Effectiveness of Physical Treatment to Inhibit Tea Cream Formation in Concentrated Black Tea

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Abstract

Tea cream (TC) is the important problem of the storage of concentrated black tea (CBT), before distribution to ready to drink industry. It has characteristics of colloidal and insoluble compounds, resulting many economic losses. A simple, convenient and effective methods are still required for CBT industry. Based on this objective, physical treatments using centrifugation and simple filtration were investigated and compared. Two groups of CBT with total solid of 35% w/w was produced from black tea. First group was centrifuged at speeds of 2,000, 4,000, 5,000 and 6,000 rpm for 20 min. The other was filtrated with 1, 2 and 3 layer of filter cloths. Furthermore, the clear fraction was stored at room temperature 25°C and periodically checked for TC formation. The untreated CBT was also performed as a control. Samples were taken to analyze caffeine (CF), total catechin content (TCC), gallated catechins (GaC), ungallated catechins (uGaC), turbidity and %TC. It was found that tea cream formed rapidly within 1 day of storage for the control. Surprisingly, centrifugation can slow down the TC formation as long as 21 days. Moreover, filtration can prolong storage time duration until 9 days. The results showed that the turbidity was increased during storage and no significant difference (p>0.05) with the control. As the TC formed, CF, TCC, GaC and uGaC significantly decreased as the amount of TC increased. The obviously decreased amount of GaC led to the assumption that it was the major component causing TC formation in CBT. Centrifugation was recommended for CBT industry which the minimum speed required for effective prevention of TC was 4,000 rpm. For the filtration the most effective was 3 layer of filter for filtration. Combination between two treatments resulted the best to inhibit TC.

Keywords: Concentrated black tea, Centrifugation, Filtration, Tea cream, Gallation

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1. Introduction

Tea (*Camellia sinensis L.*) is one of the most popular non-alcoholic beverages in the world. It has specific characteristics, such as taste, aroma, and is reported to have health effects. Tea is a rich source of polyphenols that can be used as a supplement in several products, to increase the health benefits. Tea polyphenols have a high economic value and can be applied in several areas, such as food, cosmetics and pharmaceuticals. The manufacturing process determines the type of tea produced, in general three different types are commonly consumed: green tea (unfermented), Oolong tea (partially fermented) and black tea (fully fermented) (Aoshima and Ayabe, 2007; Ashok and Upadhaya, 2012). Black tea is manufactured through fermentation of fresh tea leaves by the enzymatic polymerization of simple polyphenols using principally the catalyst polyphenol oxidase and atmospheric oxygen. Black tea concentrate, which is used to prepare both mixed tea beverage and instant tea, is massively produced throughout the world. In China, CBT is one of the main tea-extract products, with about 15,000 tons consumed per year (Xu *et al*., 2012). Most of the CBT is used for the preparation of ready to drink tea product.

A problem associated with CBT manufacturer and RTD tea production is a phenomena known as 'tea creaming' (Ishizu *et al*., 2012; Kim and Talcott, 2012; Vuong *et al*., 2011). Hot black tea infusions produce cloudy precipitation on cooling, which are known as TC, and contain up to 30% of the total solids. Tea concentrate is a colloidal dispersion that is unstable during storage. TC contains many of the compounds that provide taste and color in black tea, and its formation therefore gives rise to a cloudy appearance as well as losses of both taste and color of the CBT (Aguilar *et al*., 2007 and Jobstl *et al*., 2005. It is thus deleterious both for the consumer and for the producer, especially for producers of ready-to-drink teas, who need to prepare tea infusions at high solid concentrations and for whom cloudy solutions are undesirable. The important constituents of CBT causing the tea cream has been reported as thearubigins (TR), theaflavins (TF) and CF. The interaction among these compounds by various molecular types of interactions including polyphenol–caffeine complexation and polyphenol–polyphenol interactions plays an important role for the TC formation during storage. Polyphenol–caffeine complexation is influenced by a number of gallate and hydroxyl groups of the polyphenols (Asil *et al*., 2012; Todisco *et al*., 2002).

The factors affecting cream formation in CBT include the extraction temperature, the pH and the concentration of the infusion (Xu *et al*., 2014). The extraction of black tea at a temperature below 35°C would produce an infusion incapable of creaming. When the pH of the solvent was maintained at 4, the maximum amount of black tea cream was formed. The complexation of polyphenols by association of their galloyl groups and their limited solubility is said to be a main driver in cream formation (Chaturvedula and Prakash, 2011). Although not an initiator of TC and not an essential part, CF binds with galloyl groups and TC moieties, increasing their mass and density. There is strong positive correlation between the amount of tea sediment and the solid concentration at 5–40°Brix, as well as improvement of the colloidal stability of the tea concentrate due to an increase in viscosity (Xu *et al*., 2013). The presence of Ca^{2+} ions exacerbates cream formation through charge compensation given the negative charge carried by TF in solution at the pH of infused tea (Lin *et al*., 2014; Vuong *et al*., 2010). H^{+} is thought to encourage cream formation by stimulating polyphenols to interact with polysaccharides and nucleophilic groups on proteins.

