still be oxidized over time to the less desirable oxidized polyphenols (Ekanayake et al., 2001). Evans and Bird (2006) suggested that potentially a physical barrier could be used to separate polyphenols including TRs from the larger cream aggregates since the majority of black tea cream particles formed (84.8%) is in the size range of 0.1-1.03 μm (Liang and Xu, 2001). However, it may be desirable to employ techniques to prevent/reduce tea cream formation while retaining natural characteristics. In the last few decades, some attempts have been made employing membrane technology (pressure driven) for the clarification of extracts from black and green tea demonstrating its capability, which are discussed in this article with their relative advantages and limitations.

CLARIFICATION APPROACHES FOR GREEN TEA

Kawakatsu et al. (1995) used six MF (pore size 0.5-10 μm) and four UF (MWCO 30-100 kDa) membranes in their studies on clarification of hot water extracts of green tea (12-fold

extract). Turbidity was measured as transmittance at 660 nm for assessing the clarity. As the pore size of the MF membranes became smaller, the dry weight decreased gradually (Fig. 1) and optical transmission increased (Fig. 2). The 500 nm membrane exhibited the same level (95%) of transmittance as that of UF. However, UF offered the additional benefit of eliminating pectin to the extent of 30-50%. Partial removal of pectin retards the generation of secondary cloudiness and sedimentation in products. Optical transmission improved from 0.2% in crude to 95% after membrane filtration (Fig. 2). Although catechins and caffeine are small molecules, they were partially rejected (3-20%) by UF membranes as they might form complexes with larger particles. Prefiltration was effective in decreasing the reduction rate of flux. Cross-flow filtration was effective in keeping the flux high compared to dead-end filtration. The authors suggested UF employing a 30 kDa membrane for the clarification along with a prefiltration step. Dry weight of solids estimated in the present study was essentially the undissolved solids and not the soluble solids. Although the membrane filtration effectively removed these insolubles, in addition it has also removed some of the soluble solids as reflected by the transmittance values, which has a direct impact on the product yield. Besides, it may not be appropriate to rely upon transmittance for measuring clarity.

Ekanayake et al. (2001) proposed a clarification process involving treatment with a strongly acidic food grade cation exchange resin followed by nanofiltration (NF). Acidic extraction of green tea is preferred as described in their earlier patent (Ekanayake et al., 1995). The key aspect of the process is the cation exchange treatment which improved the clarity by removing metal cations (especially Ca and Mg) that combines with pectin or protein to form complex molecules besides acting as oxidation catalysts of catechins. During NF (MWCO 800-

2000 Da) high molecular weight compounds such as pectins, proteins, chlorophylls, TRs, some TFs and other oxidation products that form complexes with any residual metal ions in the extract are removed thus improving the clarity further. The additional benefit of the process is that the filtered extract is enriched in theanine that mellows the astringency imparted by the catechins. Theanine also suppresses the characteristic aftertaste of aspartame in diet beverages. The inventors have claimed a clarified extract with the following characteristics (on a 1% soluble solids basis): an enriched level of desired catechins, at least ~50 ppm of theanine, maximum levels of each of the metal ions (Ca, Mg, Mn, Al, Zn and Fe) <10 ppm, and with an absorbance of ≥ 0.060 at 600 nm.

Choi et al. (2005) proposed a process for reducing the bitter or astringent taste in green tea extract without a loss of catechins. The catechins in the extract are oxidized in to TFs and TRs catalyzed by metal ions. These oxidized polyphenols and the complexes formed thereof in combination with caffeine, proteins, pectins and metal ions, are responsible for the bitter taste. UF allowed permeation of catechins with only in their non-oxidized form and improved the transparency while rejecting polysaccharides along with oxidized polyphenols, polymerized intermediates and other complexes. Lower MWCO membrane (500 Da) resulted in greater removal rate of bitterness but affected the catechins transmission suggesting a preferable MWCO range of 1-3 kDa (60-95% transmission). The oxidized polyphenols or polymers display greater absorbance between 310 and 600 nm, and the spectra of UF extract showed their reduction. Sensory analysis of filtered extract showed that bitter or astringent taste was removed and, clarity and taste improved after UF. Such a process approach would allow higher concentrations of catechins in green tea beverages.

