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# PHOTO-INDUCED FEATURES OF SOME LAGER BEERS

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Luminescent beer constituents were studied using total luminescence techniques. Significant spectral variations observed between various samples including fresh and photooxidized samples confirmed the potential of the method as a tool for beer typifying and quality control. Quantitative analysis should be possible using appropriate chemometric tools. Singlet oxygen was confirmed to be an active oxygen species in the photooxidation of beers. Additional tests of beer volatiles performed using an electronic nose with subsequent principal component data analysis proved that this method can positively discriminate various beer samples and also recognize the effects of photooxidation and loss of flavour and taste in contact with the atmosphere.

## INTRODUCTION

It is well-known that beers contain a complex concoction of substances [Duarte *et al*., 2002] and are subject to oxidative degradation. A "lightstruck flavour" in beer as a result of light exposure was first noted by Lintner [Lintner, 1875], while similar effects were noted in other beverages [Spikes, 1981]. Formation of an offensive flavour and aroma is commonly referred to as "skunked" beer [Burns *et al*., 2001]. Application of fluorescence techniques in the study of beers should be interesting in view of their rapid, selective, and sensitive features. Only a limited number of papers in this respect have been published, mainly because the selectivity with respect to complex natural systems appears to be [Apperson *et al*., 2002]. Multidimensional fluorescence techniques provide more comprehensive information and, in particular, total luminescence spectroscopy (TLS) should be interesting. TLS involves simultaneous acquisition of multiple excitation and emission wavelengths in order to increase selectivity. The resulting emission-excitation data matrix (EEM) provides a total intensity profile of the sample over the range of excitation and emission wavelengths scanned [Ndou & Warner, 1991b].

The present study deals with spectroscopic and photochemical aspects of some beers available on the Polish market, including four lager beers and one imported beer readily available in the country. More specifically, the potential of TLS was explored for characterisation and differentiation of these beers and also for monitoring changes occurring upon irradiation. Moreover, light-induced changes in the composition of beer volatiles using an electronic nose were examined. Photochemical degradation of beer was studied by means of time-resolved singlet oxygen detection.

## MATERIAL AND METHODS

The beers were purchased from a local store; beer 1 – Warka, beer 2 – Lech, beer 3 – Tyskie, beer 4 – Żywiec, beer 5 – Heineken. Samples were withdrawn from freshly opened bottles. Perinaphthenone and $D_2O$ were from Aldrich.

Fluorescence spectra were obtained using a Fluorolog 3-11 Spex-Jobin Yvon spectrofluorometer. A xenon lamp was used as an excitation source. Right-angle geometry was applied for beer samples diluted in $D_2O$ (1% v/v) in a 10 mm fused-quartz cuvette. Front-face geometry was used for neat beer samples in a triangular fused-quartz cuvette. Three-dimensional spectra were obtained by measuring respectively the emission spectra in the range from 290 to 700 nm at excitation wavelengths from 250 to 500 nm, spaced by 5 nm intervals in the excitation domain. The excitation and emission slit width were 2 nm. The acquisition interval and the integration time were maintained at 1 nm and 0.1 s, respectively. A reference photodiode detector at the excitation monochromator stage compensated for the source intensity fluctuations. Individual spectra were corrected for the wavelength response of the system. Fully corrected spectra were then concatenated into an excitation-emission matrix.

UV–visible absorption spectra were obtained on a Varian Cary 5E spectrophotometer.

Electronic nose measurements were performed with a mass spectrometer equipped with a Headspace Sampler TurboMatrix HS-40 (Perkin Elmer, Norwalk) controlled by the chemometric software QMBSOFT NT (HKR Sensorsysteme).

