Journal of Technology and Value Addition, Volume 2 (2), 2020: (1-12)

Research Article

Potential of Improving Physicochemical and Organoleptic Properties of Instant Black Tea by Pre-concentration of Tea Extract Using Membrane Filtration Technique

G.A.A.R. Perera[1,3*], A.M.T. Amarakoon[2,4], D.C.K. Illeperuma[2] and E.N.U. Edirisinghe[3]

**ranjith@uwu.ac.lk*

[1]Department of Export Agriculture, Faculty of Animal Science and Export Agriculture, Uva Wellassa University of Sri Lanka, Passara Road, Badulla,90000, Sri Lanka
[2]Department of Food Science and Technology, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka
[3]Biochemistry Division, Tea Research Institute of Sri Lanka, Talawakelle, Sri Lanka
[4]Department of Chemistry, Faculty of Science, University of Kelaniya, Kelaniya, Sri Lanka

Abstract

In conventional instant black tea manufacture, tea extract is concentrated to about 25-40°Brix by thermal evaporation before spray drying. Most of the organoleptic properties of tea are destroyed at this stage. Therefore, potential of improving physicochemical and organoleptic properties of instant black tea by applying membrane-based pre-concentration technique was investigated. For membrane concentration of tea extract, a membrane was selected and operating temperature and pressure were optimized. A tea extract was prepared and a half of it was concentrated to about 12°Brix by membrane filtration technique using the selected membrane (250 Da) under optimized operating temperature (30°C) and pressure (25 bar). This pre-concentrated tea extract was further concentrated to 25°Brix by thermal evaporation and spray dried to instant black tea powder. Remaining half of the tea extract was concentrated to 25°Brix by thermal evaporation alone and it was spray dried to instant black tea powder under similar conditions. Physicochemical and sensory properties of these samples were evaluated. Theaflavin content, colour, and brightness of instant black tea prepared with the application of membrane-based pre-concentration technique (3.0±0.6 g/100 g, 3.3±0.2 and 17.2±4.0 respectively) were significantly ($P<0.05$) higher than those of control (1.7±0.2 g/100 g, 2.3±0.2, and 13.8±2.0 respectively). Furthermore, sensory properties; brightness, strength, and overall quality of instant black tea prepared with the application of membrane-based pre-concentration technique received more positive responses than those properties of control sample. Pre-concentration of tea extract using membrane filtration technique will improve physicochemical and organoleptic properties of instant black tea.

Keywords: *Chemical properties, Instant black tea, Membrane filtration, Physical properties, Sensory properties*

1. Introduction

Tender shoot of the tea plant (*Camellia sinensis* (L.) O.Kuntze) is processed into tea. Green leaf, fermented dhool, and black tea can be used as raw materials in instant black tea manufacture. Nevertheless, the rejected fractions in black tea manufacture which is collectively known as ‘Broken Mixed Fannings’ (BMF) is commonly used as raw material in instant black tea manufacture due to its low cost. Conventionally, in instant black tea manufacture, tea extract obtained by brewing tea with hot-water is concentrated to about 25-40°Brix by thermal evaporation before drying to a powder. Impairment of organoleptic properties of tea is the main problem associated with the conventional instant tea manufacture. In addition, presence of fluoride and aluminium in instant tea is considered negative because of possible health problems associated with excessive intake of them (Whyte *et al*., 2005; Walton, 2007). Several methods were described to improve the quality of instant black tea. Those methods involve use of different combinations of raw materials, together with other expensive technologies such as freeze drying (Bavan, 2003; Sinija *et al*., 2007; Yamaguchi and Kubota, 1996; Zameitat and Ridgewood, 1969). Attempts were also made to improve quality and yield of instant black tea using enzymatic treatments (Chandini, *et al*., 2011; Lehmberg *et al*., 1999).