Ramarethinam et al. (2006) used MF, UF and RO for clarification and concentration of green tea extract prior to drying as a means to reduce creaming in tea based products. A prefiltration step was employed before membrane filtration and transmittance as high as 95% (@ 660 nm) was achieved in the filtrate. UF (pore size 0.014 μm) showed higher level of clarification than MF (>1 μm). Absorbance value of permeate was lower (0.180) compared to its retentate (0.240) during UF (VCR 12). However, these measurements were not carried at uniform strength and solids concentration varied during processing. UF permeate showed a 43% reduction in polyphenols. Cold water insoluble tannins were precipitated out from retentate by centrifugation after overnight chilling and improved their solubility either by oxidation with alkali or by tannase treatment. This reclamation step reduced the loss of polyphenols in the processed stream. Total polyphenols content was high in the precipitate solubilized with enzyme than alkali treatment while catechin concentration was higher with sodium bicarbonate. The authors proposed a combination of UF and RO for clarification and concentration of green tea extracts.

Sugiyama and Ueoka (2006) developed a process for producing purified green tea extract containing greater concentration of non-polymeric catechins employing MF. The inventors proposed MF to overcome the limitations of UF (MWCO 6-100 kDa) based processes proposed earlier; although UF provided high clarity and purity, permeation characteristics especially flux decline with processing time was severe in the process. According to the process described, extract is subjected to filtration (200-mesh) and or centrifugal separation (5000 rpm for 30 min at 25°C) before MF to improve the permeation rate through the membrane. Turbidity was measured at 1% concentration of non-polymeric catechins as absorbance at 700 nm. The pore

size of the membrane is critical and selected preferably from a range, 0.08-0.5 μm. A smaller pore size could lead to reduced filtration rate whereas a larger pore size could affect the separation. During MF turbidity of prefiltered extract (0.200-2.00) could be reduced to <0.200. The purified extract is converted in to powder by known methods of concentration followed by drying. In the accompanying examples, turbidity of prefiltered extract (0.29-0.38) could be reduced to 0.03-0.06 after MF using various pore size (0.06-0.25 μm) polymeric membranes (Table 1). Using comparative examples, criticality of pore size of the membrane and prefiltration were explained. To obtain a high quality, low caffeine purified extract the inventors suggested dispersing the membrane processed extract in ethanol and water, and then treating it with activated carbon and/or acid/activated clay. The purified extract thus obtained exhibited excellent biological and chemical stability despite high concentration of non-polymeric catechins.

CLARIFICATION APPROACHES FOR BLACK TEA

Wickremasinghe (1977) developed a method for preparing cold water soluble tea concentrates and powders by selectively removing high molecular weight compounds such as chlorophyll, protein, polypeptides and polysaccharides while retaining the polyphenolic compounds by subjecting the black tea extract to filtration through a gel (polymerized dextran or polyacrylamide), porous glass granules or a UF membrane. In the UF process, extract is prefiltered through glass wool and 35 mL of ethanol (for 25 g black tea) is added to 200 mL of hot extract (60°C) to prevent membrane clogging before UF (20 kDa; 0.1 MPa). The membrane selectively removed high molecular weight compounds while allowing permeation of caffeine,

polyphenols and amino acids. The pH of the resulting extract (4.8) is adjusted to 5.1 and conventionally processed to obtain a water soluble tea concentrate or powder.

