Discrimination of teas based on total luminescence spectroscopy and pattern recognition

L. Nitin Seetohul [a], Meez Islam [a], William T O'Hare [a], Zulfiqur Ali [a]*

[a] School of Science and Technology, University of Teesside, Middlesbrough, UK TS1 3BA..* Corresponding authors Email: m.islam@tees.ac.uk

ABSTRACT

This paper reports on the application of total luminescence spectroscopy to discriminate between seven different types of tea. Bottled liquid teas; Oolong tea, Green tea, Houji tea, Japanese black tea and tea leaves (Kenya tea, Assam tea and Ceylon tea). Total luminescence spectra were recorded by measuring the emission spectra in the range 300nm to 700 nm at excitation wavelengths from 250 nm to 590nm. Discrimination of teas based on their spectral data was performed by principal component analysis (PCA) a common chemometric technique used for data reduction and visualisation. The results demonstrate the ability of fluorescence techniques to differentiate between green tea (Unfermented), black tea (fermented) and oolong tea (partially fermented).

KEYWORDS Total luminescence, tea classification, green tea, oolong tea, black tea, houji tea.

Introduction

Tea is the most widely consumed beverage aside from water [1,2] with an annual production of 1.8 million tonnes of dry leaves and a per capita worldwide consumption of approximately 40 litres of beverage per year. It is estimated that somewhere between 18 and 20 billion 6 oz. cups of tea are drunk daily on our planet. [3,4].

Tea quality mainly depends on the variety of leaf, growing environment, and manufacturing conditions, size of ground tea leaves and infusion preparation. Quality is measured on the basis of liquor (brightness, briskness, colour etc.), aroma (flavour) and leaf appearance. The production of most branded tea involves blending of many varieties of tea to maintain the consistency of taste. Assuring the optimum blend require the tea taster to taste hundreds of liquors [5,6,7,8].

Tea leaf, in common with all plant leaf matter, contains enzymes, biochemical intermediates, carbohydrates, protein, lipids, and structural elements normally associated with plant growth and photosynthesis. In addition, tea leaf is distinguished by its remarkable content of methylxathines and polyphenols. These two groups of compounds are predominantly responsible for those unique properties of tea that account for its popularity as beverage. The most important chemical constituent that influences the taste and flavour in tea infusions are polyphenols, flavonols, caffeine, sugars, organic acids, amino acids and volatile flavour compounds. Phenolic compounds of tea such as theaflavins and thearubignins are very important from an intrinsic quality point of view and constitute 60% of the total water-soluble constituents of black tea. Moreover they are responsible for the major organoleptic properties of colour, brightness (bright orange-red tone) and astringency. [5,9,10].

Researchers have endeavoured to develop methods for classifying different types of teas. Teas have been classified according to their metal content [2,11,12], chemical composition and colour difference [13] volatile components [14], physico chemical properties [15],

Fourier transform infrared spectroscopy [16], and also using an electronic nose [17] and electronic tongue [18].

In tea manufacturing the human sense of smell and sight is used as an important tool for quality diagnosis. Sensory evaluation is subjective, but with careful design of a scoring system and rigorous training of the assessors, the evaluation becomes more objective. However it remains an expensive option. Instrumental methods for the determination of odours, colour and taste such as gas chromatography – mass spectrometry (GC-MS), High performance liquid chromatograph (HPLC), colour difference meters, inductively coupled plasma- atomic emission spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS) are costly, require trained personnel and are often of limited value and time consuming. As a result there has been an effort to investigate devices or instrumental methods for more objective and inexpensive analysis of tea, which do not require specialist technicians. These devices would be less prone to drift, and more consistent than sensory assessment, but the responses must be correlated with sensory techniques. [19, 20, 21, 22]

Fluorescence spectroscopy is a well established simple, fast, accurate and non destructive technique which so far has not been used for classification of teas. Its high sensitivity and non destructive nature means this technique could be used for continuous measurement of the quality of tea during the various stages of manufacturing. Total luminescence spectroscopy (TLS) is a multi dimensional fluorescent technique that involves simultaneous acquisition of multiple excitation and emission wavelengths in order to increase the method selectivity. The total luminescence spectra are obtained from the excitation – emission data matrix; that is by plotting fluorescence intensity as a combined function of excitation and emission wavelength. The excitation emission matrices (EEM) can be used as a finger print for individual or mixtures of fluorescent components. TLS has been used for discrimination of fuels [23], water quality [24] and classification of oils [25] but not tea.

Miyazawa et al [26] reported the chemiluminescence of catechins, theaflavins and anthocyanins to have a maximum emission wavelength at 630 nm, 690 nm and 675 nm respectively. As the TLS of tea is related to the content of polyphenols and flavanoids and these compounds are responsible for the major organoleptic properties, it is proposed that TLS could be used as a measure of quality for teas. It is further proposed that, rather than calibrate the TLS data to quantify individual components and then relate these concentrations to organoleptic quality; it might be possible to relate the data more directly to quality using statistical techniques such as Principal Component Analysis (PCA). PCA is a statistical tool commonly used for the unsupervised classification of multivariate data. [27, 28].The main aim of principle component analysis is to reduce dimensionality of data, giving a small number of principle components (PCs) that represent the vast majority of variance in the data. PCA finds an alternative set of axes about which a data set may be represented and indicates along which axis there is most variation. This allows more effective visualization and classification of multivariate data [29]. This method allows natural clustering of the data to be observed as well as providing an insight into how effectively a pattern recognition system could classify the data.