The solubility of TC constituents is enhanced by the absence of CF or gallate esters (Arnas 2009; Evans *et al*., 2009). However, TF and other high molecular weight polyphenols are preferential to CF in partitioning into the cream phase. CF and TF have also been shown to associate with themselves and each other which further demonstrates the complexity of TC formation (Ishizu *et al*., 2011). CF has a bitter taste whilst TF contributes to its characteristic astringent taste. These associations cause a depletion of TF and CF which will affect the taste of tea.

De-creaming is an important step in the process to meet the cold stability requirements of RTD tea. Conventional decreaming employing any separation technique would result in loss of flavor, color, and taste including health enhancing polyphenols since TC composition is similar to tea (Wan *et al*., 2008). Besides establishing clarity, storage stability, and assessing the tea quality parameters, efforts were made to obtain maximum yield of tea solids as well as greater polyphenols content in the clarified product in the separation process.

Separation of constituents offer a means to remove haze with minimal thermal or chemical impact to the product whilst maintaining a continuous unit operation. There have been a number of studies relating to the filtration of tea for both infusions (Xu *et al*., 2015), reconstitutes (Xu *et al*., 2011) and model component mixtures (Xu *et al*., 2012). Filtration is the removal of insoluble solids from a suspension by passing it through a porous material. Filtration is used to clarify liquids by the removal of small amounts of solid particles. Centrifugation can separate the TC with normal components inside CBT. Therefore, the aim of this study was to assess the effectiveness of centrifugation and filtration to prevent TC formation in CBT and identify the characteristics of physical and chemical changes.

2. Materials and Methods

Black tea was purchased from Raming Tea®, Chiang Mai Thailand in 2016. Standards and chemicals, including gallic acid, caffeine (CF), catechin (C), catechin gallate (CG), epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), epigallocatechin gallate (EGCG), gallocatechin (GC), gallocatechin gallate (GCG), and trifluoroacetic acid (TFA) from Sigma Aldrich®, Canada. Acetonitrile and Folin-Ciocalteu's phenol reagent from LobaChemi®, India. Anhydrous sodium carbonate from Ajax Finechem®, New Zealand.

2.1 Black tea extraction and concentrated black tea preparation

CBT was prepared from black tea with a water-to-tea ratio of 20:1 for 25 min at 80°C. The extracts were cooled and filtered by using filter paper Whatman® No.4 before concentrating by rotary evaporator, at 80°C. The concentrating process was done until the total solids of 35% was obtained.

2.2 Effects of centrifugation

CBT were added into closed tubes and then centrifuged at speeds of 2,000, 4,000, 5,000 and 6,000 rpm for 20 min. After centrifugation, clear fraction was separated and stored at room temperature (25°C). The untreated CBT was done as a control. All samples were taken periodically to monitor the tea cream along with physical and chemical parameters analysis. All samples were performed in triplicate.

2.3 Effects of filtration

CBT were filtered by using three conditions of household filter cloth, there are one layer, two layers and three layers. After filtration, clear fraction was separated and stored at room temperature (25°C). The untreated CBT was done as a control. All samples were taken periodically to monitor the tea cream by analyzing physical and chemical parameters. All samples were performed in triplicate.

2.4 Analysis

2.4.1 Caffeine and total catechin content

CF and TCC in CBT samples were analyzed by High Performance Liquid Chromatography (HPLC) as ISO 14502-2:2005. The mobile phase consisted of acetonitrile and 0.05%TFA at a ratio 13:87%v/v. CBT was diluted, by a factor of 100, filtered through a 0.45 μm nylon filter and then injected to HPLC. The HPLC conditions were as follows: injection volume, 10 μL; column, temperature, 35°C, isocratic elution, flow rate 1 mL/min; Photodiode Array detector (Waters, Shanghai Corporation, Shanghai, China) at 210 nm.

2.4.2 Tea cream

CBT was centrifuged at 2,000 rpm for 10 min and the soluble solids in the supernatant were determined. The difference between the estimates of soluble solids in the primary extract and its supernatant was taken to indicate the amount of 'cream' separated on cooling. The results were shown as percent w/w.

2.4.3 Turbidity

Samples were diluted 100-times and turbidity was measured with turbidity meter in formazin turbidity unit (FTU). The method was based upon a comparison of the intensity of light scattered by the sample under defined conditions with the intensity of light scattered by a standard reference suspension. The higher the intensity of scattered lights, the higher the turbidity. The measurement was performed triplicate.

2.5 Statistical analysis

All experimental data were expressed as mean and standard deviation after that further analysis was conducted to identify different among means by using SPSS program at $p<0.05$.