Todisco et al. (2002) studied the clarification of infusions from commercial black tea leaves using a 40 kDa ceramic tubular membrane with a focus to eliminate proteins that interact with soluble tannins and precipitate in the infusion during storage. The purpose of the work was to integrate between the optimum infusion until a limiting polyphenol concentration is achieved and UF process to produce a stable tea with high polyphenols content and reproducible color quality. Flux and polyphenols rejection were studied over wide range of operating conditions (70-170 kPa; 0.49-3.20 m/s; 50ºC). Contrary to general rejection behavior, these researchers observed increased rejection of tea solids with increased pressure that stabilized only at the highest flow condition (3.20 m/s) (Fig. 3). A decreasing flux with increasing pressure was also observed. Analysis showed that the controlling resistance was only due to concentration polarization even at lower pressure and high cross flow velocity during UF. Low rejection of polyphenols (~12%) and high flux 150 LMH was observed at the highest flow velocity and 120 kPa. Polyphenols concentration and color parameters (CIE *L, a, b*) remained stable, and no visible haze was observed in the ultrafiltered product for up to 2 months stored in dark bottles at -4°C (Table 2). Corresponding untreated infusions showed a strong reduction of lightness and yellowness whereas redness increased probably as a consequence of oxidation. There was a slight decrease in the polyphenols concentration in the direct infusion owing to the precipitation. Protein content was not estimated in permeates which would have established whether proteins were eliminated during UF and their role in the cream formation.

Evans and Bird (2006) examined UF as a clarifying procedure with two flat sheet polymeric membranes of equal MWCO (30 kDa) made of fluoropolymer (FP) and regenerated cellulose (RC) in a cross-flow system using reconstituted spray dried black tea. The permeate quality was analyzed in terms of haze and color (CIE tristimulus values) at 35°C. Haze was characterized by the absorbance at 900 nm, corresponding to an absorbance minimum of a centrifuged sample. Color and haze were compared before and after UF (0.1 MPa TMP, 0.44 m/s and 50°C) at similar solids concentrations (Fig. 4). Both the membranes were effective in reducing the haze by at least an order of magnitude. Lightness and yellowness increased considerably after UF. Permeate of FP membrane showed greater haze and redness indicating its potential for transmission of larger molecular weight compounds compared to RC membrane. At 0.1 MPa TMP and after 30 min of operation, FP and RC membranes showed a steady flux of 23.0 and 32.1 LMH, rejecting 21% and 27% of solids, respectively. The membrane was effective in rejecting haze and cream aggregates, but transmitted lower molecular weight compounds that led to a relatively low overall rejection of solids. As the TMP increased, both the membranes rejected more solids.

These researchers also evaluated the solute-membrane fouling interactions during UF. Being more hydrophobic, FP membrane showed more fouling tendency than RC membrane with hydrophilic tea components that led to surface modification of FP membrane resulting in a more hydrophilic surface than the original membrane. This demonstrated the advantage of using a moderately hydrophobic membrane for tea liquor filtration in terms of greater flux following multiple fouling and cleaning cycles closer to fluxes similar to those obtained with hydrophilic

materials. Hydrophobic materials generally offer greater chemical and thermal stability compared to hydrophilic membrane materials and therefore, preferred for industrial applications.

Evans et al. (2008) in a subsequent study investigated the efficiency of separation and final product quality using different MWCO RC and FP membranes (10, 30 and 100 kDa) and demonstrated the importance of understanding the surface science in the interaction between surfaces, foulants and cleaning agents. The FP membranes generally showed lower fluxes than the RC membranes. FP-10 had the lowest steady state flux of 14 LMH and RC-100 displayed the highest with 32 LMH. All RC membranes showed similar solids (69-73%) and polyphenols (~90%) transmission. However, the FP membranes displayed greater variations in their transmission. FP-30 provided the highest solids (73%) as well as polyphenols (~90%) transmission while FP-10 (65%) and FP-100 (62.5%) gave lower solids transmissions. Caffeine transmitted through both the types of membranes easily and was thus found in higher relative concentrations in the permeated solids. The haze had been significantly removed by membrane filtration compared to unfiltered and commercial ice tea samples (Table 3). Correspondingly, lightness had also increased significantly. According to these researchers, this would enable using higher solids concentrations of ultrafiltered solutions in iced tea production. Instead, it would be a good preposition to improve the clarity without losing much of the original color of tea liquor. RC-100 gave the reddest and yellowest solution among all the membranes (Table 3). The FP membranes were significantly rougher than the RC membranes and increased fouling was present on rougher, more hydrophobic FP surfaces. The results demonstrated that flux and defined MWCO are not adequate criteria in themselves to determine membrane selection.

