# Photometric Evaluation and Statistical Analysis of Tea Infusion

By TADAKAZU TAKEO

Tea Agronomy Division, National Research Institute of Tea

## Introduction

The quality of black tea is usually evaluated and classified by the appearance of the tea leaf and by the odor, taste and color of the infusion.

On those works, the skill and experience of highly trained tastes are indispensable. Therefore, it is very useful to supplement at least some of their works by instrumental measurement. Especially the application of instrumental evaluation on tasting the tea quality is greatly demanded on the quality control of tea.

Recently the technique of separation and measurement of the colored components in the infusion remarkably progressed and some tests on using the instrumental measurement have been reported on the evaluation of tea quality[1].

In our laboratory, the colored components in the infusion separated into three fractions (theaflavin, thearubigin and high polymerized substances) by using Sephadex LH-20 column chromatography and the influences of their fraction on the color of the infusion have been revealed[2]. Then, we have also developed the statistical analysis method of the quality of the infusion by using the photometric technique[3].

## Separation of colored components in tea infusion by chromatography on Sephadex LH-20

The colored components in the infusion were separated into nine components by using Sephadex LH-20 column chromatography in aqueous acetone as shown in Fig. 1.

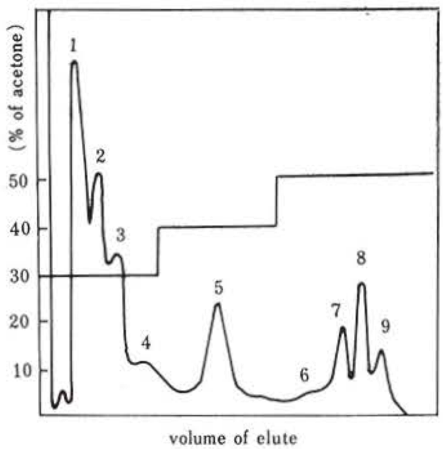


Fig. 1. Elution pattern of tea infusion

Among those components, the early eluted components (1–4) consisted of brown colored substances eluted by aqueous acetone of 30%. Their visible spectra showed the concave curves drew down from short-wave length to long-wave length. We classified those components to the water-soluble high polymerized substances. Usually, concentrations of those components showed high levels in the infusion of low-grade tea.

Component 5 mainly consisted of thearubigin. This component was deep red and eluted by aqueous acetone of 40%. The visible spectrum of this elute showed a peak at 355 mμ, as reported by Roberts.

The last components (7–9) consisted of theaflavins. These components were light orange red and the spectra had two peaks at

375 and 460 mμ.

Generally the concentrations of theaflavins and thearubigin indicated high values in the infusions of high-grade tea.

Before the last three components, orange colored substance was eluted (component 6). In this component, some anthocyanins were revealed.

## Separation and determination of theaflavins

Theaflavins have been resolved in the order of theaflavin, theaflavin-monogallates and theaflavin-digallate from Sephadex LH-20 column with gradient elution of aqueous acetone from 40 to 80%, as shown in Fig. 2.

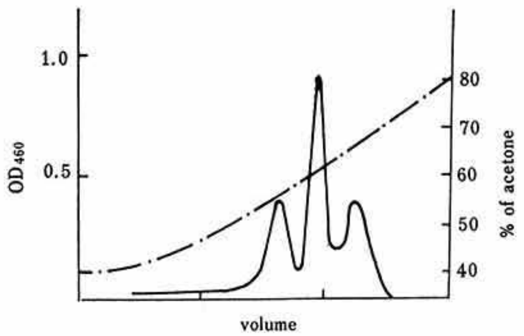


Fig. 2. Elution pattern of theaflavins in tea infusion

The contents of three theaflavins were determined colorimetrically from the molar extinction coefficient of authentic theaflavin at 460 mμ after separating each component by Sephadex LH-20.

From the results of the determination of each theaflavin in the infusion of packed tea in Table 1, it has been recognized that the content rates of theaflavin, theaflavin-monogallates and theaflavin-digallate in total theaflavins were about 35%, 46% and 19% respectively[4].

