



# **Polyphenolic Retention During** **UF-Membrane Clarification -** **Temperature**

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## **ABSTRACT**

Because high-clarity is a significant driver of consumer acceptance in today's beverage market, ultrafiltration (UF) membrane technology has been implemented at Sensus for higher clarification processing. UF membrane processing is known to be a temperature and pH sensitive method, so polyphenolic retention will vary depending on processing conditions. Since polyphenolic compounds contribute to many aspects of beverage products including taste, flavor, turbidity, and color, the maximum retention of polyphenolic compounds must be considered for the highest quality of beverage products. In the present study, one important factor (temperature) influencing polyphenolic retention during clarification has been investigated and the currently utilized temperature (10-15°C) is the optimum.

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## **INTRODUCTION**

Ultrafiltration was introduced to Sensus tea processing as part of a two stage clarification process, centrifugation followed by ultrafiltration to maximize retention of bioactive compounds including polyphenolics. Temperature and pH significantly affect the solubility of the compounds responsible for the tea creaming phenomenon as well as other tea phytochemicals. By using the optimized treatment, one can obtain a concentrate with optimal polyphenolic and antioxidant content while reducing chemical complexation (tea creaming).

## **MATERIALS AND METHODS**

### Material:

Four black tea liquors at four different temperatures were obtained after UF membrane filtration. The temperatures were 1. 7.5°C, 2. 9.2°C, 3. 12.3°C and 4. 15.0°C. Raw tea material used for this study was Indonesian black tea.

### Chemical analysis:

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1. Total Phenolics (TP) (also known as total soluble phenolics) was determined using Folin-Ciocalteu assay as early described by Swain and Hillis (1959) and expressed as gallic acid equivalent.
2. °Brix was determined by AR200 digital hand-held refractometer at room temperature after 5-fold dilution.
3. Turbidity of each tea liquor was measured using 2100P turbidimeter (Hach) at room temperature.
4. Total liquor color was measured at 460nm using Genesis 6 spectrometer (Thermo Inc.). The absorbance of each tea infusion was recorded and the color value was calculated as described by Obanda et al., (2004).
5. Total Theaflavin (TF) was determined by Flavognost method modified by Obanda et al., (2001). Briefly, 10mL of tea liquor was mixed with 10mL of isobutylmethylketone (IBMK) solution and the mixture was allowed to sit for 15min for complete separation after shaking for 10min. Two milliliters of IBMK layer (upper layer) was taken into test tube which containing 4ml of ethanol and 2ml of flavognost reagent. After shaking, the mixture stayed at room temperature for 15min for complete color development and it was measured at 625nm using Genesis 6 spectrometer (Thermo Inc.). IBMK solvent was procured from Sigma-Aldrich. Flavognost reagent was prepared by dissolving 2g or diphenylboric acid-2-aminoethyl ester in 100mL of distilled water. The measured absorbance was introduced into following equation for total TF.

$$TotalTF = A_{625} \times 47.9 \times \frac{DM}{100}$$

Where: DM – dry matter

6. Total thearubigin (TR), thearubigin type SI (TRSI), and thearubigin type (SII) were quantified by spectrometric assay as described by Obanda et al., (2004). A solution was prepared by mixing 10mL of tea liquor with 50mL of IBMK solution. The mixture stayed at room temperature for 10 min for complete separation after shaking for 10min. Four milliliters of IBMK layer (upper layer) was taken into beaker and the volume was made up to 25mL with HPLC grade methanol (A). Two milliliters of aqueous layer (bottom layer) from (A) was taken into test tube and diluted to 10mL with distilled water. The volume was made up to 25mL with HPLC grade methanol (B). Twenty five milliliters of IBMK layer from (A) was mixed with 2.5% aqueous sodium hydrogen carbonate (sodium bicarbonate) and shaken for 10min in separatory funnel. After discard aqueous layer, 4mL of IBMK was taken into beaker and the volume was made up to 25mL with HPLC grade methanol (C). Two milliliters of aqueous layer from (A) was taken into beaker and mixed with 2mL of saturated oxalic acid solution and 6mL of distilled water. The volume was made up to 25mL with HPLC grade methanol (D). Four obtained solutions were measured at 280 and 460nm using Genesis 6 spectrometer (Thermo Inc.). Total TR, TRSI, TRSII, and brightness were calculated using following equations.

$$\%TotalTR = \frac{375 \times 0.02 \times 6.25 \times (2D + A - C)}{0.733 \times 9 \times \frac{DM}{100}}$$

$$\%TRSI = \frac{375 \times 0.02 \times 6.25 \times (A - C)}{0.138 \times 9 \times \frac{DM}{100}}$$

$$\%TRSII = \frac{375 \times 0.02 \times 12.5 \times D}{0.233 \times 9 \times DM / 100}$$

$$Darkness = \frac{100 \times C}{A + 2B}$$

7. Ratings and scales test was conducted for liquor color perception by tasters. Seven randomly selected and trained participants picked one darkest and one lightest samples out of 4 samples. During sensory analysis, enough back light was provided for accurate testing with white background to avoid background effect.

Data represent the mean triplicate analysis using ANOVA (analysis of variance) with JMP 5 statistical software. Mean separation was conducted using the LSD test ( $P < 0.05$ ). Correlations ( $r$ ) of data was evaluated using Pearson's correlation analysis.

## **RESULTS AND DISCUSSION**

°Brix and Turbidity are displayed in Table 1. No remarkable difference was found between different temperatures for °Brix except for #2 which was slightly higher than the others while the turbidity varied somewhat, the error of the instrument becomes significant at such low NTU values and we are reluctant to claim any significance.