3. Results and Discussion

3.1 Changes of CF, TCC, GaC and uGaC of CBT treated by centrifugation and filtration

Figure 1 showed that CF, TCC, GaC and uGaC determination were analyzed in order to investigate the effect of these compounds with TC formation. Figure 1A showed that CF content of control at initial day until second day was significant difference with treated sample 2,000, 4,000 and 5,000 rpm ($p<0.05$). The values of control and 6,000 rpm treatment was lower than other treated sample in the beginning of observation days, that mean centrifugation could reduce CF loss in CBT, but if the speed of centrifugation reached 6,000 rpm the content loss will be same with control. It was probably due to the CF compound was separated during centrifugation, although the value of TC was lower than control but the content inside was already separated from the beginning. CF content in control response and 6,000 rpm treatment is decreased significantly start from the second day, compared with the other treated sample it was started from the first day ($p<0.05$). In day 9 and 21 of observation the value of CF content was not different among control and all treated samples. It can be assumed that CF decreased steadily as a result of complexation with other compounds content will be decreased steadily and then TC formed.

Regarding by the principal of tea cream formation affected mostly by gallated catechin, Figure 1B, 1C and 1D showed TCC, GaC, uGaC results classified the group of C which had effect to TC formation. Another compound like TCC content in control is significantly different with treated sample (p<0.05). Figure 1B showed the value of TCC in the control sample at all days of observation was lower than the treated samples. The result proved that centrifugation could reduce TCC loss in CBT during observation. TCC in control and treated samples were decrease significantly start from initial day until second day, after that the slope of graph was reduced until steady. It showed that TCC would decreased significantly until equilibrium state was reached. TCC in control response was decreased significantly, starting from the second day to 14 days, compared with the treated sample starting from the first day to 14 days (p<0.05).

Basically catechins in tea can be divided into GaC and uGaC based on chemical structures. It has been reported that GaC played an important role in the TC formation (Lin *et al*., 2014). Figure 1C showed that, GaC had similar result with TCC (Ashok and Uphandaya, 2012), the content of GaC of the control at initial day until the last day of observation was significantly different with treated sample (p<0.05).

GaC content of the control was lower than treated sample, this indicated that GaC might participate in the TC formation, resulting lower content in the clear fraction. The GaC of untreated CBT was lower than treated sample, it means centrifugation also can reduce GaC participating in TC which cause the loss in CBT. Value of GaC in control and untreated was decreased significantly until the second day, after that decreased steadily as found in TCC. It showed that GaC was a component involving in TC based on the similarity as found for CF and TCC. However, GaC could be the important compound due to the trend of reduction was higher and sharper than CF and TCC.

To prove the statement was mentioned above, uGaC the last C group was determined. Figure 1D showed that the uGaC decreased during storage. This result was similar to the results of CF, TCC and GaC. Value of uGaC in control was significant difference when compared to treated sample (p<0.05). It showed that GaC might be the important component to participating in TC during storage. The content of uGaC in the untreated samples was lower than treated samples. It indicated that centrifugation can reduce uGaC participating in TC which cause the loss in CBT. Other group of CBT was using filtration as physical treatment to inhibit TC formation. Besides centrifugation filtration treatment was commonly used in tea company for many purposes.

Similar with Figure 1, Figure 2 also showed that CF, TC, GaC, and uGaC content were expressed on relative graph and being classified into initial day until day 9 with interval every 1 day. The graph was expressed on g/100mL unit. Figure 2A showed that CF content in control and all treated samples on initial day was significant different, after that during observation days until day 5 there were no difference among them, but on day 6 value in control significant different compared with 1, 2 and 3 layer of filter treatment which used. CF content in control and all treated samples at initial day, day 3 and day 6 was significant difference with treated sample 1 layer, 2 layer and 3 layer of filter materials (p<0.05). The CF in control treatment was lower than other treated sample, that mean filtration could reduce CF loss in CBT, but in the last day of observation days CF content in control and all treated samples were not significant difference. It was due to the CF compound inside was separated during filtration, although the value of TC was lower than control but the content inside was already separated from the beginning. In the last 3 days of observation the value of CF content was not different among control and all treated samples (p<0.05). It showed that the CF content will be decreased steadily and reached the equilibrium state along with TC formation. Figure 2B showed 3 layer of filter treatment started from initial day until day 8 was not significant different compared with control and other treated samples, thus in the last of observation days there were significant difference between control and all treated samples statistically (p<0.05). Otherwise TCC was the combination between GaC and uGaC so the graph will be the representative for GaC and uGaC. Figure 2C explained that GaC value in control and all treated samples were decrease steadily from initial day until second day of observation. Furthermore on day 3 GaC decrease sharply after that steady until the last day of observations. Hence CG, ECG, EGCG and GCG were one of gallated catechin which main constituent for tea cream formation. Figure 2D showed value of uGaC results quite similar with Figure 2B. Starting from initial day until the last day of observation, uGaC was decreased steadily. Especially for the control uGaC decreased steadily very smooth compared with 1 layer treated samples uGaC descending roughly.