Singlet oxygen luminescence experiments were carried out by excitation of the sample with a third harmonic (355 nm)

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of a Q-switched Nd:YAG laser (Spectron Laser Systems, UK; 20 mJ per pulse, *ca.* 9 ns FWHM). The excitation energy was attenuated using solutions of sodium nitrite in water. Emission at 1270 nm from singlet oxygen was measured with a liquid-nitrogen-cooled *Edinburg EI-P* Ge detector. The luminiscence was detected after passing through Spectrogon LP-1000 and BP-1275-080-B filters. The signal kinetics were recorded by an Infinium oscilloscope. Perinaphthenone (Aldrich) was used as a reference for singlet oxygen quantum yield, $\varphi_{\Delta} = 0.95 \pm 0.05$, [Schmidt *et al*., 1994].

## RESULTS AND DISCUSSION

### Total luminescence spectra of beers

Since emission spectroscopy requires little or no sample preparation, it is an attractive method for routine measurements. However, single-wavelength analysis is hardly applicable to complex multi-component samples, that are characterized by overlapping emission and/or excitation spectra. Several techniques have been examined to overcome the problem including synchronous luminescence, phase-resolved luminescence, and multidimensional luminescence. Total luminescence spectroscopy (TLS) appeared to be especially successful [Ndou & Warner, 1991a]. Total luminescence spectroscopy gives a complete description of fluorescent components in complex mixtures. It incorporates all information present in the excitation and fluorescence spectra of each compound. The spectral resolution of the method depends on the number of conventional emission scans at different excitation wavelengths used to construct the contour plot. Such a contour map may be obtained using a conventional scanning spectrofluorometer by recording a series of emission spectra at different excitation wavelengths. The total fluorescence contour map serves as a unique fingerprint for the luminescent content. Since most food products are opaque or highly absorptive, front-face geometry is a preferred choice. Thus, the fluorescence is collected from the surface of the sample on which the exciting radiation also impinges. Front-face illumination differs from the more common right-angle arrangement, where fluorescence is collected at 90 degrees relative to the direction of the excitation light beam. This geometry, however, is only suitable for transparent, weakly absorbing liquid samples. Both arrangements, right-angle for diluted samples and front-face for neat beer samples, were used in the present study (Figure 1).

The weaker band observed on excitation at 300–350 nm and emission at 410–470 nm disappeared upon dilution with water. Recently similar fluorescence behaviour has been observed using single fluorescence scans, with the long-wavelength fluorescence tentatively attributed to iso-α-acids and, to a small extent, polyphenols [Apperson *et al*., 2002]. Our results, beside supporting this observation, give a complete profile of the fluorescent components in neat and diluted beer samples. Differences may result from molecular interactions in concentrated samples and, also, from screening, inner filter, and self-quenching effects. Beer is a complex mixture of non-fluorescent and fluorescent compounds, hence, intermolecular interactions between components involving energy transfer or self-quenching *via* solvent collisions are expected to occur [Apperson *et al*., 2002]. These interactions may distort the characteristic fluorescence patterns of individual components, especially in concentrated samples.

Fluorescence EEMs of all undiluted beer samples are shown in Figure 1 (left panel) and Figure 2. A strong characteristic band appears on excitation at 250–280 nm and emission at 290–360 nm. This band is, however, difficult to interpret due to the high intensity of scattered light in the region close to the excitation wavelength. A second band is present with emission at 410–470 nm and excitation at 330–370 nm, and its identification remains currently elusive. Based on a recent study, that used conventional single fluorescence scans, the fluorescence at about 315 nm has been assigned to tyrosine [Apperson *et al*., 2002]. A long-wavelength emission at 500–600 nm on excitation at 400–450 nm could be attributed to the emission of flavins present in beer. Beer is known to contain different flavins, in particular riboflavin [Andres-Lacueva *et al*., 1998]. As expected, all beers possess similar fluorescence EEMs subtle differences being noticeable at close examination, *e.g.* slight changes in the position of maxima and intensity ratios. Such variations are too small for visual inspection, hence more advanced methods should be applied [Siebert, 2001]. To avoid fluorescence changes upon dilution, most measurements were done using undiluted samples. From comparison of spectra for undiluted and diluted samples, it is evident that measurements of EEMs of undiluted beer, despite their complex nature, are more useful for differentiation and characterisation purposes.

