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Differential spectroscopy and beer ageing

The role of quinones in the beer oxidation was studied by both differential spectroscopy and photobleached methylene blue reoxidation. Juglone, p-benzoquinone and ubiquinone 0 were added to beer that was pasteurised in the presence or absence of oxygen. Quinones had catalytic effect in the course of beer oxidation by air. Oxygen is consumed by beer in a short time but beer ageing continues in the absence of oxygen. Possible anaerobic mechanism of beer ageing may involve oxidative action of quinones or peroxides, anaerobic Fenton reaction or additional effect of oxygen ingress through crown.

BC 25 Bier/36 Bier

(Descriptors: beer ageing, differential spectroscopy, quinones, oxygen radicals, Fenton reaction.

Deskriptoren: Bieralterung, Differentialspektroskopie, Chinone, Sauerstoffradikale, Fenton-Reaktion).

1 Introduction

Beer colour has often been used as an important beer quality attribute. Spectroscopy measurement at 430 nm in 1 cm glass cuvette against distilled water was recommended for laboratory routine obtaining beer colour by standard reference method (SRM). The beer colour value in EBC units has been obtained multiplying absorbance by 25.

This value is being replaced by some form of tristimulus measurement as a mean of colour interpretation. The colour is conceived as a product of three distinguishable properties, namely hue, value and chroma to be able to express consumer's view more exactly.

Visual tests revealed that beers with the same EBC colours could be distinguished by observers as well as with the help of CIELAB colour space coordinates. *Smedley* measured visible spectra of bright beers within 350 – 750 nm comparing CIE L*a*b* values and their difference D Ea,b to discriminate similar beers with the same EBC colours (1).

It has commonly been believed that beer colour reading at 430 nm responds mostly to melanoidins while polyphenols absorb at long wavelengths and that beer colour increases during ageing.

A ratio of absorbance at 465 nm to 550 nm (A465:A550) was reported to be a useful index of wort and beer oxidation and sensitivity of the method increased by long optical path in cylindrical 5 or 10 cm cuvette. Decrease in colour ratio reflected the extent of cumulative beer oxidation. Colour ratio has proven to be a sensitive index of beer oxidation as well. SRM beer colour at 430 nm measured in long path cuvette was also shown to be a very useful parameter increasing with beer age (2).

Pfenninger and *Meir* proved close correlation (r = 0.891) between increasing beer colour obtained by beer heating with radical initiator peroxodisulfate and beer haze resulting from laboratory forcing test (3).

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Figures see Appendix

In the previous study we used differential spectroscopy between 380 and 600 nm for the beer compounds resistance against natural as well as radical oxidation (4). This technique allowed to recognise very small changes of the beer absorption spectra.

In the course of beer oxidation the catalysers enable the oxygen reduction resulting in free oxygen radicals formation. The new efficient method was developed to determine radicals generation using photobleached methylene blue reoxidation (5).

2 Experimental procedures

2.1 Chemicals

Stock solutions: methylene blue (1000 mg/l), ascorbic acid (1 %) and hydrogen peroxide (1000 mg/l) were prepared by dissolving of the components in deionised water. Hydrogen peroxide stock solution was further diluted by deionised water to get other stock solutions (10 and 100 mg/l) before their using. The potassium dichromate (0.2 g) was dissolved in deionised water, concentrated sulphuric acid (0.245 ml) added and filled with deionised water up to 1000 ml. Ubiquinone 0 (2,3-dimethoxy-5-methyl-1,4-benzoquinone), p-benzoquinone and juglone (5-hydroxy-1,4-naphthoquinone) were prepared by dissolving in 96% ethanol (1000 mg/l). All chemicals were obtained from Sigma Aldrich.