## Application of photometric evaluation of tea infusion

Each optical density at 380 mμ ($OD_{380}$) of theaflavin, thearubigin and high polymerized substance in the infusion has been determined by the procedure described in Fig. 3[5], respectively.

The $OD_{380}$ values of theaflavin, thearubigin and especially theaflavin plus thearubigin showed high positive correlations with the evaluation of the quality of tea leaf, while high polymerized substance showed negative correlation, as shown in Tables 2 and 3. From these results, it was revealed that the rates of $OD_{380}$ values of theaflavin plus thearubigin and high polymerized substance in total color of the infusion were very effective to evaluate

**Table 1. Contents of theaflavins in packed tea**

(mg/g)

| Tea | Source of infusion | Theaflavin | Theaflavin-monogallate | Theaflavin-digallate | Total Theaflavin |
|---|---|---|---|---|---|
| Nitto (Green) | 10 | 0.48 | 0.64 | 0.24 | 1.36 |
| Lipton (Early Gray) | 10 | 0.52 | 0.64 | 0.28 | 1.46 |
| Nitto (Violet) | 12 | 1.44 | 1.68 | 9.72 | 3.84 |
| Ridgways (Darjeeling) | 12 | 1.00 | 1.58 | 0.70 | 3.28 |
| Lipton (Darjeeling) | 15.5 | 1.08 | 2.08 | 1.00 | 4.16 |
| Lipton | 16.5 | 1.52 | 1.76 | 1.20 | 4.48 |
| Ridgways (Five O'clock) | 18 | 1.52 | 2.32 | 1.16 | 5.00 |
| Ridgways (Breakfast) | 18.5 | 1.56 | 2.14 | 0.90 | 4.69 |
| Brooke Bond | 18.5 | 2.32 | 2.56 | 0.56 | 5.44 |
| Nitto (White) | 19 | 1.76 | 2.04 | 0.72 | 4.52 |
| Lipton (London) | 19 | 2.38 | 2.56 | 0.82 | 5.76 |