Several applications of PCA may be found in literature with respect to establishing the difference between dissimilar teas using quality control data, trace metal content [11], colour [13], volatile components [14] and organic compounds [16].

In this study total luminescence spectroscopy has been used as a fingerprint and pattern recognition applied to these fingerprints allowed the discrimination of different types of tea. This objective instrumental method is inexpensive, sensitive, relates to the quality of tea has potential for automation and also it is not subjective as sensory evaluation by tea graders.

Materials and methods

Apparatus

Total luminescence measurements were carried out using a Hitachi F-2000 spectrofluorimeter, with a Quartz cell (VWR) of 10 mm path length. The apparatus was remotely operated using the F3D software (Hitachi) on a PC connected via an RS232 port. The light source was a long life 150 W xenon lamp with ozone self dissociation function. The wavelength scan rate and the band pass were maintained at 1200 nm/min and 10 nm respectively.

Seven different types of tea were used in this study. Three tea leaves; Assam (Sainsbury) Kenya (Sainsbury), Ceylon (Tea Plucker, pure Ceylon tea) and four PET bottled Japanese tea; black tea (Kirin Beverages), oolong tea (Suntory), green tea (Ito – En Beverages), Houji tea (Ito – En Beverages). The Japanese teas were donated courtesy of the GEN foundation - UK. Water purified by reverse osmosis was used throughout.

Statistical analysis

The data were standardised using Minitab (Version 14). The 3D excitation – emission matrix (EEM) and the principal component analysis (PCA) were constructed using the Unscrambler software (version 9).

Sample preparation for fluorescence measurement of leaf teas (Assam, Kenya and Ceylon)

Tea samples (0.5 g) were weighed and transferred into 60ml brown glass bottles. Hot water (30 ml at 80 ^{0}C) was added to the bottles by means of a pipette. The tea was allowed to infuse for 5 min and the liquor was then filtered through Whatman® filter papers (number 1 of 125mm diameter). The filtration process removes, tea particles that produces scattering of the incident excitation light source. The filtrate was collected and stored in brown glass bottles flask for 15 min to allow the brew to cool down to room temperature before being analyzed using the fluorimeter. This simple procedure was repeated for each tea sample.

Sample preparation of PET bottled tea for fluorescence analysis.

The PET bottled teas were used without further filtration. It was found that they did not contain any particles that might interfere with the fluorescence analysis as they are filtered during the manufacturing process. The bottled tea available for this study was found to be too concentrated as self quenching was observed when the EEM was recorded. Hence the teas were diluted 2 fold with reverse osmosis purified water and then stored in brown glass bottles before analysis with the fluorimeter as this produced an active concentration similar to the leaf infusions.

Sampling procedure

The diluted solutions were placed in quartz cuvettes of 10 mm path length and the total luminescence measurement was carried out using the fluorimeter. The temperatures of the samples were recorded before each run, to ensure that they were at room temperature. The excitation wavelength was set from 250nm to 590nm with interval of 20nm. The emission was set from 300nm to 700nm with an interval of 5nm. The contour interval was set at 10nm.

Results and discussion

The total luminescence spectra were recorded for the different teas. Residual excitation peaks were observed in the EEM's for excitation wavelengths at 20nm intervals between 350nm to 590nm and emission wavelengths at 5nm intervals between 340nm and 605nm. It can be observed from Fig.1 which shows the EEM of Japanese black tea that, as the residual excitation peaks have high intensity, the fluorescence from the tea is therefore masked. The residual excitation peaks were removed from all the data collected and Fig.2 shows the EEM of the same black tea that was used to produce the EEM in Fig.1 but without the excitation peaks.

The data collected were standardized from 1 to -1 using Minitab 14. Hence the highest peak was given a score of 1 and the lowest peak a score of -1. This process was carried out in order to standardise all the samples according to ratio of the chemical component responsible for the luminescence in the tea samples. The standardisation reduces the problems due to slight variations in infusion strength and it is also a better strategy rather than trying to standardise infusion strengths.

Fig. 3 shows the EEMs for the standardized data. The x axis represents the emission wavelength and y-axis represents the excitation wavelength, while the z axis represents the fluorescence intensity

The total luminescence spectra of the black teas are shown in figure 3 (a) to (d).The 3D fluorescence spectra show different emission spectrum for the different type of teas. The distinct shapes can be visually analyzed for discrimination between Assam, Kenya and Japanese black tea as they have completely different EEM patterns. However the small difference between Assam and Kenya tea is less obvious apart from the difference in intensity.

The total luminescence spectra of the bottled Japanese teas are shown in figure 3 (e) to (g).The 3D fluorescence spectra show different emission spectrum for the different type of teas. The small difference between green, Houji and Oolong tea is less obvious by visual inspection of the EEM's. Hence the need to use a statistical tool such as PCA to differentiate between them.

A PCA was carried out on the data from the 7 different types of tea. This procedure was used as it extracts the dominant patterns in the data and permits us to find the correlation structure of the variables and investigating how many components are necessary to explain the greater part of variance with a minimum loss of information. Several techniques other than TLS have been used in conjunction with PCA to discriminate between teas [2, 11, 14, 17, 18]. Electronic nose and electronic tongues have been use in conjunction with PCA for

food applications without having to know the specific compounds responsible for differences [17, 18, 29, 30].

As PCA is powerful technique for visual comparison, knowledge of the chemical properties of the different analytes responsible for the difference in TL is not required.