It was reported that membrane filtration techniques could be used successfully to concentrate cactus pear, orange, lemon, carrot and melon juices while preserving their original organoleptic and nutritional properties (Cassano *et al*., 2003; Cassano *et al*., 2007; Vaillant *et al*., 2005). Further, studies were undertaken to investigate possibility of applying membrane filtration techniques in clarification and concentration during ready-to-drink tea manufacture (Long-xin, 2002) and in the production of cold-soluble instant green tea (Wen-jun *et al*., 2005). Wen-jun *et al*. (2006) studied yield recovery in membrane concentration of tea extract. Nevertheless, potential of applying membrane filtration technique to concentrate tea extract during instant black tea manufacture has not yet been studied in detail. Therefore, the present study was undertaken with the main objective of investigating potential of applying membrane-based pre-concentration technique in instant black tea manufacture to improve physicochemical and organoleptic properties of instant black tea.

2. Materials and Methods

2.1 Screening Membranes for Pre-concentration of Tea Extract

A tea extract was prepared by brewing BMF with boiling water at the ratio of 1:10 (w/v) for 10 minutes followed by filtering through a 45 µm sieve. Then it was passed through flat sheet membranes with four different molecular weight cut-off points (MWC): 1000, 5000, 10,000, and 20,000 Da as treatments using a membrane filtration pilot plant (Model–L, GEA Filtration, USA) while maintaining operating temperature at 40°C and inlet and outlet pressures at 10 and 7 bar respectively. Six flat sheet membranes (area of a sheet is 0.018 m^2) were used for each treatment. Permeates were collected and flux rates through each of the membrane were recorded.

Initial tea extract and permeates were analyzed in duplicate for total polyphenol (TPP), caffeine, theaflavins (TF) and thearubigins (TR) contents. Total polyphenol content was determined by Folin-Ciocalteu colorimetric assay (ISO 14502-1, 2005). One millilitre (1 ml) of sample was firstly, mixed with 5 ml of 10% Folin Ciocaltue Phenol reagent and then, after 3-8 min, with 4 ml of 7.5% Sodium Carbonate. Distilled water (1 ml) was used as the blank. Samples were allowed to stand at room temperature for 60 minutes and absorbance was measured at 765 nm using a UV-Visible spectrophotometer (Cary 50, Varian, Australia). Similar procedure was followed with a standard series of gallic acid. A calibration graph was constructed and total polyphenol content was calculated as gallic acid equivalent.

Caffeine content was determined by using a High-Performance Liquid Chromatograph (HPLC) (Alliance 2690, Waters, USA) fitted with a phenyl-hexyl column (5 µm, 25×0.5 cm). Using two solvents; (A) 9% (v/v) acetonitrile with 2% (v/v) acetic acid and 20 µg/ml EDTA and (B) 80% (v/v) acetonitrile with 2% (v/v) acetic acid and 20 µg/ml EDTA, gradient elution was done over a period of 45 minutes with a flow rate of 1 ml/min while maintaining column temperature at 35±0.5°C and UV detection was done at 278 nm (ISO 14502-2, 2005).

TF and TR contents were determined by following the spectrophotometric method described by Roberts and Smith (1963). Twenty-five millilitres of sample was mixed with 25 ml of isobutyl methyl ketone (IBMK) and centrifuged at 2500 rpm for 2 minutes. Then the aqueous and the organic (IBMK) layers were separated using a separating funnel. Solution ‘A’ was prepared by diluting 4 ml of the organic layer to 25 ml with absolute methanol. Solution ‘B’ was prepared by mixing 2 ml of the aqueous layer with 8 ml of distilled water and then diluting to 25 ml with absolute methanol. Two millilitres (2 ml) of the aqueous layer was mixed with 2 ml of saturated oxalic acid and 6 ml of distilled water and the mixture was diluted to 25 ml with absolute methanol to prepare the solution ‘D’. Ten millilitres (10 ml) of the organic layer was mixed with 10 ml of 2.5% $NaHCO_3$ and the organic layer was obtained after centrifuging the mixture at 2500 rpm for 2 minutes. Solution ‘C’ was prepared by diluting 4 ml of this clarified organic layer to 25 ml with absolute methanol.