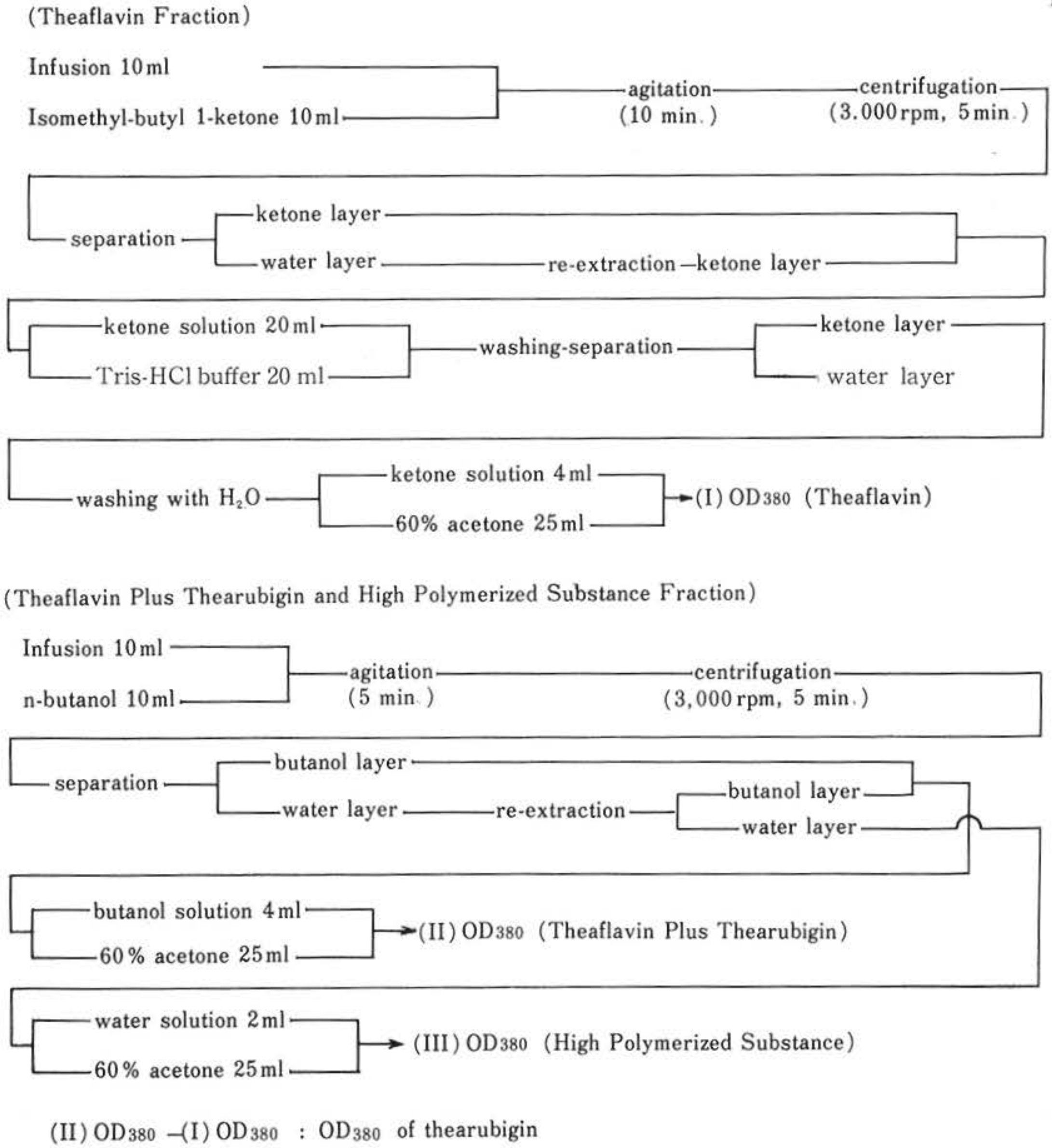


Fig. 3. Separation of colored components

and classify the quality of tea.

## Statistical analysis of tea quality

In the foregoing paragraph, we have proposed that the three color components in the infusion were very effective as the factors expressing the quality of the infusion. So we have tried to apply the above mentioned photometric analysis on the quality control of tea.

Among the high, middle and low-grade tea packed by a commercial company, the $OD_{380}$ values of high polymerized substance (Factor X) and theaflavin plus thearubigin (Factor Y) in the infusions were surveyed by using the methods indicated in Fig. 3.

The high-grade tea showed the low values of Factor X and the high values of Factor Y, while the reversed results were obtained from low grade tea (Table 4).

**Table 2. $OD_{380}$ value in tea infusion**

| Group | | Price (yen/kg) | Thea-flavin (%) | Thea-rubigin (%) | Theaflavin + Thearubigin (%) | High polymerized substance (%) | Thea-flavin (%) | Thea-rubigin (%) | High polymerized substance (%) | Theaflavin + Thearubigin (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| Low | 1 | 365 | 13.1 | 44.3 | 57.4 | 42.6 | | | | |
| | 2 | 398 | 16.6 | 47.2 | 63.8 | 36.2 | | | | |
| | 3 | 424 | 11.3 | 47.4 | 58.7 | 41.3 | 13.3 | 45.1 | 41.6 | 58.4 |
| | 4 | 440 | 13.2 | 42.1 | 55.3 | 44.7 | ±1.7 | ±2.3 | ±3.4 | ±3.4 |
| | 5 | 442 | 11.4 | 43.1 | 54.5 | 45.5 | | | | |
| | 6 | 449 | 12.6 | 43.4 | 56.0 | 44.0 | | | | |
| | 7 | 455 | 14.6 | 48.2 | 62.8 | 37.2 | | | | |
| Middle | 8 | 513 | 20.3 | 46.6 | 66.9 | 33.1 | | | | |
| | 9 | 515 | 19.3 | 52.1 | 61.4 | 38.6 | 17.5 | 49.8 | 38.7 | 65.3 |
| | 10 | 531 | 15.3 | 51.1 | 66.4 | 33.6 | ±2.1 | ±2.4 | ±2.1 | ±2.1 |
| | 11 | 559 | 15.2 | 51.9 | 67.1 | 32.9 | | | | |
| | 12 | 579 | 17.5 | 47.3 | 64.8 | 35.2 | | | | |
| High | 13 | 625 | 19.2 | 52.5 | 71.7 | 28.3 | | | | |
| | 14 | 630 | 19.3 | 53.6 | 72.9 | 27.1 | | | | |
| | 15 | 658 | 18.6 | 51.3 | 69.9 | 30.1 | 19.0 | 52.6 | 28.3 | 71.7 |
| | 16 | 682 | 18.8 | 53.5 | 72.3 | 27.7 | ±0.3 | ±0.8 | ±1.0 | ±1.0 |
| | 17 | 700 | 19.3 | 52.3 | 71.6 | 28.4 | | | | |

