

## FLUORESCENCE SPECTROSCOPY FOR ANALYSIS OF BEER

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The purpose of the present work was to assess the fluorescence as a technique for food quality evaluation, using samples of two brands of beer exposed to a forced ageing process as a test case. Results of the emission measurements obtained for these samples are presented and discussed. The present research regards the beer luminescence as its inherent property, permitting to monitor the state of beer within the technological production cycle and during storage. Supplemented by appropriate chemometric tools, this method can be used to identify beer brands and brews.

### INTRODUCTION

Quality control and monitoring of chemical composition of food products during processing and storage are very important tasks. Spectroscopic methods that have been employed to monitor quality and condition of foodstuffs include fluorescence spectroscopy, UV-Vis, NIR and IR absorption spectroscopy, NMR, and Mass Spectrometry [Almeida *et al.*, 2006; Duarte *et al.*, 2003; Duarte *et al.*, 2004; Vanhoenacker *et al.*, 2004; Ventura *et al.*, 2006]. Recently, special attention has been given to luminescence methods, which allow to characterize and analyze foods without any special sample preparation [Christensen *et al.*, 2005, 2006; Sikorska *et al.*, 2004, 2006; Sikorska, 2007]. In general, luminescence methods offer important advantages over other techniques, being very sensitive, comparatively fast and cheap. In our previous research the single fluorescence spectra and the single synchronous spectra were used to characterize beer before and after the exposure to irradiation process [Sikorska *et al.*, 2003, 2004, 2006; Sikorska, 2007].

In this paper there have been reported the results concerning beer samples exposed to forced ageing process. In order to compare the spectra of the samples before and after the process mentioned above and to characterize, in further steps of the study, the changes occurring in beer after forced ageing, there have been used three-dimensional fluorescence technique and three-dimensional synchronous scan method.

### MATERIAL AND METHODS

The beers were acquired from a brewing company in Poland. Samples were degassed in an ultrasonic bath before measurements to avoid light scattering on gas bubbles.

Fluorescence spectra were obtained on a Fluorolog 3-11 Spex – Jobin Yvon spectrofluorometer. Xenon lamp source was used for excitation. Excitation and emission slit width was 2 nm; the acquisition interval was 1 nm and the integration time was maintained at 0.1 s for the total luminescence spectra and 0.05 s in the synchronous scan method. Individual spectra were corrected for the wavelength response of the system. Front-face geometry was used for bulk beer samples in a 10 mm fused silica cuvette. Fluorescence measurements have been made using total luminescence spectroscopy method (TLS), synchronous three-dimensional fluorescence spectroscopy, and single synchronous scan.

The emission spectra in the total luminescence method were collected between 290 to 700 nm in the range from 250-550 nm at excitation wavelengths, spaced by 10 nm intervals in the excitation domain. The single emission spectra were then folded into an excitation-emission matrix (EEM).

Synchronous fluorescence spectra were collected by simultaneously scanning the excitation and emission monochromator in the 240-700 nm range in the entire range of offsets between the excitation and emission wavelengths ( $\Delta\lambda = 10-100$  nm), with 10 nm step. The single synchronous fluorescence spectra were folded into 3-D spectrum.

### RESULTS AND DISCUSSION

Concentrations of the beer components depend on the type of beer, brewing conditions and the raw materials used. In general, there are three groups of fluorescent components in beers – amino acids, vitamins and polyphenolics. The measurement of their concentrations gives a new approach to evaluation of the product quality. The fluorescence spectroscopy can serve as the first step of a larger analytic effort, allowing to note differences in beer composition.

We studied beers of two distinct brands exposed to a forced ageing process, achieved by a thermal treatment imitating long-term storage. For this purpose, bottled beer was stored alternately for 24 h at 60°C and 24 h at 0°C. Such thermal cycling allows to determine the anticipated period of beer stability, with the cycles repeated until beer turbidity appears in the bottles. The respective turbidity stage (2 Units EBC) has been established by the European Brewing Convention [Polish Standard PN-A-790939:2000]. Thus, one thermal cycle corresponds to 30 days of expected beer stability, used in the “best before” date marked on the bottle.

The samples of the first beer brand (Beer A) were exposed to 9 cycles of forced ageing, while those of the second one (Beer B) were exposed to 7 ageing cycles. According to the data furnished by the brewery, these artificially aged samples were over-aged, implying that after 9 (A) and 7 (B) months of conventional storage the product would be over-stored and could lose its freshness and quality.