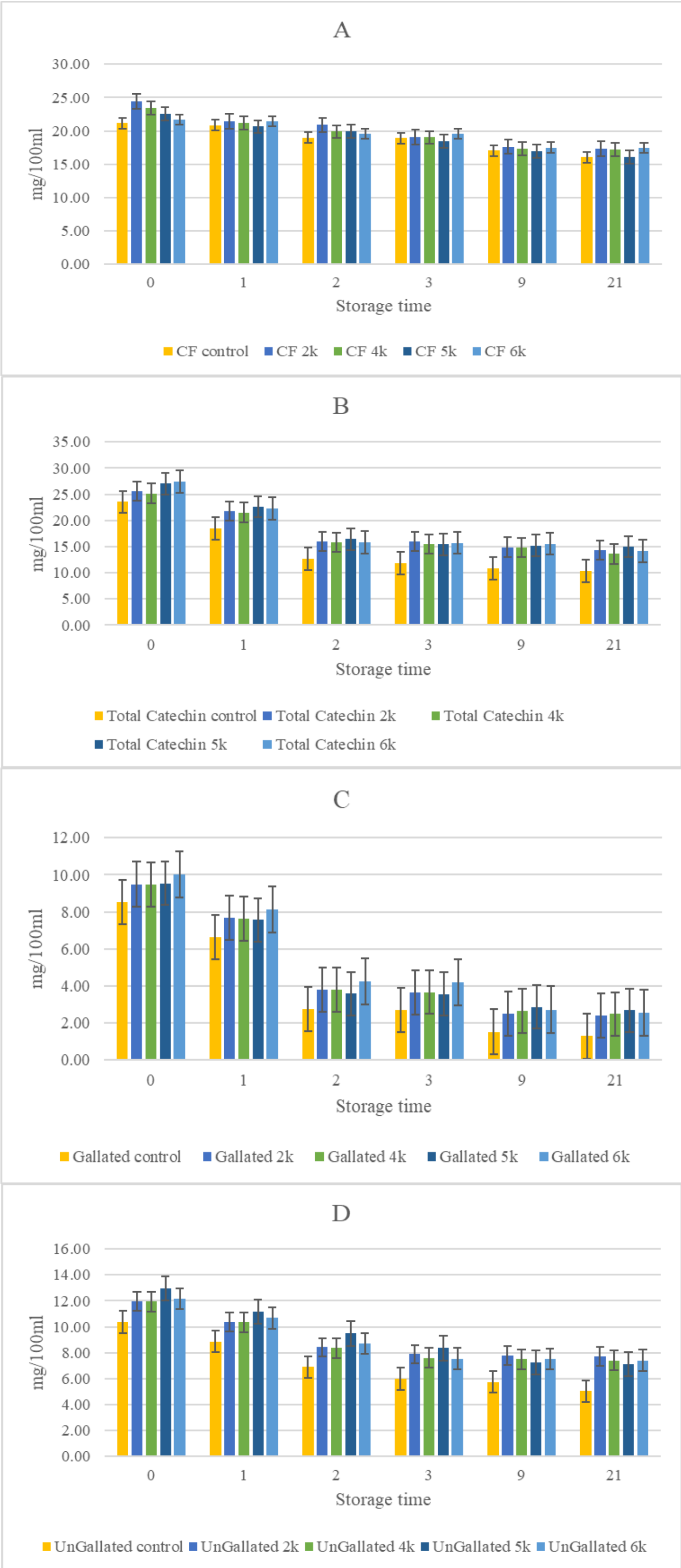

Figure 1 Chemical parameter changes on centrifugation treatment during storage time: (A) CF, (B) TCC, (C) GaC and (D) UGaC.