Optical densities; E_A, E_B, E_C and E_D of ‘A’, ‘B’, ‘C’ and ‘D’ solutions respectively were measured at 380 nm in 1 cm cell using a spectrophotometer (Cary 50, Varian, Australia).

TF and TR contents were calculated using following equations.

TF (%) = 6.25 x Ec, x 0.36

TR (%) = [(12.5ED + 6.25(EA - Ec)] x 1.13

where,

EA, EC and ED are optical densities of solutions ‘A’, ‘C’ and ‘D’ at 380 nm respectively.

Percentages of constituents retained by each of the membranes were calculated by using the following equation.

Percentage of constituent retained (g/100 g) = ((TE-PE)/TE) x 100

Where, TE is the concentration of the constituent in the initial tea extract (g/100 ml) and PE is the concentration of the constituent in the permeate (g/100 ml).

This experiment was repeated thrice and data were statistically ($P<0.05$) analyzed by performing Duncan's multiple range test using SAS statistical package (Version 13.1).

Further, the above experiment was repeated using Reverse Osmosis (RO), Nano and 250 Da membranes maintaining feed temperature at 40^{o}C and inlet and outlet pressures at 16 and 7 bar respectively using the same membrane filtration pilot plant.

2.2 Optimization of Operating Parameters

A tea extract prepared as described above (Section 2.1) was passed through the 250 Da membrane (Selected from the above experiment) at different combinations of operating temperature (20, 30, 40 and 50°C) and outlet pressure (10, 15, 20, and 25 bar). Permeates were collected and flux rates were recorded. Initial tea extract and permeates were analyzed for total polyphenol, TF and TR using the methods described above (Section 2.1).

Caffeine and gallic acid contents were analyzed by the HPLC method (ISO 14502-2, 2005) described above. Percentages of constituents retained at different combinations of temperature and pressure were calculated using the above equation. Data were statistically analyzed ($P<0.05$) by performing Duncan's multiple range test using SAS statistical package (Version 13.1).

2.3 Preparation of Instant Black Tea by Conventional Method

A tea extract was prepared as described above (Section 2.1) and centrifuged while hot (75°C) at 9300 x g using a liquid discharging centrifuge (Model ASlb–Tubular, Sharples-super centrifuge, Penwalt India Ltd) to remove suspended solids. A portion (about 19 L) of the clarified tea extract (~3°Brix) was taken and concentrated to about 25°Brix using an evaporation pilot plant (Type CT-1B, α-ALFA LAVAL) while maintaining vacuum pressure and steam pressure at 75 bar. The concentrated tea extract was spray dried to instant black tea powder using a spray drying pilot plant (Niro, Denmark) operated at inlet and exhaust temperatures of 145-150°C and 95-100°C respectively. Instant black tea prepared by using tea extract concentrated by thermal evaporation alone was coded as IBT-C.

2.4 Preparation of Instant Black Tea Using Tea Extract Pre-concentrated by Membrane Filtration Technique

The remaining portion (about 19 l) of the clarified tea extract prepared above (Section 2.3) was concentrated to about 10-12°Brix by filtering through a 250 Da spiral membrane under the optimized operating temperature (30°C) and pressure (25 bar). This pre-concentrated tea extract was further concentrated to about 25°Brix by thermal evaporation and spray dried to instant black tea powder as described above (Section 2.3). Instant black tea prepared by using tea extract concentrated by membrane filtration followed by thermal evaporation was coded as IBT-M. Instant black tea samples were prepared in triplicate.