Surface science parameters are important both to the filtration properties of real liquors, and the resulting fouling and cleaning mechanisms.

Pierre (2008) proposed a process for making a cold water soluble tea extract with good colour, low or no haze and acceptable yield, without addition of any chemicals or enzymes. According to the process, the cream fraction obtained after chill decreaming is solubilized in boiling water (1-15% concentration) and ultrafiltered (50-200 kDa) at ~45ºC. The permeate fraction upon cooling to room temperature was found to be free from haze, which may be combined with the decreamed fraction obtained earlier from chill-decreaming step before or after concentration/drying. In the accompanying example, the above UF process (100 kDa) greatly reduced the turbidity (0.65 NTU) compared to mere centrifugation (42.2 NTU) while processing solubilized primary cream fraction.

ENZYMATIC-MEMBRANE CLARIFICATION APPROACHES

Tannase is known to hydrolyze the galloyl ester linkages of polyphenolic substrates such as tannic acid, tea polyphenol gallates and the like including gallic acid methyl esters. Although the tannase treatment gives a certain amount of solubilization of cream constituents, the extract is still turbid at 5°C. Besides, tannase comes into the tea which needs to be inactivated by thermal means or removed by a precipitation method and both the methods impair the quality of the tea. In the case of UF, cream constituents are not optimally eliminated and the extract is still turbid at 5°C. Moreover, the yield is very low. To overcome the disadvantages associated with the above two decreaming methods, Barmentlo et al. (1993) proposed a method in which a hot tea extract is first treated with tannase and subsequently subjected to UF, optionally followed by a second filtration, preferably MF. The tannase treatment may be carried out using a range of enzyme

concentrations, temperatures and incubation times. Before the conversion step, pH is adjusted preferably between 4.5 and 6.0 as it drops during the tannase treatment. UF is carried out employing a 30-500 kDa membrane, preferably 50 kDa, at 5-40°C. To improve the quality further, the ultrafiltered extract is subjected to MF (0.01-100 μm) at a low temperature (0-20°C). MF treatment given to the extract is just an alternative to the traditional centrifugal polishing step. The tea extract thus obtained is then concentrated or dried. In the accompanying example, samples treated only with enzymes and only with UF did not yield satisfactory products. The clear solution obtained subsequent to enzyme treatment and UF formed a precipitate upon cooling to 5°C. Whereas the sample that was subjected to an additional filtration (filter paper) at 5°C remained crystal clear. The tea solids recovered in the UF permeate was only ~63%. Long et al. (2008) used papain from *Rhizopus cohnii* to react with green tea extract at 50-60°C for 30-60 min followed by MF (0.1-100 μm) to obtain a clarified extract for further use in the preparation of a green tea beverage. The method is claimed to be simple that decreases the damage to various active substances without any side effects.

FUTURE TRENDS

Considering the increasing market demand for RTD teas, there is a great potential for adopting membrane technology in the production process to improve their stability and decrease the haze developed during refrigerated storage while retaining most of its natural characteristics. Although the above research works advanced the application of membrane technology for clarification of tea extracts its efficacy has not been completely tested. For instance, none of the above researchers have studied polyphenols and solids recoveries in the process. Besides, retentate stream is a very rich source of polyphenols and there are no attempts towards its

recovery. It may be desirable to introduce this clarification technique to the primary extract considering the fact that RTD tea beverages are generally produced from reconstituted spray dried tea powder. However, majority of the earlier researchers have used reconstituted tea, which would have gone through a primary clarification process and may not be a representative sample for carrying out studies. Besides, most of the researchers relied upon transmittance as a measure of clarity which could be misleading instead it may be desirable to assess in terms of direct turbidity units. It is also necessary to measure these quality parameters at uniform strength of samples for meaningful comparison. The clarification process needs are to be benchmarked in terms of low turbidity, high retention of polyphenols, high recovery of solids and storage stability. Future attempts on membrane clarification of tea extracts shall address these issues for the better success of the process.