Absorption and emission spectra were obtained for three samples of each of the two beer brands. The total luminescence spectra were collected for all samples in order to look for differences in beer composition. Figure 1 shows the total

luminescence spectra of the fresh samples of Beers A and B. The two spectra show significant differences in two separate zones: one at 300-400 nm and another at 400-550 nm in the emission scale. Namely, the fluorescence of the Beer A sample is more intensive than that of the Beer B in the 300-400 nm range, while the sample of Beer B has higher fluorescence intensity in the 400-550 nm range.

These results show that the luminescence method allows to notice changes in beer composition. Moreover, the contour maps of the respective spectra present a very detailed image of the sample emission, which can be used as a “fingerprint” of the respective beer sample and/or the respective beer brand.

Synchronous fluorescence scan is another fluorescence method useful in monitoring condition and quality of beers. Synchronous fluorescence spectra were collected by simultaneously scanning the excitation and emission monochromators in the 240-700 nm range in the entire range of offsets between the excitation and emission wavelengths ( $\Delta\lambda = 10-100$  nm) with 10 nm step. The final result is a 3-D data matrix, which includes all the measured synchronous spectra (Figure 2). In this figure we compare the spectra obtained for Beer B samples before any thermal treatment (a) with the spectra of the

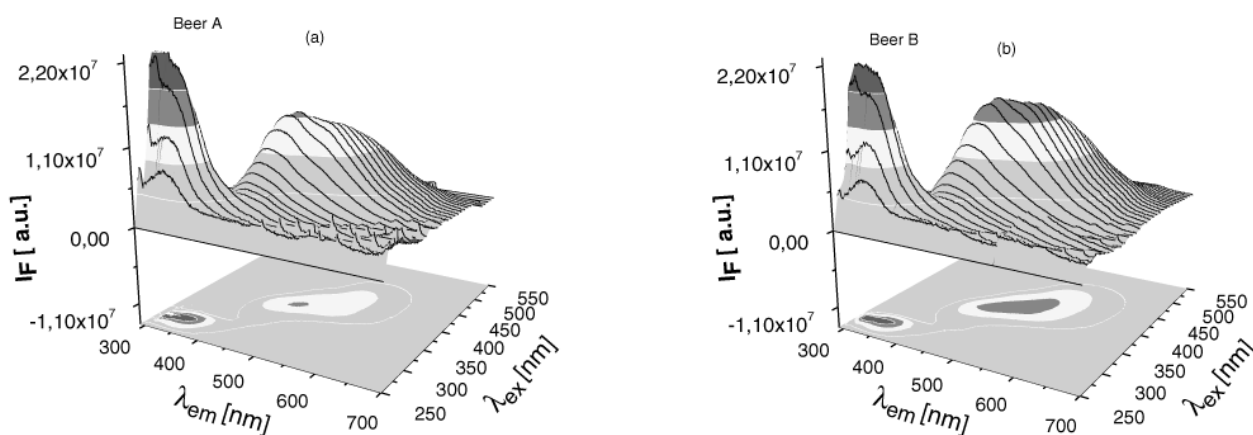


FIGURE 1. Total luminescence spectra of fresh samples of Beer A and B with the respective contour maps.

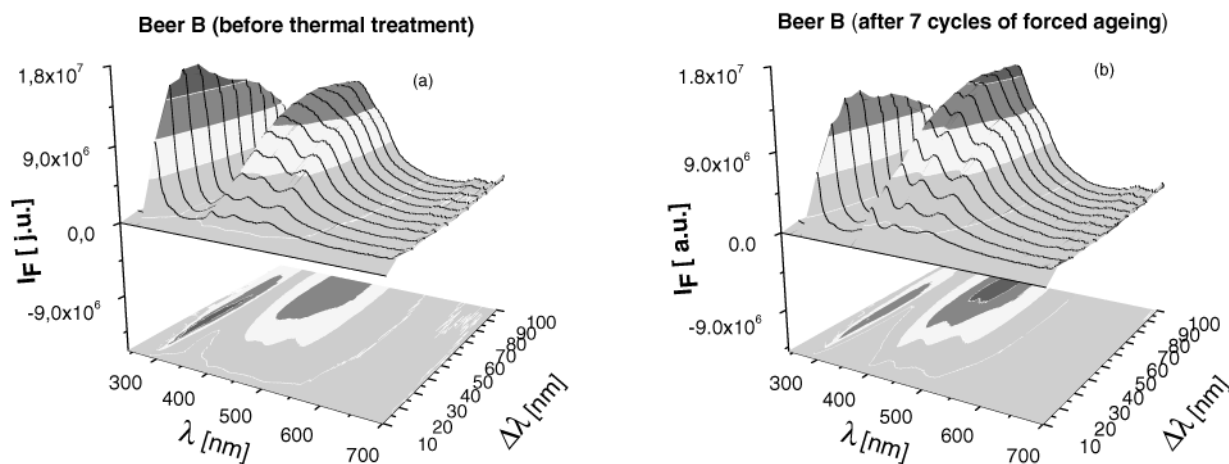


FIGURE 2. Synchronous 3-D fluorescence spectra of Beer B samples: (a) before thermal treatment and (b) exposed to 7 cycles of forced ageing.