**Table 3. Correlation coefficient between price and color content**

| Theaflavin | Thearubigin | Theaflavin + Thearubigin | High polymerized substance | Price |
|---|---|---|---|---|
| Theaflavin | 0.442 | 0.172 | −0.844** | 0.787** |
| Thearubigin | | 0.925** | −0.244 | 0.475* |
| Theaflavin+Thearubigin | | | −0.537* | 0.683** |
| High polymerized substance | | | | −0.773** |

*,** Indicates significant coefficients of 5 or 1 per cent

The colors of the infusion of each grade tea were divided in the ellipses controlled by the bivariate of the $OD_{380}$ values of Factors X and Y and restricted within (2; 0.05), respectively. These ellipses were termed the quality control diagram using bivariate and drawn as follows.

That is;
the circumscribed rectangle in Fig. 4 is obtained by the following equations:

$$x = x \pm Sx\chi(2;\ 0.05) = x \pm dx$$
$$y = y \pm Sy\chi(2;\ 0.05) = y \pm dy$$

$x, y$; $OD_{380}$ of $X$ or $Y$
$x, y$; mean value of determined $OD_{380}$ values
$Sx, Sy$; standard deviation of $OD_{380}$ values

The points of contact of ellipse with the rectangle.

$$(x \pm r dx,\ \ y \pm dy)$$
$$(x \pm dx,\ \ y \pm r dy)$$

$r$: correlation coefficient between $x$ and $y$
The crossing points of ellipse on $x$ and $y$ axis.

$$(x \pm \sqrt{1-r}\, dx)$$
$$(y \pm \sqrt{1-r}\, dy)$$

Moreover, each adjoining group between high and middle or middle and low-grade tea was separated by the discriminant function made from bivariate as follows:
the discriminant function is

$$Z = a_1(x - \bar{x}) \cdot a_2(y - \bar{y})$$
$$\bar{x} = (\bar{x}_1 + \bar{x}_2)/2, \quad \bar{y} = (\bar{y} + \bar{y})/2$$

**Table 4. Concentration of color component**

($-\log T_{380nm}$)

| Tea grade | No. | Theaflavin | Thearubigin | Theaflavin + Thearubigin | High polymerized substance |
|---|---|---|---|---|---|
| High | 1 | 0.162 | 0.391 | 0.553 | 0.236 |
| | 2 | 0.166 | 0.376 | 0.542 | 0.234 |
| | 3 | 0.158 | 0.404 | 0.562 | 0.208 |
| | 4 | 0.161 | 0.416 | 0.577 | 0.245 |
| | 5 | 0.148 | 0.393 | 0.541 | 0.219 |
| | 6 | 0.144 | 0.423 | 0.567 | 0.244 |
| | 7 | 0.141 | 0.387 | 0.528 | 0.218 |
| | 8 | 0.135 | 0.403 | 0.538 | 0.199 |
| | 9 | 0.133 | 0.422 | 0.555 | 0.189 |
| Middle | 1 | 0.130 | 0.385 | 0.515 | 0.231 |
| | 2 | 0.128 | 0.361 | 0.489 | 0.235 |
| | 3 | 0.129 | 0.361 | 0.490 | 0.248 |
| | 4 | 0.130 | 0.401 | 0.531 | 0.263 |
| | 5 | 0.115 | 0.382 | 0.497 | 0.263 |
| | 6 | 0.132 | 0.376 | 0.508 | 0.250 |
| | 7 | 0.127 | 0.403 | 0.530 | 0.265 |
| | 8 | 0.115 | 0.404 | 0.519 | 0.257 |
| | 9 | 0.138 | 0.396 | 0.534 | 0.270 |
| Low | 1 | 0.107 | 0.341 | 0.448 | 0.283 |
| | 2 | 0.114 | 0.354 | 0.468 | 0.269 |
| | 3 | 0.143 | 0.324 | 0.467 | 0.299 |
| | 4 | 0.105 | 0.333 | 0.438 | 0.292 |
| | 5 | 0.094 | 0.359 | 0.453 | — |
| | 6 | 0.103 | 0.325 | 0.428 | 0.291 |
| | 7 | 0.091 | 0.332 | 0.423 | 0.284 |
| | 8 | 0.094 | 0.332 | 0.426 | 0.300 |
| | 9 | 0.114 | 0.351 | 0.465 | 0.329 |