Some intrinsic experimental difficulties may be overcome by using CCD fluorometers or video fluorometers in order to accelerate data acquisition. Clearly, the total luminescence

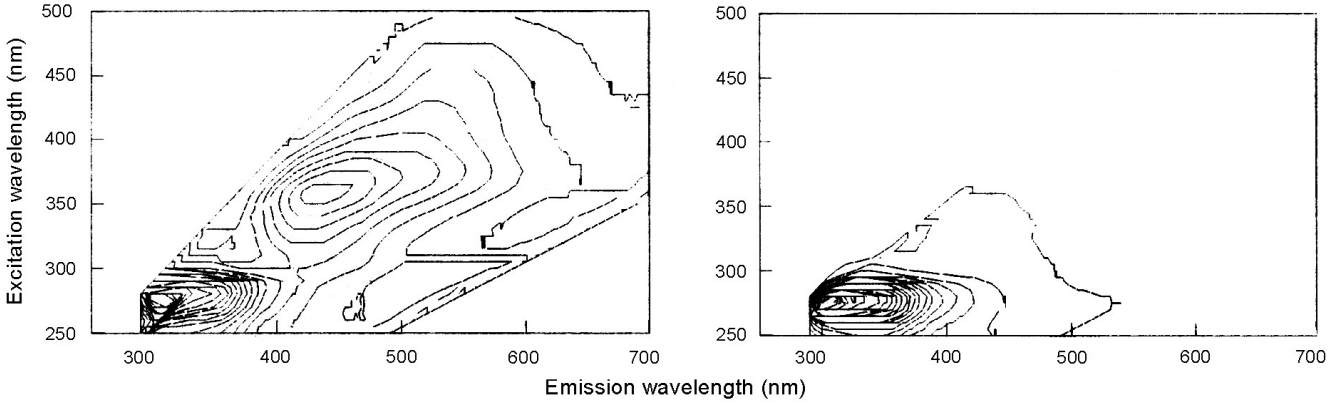


FIGURE 1. Contour maps of total luminescence of undiluted (left panel) and diluted (eater) (right panel).