samples after 7 cycles of forced ageing (b). In the 250-350 nm range, characteristic for amino acids and some polyphenolics emission, more intensive fluorescence is observed for the fresh beer samples as compared to samples after a long thermal treatment (7 cycles), while in the 400-550nm range, corresponding to the emissions of vitamins and polyphenolics and other unidentified compounds, the fluorescence intensity increases for the samples after forced ageing. These data allow to choose the best value of the offset between the excitation and the emission wavelengths in order to observe the most significant differences between the particular samples. Additionally, the usage of the entire data matrix may provide additional advantages in optimising the sensitivities for each specific constituent or group of constituents by choosing an appropriate  $\Delta\lambda$  value.

Figure 3 shows the results obtained for the samples of Beer A. We observe that the changes of fluorescence intensity between the samples before and after forced ageing are slight but observable in the 250-350 nm range, while the fluorescence intensity increases quite significantly in the 400-550 nm range.

Figure 4 shows the single synchronous fluorescence spectra for the samples of both beer brands, Beers A and B. There

are significant differences in the fluorescence spectrum of fresh samples of these two brands, probed at either  $\Delta\lambda = 30$  nm or  $\Delta\lambda = 60$  nm. The fluorescence of beer B is more intensive in the 350-550 nm range, with the difference becoming even more significant when using the offset  $\Delta\lambda = 60$  nm. A different offset between the excitation and the emission wavelengths allows to observe different changes in the emission spectra. Comparing the two cases (Figures 4a and b) we can choose the best value of the offset, allowing to follow the differences we consider the most important.

Synchronous fluorescence method allows to notice specific changes in composition of beer after forced ageing treatment. Figure 5 shows synchronous emission spectra of Beer A after 9 cycles of forced ageing compared with the spectrum of the same beer before thermal treatment. Significant changes in shape, absolute intensity and relative contributions of the individual fluorescence bands are observed, particularly when the offset  $\Delta\lambda = 60$  nm is used.

As we already mentioned, beer contains at least three characteristic groups of fluorescent species. The changes observed in the emission spectrum of beers correspond to the changes in the concentrations of these compounds. In our opinion, despite the complex composition of the system stud-

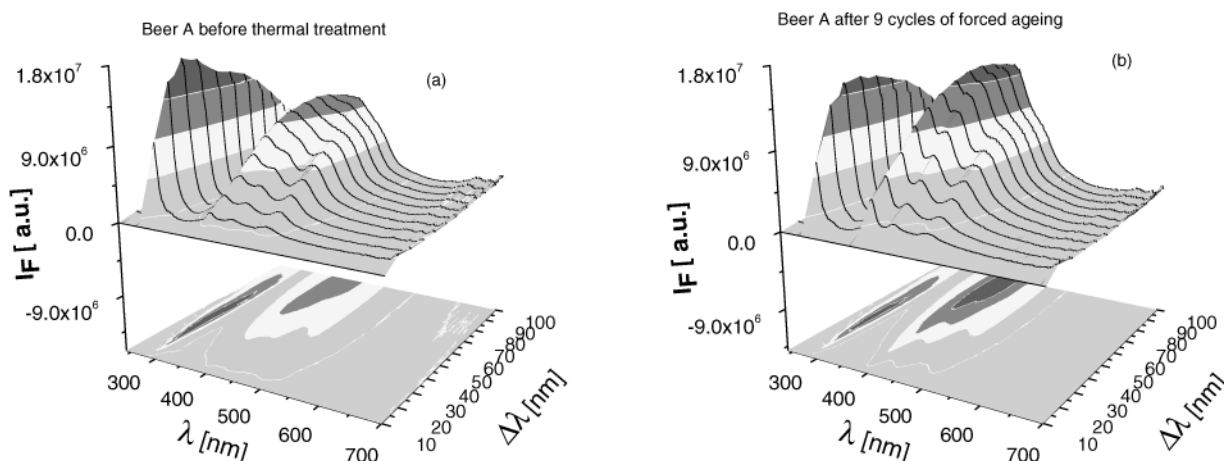


FIGURE 3. Synchronous 3-D fluorescence spectra of Beer A samples: (a) before thermal treatment and (b) exposed to 9 cycles of forced ageing.