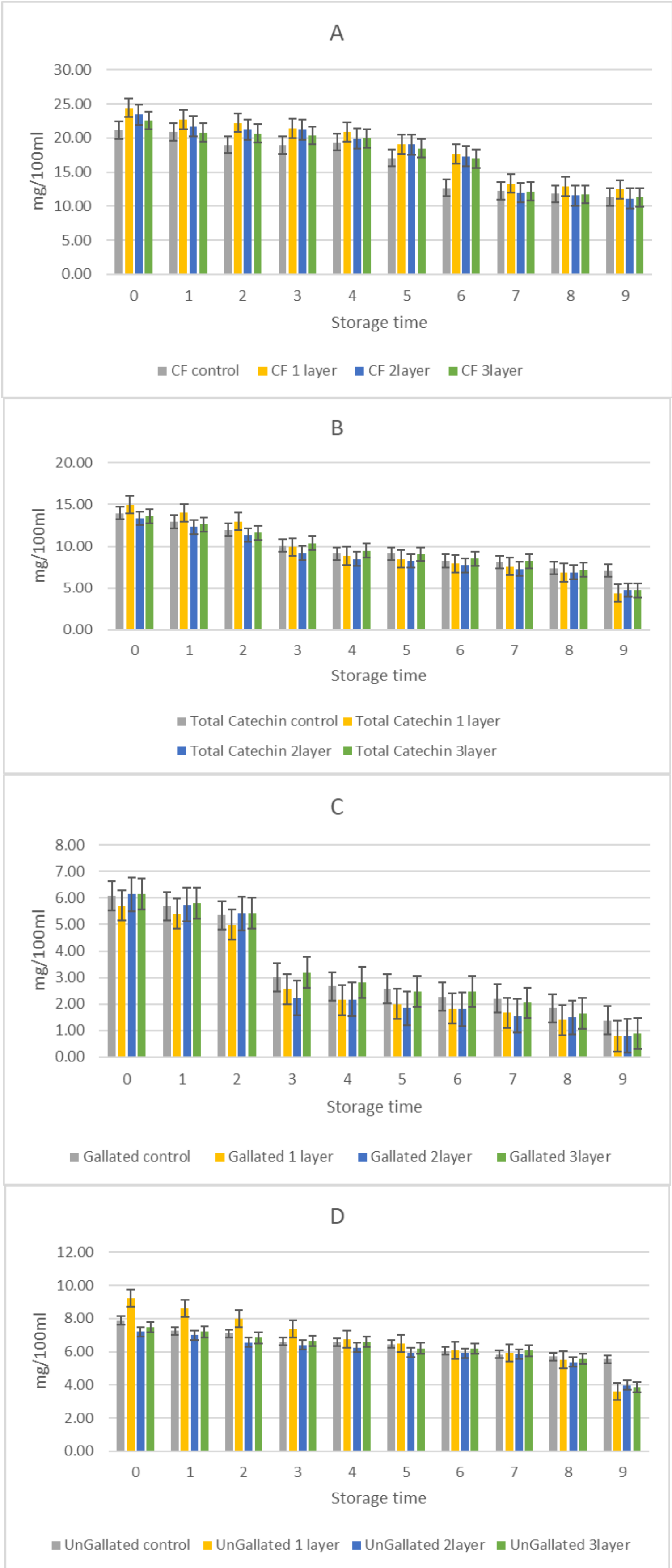

Figure 2 Chemical parameter changes on filtration treatment during storage time: (A) CF, (B) TCC, (C) GaC and (D) UGaC.

3.2 Turbidity of CBT treated with centrifugation and filtration

Centrifugation and filtration affected the turbidity in CBT. Turbidity was one of main physical quality parameter of CBT or RTD tea. The turbidity normally increased during storage time, especially under cold environment. Figure 3 indicates that the turbidity of the control at all days of observation was significantly lower than that of treated samples. Different with treated sample, control have highest tea cream formed and it affects turbidity. Variables which affect turbidity are many components that spread in CBT solution, otherwise it was attracted into TC layer. In the last day of observation (day 21), the value of turbidity in treated samples besides 6,000 RPM treatment sample were not significantly different with control. This phenomenon happens due to many components inside already separated during treatment, higher speed of centrifugation affects the separation. Similar reason with control which have lower turbidity compare with treated samples. Higher component separation means lower turbidity will be got. Trend of turbidity values in this research was decrease steadily, it had opposite effect with normal black tea. Normally turbidity of CBT will be increased during storage time, regarding the hazy color will be occurred. Because during storage time, TC was formed. TC would attract many components inside and precipitation would be occurred, resulting in decrease of turbidity along with observation days.

Centrifugation speed had no effect to reduce turbidity of CBT, due to its principal was to separate heavy molecule weight which contributed on TC formation. Other physical treatment was filtration; the principal was separation based on molecule size. Turbidity of filtration treatments had different results. Figure 4 showed that turbidity of treated samples which using 3 layer of filter cloth and control had lower turbidity value compared with other treated samples in the end of observation days. Starting on day 3 afterwards, turbidity of the control was significantly higher with 3 layer of filter cloth sample. Otherwise it was significantly lower than those of 1 layer and 2 layers of filter cloth sample. This was because the mechanism of filtration was to separate many components like polyphenol and caffeine which have big molecular size. That components which contributed with turbidity and the material of filter absorbed some coloring components inside. Filtration might be better treatment compared with centrifugation to reduced turbidity of CBT regarding with the characteristic of filter cloth which can absorbed hazy components. Since higher turbidity will reduced the quality of the tea.