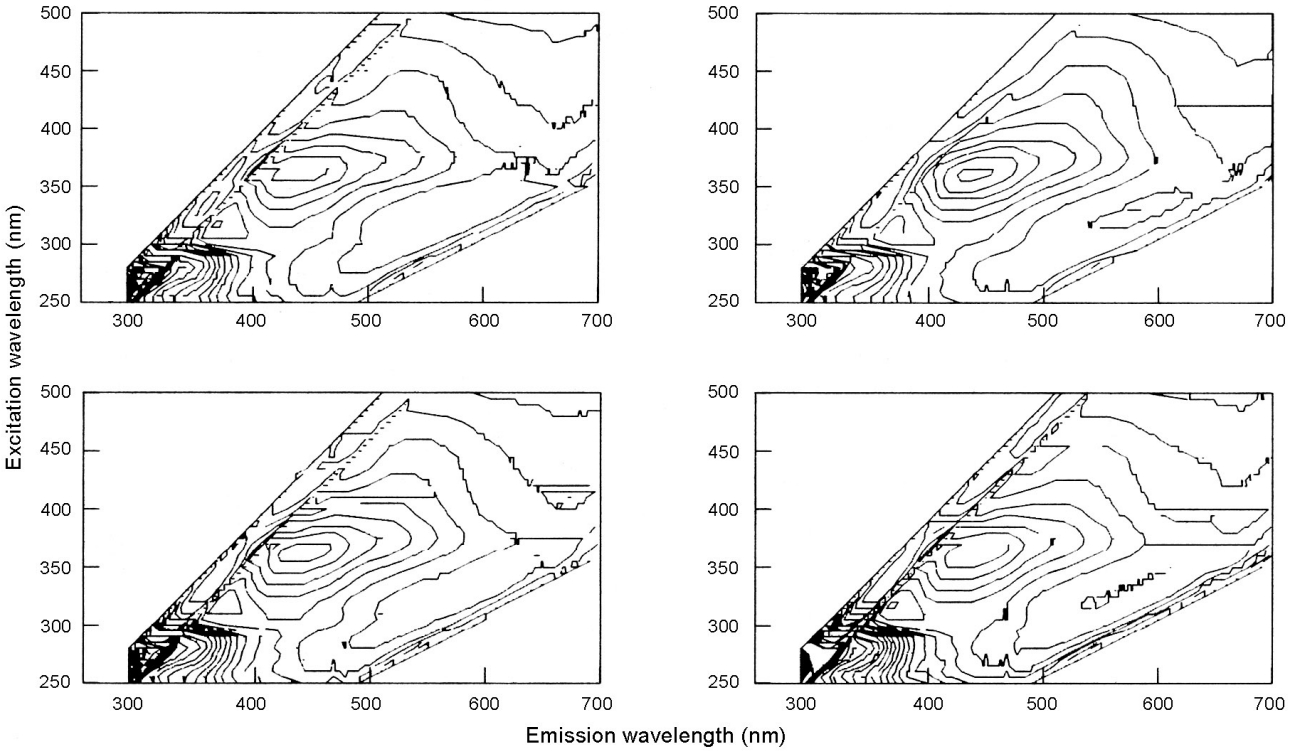


FIGURE 2. Contour maps of total luminescence of undiluted beer samples.

contour map serves as a unique fingerprint. However, further studies are needed to identify the fluorophores responsible for the observed emission bands.

Exposure of beer to light causes undesirable changes, among others developments of an offensive taste and a 'skunky' odour [Burns *et al*., 2001; Laane *et al*., 1999]. We studied alternations in EMMs characteristics of beers under light exposure. Figure 3 shows typical contour maps of total luminescence of a beer exposed to light and of a test sample stored in the dark. Changes are apparent in particular, a decreased intensity of the emission band at 400–450 nm in excitation and at 500–600 nm in emission. Additionally, selected single scans are presented in Figure 4 for comparison. Disappearance of the fluorescence maximum at 530 nm can be tentatively interpreted as resulting from photodecomposition of riboflavin or other flavins. A simultaneous increase of fluorescence with maximum at about 430 nm may, thus, result from formation of lumichrome, a well-known photoproduct of riboflavin. The results presented clearly demonstrate the effect of beer exposure to light and show that such effect can be monitored by TLS.

**Electronic nose experiments**

The system for analysis of volatile compounds (electronic nose) utilized a TurboMatrix HS-40 Headspace Sampler and a mass spectrometer as detector. This configuration enabled discrimination between samples on the basis of mass spectra of volatiles contained in the beers. The concentrations of constituents were subjected to Principal Component Analysis, (PCA). These mass spectra representing various chemical compounds may be used as fingerprints (Figure 5). Nevertheless the presence of certain compounds may be verified. Based on results reported by [Kolaghar *et al*., 2002] ethyl caproate (m/z: 55), ethyl acetate (m/z: 61), ethyl octanone (m/z: 88), and