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Figure Captions

Figure 1 Effects of membrane pore size on dry weight in permeate of crude green tea extract (dead-end filtration). (Source: Kawakatsu et al., 1995)

Figure 2 Effects of membrane pore size on optical transmission in permeate of crude green tea extract (dead-end filtration). (Source: Kawakatsu et al., 1995)

Figure 3 Observed rejection coefficient (R_o) as a function of applied Trans Membrane Pressure (TMP). (Source: Todisco et al., 2002)

Table 1 Effect of membrane pore size and feed pretreatment on the clarification of green tea

Description	Invention examples			Comparative examples				
	1	3	5	1[a]	2[b]	3[c]	4[d]	5[e]
Membrane - Pore size (μm)	0.25	0.1	0.06	1	0.45	-	0.1	0.1
MWCO	-	-	-	-	-	13000	-	-

Thickness (mm)	0.5	0.9	0.5	-	-	0.6	0.9	0.9
Membrane material	Polyolefin	PVDF	PES	PTFE	CA	PAN	PVDF	PVDF
Turbidity before membrane filtration[f] (700 nm)	0.29	0.31	0.32	0.30	0.28	0.30	4.0	0.01
Filtration efficiency (catechins) (%)	100	98	98	100	100	93	98	98
Turbidity after membrane filtration[f] (700 nm)	0.04	0.03	0.06	0.89	0.32	0.03	0.03	0.01
Processing time, 100 L/m^2 (hr)	3	2	7	-	-	16	10	2

Assessment of membrane filtered products

Immediately after membrane filtration	Good color & stability	Good color & stability	Good color & stability	Poor color & flocculates suspended	Poor color & flocculates suspended	Good color	Good color	Good color
After 3 days	Good color & stability	Good color & stability	Good color & stability	Poor color& stability	Poor color & stability	Poor color & stability	Good color & stability	Poor color & stability
Biological stability	No fungal growth	No fungal growth	No fungal growth	Slight fungal growth	Very slight fungal growth	Slight fungal growth	No fungal growth	Slight fungal growth

[a] Pore size > invention range; [b] Non-preferred membrane material; [c] Pore size < invention range;

[d] Without prefiltration/centrifugation; [e] Excessive centrifugation;

[f] 1 wt% aqueous solution of non-polymeric catechins (Source: Sugiyama & Ueoka, 2006

Table 2 Comparison of parameters of color and polyphenols concentrations between permeate and infusion at different days of storage

Parameter		Sample	Time (days)			
			0	7	30	60
Colour	L	I	56	49	21	20
		P	59	61	62	62
	a	I	14	16	18	21
		P	12	11	10	11
	b	I	34	31	14	12
		P	36	36	36	36
Polyphenols (g/l)		I	1.97	1.88	1.84	1.80
		P	1.74	1.73	1.74	1.72

I – Infusion; P - Permeate

(Source: Todisco et al., 2002)

Table 3 CIE color parameters and haze of tea solutions[a]

Experiment				Chroma	Haze
	L	a	b	C*	Absorbance (900 nm)
FP10	92.92	-3.32	32.54	32.71	-0.003

FP30 no initial clean. 1st cycle	86.35	0.83	47.54	47.55	-0.002
FP30 no initial clean. 2nd cycle	85.89	1.19	48.19	48.20	-0.002
FP30 multicleaned	86.99	0.34	45.86	45.86	-0.001
FP100	90.86	-2.14	37.65	37.71	-0.003
RC10	87.79	-0.06	45.89	45.89	0.001
RC30	88.36	-0.39	43.46	43.46	0.001
RC100	85.16	1.90	49.32	49.36	0.002
PS100	88.09	-0.29	44.51	44.51	0.001
No treatment (0.2 wt %)	64.16	12.03	59.83	61.03	0.074
No treatment (0.14 wt %)	71.97	7.85	55.48	56.03	0.043
Liptons ice tea (0.14 wt %)	79.97	2.63	41.30	41.38	0.025

[a]Measured at 0.2 wt% and 35ºC

(Source: Evans et al., 2008)

Figure 4 Comparison between reconstituted black tea (feed) and ultrafiltered permeates using fluoropolymer (FP) and regenerated cellulose (RC) membranes at 50°C. (Source: Evans and Bird, 2006)