2.5 Analysis of Physicochemical Properties of Instant Black Tea Samples

Instant black tea samples (IBT-C and IBT-M) were analysed for total polyphenol, caffeine, TF and TR using the methods described above (Section 2.1). Instant black solutions were prepared (0.5 g of instant black tea in 100 ml of boiled distilled water) and their antioxidant activities were estimated using the stable free radical diphenylpicryl-hydrazyl (Brand-Williams *et al.,* 1995) and expressed as trolox equivalent (mg) per g of instant black tea. Solutions ‘A’, ‘B’, ‘C’ and ‘D’ were prepared using instant tea samples and their optical densities were measured at 460 nm as explained above. Colour and brightness of instant black samples were estimated using following equations (Roberts and Smith, 1963).

Total colour =6.25(EA+ 2EB)

where,

EA and EB are optical densities of solutions ‘A’ and ‘B’ at 460 nm respectively.
Brightness (%) = (100 x EC) / (EA+ 2EB)

where,

EA, EB and EC are optical densities of solutions ‘A’, ‘B’ and ‘C’ at 460 nm respectively.

Aluminium contents of the instant tea samples were determined by standard colorimetric method using aluminon reagent (Chenery, 1948) and fluoride content was determined by measuring the potential of a solution of instant black tea (0.5 g in 100 ml) in the presence of total ionic strength adjustment buffer using a pH meter (Orion - 420 A) coupled with ion selective electrode (Orion – ionplus) (Tokalioglu *et al*., 2004). Analyses were carried out in duplicate. Data were subjected to analysis of variance ($P<0.05$) and mean separation (Dunnett's t- Test). All chemicals used for chemical analysis were of analytical grade.

2.6 Sensory Evaluation

A paired comparison test was conducted to compare organoleptic properties of instant black tea samples (IBT-C and IBT-M) using a tasting panel consisted of six expert panellists (trained in tea tasting and quality control at the Kothari Agricultural Management Centre, India). Instant black tea solutions were prepared by dissolving 0.5 g of IBT-C and IBT-M separately in 150 ml of boiled distilled water and allowed to cool to 45^{o}C.

These instant black tea solutions were served as pairs in white ceramic tea tasting cups, labeled with random 3 digits codes to each panelist separately and asked to indicate the sample which has more designated characteristics (color, brightness, strength, and overall quality) in the given score card. This sensory evaluation was undertaken in a purposely built sensory lab. Sensory data were evaluated for statistical significance using a bilateral table ($p\leq0.05$).

3.Results and Discussions

3.1 Screening Membranes for Pre-concentration of Tea Extract

Polyphenols and caffeine are responsible for astringency, briskness and color of tea brew (Engelhardt, 2010). Furthermore, TF contributes to brightness and briskness of tea infusion whereas TR contributes to color and body of tea infusions (Obanda *et al*., 2004). Therefore, in membrane concentration of tea extract, maximum retention of tea constituents is important to preserve physicochemical and organoleptic properties of initial tea extract.

Moreover, higher flux rate will reduce energy required for membrane concentration process. Therefore, membrane with higher flux rate will reduce cost of production. Retentions of polyphenols, caffeine, TF and TR by the reverse osmosis (RO) membrane (99.8±0.1, 99.3±0.4, 99.9±0.1 and 100±0.1% respectively) and the 250 Da membrane (99.4±0.1, 98.3±0.3, 99.8±0.0 and 100±0.0% respectively) were not significantly different (Table 1). Nevertheless, flux rate of the 250 Da membrane (23.7±4.2 L/m^2/h) was significantly higher than that of the RO membrane (13.0±3.0 L/m^2/h). Therefore, considering flux rate and percentages of tea constituents retained, the 250 Da membrane was selected for concentration of tea extract.

Table 1: Separation characteristics and flux of membranes.