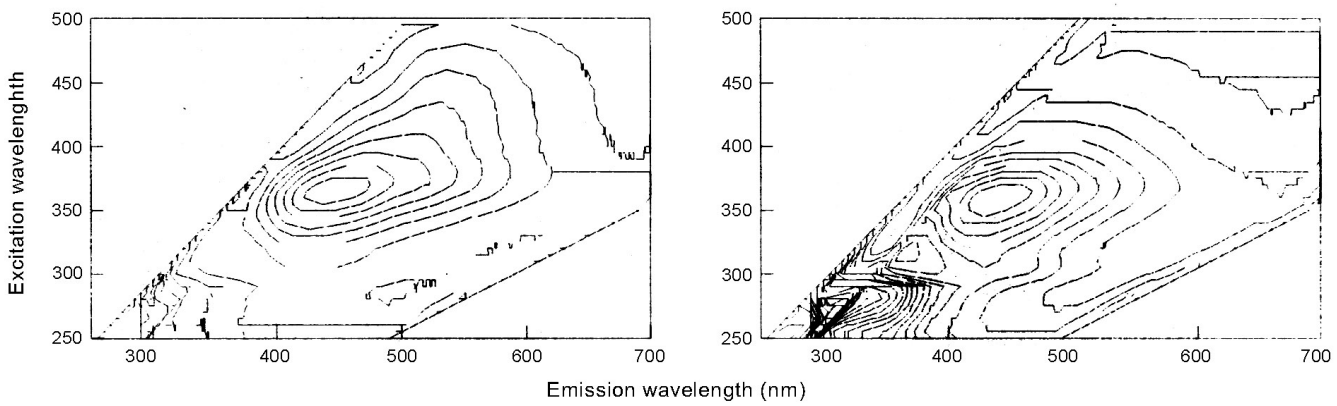


FIGURE 3. Contour maps of total luminescence of undiluted beer 3 stored in a transparent glass bottle in the dark (left panel) and exposed to daylight (right panel).

2-phenylethyl actate (m/z: 104) were identified. For PCA, six characteristic ions with m/z = 57, 61, 74, 84, 100 and 114 were selected. Figure 6 shows the PCA plot of the beers. Significant differences between the samples were noted and all beers could be positively discriminated using the six MS peaks selected.

The effect of light on beer can also be monitored using PCA. Figure 7 shows the PCA plot of the mass peaks of a freshly opened beer, the beer exposed to light, and a control sample kept in the dark. Alterations in the composition of beer volatiles due to irradiation are evident.

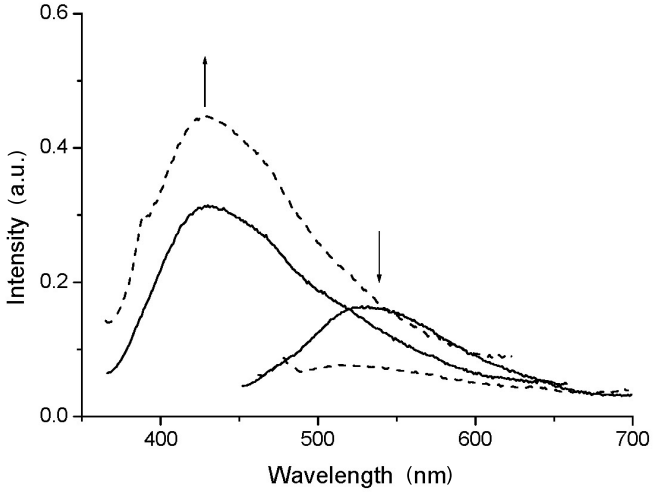


FIGURE 4. Fluorescence spectra of beer 3 stored in the dark (solid line) and exposed to light (dashed line).

**Singlet oxygen measurements**

Reactive oxygen species may attack beer, but the exact nature of such species that oxidize beer is unknown [De Keukeleire *et al*., 1976]. We measured emission at 1270 nm, which is highly specific to the $O_2(^1\Delta_g) \rightarrow O_2(^3\sum_g^-)$ transition, under laser excitation at 355 nm in air-equilibrated samples of all the beers. The first series of measurements was performed for neat beer samples without any preparation using the front-face geometry. The lifetime of singlet oxygen phosphorescence was obtained by fitting the decay curve to a single-exponential function. No attempt was made to estimate the singlet oxygen quantum yields for this series.