Fig. 4 shows the PCA scores plot, the seven tea groups occupy seven regions within the variance space. It is clearly shown that the different teas Oolong, Green, Houji, Kenya, Assam, Ceylon and black are sufficiently separate, without any classification error. The Black teas are found on the right quadrants with Kenya tea on the top (Positive score for PC1 and positive score for PC2), Japanese black tea on the bottom right (positive score for PC1 and negative score for PC2) and Assam and Ceylon in the mid right portion (positive score for PC1) of the variance space. In the first principal component (PC1) the Black teas (Kenya, Assam, Ceylon, Japanese PET black tea) shows positive scores in contrast to unfermented teas (Green, Oolong and Houji Tea) which are found on the left quadrant. Oolong tea showed a positive score for both PC's whereas Green tea and Houji tea showed negative score for both PC's

According to the results obtained PCA provides very good discrimination between the different classes of tea. From the PCA it can also be observed that Assam and Ceylon tea are found very close in the variance space perhaps reflecting the fact that they are obtained from geographically the closest regions whereas Kenya and the Japanese PET black tea are cultivated in regions with considerable difference in their climatic conditions as compared to Assam and Ceylon.

This work has shown the potential of fluorescence spectroscopy to distinguish between seven types of tea. Visual inspection of the EEMs of tea samples makes it possible to assign a large number of samples to the expected type. This study demonstrates that TLS could potentially form the basis of a simple method to discriminate between different types of teas in the tea manufacturing industry.

According to the statistical analysis the Green, Houji and Oolong tea are different from black teas. This could be explained by the fact that green tea is unoxidised as they are steamed immediately after picking in order to avoid fermentation, brown teas (Oolong tea and Houji tea) are not fully oxidized as the fermentation process utilises oxidising enzymes originally contained in tea leaves and black tea is fully oxidized as it is produced by full fermentation where various compounds are produced. The black tea also shows a broader emission spectrum possibly due to the formation of more compounds produced in the manufacturing process that involves the intensive oxidation of green tea catechins to catechin quinines and eventually leading to the formation of coloured complex polyphenols known as theaflavins and thearubignins [7]. The data from the PCA plot could be of use as a tool in the tea industry for discriminating between different types of teas and also as a monitoring tool within the processing of tea.

This work lays the foundation for the further work which is required to develop a cheap, robust, objective device to determine the quality of tea rather than the costly subjective assessment by tea tasters and tea graders. This work will be extended to classification of leaf teas from a single region, hopefully allowing prediction of quality allocations made by trained by tea tasters, and also to classification of blended teas.

REFERENCES

1. Harbowy ME; Balentine, DA Tea Chemistry. *Critical Reviews in Plant Sciences* **16:**415-480 (1997**)**

2. Fernandez-Caceres PL; Martin MJ; Pablos F. and Gonzalez AG, Differentiation of Tea (Camellia sinensis) Varieties and Their Geographical Origin According to their Metal Content *J. Agric. Food chem* **49:**4775-4779 (2001**)**

3. Weisburger JH, Tea and health: a historical perspective, *Cancer Letters* **114:**315-317 (1997)

4. London: International Tea Committee *Annual bulletin of Statistics*. pp123 (1995**).**

5. Astill C; Birch MR; Dacombe C; Humphrey PG and Martin PT, Factors Affecting the Caffeine and Polyphenol Contents of Black and Green Tea Infusions *J. Agric. Food chem* **49:**5340-5347 (2001)

6. Englehardt.U, Caffeinated beverages symposium, 219th American chemical meeting, Anahein, USA (1999)

7. Ponce E; Taylor AJ, Phenolic Compounds as Astringent Factors in Black Tea Liquors, *Flavour chemistry of ethnic foods edited* by Shahidi F and Ho C T 197-210 (1997)

8. Ramadurai N, Global Trade Trends in Tea /. *Planters Chronicle* 537-545 (2000)

9. Larger PJ; Jones AD and Dacombe C, Separation of tea polyphenols using micellar electrokinetic chromatography with diode array detection *J of C*hromatography A **799:**309-320 (1998)

10. Obanda M; Owuor PO; Mang'oka R; Kavoi MM, Changes in thearubigin fractions and theaflavin levels due to variations in processing conditions and their influence on black tea liquor brightness and total colour, *Food Chemistry* **85:**163-173 (2004)

11. Moreda-Pineiro A; Fisher A and Hill SJ, The classification of tea according to region of origin using pattern recognition techniques and trace metal data, *J Food Composition and Analysis*, **16:**195-212 (2003)

12. Fernandez PL; Pablos F; Martin MJ and Gonzalez AG. Multi-element analysis of tea beverages by inductively coupled plasma atomic emission spectrometry, *Food Chemistry* **76:**483-489 (2002)

13. Liang Y; Lu J; Zhang L; Wu S, Estimation of black tea quality by analysis of chemical composition and colour difference of tea infusions, *Food Chemistry* **80:**283-290 (2003)

14. Togari N; Kobayashi A and Aishima, Pattern recognition applied to gas chromatographic profiles of volatile components in three tea categories, *T. Food Research International* 495-502 (1995)

15. Borse B B; L Jagan Mohan Rao; Nagalakshmi S and. Krishnamurthy N, Fingerprint of black teas from India: identification of the regio-specific characteristics, *Food Chemistry* 419-424 (2002)

16. Budinova G; Vlacil D; Mestek O and Volka K, Application of infrared spectroscopy to the assessment of authenticity of tea, *Talanta* -Oxford then Amsterdam **47:** 255-260 (1998)

17. Dutta R; Hines EL; Gardner JW; Kashwan KR and Bhuyan M, Tea quality prediction using a tin oxide-based electronic nose: an artificial intelligence approach, *Sensors and Actuators B* **94:**228-237 (2003)