Membrane	Pressure (bar)		Mean values of constituents retained (%)				Flux ($L/m^2/h$)
	Inlet	Outlet	Total Polyphenol	Caffeine	TF	TR	
RO	16	7	99.8 ± 0.1^{a}	99.3 ± 0.4^{a}	99.9 ± 0.1^{a}	100 ± 0.1^{a}	13.0 ± 3.0^{c}
250 (Da)	16	7	99.4 ± 0.1^{a}	98.3 ± 0.3^{a}	99.8 ± 0.0^{a}	100 ± 0.0^{a}	23.7 ± 4.2^{a}
Nano	16	7	98.4 ± 0.3^{b}	92.9 ± 1.8^{b}	99.6 ± 0.2^{a}	99.9 ± 0.0^{a}	9.5 ± 1.3^{d}
1000 (Da)	10	7	95.3 ± 0.3^{c}	62.5 ± 4.0^{c}	99.2 ± 0.3^{a}	98.5 ± 0.3^{b}	8.7 ± 0.5^{d}
5000 (Da)	10	7	93.5 ± 0.7^{d}	61.8 ± 3.9^{d}	98.3 ± 0.3^{b}	97.1 ± 0.6^{c}	13.7 ± 0.6^{c}
10,000 (Da)	10	7	93.1 ± 0.7^{e}	59.9 ± 3.6^{ed}	97.3 ± 1.0^{c}	96.6 ± 0.2^{d}	15.7 ± 1.6^{c}
20,000 (Da)	10	7	89.7 ± 0.3^{f}	57.8 ± 3.4^{e}	94.4 ± 1.6^{d}	94.3 ± 0.4^{e}	16.8 ± 1.2^{b}

Figures are mean ± Standard Deviation (n=6); RO, reverse osmosis; TF, Theaflavins; TR, Thearubigins; Figures with different superscript in a column differs significantly ($p\leq0.05$)

3.2 Optimization of Operating Parameters

Retention of polyphenols did not vary significantly with temperature owing to its higher molecular weight. Nevertheless, retention of caffeine and gallic acid were highest at 20°C and their retention decreased with increasing temperature (Table 2). Upon cooling, phenolic constituents and caffeine tend to bind with each other making a precipitate (Roberts, 1963). This may be the reason for higher retention of tea constituents with lower molecular weight such as caffeine and gallic acid during filtration at low temperature. However, membrane concentration at 20°C requires additional energy for cooling which in turn increases cost of production. Therefore, operating temperature of 30°C at which second highest percentages of caffeine and gallic acid were retained can be considered as the optimum temperature for membrane concentration of tea extract.

Retention of polyphenols did not vary significantly with pressure but retention of caffeine and gallic acid increased with increasing pressure (Table 3). Protein present in tea extract can be denatured due to high pressure (Shen *et al.*, 2008). Higher retention of caffeine and gallic acid during filtration at higher pressure may be due to possible cross linking of them with denatured protein molecules and formation of large clusters. Highest retention of caffeine and gallic acid were observed at 25 bar.

Therefore, operating outlet pressure of 25 bar can be considered as the optimum pressure for membrane concentration of tea extract.

Table 2: Effect of temperature on separation characteristics and flux of 250 Da membrane.

Temperature (°C)	Mean values of constituents retained (%)			Flux (L/m^2/h)
	Total Polyphenol	Caffeine	Gallic acid	
20	99.6±0.1^a	99.3±0.3^a	97.3±1.5^a	17.4±5.1^b
30	99.5±0.1^a	98.4±0.4^b	95.7±1.8^b	16.2±5.1^c
40	99.4±0.2^a	97.0±1.0^c	94.6±2.1^c	16.5±7.6^c
50	99.2±0.2^a	94.4±2.0^d	93.1±2.8^d	36.2±8.1^a

Figures are mean ± Standard Deviation (n=6)
Figures with different superscript in a column differs significantly ($p \leq 0.05$)

Table 3: Effect of pressure on separation characteristics and flux of 250 Da membrane.