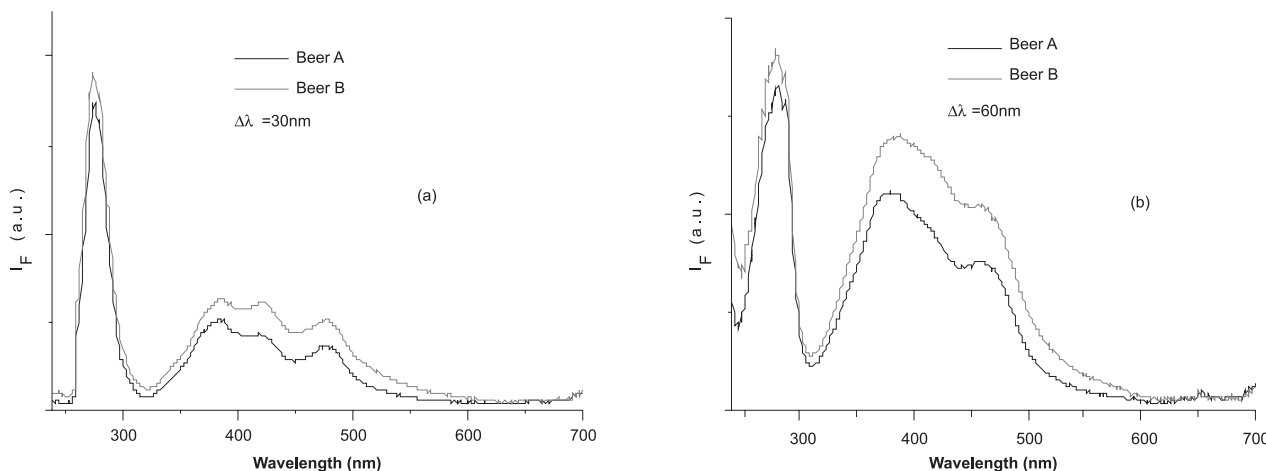


FIGURE 4. Synchronous fluorescence spectra of Beer A and B fresh samples: (a)  $\Delta\lambda = 30$  nm and (b)  $\Delta\lambda = 60$  nm.

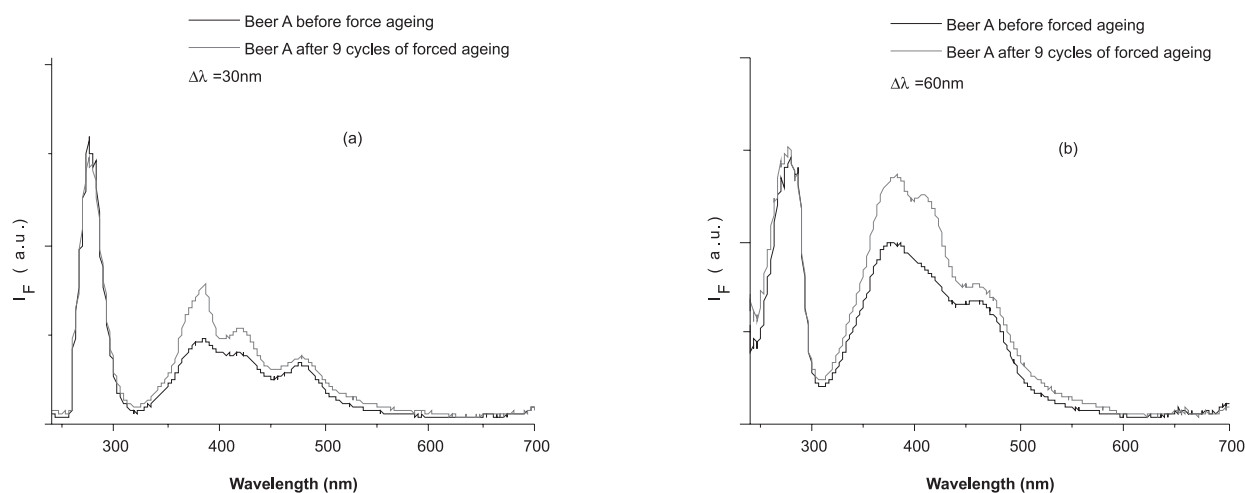


FIGURE 5. Synchronous fluorescence spectra of Beer A samples before the forced ageing treatment compared to spectra of the samples exposed to 9 cycles of forced ageing: (a)  $\Delta\lambda = 30$  nm and (b)  $\Delta\lambda = 60$  nm.

ied, the changes in the specific groups of compounds are very marked, what allows to use the fluorescence spectroscopy as a method for beer analysis and quality monitoring.