18. Ivarsson P; Holmin S; Hojer NE; Krantz-Rulcker C and Winquist F, Discrimination of tea by means of a voltammetric electronic tongue and different applied waveforms *Sensors and Actuators B* **76:**449-454 (2001)

19. Pecore, S; Kellen, L, A consumer-focused QC/sensory program in the food industry *Food Quality and Preference* **13:**369-374 (2002)

20. Annor-Frempong IE; Nute GR; Wood JD; Whittington FW and West A, The measurement of the responses to different odour intensities of 'boar taint' using a sensory panel and an electronic nose *Meat Science* **50:**139-151 (1998)

21. Frank M; Ulmer H; Ruiz J; Visani P; Weimar U, Complementary analytical measurements based upon gas chromatography-mass spectrometry, sensor system and human sensory panel: A case study dealing with packaging materials *Analytica Chimica Acta* **431:**11-29 (2001)

22. Wittig De Penna E; Zúñiga M.J; Fuenzalida R and López-Planes R, Chemical and sensory characterization of tea (Thea sinensis) consumed in Chile, *Archivos Latinoamericanos de Nutricion* **55:**93-100 (2005)

23. Patra D; Lakshmi Sireesha K and Mishra AK, Characterization and investigation of polycyclic aromatic compounds present in petrol, diesel, kerosene and 2T oil using excitation emission matrix fluorescence *Indian Journal of Chemistry A*. **40:**374-379 (2001)

24. Baker A, Fluorescence properties of some farm wastes: implications for water quality monitoring *Water Research* **36:**189-195 (2002)

25. Scott SM; James D; Ali Z; O Hare WT and Rowell FJ, Total luminescence spectroscopy with pattern recognition for classification of edible oils. *Analyst* 966-973 (2003)

26. Miyazawa T; Nakagawa K, Structure-related Emission Spectrometric Analysis of the Chemiluminescence of Catechins, Theaflavins and Anthocyanins. *Bioscience, Biotechnology and Biochemistry* **62:** 829-832 (1998)

27. Pearson K, On lines and planes of closest fit to systems of points in space. *London Edinburgh Philosophical Magazine* **2:**559–572. (1901)

28. Gardiner WP, Statistical analysis methods for chemists, Multivariate analysis methods in chemistry. The Royal society of chemistry 293-307 (1997)

29. Dorothy D H Boothe and Judy W Arnold, Electronic nose analysis of volatile compounds from poultry meat samples, fresh and after refrigerated storage. *J Sci Food Agri*. **82:** 315-322 (2002)

30. Legin A; Rudnitskaya A; Vlasov Y; Di Natale C; Davide F and D'Amico Arnaldo,

Tasting of beverages using an electronic tongue, Sensors and Actuators B **44:** 291-296 (1997)