Pressure (bar)		Mean values of constituents retained (%)			Flux (L/m^2/h)
Inlet	Outlet	Total Polyphenol	Caffeine	Gallic acid	
12	10	99.2±0.2^a	96.2±3.0^d	92.4±2.8^d	10.9±2.1^d
18	15	99.4±0.1^a	97.1±1.8^c	94.7±1.5^c	19.3±9.6^c
24	20	99.5±0.1^a	97.6±1.7^b	96.4±1.5^b	22.9±8.5^b
30	25	99.6±0.1^a	98.3±1.3^a	97.1±1.0^a	33.3±9.7^a

Figures are mean ± Standard Deviation (n=6)
Figures with different superscript in a column differs significantly ($p \leq 0.05$)

3.3 Comparison of Physicochemical Properties of Instant Black

Instant black tea prepared by applying membrane pre-concentration technique (IBT-M) contained significantly higher amount of TF (Table 4). TF contributes positively to the bright color and brisk taste of tea brews (Obanda *et al.*, 2001).

Therefore, it can be expected that liquors of the IBT-M to be better in quality and brighter in color.

Table 4: Physicochemical properties of instant black tea prepared by conventional method and membrane pre-concentrated method.

Physicochemical property	Instant black tea prepared by	
	Conventional method (IBT-C)	Membrane pre-concentration method (IBT-M)
Total polyphenol (g/100 g)	27.2±0.42	27.2±0.48
Theaflavins (g/100 g)	1.7±0.2	3.0±0.6*
Thearubigins (g/100 g)	38.5±1.9	39.6±1.7
Total colour	2.3±0.2	3.3±0.2*
Brightness (%)	13.8±2.0	17.2±4.0*
TR/TF	23.3±2.9	13.8±3.5*
Caffeine (g/100 g)	8.7±0.1	8.7±0.5
Antioxidant activity (TE g/100 g)	47.15±1.3	48.0±0.5
Fluoride(µg/g)	176.1±5.0	154.8±4.1*
Al (mg/g)	0.97±0.13	0.96±0.11

Figures are mean ± Standard Deviation (n=6); * Denotes a statistically significant difference ($p \leq 0.05$)

Results of sensory evaluation showed a similar trend as reflected by more positive responses given by tasters to liquor brightness, strength and overall quality of IBT-M (Table 5). TR/TF ratio within 10 to 15 is reported to be a key factor, which contributes to an ideal liquor color, brightness and strength (Roberts, 2008). Therefore, the TR/TF ratio of IBT-M (13.8) may be the reason for its higher estimated liquor color and brightness and superiority in sensory properties as compared to IBT-C (Table 4 and 5). As consequences of low temperature involvement, membrane concentration minimizes undesirable changes of tea constituents during concentration of tea infusion. Therefore, physicochemical and sensory properties of IBT-M were superior to those of IBT-C.

Table 5: Sensory properties of instant black teas.

Sensory Character	Number of positive responses for the question; of two samples which one has more designated characteristic	
	IBT- C	IBT-M
Colour	10	5
Brightness	2	13*
Strength	3	12*
Overall Quality	2	13*

*Comparison is significant ($p \leq 0.05$)

Tea is considered as a significant dietary source of fluoride. Presence of fluoride in instant tea is considered negative because of possible health problems associated with excessive intake of it (Whyte *et al.*, 2005**)**. Fluoride content of IBT-M (154.8±4.1 µg/g) was significantly less than that of IBT-C (176.1±5.0 µg/g). Some amount of fluoride might have passed through the membrane during membrane concentration resulting less amount of fluoride in IBT-M. Therefore, lowering fluoride content of instant black tea would be an added benefit of applying membrane pre-concentration in instant black tea manufacture.

4. Conclusions

Membrane with 250 Da molecular weight cut-off point is most suitable for pre-concentration of tea extract during instant black tea manufacture and operating temperature of 30°C and outlet pressure of 25 bar can be considered as optimum for this process. It can be concluded that membrane-based pre-concentration of tea extract will improve the physicochemical and sensory properties of instant black tea.

5. Acknowledgement

Authors wish to thank the management of the Tea Research Institute of Sri Lanka for granting facilities to undertake this research.

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