The results obtained by fluorescence spectroscopy can be analysed and interpreted by using appropriate chemometric tools, allowing to identify beer brands and brews, and to analyse the contents of specific groups of minor fluorescent constituents in beer samples.

## CONCLUSIONS

The quality, freshness and nutritive value of foods remain very important issues in the contemporary context. The concerns involve adulteration of food products along with the food processing and storage chain, and, as a consequence, the consumer safety.

The present results confirm that advanced techniques of fluorescence spectroscopy may be used to identify differences between beer samples, brews and brands. The methods used in our research, the *Total Luminescence Spectra (TLS)* and the *Synchronous Fluorescence Spectroscopy*, are very accurate and sensitive. Moreover, tests may be run without any special sample preparation, which simplifies and accelerates the analyses.

## REFERENCES

- Almeida C., Duarte I.F., Barros A., Rodrigues J., Spraul M., Gil A.M., Composition of beer by H-1 NMR spectroscopy: Effects of brewing site and date of production. *J. Agric. Food Chem.*, 2006, 54, 700-706.
- Christensen J., Ladefoged A.M., Norgaard L., Rapid determination of bitterness in beer using fluorescence spectroscopy and chemometrics. *J. Institute of Brewing*, 2005, 111, 3-10.
- Christensen J., Norgaard L., Bro R., Engelsen S.B., Multivariate autofluorescence of intact food systems. *Chem. Rev.*, 2006, 106, 1979-1994.
- Duarte I.F., Barros A., Almeida C., Gil A.M., Spraul M., Multivariate analysis of NMR and FTIR data as a potential tool for the quality control of beer. *J. Agric. Food Chem.*, 2004, 52, 1031-1038.
- Duarte I.F., Godejohann M., Braumann U., Spraul M., Gil A.M., Application of NMR spectroscopy and LC-NMR/MS to the identification of carbohydrates in beer. *J. Agric. Food Chem.*, 2003, 51, 4847-4852.
- Polish Standard PN-A-700939:2000. Sprawdzanie napelniania butelek i puszek, oznaczanie klarownosci, oznaczanie trwałości metodą forsowania.
- Sikorska E., Analysis of vitamin B2 using front-face intrinsic beer fluorescence. *Eur. Food Res. Technol.*, 2007, 225, 43-48.
- Sikorska E., Chmielewski J., Koziol J., Khmelinskii I.V., Herance R., Bourdelande J.L., Sikorski M., Photo-induced features of some lager beers. *Pol. J. Food Nutr. Sci.*, 2003, 53, 113-118.
- Sikorska E., Gorecki T., Khmelinskii I.V., Sikorski M., De Keukeleire D., Fluorescence spectroscopy for characterization and differentiation of beers. *J. Institute of Brewing*, 2004, 110, 267-275.
- Sikorska E., Gorecki T., Khmelinskii I.V., Sikorski M., De Keukeleire D., Monitoring beer during storage by fluorescence spectroscopy. *Food Chem.*, 2006, 96, 632-639.
- Vanhoenacker G., De Keukeleire D., Sandra P., Analysis of iso-alpha-acids and reduced iso-alpha-acids in beer by direct injection and liquid chromatography with ultraviolet absorbance detection or with mass spectrometry. *J. Chromatogr. A*, 2004, 1035, 53-61.
- Ventura M., Guillen D., Anaya I., Broto-Puig F., Lliberia J.L., Agut M., Comellas L., Ultra-performance liquid chromatography/tandem mass spectrometry for the simultaneous analysis of aflatoxins B1, G1, B2, G2 and ochratoxin A in beer. *Rapid Commun. Mass Spectrom.*, 2006, 20, 3199-3204.

**BADANIA PIWA METODĄ SPEKTROSKOPII FLUORESCENCYJ**

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W pracy przedstawiono wyniki badań nad możliwością wykorzystania naturalnej fluorescencji piwa do oceny jego jakości. Emisję prób piwa dwóch różnych gatunków, świeżych oraz poddanych procesom starzenia, badano z wykorzystaniem metod pomiaru całkowitej fluorescencji oraz synchronicznego pomiaru fluorescencji. Wykazano przydatność widm fluorescencji do rozróżnienia piw różnych gatunków i różnej świeżości.