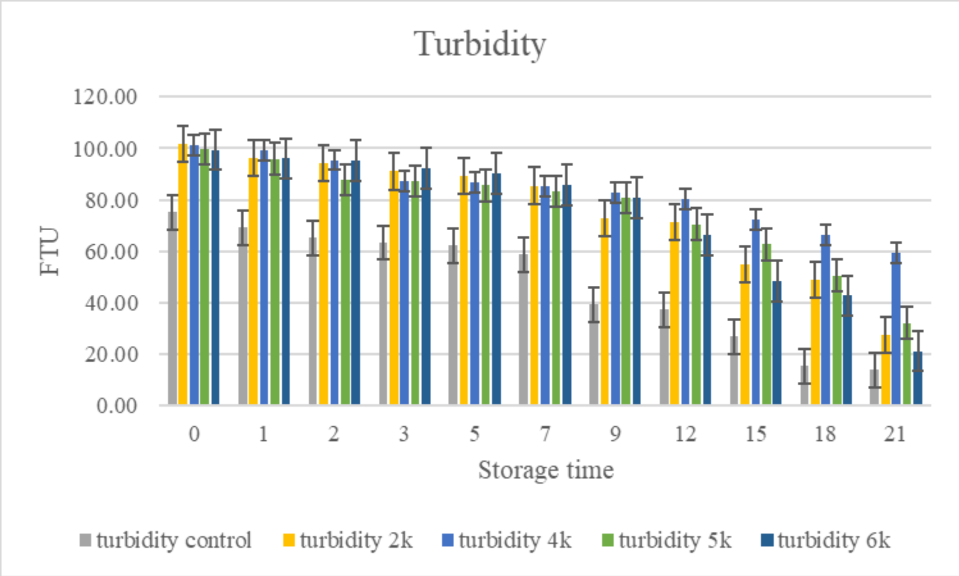

Figure 3 Turbidity changes on centrifugation treatments during storage time

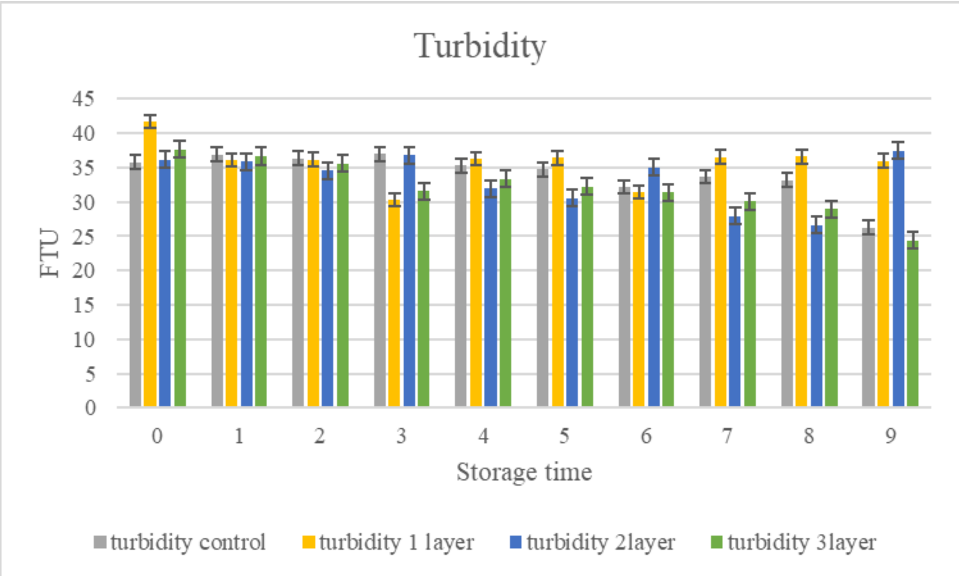

Figure 4 Turbidity changes on filtration treatments during storage time.

3.3 Tea cream

TC was a finely divided colloidal precipitate which imparts a distinct opacity compared with clear phase of CBT. Generally, TC would increase during storage time along with proper condition. There were several components which contributed to TC formation such as, CF, TCC, GaC and uGaC. TC content increased, therefore turbidity decreased due to many components attracted into TC layer. Figure 5A-5B show that tea cream of centrifugal treatments increased during storage time. The control (untreated CBT: I) remarkably showed higher TC than treated CBT (II, III, and IV). The longer storage time than higher TC (Figure 5A and Figure 5B). The higher speed of centrifugation the lower TC formed (Figure 5B: II, III and IV).

Centrifugation and filtration were categorized as physical treatment but the principals were different. Resulting in different impact on TC formation during storage. Similar to Figure 5, Figure 6 shows that tea cream formed and increased during storage time. The control (untreated CBT: I) remarkably showed TC higher than (treated CBT: II, III, and IV). The longer storage time then higher TC (Figure 6A and 6B). Layer of filter that used increasing the lower TC formed (figure 6A and 6B: II, III and IV).