2.2 Aerated and deaerated beer

A cold bottle of beer (5 ºC) from the bottling line was opened and the unpasteurized beer poured down along the wall into 150 ml Erlenmeyer flask prefilled with nitrogen entering through a hypodermic needle placed between the flask neck and the rubber stopper. The beer (100 ml) was purged by nitrogen bubbling for 30 min, the needle removed and the flask tightly closed. This sample was stored at 5 ºC and used as a blank for several hours. The content of dissolved oxygen in deaerated beer was less than 0.05 mg/l.

The aerated beer containing about 7 mg/l of oxygen was prepared by purging of the same beer with air instead of nitrogen.

2.3 Differential beer spectra

The starting beer spectrum (baseline) was measured in rectangular glass cuvette (5 cm) between 380 and 580 nm against distilled water, the absorption curve recorded and wavelength/absorbance pairs loaded into database. The same procedure was used to obtain wavelength/absorbance values of sample. Differential spectrum

was obtained as the difference between sample and baseline absorbance values transformed into graph.

2.4 Spectral changes during beer pasteurisation

The flasks with aerated or deaerated beers were placed into water bath and heated at 60 ºC for 1 to 4 hours, then removed and cooled by cold tap water. Differential spectra were measured against nitrogen beer purged by nitrogen (Fig. 1).

2.5 Spectral changes during beer pasteurisation with the hydrogen peroxide addition

Stock solutions of hydrogen peroxide as well as the same amount of deionised water were added to beer (1:100) before pasteurisation to get final concentration 0.1, 1.0 and 10.0 mg/l of H_2O_2. The flasks with aerated or deaerated beers were placed into a water bath and heated 1 h at 60 ºC, then removed and cooled by tap water. Differential spectra were measured against beer purged by nitrogen (Fig. 2).

2.6 Spectral changes during beer pasteurisation with the quinone addition

Stock solutions of quinones in ethanol as well as the same amount of 96 % ethanol were added to beer (1:100) before pasteurisation to get final concentration 10.0 mg/l of quinone. The flasks with aerated or deaerated beers were placed into water bath and heated 1 h at 60 ºC, then removed and cooled by tap water. Differential spectra were measured against beer purged by nitrogen (Fig.3).

2.7 Spectral changes during bottled beer ageing

Bottled unpasteurized beer from the bottling line (total oxygen content in a package about 50 ppb) was stored at 20 and 45 ºC for 1 to 7 weeks, then the bottles were opened and differential spectra measured against blank purged by nitrogen purged immediately after bottling (Figs. 4, 5).

2.8 Differential spectra measurement repeatability

The starting spectrum (baseline) of potassium dichromate stock solution was measured in rectangular glass cuvette (5 cm) between 380 and 580 nm against distilled water and the measurement repeated during storage of this solution at 20 ºC in dark. The starting beer spectrum is also showed for a comparison (Fig. 6).

2.9 Reoxidation of the photobleached methylene blue

The beer or deionised water (4.9 ml) with Cu^{2+} or quinone addition was bubbled by nitrogen or air (30 min). The nitrogen was introduced into round cuvette through the syringe needle placed between the rubber stopper and the cuvette neck. The ascorbic acid (50 µl) and methylene blue (50 µl) stock solutions were added then another minute bubbled, the needle removed and cuvette tightly sealed. In parallel trial was nitrogen replaced by air.

The cuvettes were illuminated with a halogen bulb (50 W) from the distance of 5 cm for 5 to 10 s then moved into spectrophotometer chamber and the absorbance measured at 666 nm recorded in one-minute intervals (Figs. 7,8). This procedure differs slightly from that previously designed using 2.5 min time intervals (5).

2.10 Instruments

CADAS 200 spectrophotemeter (Dr. Lange, Germany) was used with 1 cm round cuvette or 5 cm rectangular glass cuvette using distilled water as a blank.

3 Results and discussion

Differential spectroscopy is a useful technique for recognizing small colour changes during beer processing and ageing. In our experiments 5 cm optical path offered much greater sensitivity and reproducibility than standard 1 cm. The method was able to differentiate between aerated and deaerated beer in the course of laboratory pasteurisation. The differential spectra showed the increase of absorbance at 410 – 420 nm (first differential maximum – FDM) and second differential maximum (SDM) at 500 – 520 nm (Fig. 1).