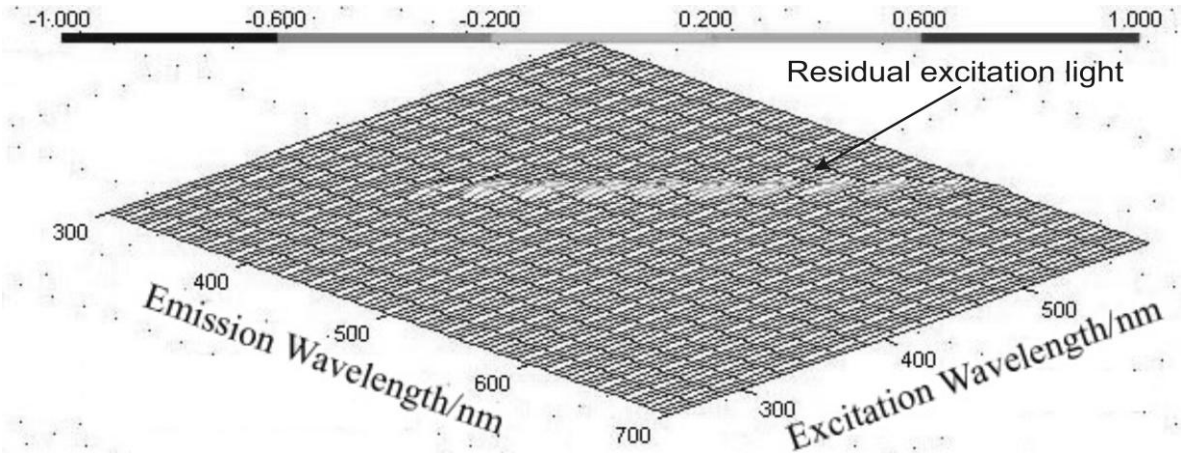


Fig.1. EEM of bottled black tea

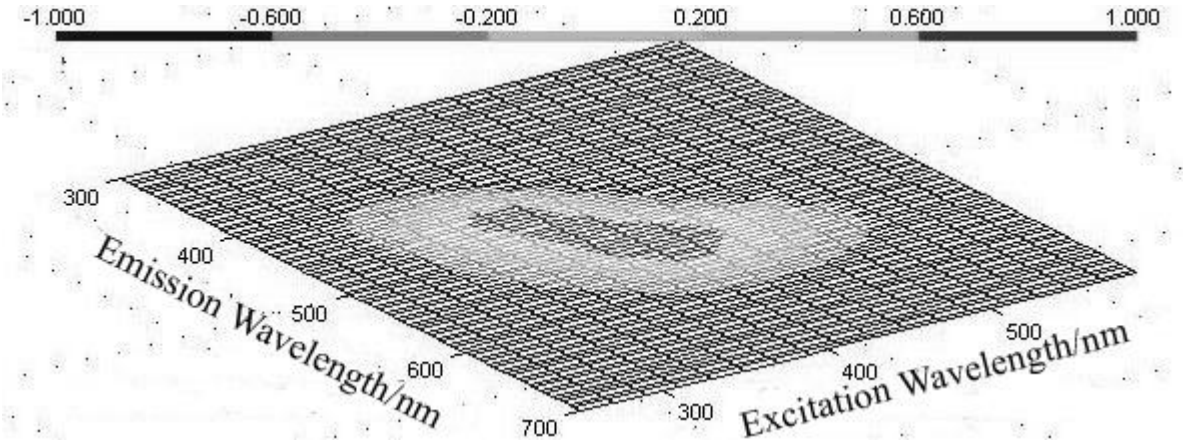
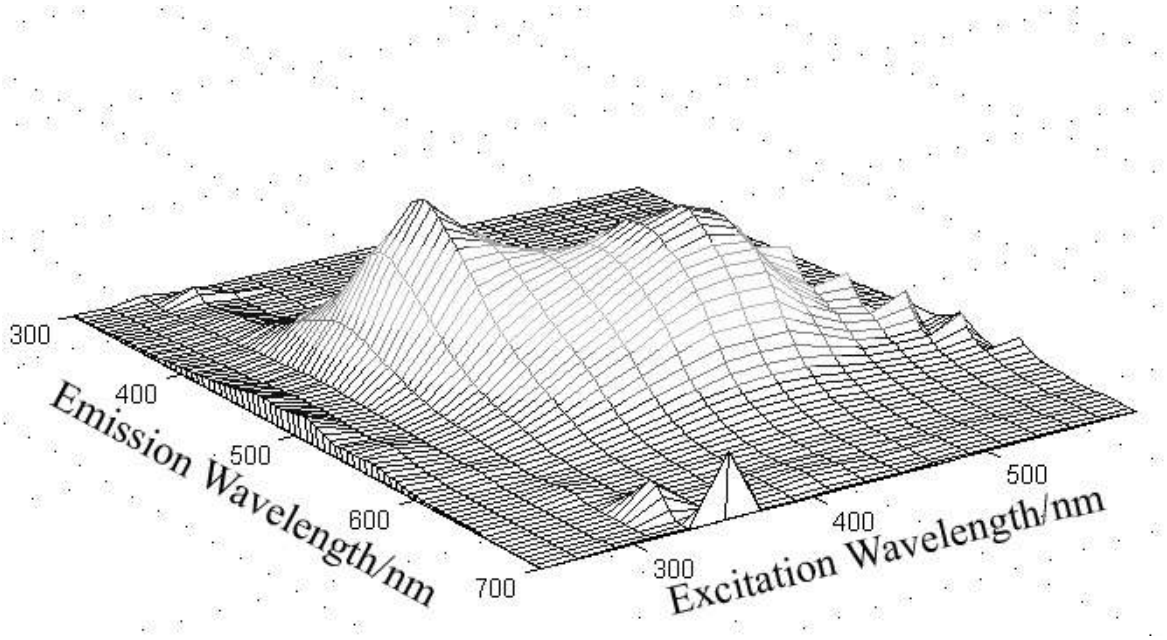
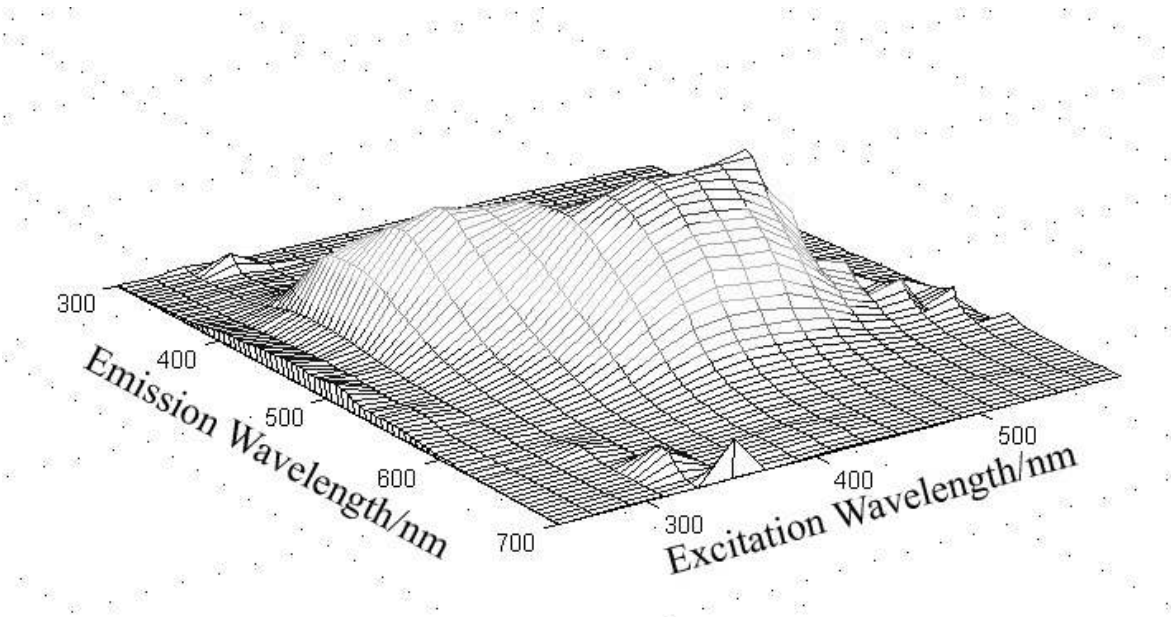


Fig.2. EEM of bottled black tea without residual excitation light.