Table 1 showed that centrifugation could be effectively used to inhibit TC during storage. %TC during storage for 21 days of control was significantly different (p<0.05) with other treatments. At the last day of observation, the value of TC for all treated sample was not significant different with control (p>0.05), it means the TC increases during storage and reach the same value with control at 21 days. Control at initial day and first day had around 22 percent TC, for treated sample the value only around 3 percent TC. Treated sample need 21 days of storage time to reached the same value with control. It showed that centrifugation can be used effectively to reduce TC formation occurred. TC content at centrifugation speed 2,000 RPM increased faster than other treated sample. Started from second days, for 2,000 RPM treated sample, the TC percentage higher than other treated sample. From graph the trend of percentage of TC for all treated response was increased, compared with control which stabile in high value.

Even though the purpose of filtration was different with centrifugation otherwise in term of separation it was similar. It can be used to inhibit TC but it less effective compared with centrifugation. Centrifugation can effectively separate TR from CBT due to the molecular weight of TR was higher than water and other components. TR was one of the most components which contributed in TC formation. Table 2 showed that TC of control at initial day until 6 days was significant difference (p<0.05) with other treatment. Furthermore, at day 6 until day 8 the value of control was not different with 1 and 2 layer of filter treated samples, but different significantly with 3 layers of filter treated samples. At the last day of observation, TC for all treated sample was not significant difference with control, it means the TC would increase during storage time and reach the same value with control at 9 days. Control at initial day and first day had around 19 percent TC, for treated sample the value only around 8 percent TC. Treated sample need 9 days of storage time to reached the same value with control. It showed that filtration can be used effectively to reduce TC formation occurred. TC content at 1 layer of filter which used for filtration increased faster than other treated sample. Started from initial days, for 1 layer of filter treated sample, the TC percentage higher than other treated sample. Centrifugation could inhibit TC formed until 21 days, otherwise filtration only could inhibit until 9 days of storage time.

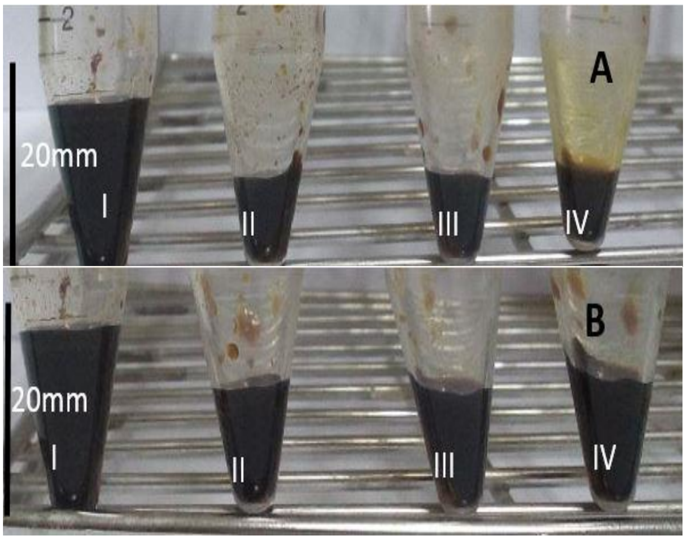

Figure 5 TC increased during observation days on centrifugation treatment: (A) 5 days, (B) and 15 days; (I) control, (II) 4,000RPM, (III) 5,000RPM and (IV) 6,000RPM.

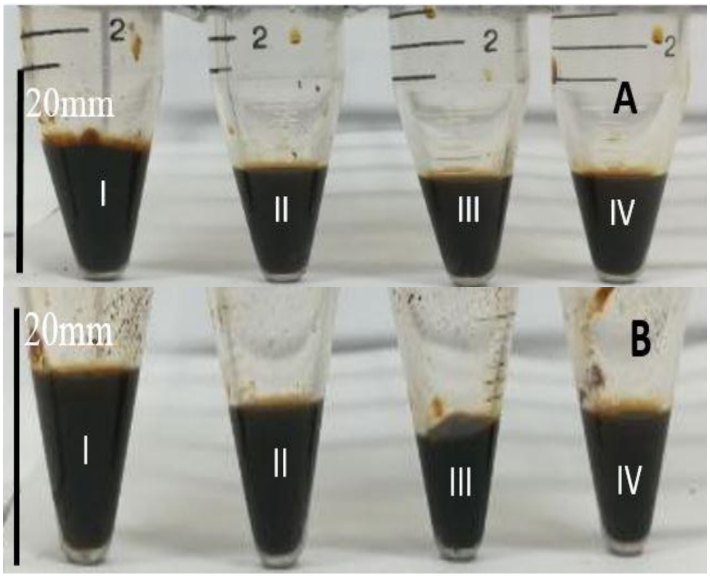

Figure 6 TC increased during observation days on filtration treatment: (A) 1 days and (B) 5 days; (I) control, (II) 1 layer, (III) 2 layer and (IV) 3 layer.