The formation of hydrogen peroxide during beer oxidation/heating has often been reported (6). The addition of the hydrogen peroxide made the spectra more distinctive increasing both maxima at heated beer with 0.1 and 1.0 mg/l hydrogen peroxide but decreasing the FDM in deaerated beer with high hydrogen peroxide dosage (10 mg/l). In the presence of air both maxima increased even at high H_2O_2 dosage, which witnesses the different mechanism of H_2O_2 acting.

On the other hand the FDM decrease could also been recognised after addition of reducing compounds such as ascorbic acid into aerated or deaerated beer. It is difficult to differentiate between reduction and oxidative degradation of colour compounds taking part in FDM and SDM without knowing their composition.

Some organic dyes (e.g. indigo carmine) added to beer can be destroyed only on condition that oxygen is present while other dyes (e.g. methyl red) needed anaerobic conditions. The presence of hydrogen is supposed in both cases (7). It seems that there are two mechanisms of hydrogen peroxide action depending on the presence of oxygen. In the absence of oxygen the methyl red might undergo radical reduction. Some reduction products (e.g. furfurylacohol) have often been found together with oxidation ones (e.g. furfuraldehyde) in aged beer.

Hydrogen peroxide can be formed during air oxidation of many natural compounds such as ascorbic acid or quinones. It is surprising that even industrial production of hydrogen peroxide uses processes featuring catalytic hydrogenation followed by autooxidation of a suitable organic carrier molecule, predominantly an alkylated anthroquinone, although an efficient natural catalyser juglone containing naphthoquinone is not present in beer (8).

The oxidation pathway of the volatile aldehydes formation is well documented now. Oxidation of phenylalanine in a solution containing ascorbic acid as an example of reductone indicated that effective oxidation under the experimental condition requires not only oxygen but also an activating agent such as transition metals. It was suggested that similar mechanisms might be involved in the oxidation of amino acids in ageing beer leading to the formation of volatile aldehydes associated with off-flavour (9). This mechanism is able to explain both hydrogen peroxide formation and aerobic Fenton reaction providing volatile carbonyl compounds from aminoacids and/or alcohols.

Recently *Qian* and *Buettner* discovered that pre-existing hydrogen peroxide is only a minor initiator of free-radical oxidation and that the major initiators of biological free-radical oxidation are the oxidising species formed by the reaction of Fe^{2+} with dioxygen.

They also confirmed different mechanism of aerobic or anaerobic Fenton reaction, which depends on O_2/H_2O_2 concentration ratio (10).

The Fenton reaction catalysed by transition metals plays an important role in beer ageing but other catalysers can also occur. The quinones have been reported as natural catalysers accelerating ascorbic acid destruction.

Quinones are important natural compounds having the ability to damage biological targets. The reduction of quinones results in the formation of semiquinones followed by reactive oxygen species generation. This non-enzymatic redox cycling may occur due to quinones interaction with ubiquitous bioreductants most notable ascorbic acid (11).

Ascorbic acid can serve as an example of reductones, which are compounds naturally present in beer. The addition of quinones showed similar changes to those observed during beer pasteurisation in the presence or absence of hydrogen peroxide (Fig.1 – 3).

The possible mechanism of quinones action can comprise:

- Catalytic effect in the course of oxygen reduction;
- direct oxidation of natural beer compounds;
- hydrogen peroxide formation.

The ratio between quinones/semiquinones/hydroquinones can be changed during beer production including yeast reduction or beer oxidation.

Quinones can be formed by natural oxidation of polyphenols present in beer, which could explain possible positive or negative role of polyphenols in beer. In a model solution photocatalytic oxidation of p-benzoquinone provided partly hydroquinone, partly unidendified oxidised products (12).