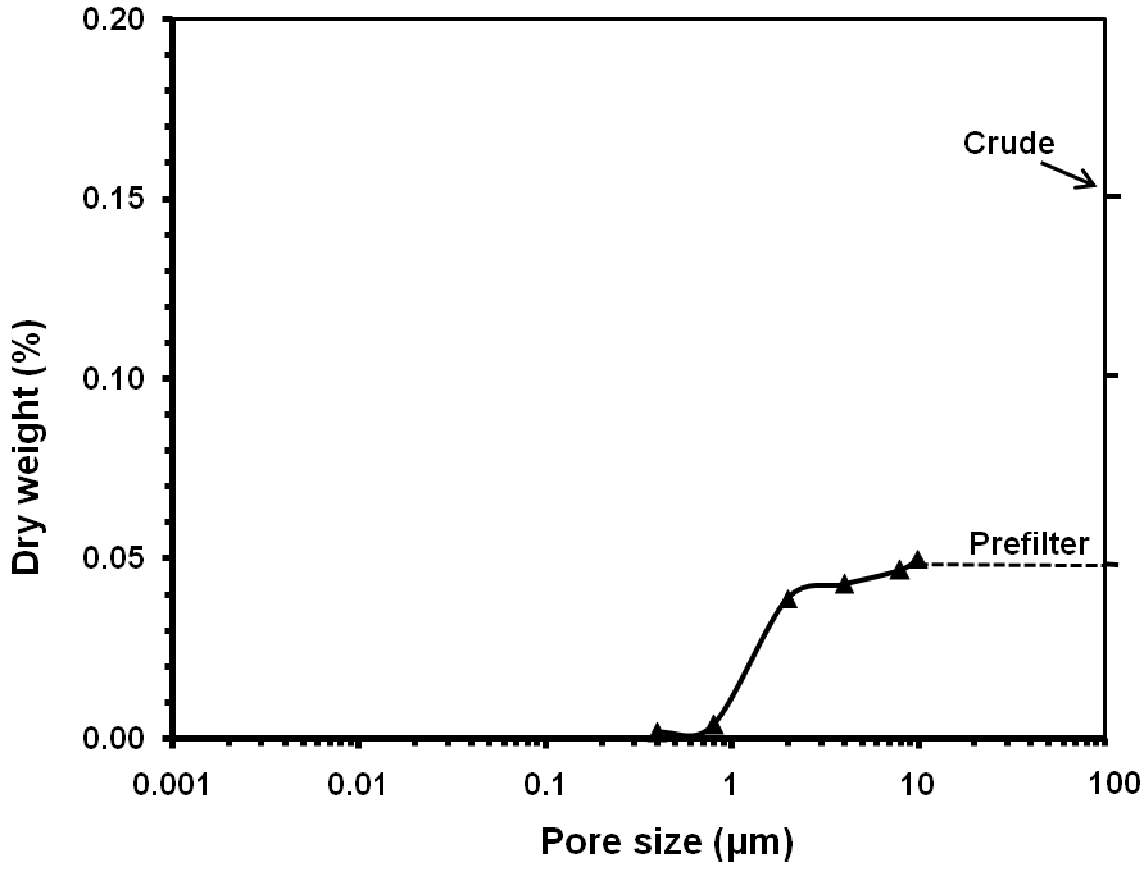

Figure 1