Table 1 Tea cream percentage changes on centrifugation treatment during storage time

Parameter	Centrifuge speed (rpm)	Storage (days)										
		0	1	2	3	5	7	9	12	15	18	21
% Tea cream	Control	21.5±4^{Aa}	22.0±4^{Aa}	21.5±4^{Aa}	22.0±5^{Aa}	22.5±4^{Aa}	22.5±4^{Aa}	22.5±4^{Aa}	22.5±5^{Aa}	22.5±5^{Aa}	23.0±4^{Aa}	23.0±4^{Aa}
	2,000	3.0±0^{Bf}	6.6±0^{Be}	7.1±2^{Be}	9.2±1^{Bd}	9.3±1^{Bd}	11.1±1^{Bc}	12.1±2^{Bc}	14.1±1^{Bc}	16.6±2^{Bb}	20.2±1^{Bb}	23.1±1^{Aa}
	4,000	3.0±0^{Bf}	6.6±1^{Be}	6.1±1^{Be}	8.2±2^{Bd}	8.3±2^{Bd}	10.1±1^{Bc}	12.1±2^{Bc}	14.1±1^{Bc}	16.6±2^{Bb}	19.2±1^{Bb}	22.1±1^{Aa}
	5,000	3.0±0^{Bf}	6.6±2^{Be}	6.1±1^{Be}	8.2±1^{Bd}	8.3±1^{Bd}	10.1±2^{Bc}	12.1±2^{Bc}	14.1±1^{Bc}	16.6±2^{Bb}	19.2±1^{Bb}	22.1±1^{Aa}
	6,000	3.0±1^{Bf}	5.6±1^{Be}	5.1±2^{Be}	7.2±1^{Bd}	8.3±1^{Bd}	10.1±2^{Bc}	11.1±2^{Bc}	14.1±1^{Bc}	16.6±1^{Bb}	19.2±1^{Bb}	21.1±1^{Aa}

Note: Each value is the mean of two independent measurements.

Values in the same column with the different superscript lowercase letters are statistically different (p<0.05).

Values in the same row with the different superscript uppercase letters are statistically different (p<0.05).

Control means untreated sample; 2,000 means speed of centrifugation is 2,000RPM; 4,000 means speed of centrifugation is 4,000RPM; 5,000 means speed of centrifugation is 5,000RPM; and 6,000 means speed of centrifugation is 6,000RPM.

Table 2 Tea cream percentage changes on filtration treatment during storage time

Parameter	Filtration level (filter cloth)	Storage (Days)									
		0	1	2	3	4	5	6	7	8	9
% Tea cream	Control	19.0±4.3^{Aa}	19.9±3.0^{Aa}	20.5±2.1^{Aa}	20.8±1.7^{Aa}	20.7±1.8^{Aa}	21.1±2.0^{Aa}	21.6±1.2^{Aa}	21.6±1.2^{Aa}	21.5±1.4^{Aa}	21.7±1.1^{Aa}
	1 layer	9.8±1.1^{Bcd}	11.3±0.5^{Bc}	13.9±1.6^{Bc}	16.1±2.7^{Bb}	17.1±2.8^{Bab}	18.9±3.0^{ABab}	20.7±1.9^{Aa}	21.0±1.5^{Aa}	21.2±1.1^{Aa}	21.4±0.8^{Aa}
	2 layer	8.6±0.9^{Bd}	10.5±0.7^{Bc}	12.2±1.1^{Bc}	14.1±2.6^{Bc}	15.5±3.6^{Bb}	18.0±4.3^{ABab}	19.6±2.1^{ABa}	20.5±2.1^{Aa}	20.8±1.7^{Aa}	21.2±1.1^{Aa}
	3 layer	7.5±2.1^{Bd}	9.2±1.6^{Bd}	9.8±1.1^{Bd}	11.8±1.7^{Bc}	12.9±1.6^{Bc}	13.4±2.3^{Bc}	14.5±3.5^{Bb}	16.1±4.2^{Bb}	18.1±2.8^{ABab}	21.2±1.2^{Aa}

Note: Each value is the mean of two independent measurements.

Values in the same column with the different superscript lowercase letters are statistically different (p<0.05).

Values in the same row with the different superscript uppercase letters are statistically different (p<0.05).

Control means untreated sample; 2k means speed of centrifugation is 2,000RPM; 4k means speed of centrifugation is 4,000RPM; 5k means speed of centrifugation is 5,000RPM; and 6k means speed of centrifugation is 6,000RPM.

4. Conclusion

This research indicates that centrifugation and filtration can be effectively applied to reduce the tea cream formation in concentrated black tea. The most effective centrifuge speed was at least 4,000 RPM for 20 minutes moreover the effective layer of filter in filtration was 3 layer. This treatment provides the stable CBT as long as 21 days of storage for centrifugation treatment and 9 days of storage for filtration treatment at room temperature (25°C). In conclusion, both simple and convenient centrifugation and filtration can be applied effectively to CBT manufacturer in order to solve the TC problem before distribution to RTD industry, providing the reduction of economic loss due to TC. Combination between centrifugation and filtration in CBT company commonly used due to the convenient application for continuous production.

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