The beer spectral changes were followed during beer ageing. The differential spectroscopy was able to recognise colour changes during beer ageing at 20 and 45 ºC (Figs. 4, 5).

The repeatability of the differential spectroscopy was studied using comparative measurement of the potassium dichromate solution. The repeatability of the differential technique was excellent in the range above 400 nm although the unexpected decreases between 380 to 400 nm could also been observed similar to those of beer. There are two possible reasons for the explanation of this phenomenon: (i) low sensitivity of spectrophotometer at high level of the absorbance, (ii) changes during potassium dichromate storage. Other experiments will be needed to recognise between (i) and (ii).

There are two common techniques used in beer differential spectroscopy. Our procedure is based on digit operation with wavelength/absorbance pairs from the database. We used the starting spectrum (baseline) recorded against distilled water with the following computer subtraction from the sample spectrum digital record.

The second technique is based on starting beer spectrum measurement, which is used as a blank zeroing the spectrophotometer against beer instead of distilled water. The all-absorbance values in beer spectrum obtain zero absorbance values and the changes are measured against them. This more sensitive procedure is more suitable for short time measurement not allowing to obtain the starting spectrum curve.

The catalytic effect of quinones was also studied with photobleached methylene blue reoxidation (Fig. 7, 8). Visible light strongly accelerates conversion of methylene blue into its leuco-form, which is reoxidised by oxygen radicals formation caused by oxygen reduction present in the sample (5).

The range of photobleached methylene blue reoxidation was dependent on the kind of quinones, some of them being comparable with the addition of Cu^{2+} (0.1 mg/l) or natural beer reoxidation activity (5,9). The higher effect of other quinones could be reached by increasing their concentration up to 20 – 50 mg/l. The reoxidation activity of the beer exposed to air also gradually increased (data not shown).

We believe that this aerobic mechanism comprises the substantial part of beer ageing. Beer reductones are oxidised by oxygen providing oxygen radicals taking part in oxidation of amino-acids, alcohols, sugars and other compounds. This reaction can be catalysed by anorganic or organic catalysers presented in beer. We have also found that the effect of catalysers can combine mutually.

On the other hand the oxygen is consumed by beer in relatively short time but the ageing continues after oxygen consumption. Anaerobic ageing must be taken into account based on compounds storing active oxygen such as organic peroxides or quinones, which can act as catalysers or oxidating agents as well.

The hydrogen peroxide can easily degrade various organic compounds providing other accelerators or inhibitors of the radical oxidation (7). The other explanation of the anaerobic ageing is based on the oxygen ingress through beer package closures, which has been repeatedly proved. The daily intake of oxygen through commercially produced crowns is approximately about 0.001 – 0.002 ml O_2/day, which presents 0.28 – 0.56 µg O_2/day(13).

4 Conclusions

- Differential spectroscopy is a useful technique recognising small colour changes during beer processing and ageing.
- Hydrogen peroxide is supposed to be formed during air oxidation of natural compounds such as reductones or quinones.
- The volatile aldehyde formation requires oxygen and activating agent such as transition metal or an organic catalyser.
- The quinones action can comprise catalytic effect in the course of oxygen reduction, direct oxidation of natural beer compounds or hydrogen peroxide formation.
- The balance between quinones/semiquinones/hydroquinones can play an important role in beer ageing.
- Beer ageing is based on oxygen oxidation of reductones, which is accelerated by anorganic or/and organic catalysers.
- Beer ageing continues after oxygen consumption. Anaerobic ageing may be based on oxygen binding compounds such as organic peroxides or quinones.
- The additional effect of oxygen ingress through crown closure can also be involved
- There are different mechanisms of the aerobic or anaerobic Fenton reaction. Radical reduction could also participate.