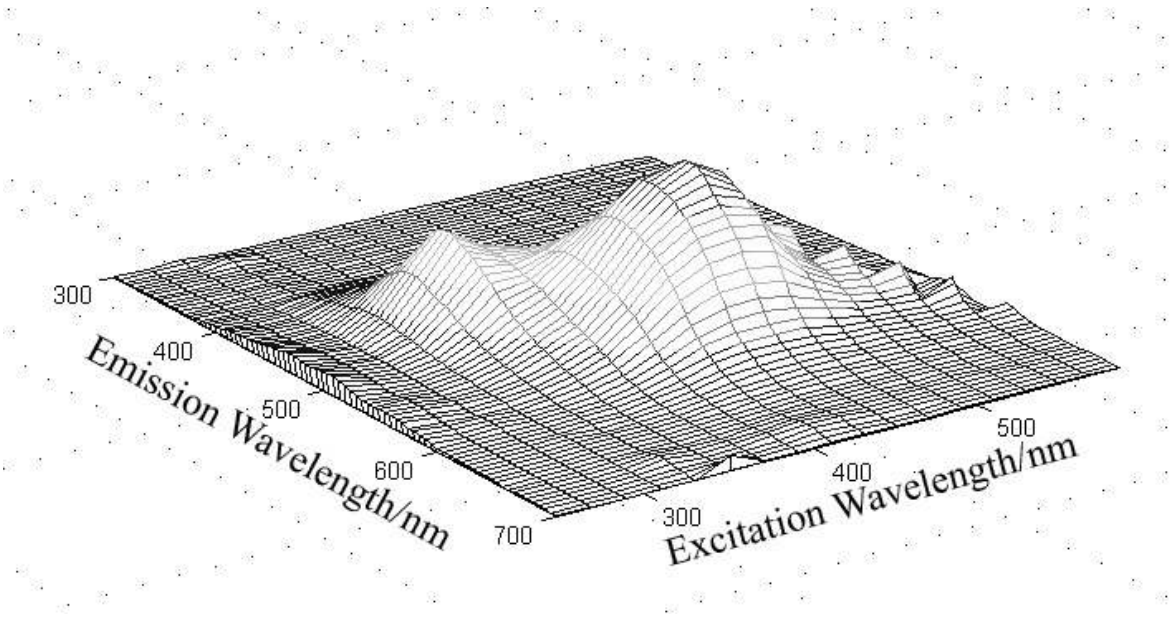
a)


300
400
500
600
700
Emission Wavelength/nm
300
400
500
Excitation Wavelength/nm

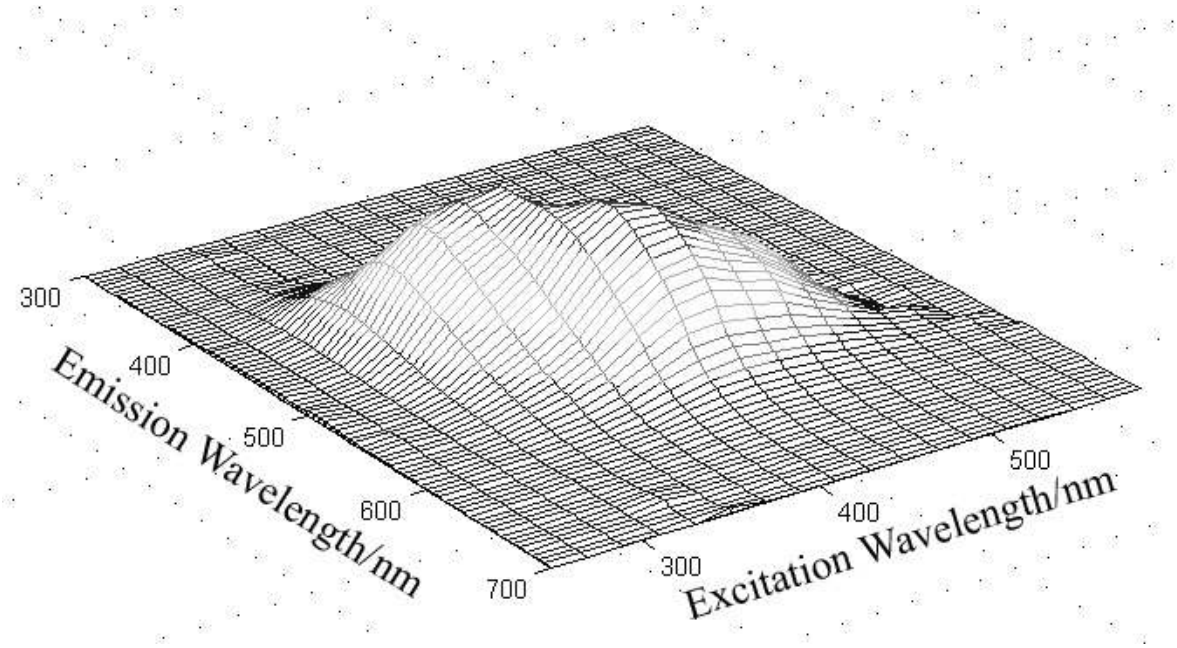
b)