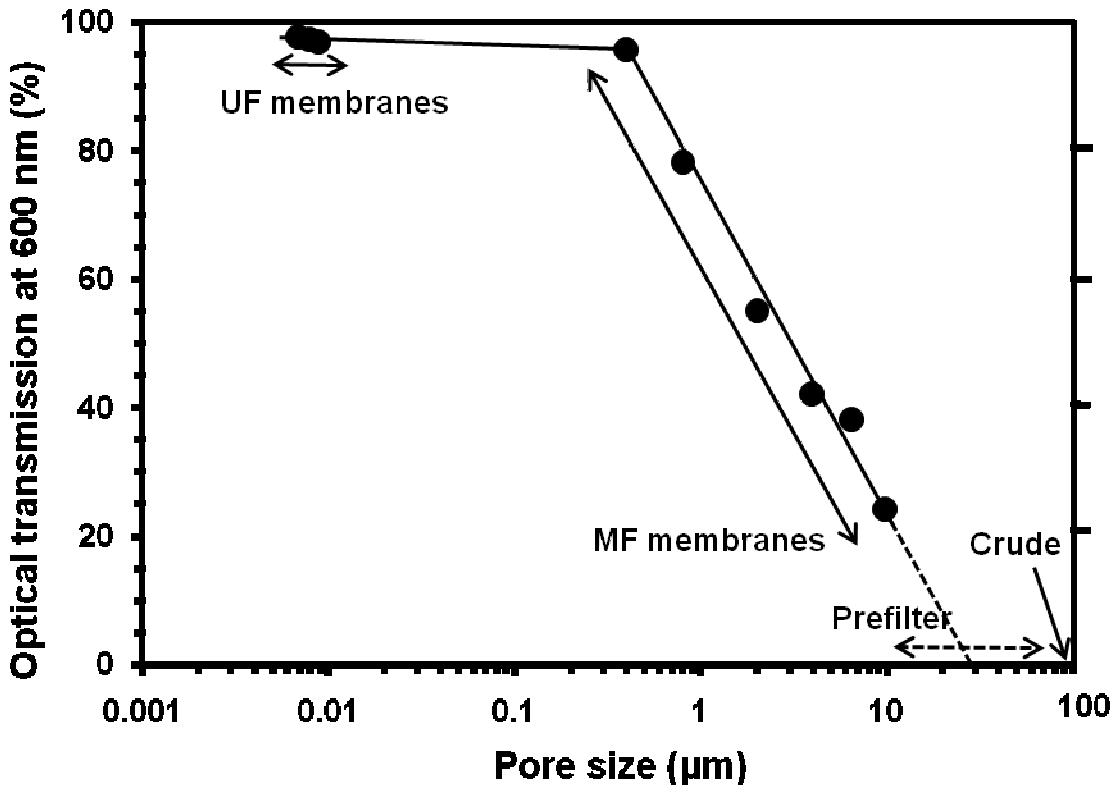

Figure 2

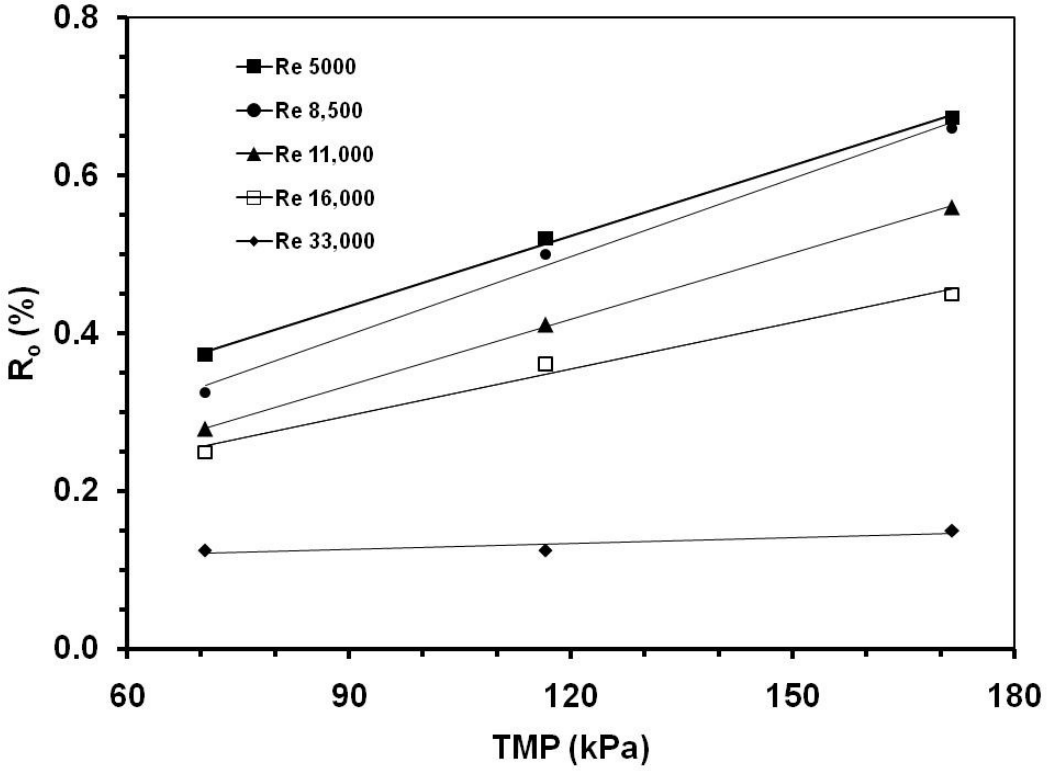