5 Summary

The role of quinones in the beer oxidation was studied by both differential spectroscopy and photobleached methylene blue reoxidation. Juglone, p-benzoquinone and ubiquinone 0 were added

to beer that was pasteurised in the presence or absence of oxygen. Quinones had catalytic effect in the course of beer oxidation by air. Oxygen is consumed by beer in a short time but beer ageing continues in the absence of oxygen. Possible anaerobic mechanism of beer ageing may involve oxidative action of quinones or peroxides, anaerobic Fenton reaction or additional effect of oxygen ingress through crown.

6 Zusammenfassung/Résumé

Savel, J.: Differential-Spektroskopie und Bieralterung — Monatsschrift für Brauwissenschaft 57, February, 1– 7, 2003.

BC 36 Bier/25 Bier

Die Rolle der Quinone bei der Bieroxidation wurde sowohl anhand von Differential Spektroskopie als auch mit der Methylenblau-Reoxidation untersucht. Juglon, p-Benzoquinon und Ubiquinon 0 wurden dem Bier zugefügt, welches in Gegenwart oder Abwesenheit von Sauerstoff pasteurisiert wurde. Quinone übten einen katalytischen Effekt während der Bieroxidation mit Luft aus. Sauerstoff wird innerhalb kurzer Zeit von Bier verbraucht, jedoch geht die Bieralterung auch ohne Sauerstoff weiter. Mögliche anaerobe Mechanismen der Bieralterung könnten oxidative Reaktionen von Quinonen oder Peroxyden einschließen oder auch eine anaerobe Fenton-Reaktion oder Zusatzeffekte durch Sauerstoffeintrag über den Kronkorken.

Savel, J.: Spectroscopie différentielle et vieillissement de la bière — Monatsschrift für Brauwissenschaft 57, February, 1 – 7, 2003.

BC 36 Bière/25 Bière

On a examiné le rôle des quinones au cours de l'oxydation de la bière d'une part avec la spectroscopie différentielle et d'autre part avec ré-oxydation au bleu de méthylène. On a ajouté de la jugulone, de la p- benzoquinone et de l'ubiquinone à la bière qui a été pasteurisée en présence et en absence d'oxygène. Les quinones exerçaient un effet catalytique pendant l'oxydation à l'air de la bière. L'oxygène est consommé rapidement par la bière ; toutefois le vieillissement de la bière se poursuit sans oxygène. Il y a d'autres mécanismes anaérobies possibles du vieillissement de la bière, par exemple les réactions d'oxydation de quinones ou de peroxydes ou une réaction anaérobie de Fenton ou effet d'addition par introduction d'oxygène par le bouchon couronne.

7 Literature

1. Smedley, S.M., Discrimination between beers with small colour differences using the CIELAB colour space. J. Inst. Brew., **101** (3), 195 – 201, 1995.
2. Pflugfelder, R., Assesment of beer oxidation using long-path color techniques. Poster 33, World Brewery Congress 2000, Orlando, USA.
3. Pfenninger, H.B., Meier, H.U., Some experience with the Savel peroxodisulfate test for the prediction of beer instability. Brewing Science Group Bulletin 1998, Edinburough, 235-240.
4. Savel, J., Zdvihalova, D., Prokopova, M.: Colour changes during chemical and photochemical oxidation (In Czech). Kvasny Prum., **43** (3), 67 – 70, 1997.
5. Savel, J.: Electron transport influencing species in beer (submitted for publications).
6. Uchida, M., Ono, M., Determination of hydrogen peroxide in beer and its role in beer oxidation. J. Am. Soc. Brew. Chem. **57** (4), 145 – 150, 1999.
7. Savel, J., Fenton reaction acceleration using maltose and ascorbic acid. Mschr. Brauwissenschaft, **56** (1/2), 4 – 8, 2003.
8. Chapon, L., Chapon, S.: Peroxidatic step in oxidation of beer. J. Am. Soc. Brew. Chem., **37** (2), 1979, 96 –104.
9. Savel, J., Aerobic and anaerobic beer ageing. (InCzech). Kvasny Prum. **49** (5), 115 – 118, 2003.
10. Qian, S., Y., Buettner, G., R., Iron and dioxygen chemistry is an important route to initiation of biological free radical oxidations: an electron para,agnetic resonance spin trapping study. Free Radic. Biol. Med., **26** (11/12), 1447 – 1456, 1999.
11. Roginsky, V., A., Bruchelt, G., Bartuli, O.: Ubiquinone-0 (2,3-dimethoxy-5-methyl-1,4-benzoquinone as effective catalyzer of ascorbate and epinephrine oxidation and damager of neuroplasma cells. Biochemical. Pharmacol., **55** (1), 85 – 91, 1998.
12. Sobczynski, A., Duczmal, L., Dobosz, A., Photocatalysis by illuminated titania: oxidation of hydroquinone and p-benzoquinone. Monatshefte f. Chemie, **130** (3), 377 – 384, 1999.
13. Teumac, F., N., Ross, B., A., Rassouli, M.R., Air ingress through bottle crowns. MBAA Technical Quart., **27** (4), 122-126, 1990.