$$a_1 = \frac{\bar{x}_1-\bar{x}_2}{Sx^2} + r\,\frac{\bar{y}_1-\bar{y}_2}{Sx\cdot Sy}$$

$$a_2 = \frac{\bar{y}_1-\bar{y}_2}{Sy^2} + r\,\frac{\bar{x}_1-\bar{x}_2}{Sx\cdot Sy}$$

exponent 1 or 2 indicates the grade number of tea.

The line made from substituting zero to $Z_k$ in the discriminant function terms the discriminant line between the adjoining groups.

The discriminant lines among three grade tea made from the data in Table 3 were drawn in Fig. 4, respectively; furthermore, the adjoining groups separated by the discriminant line were divided from each other at the significant levels of 1 or 5% by the $F$-distribution test, as shown in following figures, respectively:

$F_2^{17}=6.33^{**}$: between high and middle grade.

$F_2^{16}=4.23^{*}$ : between middle and low grade.

(* and ** show the significant levels of 5 or 1%)

the significant levels are estimated from the following equation:

$$F=\frac{f-p+1}{Pf}\left(\frac{n_1n_2}{n_1+n_2}\right)(D_1^2-D_2^2)$$

$p$: the number of groups

$f$: the total number of tea in two groups

$n_1$ or $n_2$: the number of tea in group 1 or 2, respectively

$D_1$ or $D_2$ was calculated from the discriminant function by substituting $\bar{y}_{1or2}$ to $y$ and $\bar{x}_{1or2}$ to $x$, respectively.

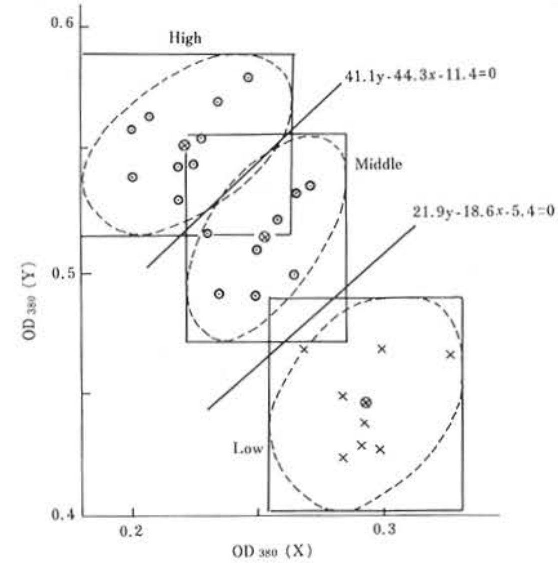

Fig. 4. Quality control diagrams and discriminant lines among three grade teas

From those results, it was revealed that the colors of the infusion among the three grade tea were controlled in the quality control diagrams made from the $OD_{380}$ values of theaflavin plus thearubigin and high polymerized substance at the significant level of 5%, respectively, and then the adjoining grades were separated from each other at the significant levels of 1 or 5%.

Thus it has been explained that the statistical analysis by the quality control diagram and the discriminant function made from the bivariate of $OD_{380}$ values of theaflavin plus thearubigin and high polymerized substances is very effectual on evaluating and classifying the quality of the infusion.

## References

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