Figure 3

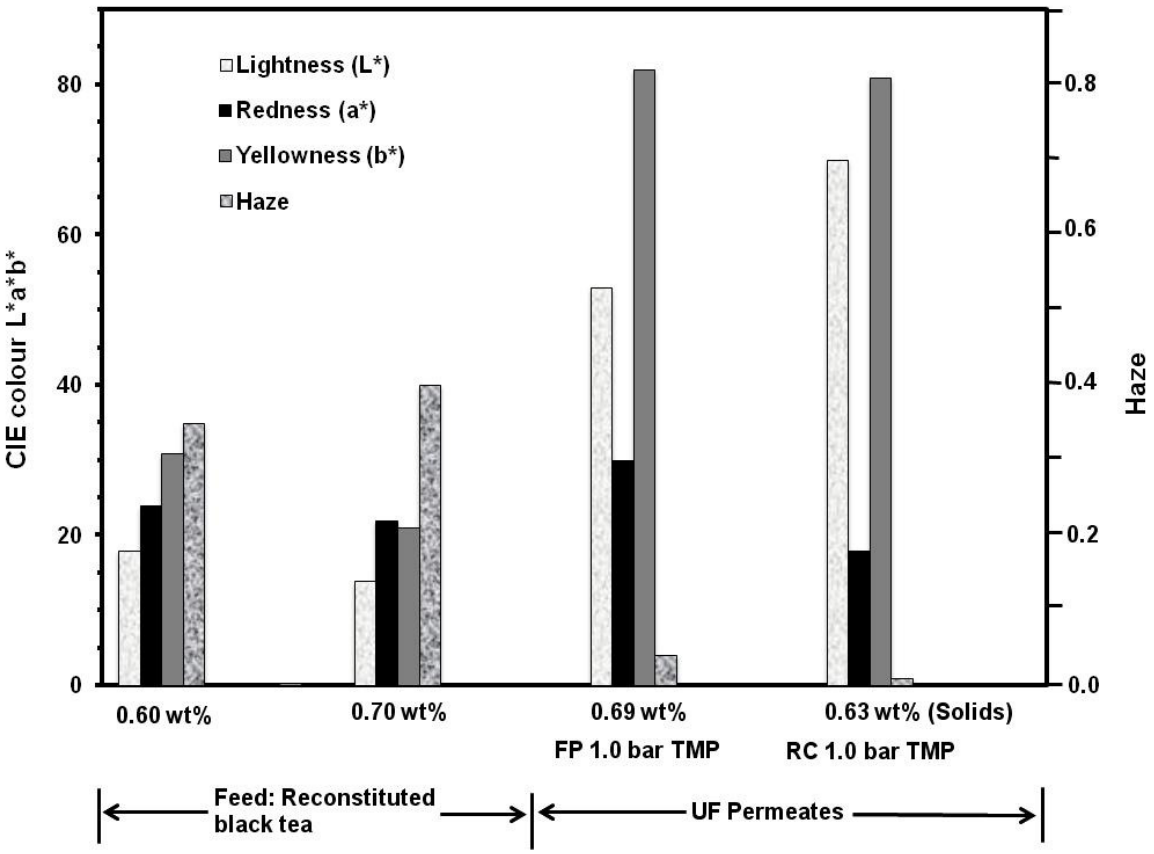

Figure 4