Manuscript received August 19, 2003

Appendix

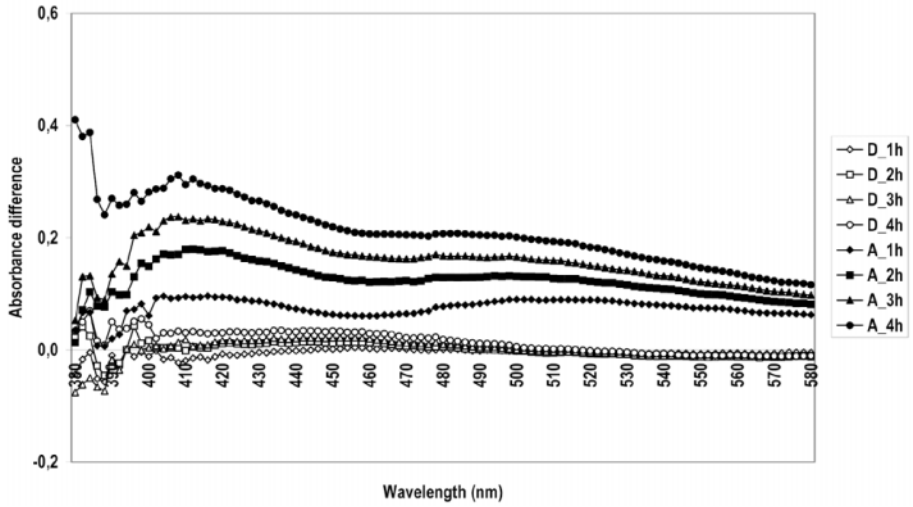

Fig. 1 Differential spectra of the aerated (A) and deaerated (D) beer heated at 60 °C for 1 – 4 h

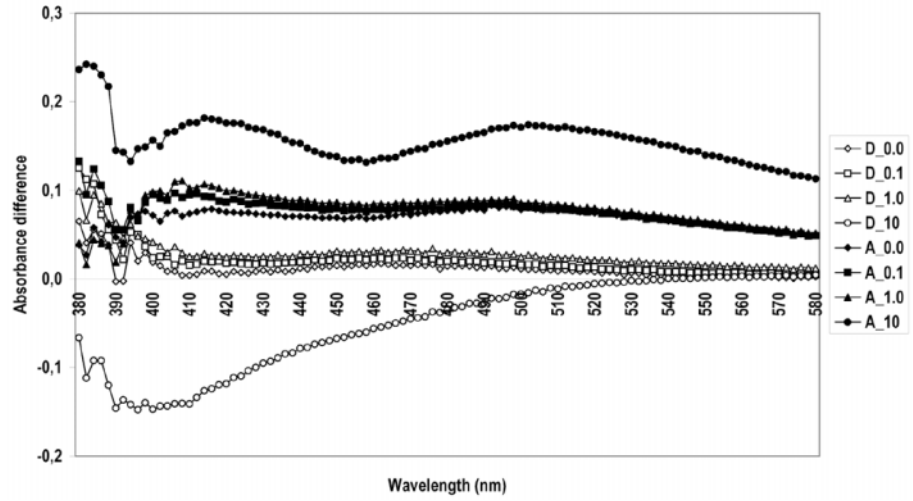

Fig. 2 Differential spectra of the aerated (A) and deaerated (D) beer with hydrogen peroxide addition (0 – 10 mg/l) heated 1 h at 60 °C