300
400
500
600
700
Emission Wavelength/nm
300
400
500
Excitation Wavelength/nm

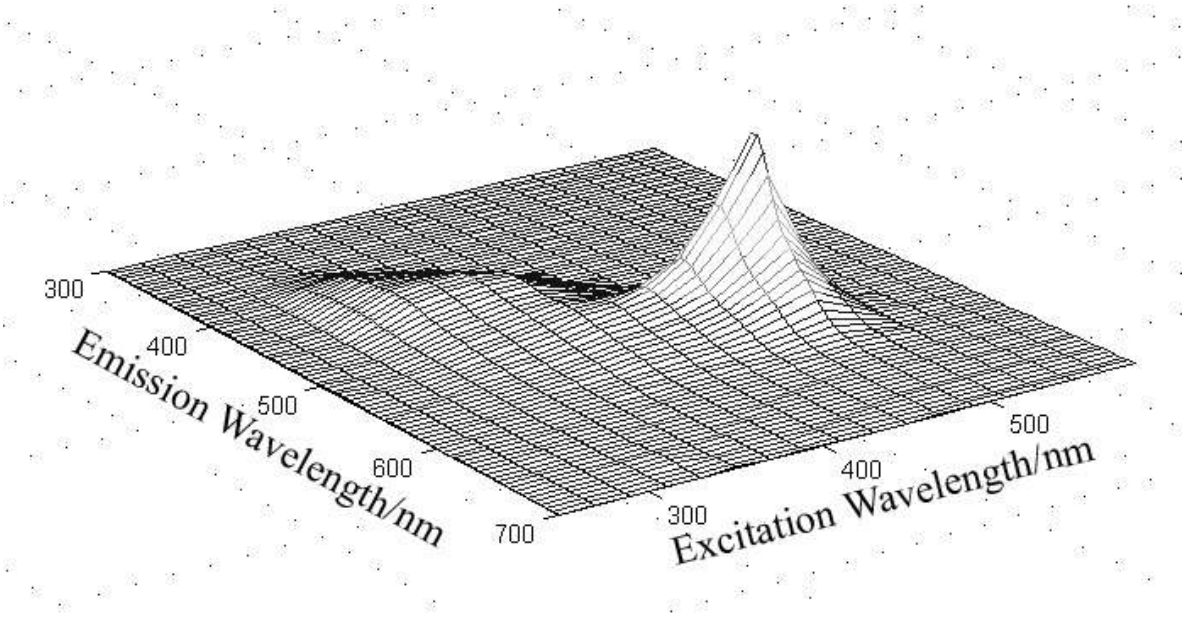
c)


300
400
500
600
700
Emission Wavelength/nm
300
400
500
Excitation Wavelength/nm

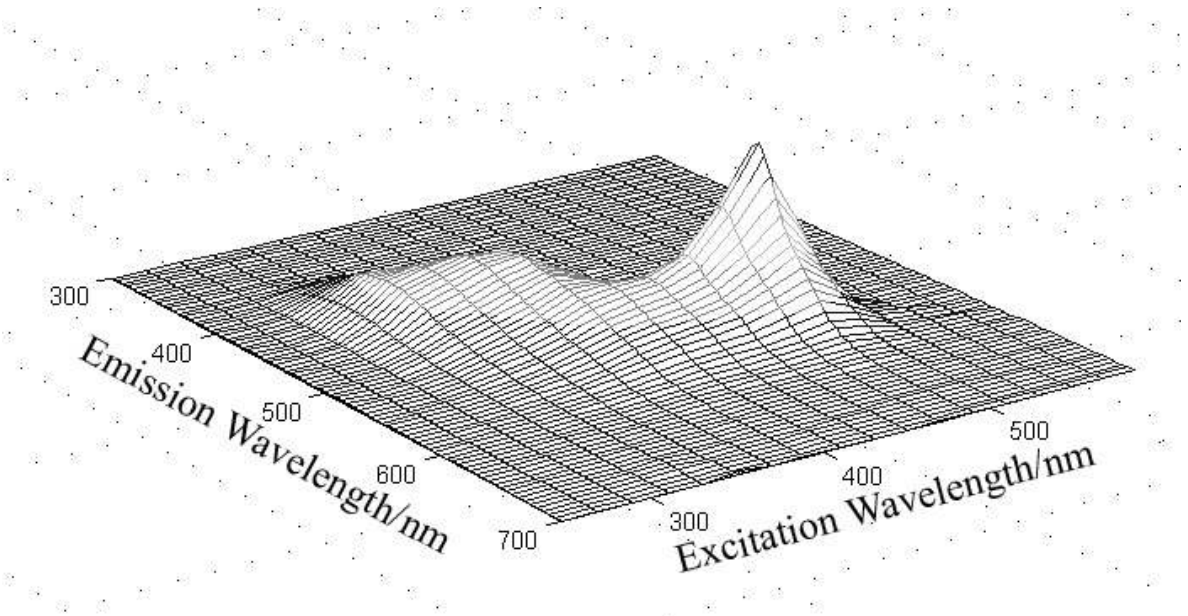
d)


300
400
500
600
700
Emission Wavelength/nm
300
400
500
Excitation Wavelength/nm

e)


300
400
500
600
700
Emission Wavelength/nm
300
400
500
Excitation Wavelength/nm

f)


300
400
500
600
700
Emission Wavelength/nm
300
400
500
Excitation Wavelength/nm

g)