Total phenolic content, total theaflavin, total thearubigin, thearubigin (type SI), thearubigin (type SII), liquor darkness, and taster's color reception were illustrated in Table 2.

Total phenolic content was highest in no.2 (9.2°C) and it was higher by 8, 3, and 4% than no.1, 3, and 4 tea liquors, respectively, which is not significantly different ( $P < 0.05$ ).

Total theaflavin content was very well correlated ( $r=1$ ) with total liquor color indicating that total theaflavin content fully contribute to color of tea product (Table 3). Total theaflavin content was highest in no.4 tea and lowest in no. 1 tea. There was no difference between no. 2 and 3 teas in total TF. Thearubigins (TR and TRSII) contributed to color strength but the degree of contribution was lower than that of theaflavin ( $r=0.77$  and  $0.75$ , respectively). The other color value "darkness" showed how dark the samples were. This value was well correlated with total liquor color. Darkness was also directly affected by the concentration of total TF. Taster's color perception was somewhat different from actual color value measured by spectrometer. About 86% of participants indicated no.2 is the darkest but 71% chose no.1 sample as a lightest sample.

According to the data from the present study, the liquor temperature passing through UF membrane is already optimized from previous trials in terms of polyphenolic retention and resultant color strength. The temperature range (10-15°C) should be kept for highest polyphenolic retention. By using this temperature, the product from Sensus processing line would fit well to bag in the box products.

**Table 1. Total phenolics and °Bx of tea liquors from different membrane filtration temperature.**

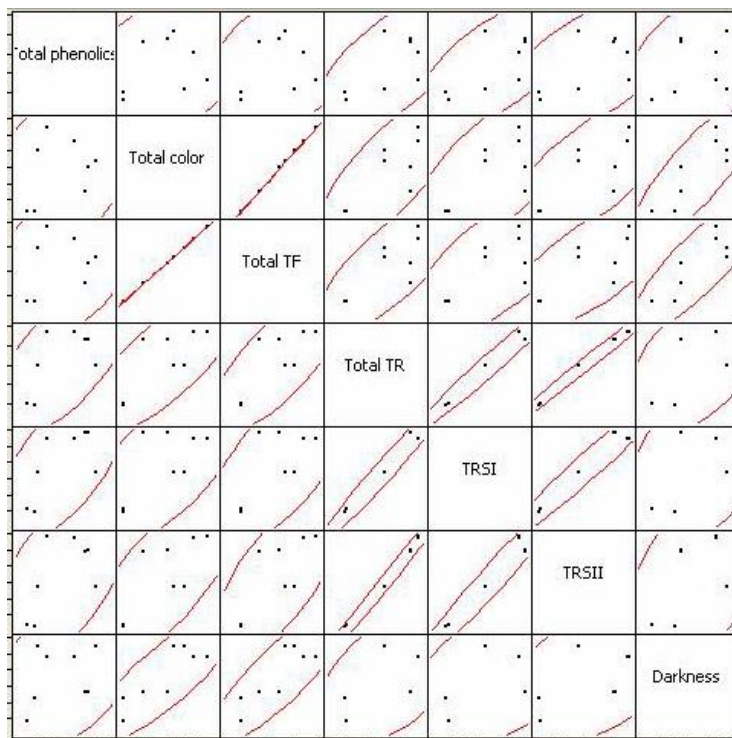
	<b>1. 7.5°C</b>	<b>2. 9.2°C</b>	<b>3. 12.3°C</b>	<b>4. 15.0°C</b>
°Brix	3.8	4.2	3.7	3.5
Turbidity (NTU)	0.61	0.49	0.59	0.45

**Table 2. Total phenolic content, total liquor color, total theaflavin, total thearubigin, thearubigin (SI), thearubigin (SII), liquor darkness, and taster's color perception on four different UF membrane temperatures.**

<b>Temperature</b>	<b>1. 7.5°C</b>	<b>2. 9.2°C</b>	<b>3. 12.3°C</b>	<b>1. 15.0°C</b>
<b>Results</b>				
1. Total phenolic content (mg/L)	3279.3	3565.5	3465.52	3434.48
1. Total liquor color (no unit)	1.56	2.06	2.42	2.75
2. Total Theaflavin (mg/kg)	745.63	981.75	1155.73	1317.28
3. Total Thearubigin (%)	34.98	43.21	39.98	44.18
4. Thearubigin (SI) (%)	10.50	12.92	11.69	12.72
5. Thearubigin (SII) (%)	9.65	11.98	10.84	12.37
6. Liquor Darkness (%)	0.88	1.16	1.86	1.70
7. Taster's color reception (%)	0/71	86/0	0/0.14	14/0.14

**Table 3. Corelation between color and color contributing compounds and scatterplot matrix.**

Correlation	Total phenolics	Total color	Total TF	Total TR	TRSI	TRSII	Darkness
Total phenolics	<b>1.00</b>	0.29	0.29	0.63	0.67	0.59	0.31
Total color	0.29	<b>1.00</b>	1.00	0.77	0.66	0.75	0.85
Total TF	0.29	1.00	<b>1.00</b>	0.77	0.66	0.75	0.85
Total TR	0.63	0.77	0.77	<b>1.00</b>	0.98	0.99	0.52
TRSI	0.67	0.66	0.66	0.98	<b>1.00</b>	0.98	0.39
TRSII	0.59	0.75	0.75	0.99	0.98	<b>1.00</b>	0.46
Darkness	0.31	0.85	0.85	0.52	0.39	0.46	<b>1.00</b>



## **REFERENCES CITED**

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