In the second series, quantum yields and lifetimes of singlet oxygen were determined by exciting air–saturated beer samples dissolved in $D_2O$, all optically matched (OD=0.5) at the excitation wavelength (355 nm). Singlet oxygen was detected by monitoring the 0.0 vibronic band of its phosphorescence centered at 1270 nm using a germanium photodiode detector. The phosphorescence was detected at right angles to the exciting beam. The intensity of singlet oxygen phosphorescence ($I_0$) at time $t = 0$ was obtained by fitting the decay curve to a single-exponential function. For each sample, $I_0$ values were plotted against the relative laser fluence; such plots were linear below 0.5 mJ per pulse of incident energy. All results were compared to those obtained for the reference air–equilibrated solution of perinaphthenone, also optically matched at the excitation wavelength. Quantum yields of singlet oxygen production, $\varphi_\Delta$, were determined from the ratios of the slopes of the plots of $I_0$ *vs*. the relative laser

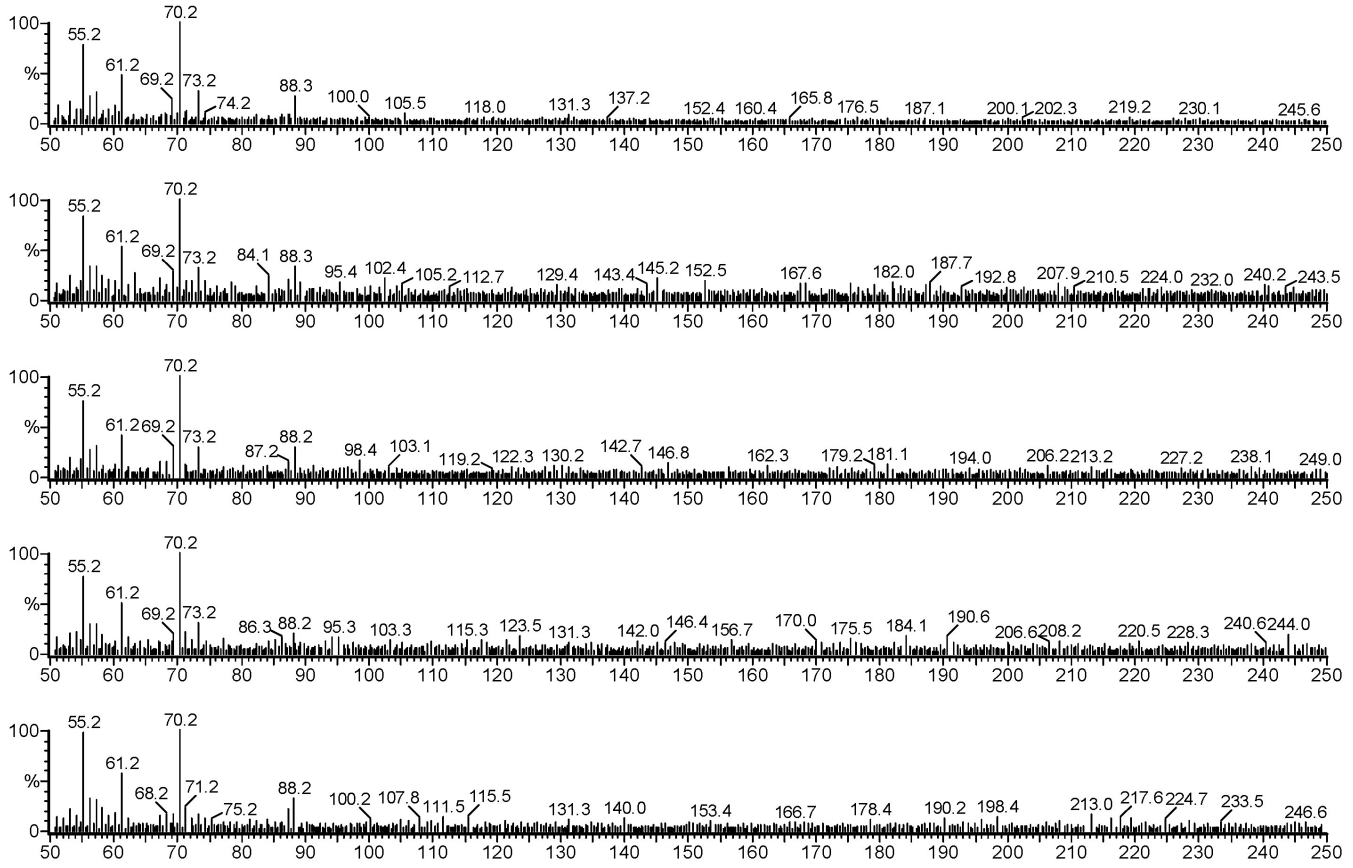


FIGURE 5. GC-MS total ion chromatograms of (from top to bottom) beer 1 to beer 5.