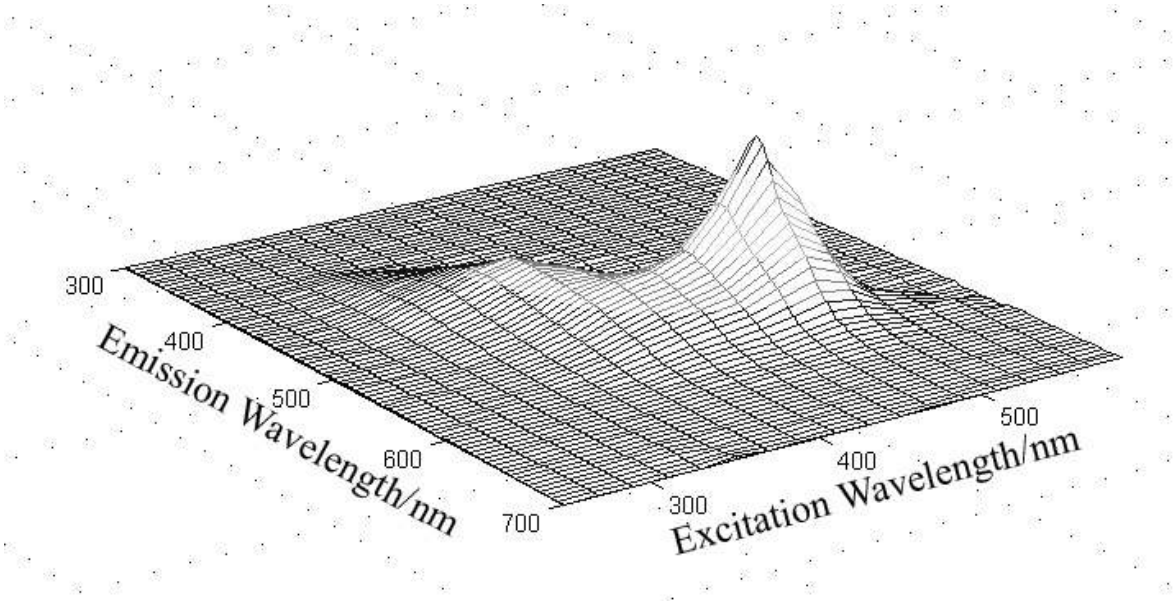


Fig. 3. EEMs of teas (a) Assam tea, (b) Ceylon tea (c) Kenya tea (d) Japanese Black tea (e) Japanese green tea (f) Japanese Houji tea (g) Japanese Oolong tea..

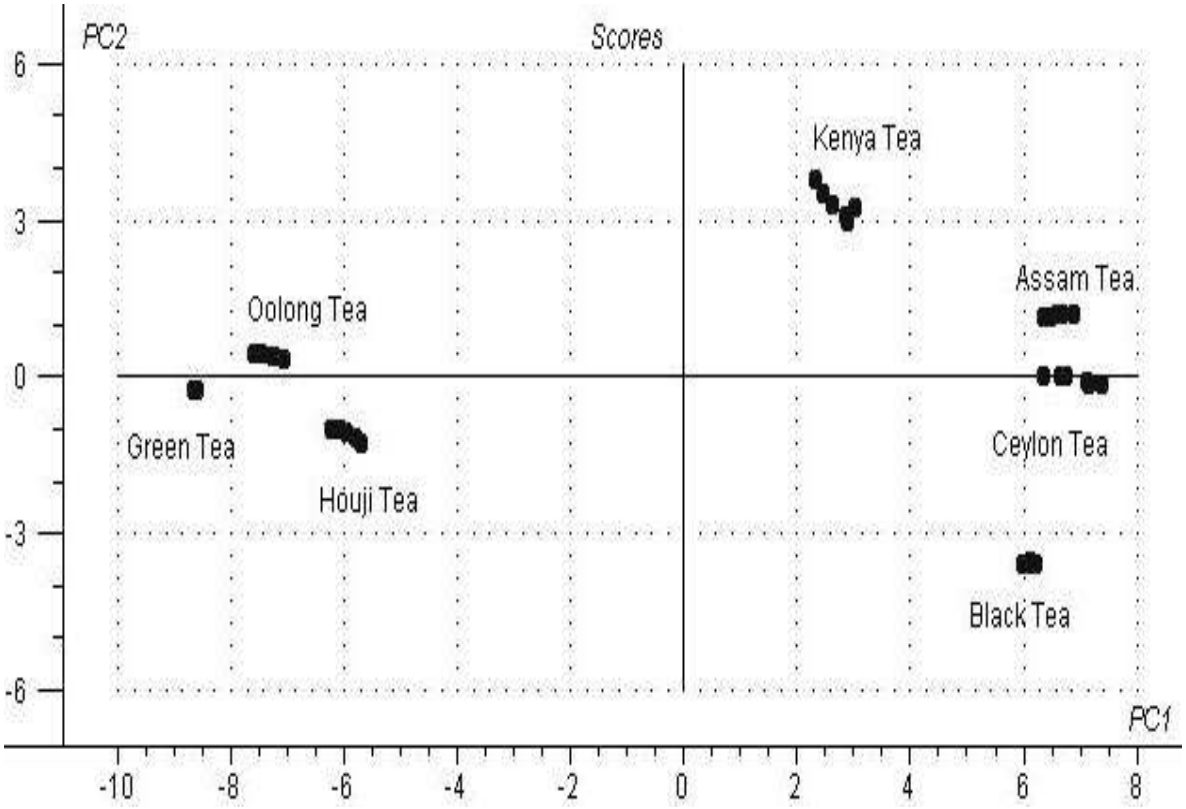


Fig. 4 PCA scores plot for total luminescence of Teas.