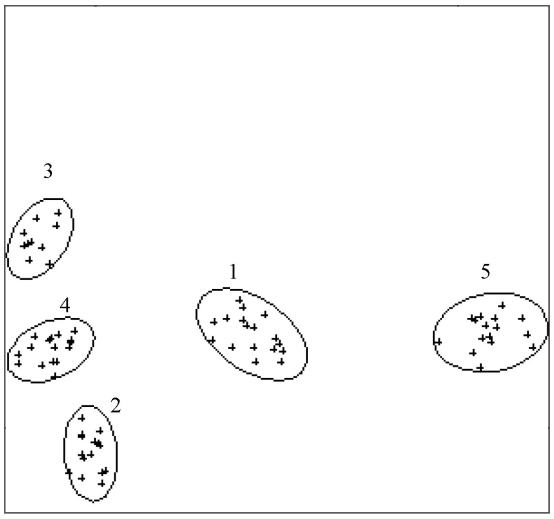


FIGURE 6. Principal component analysis (X=PC1 = 83.2%, Y=PC2 = 15.5%) of beers 1 to 5.

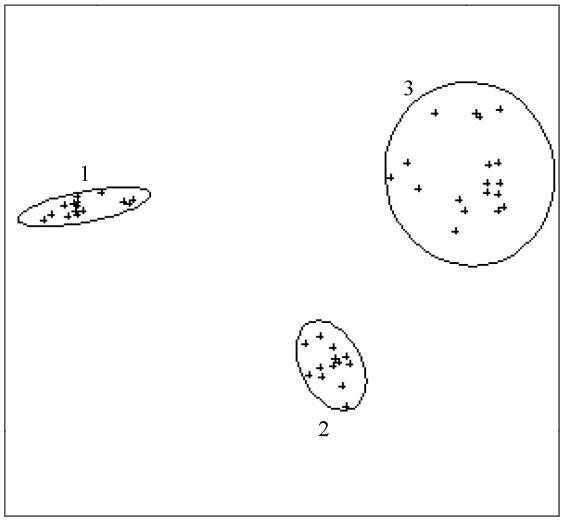


FIGURE 7. Principal component analysis (X=PC1 = 80.2%, Y=PC2 = 19.2%) of beer 3, freshly opened (1), after exposure to light (2), and of a control kept in the dark (3).

intensity for beers and perinaphthenone solution in $D_2O$, for which the quantum yield value of 0.95±0.05 has been measured [Schmidt *et al*., 1994].

Quantum yields of singlet oxygen formation were very low for all beers, with the highest value being 0.004. The emission lifetimes recorded at 1270 nm in air-equilibrated solutions were in a range of 58 to 90 μs, the lifetime for the perinaphthenone experiments being 60 μs, typical of singlet oxygen in $D_2O$ [Wilkinson *et al*., 1995]. The singlet oxygen data confirm that involvement of singlet oxygen may be important in the photo-oxidation of beer [De Keukeleire *et al*., 1976].

## CONCLUSIONS

Luminescent beer constituents were examined using total luminescence techniques. Significant spectral variations observed between various beers samples and, also, between fresh and photo-oxidized samples confirmed the utility of the method as a tool for beer typifying and quality control. Appropriate chemometric analysis allows fuller probing.

Singlet oxygen measurements in diluted beer samples confirmed the role of singlet oxygen an active oxygen species in the photooxidation of beers.

Analysis of beer volatiles performed using an electronic nose with subsequent principal component analysis demonstrated discrimination between various beers is feasible, while effects of photo-oxidation and loss of flavour and taste in contact with the atmosphere can be recognized.

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