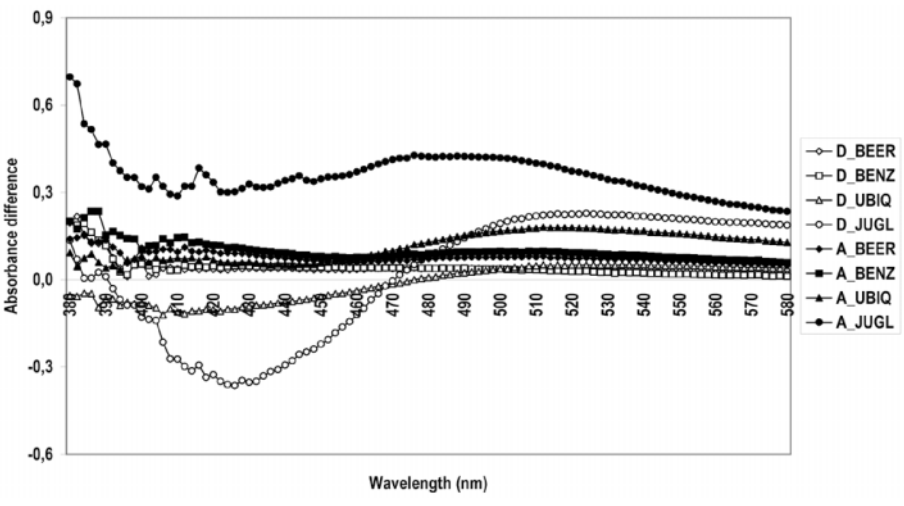

Fig. 3 Differential spectra of the aerated (A) and deaerated (D) beer containing p-benzoquinone, ubiquinone 0 and juglone

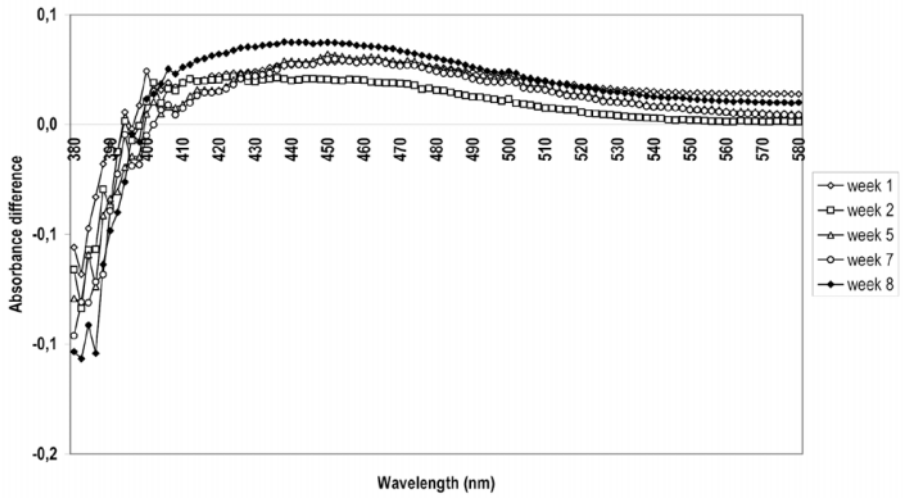

Fig. 4 Differential spectra of the bottled beer stored 1 – 8 weeks at 20 °C

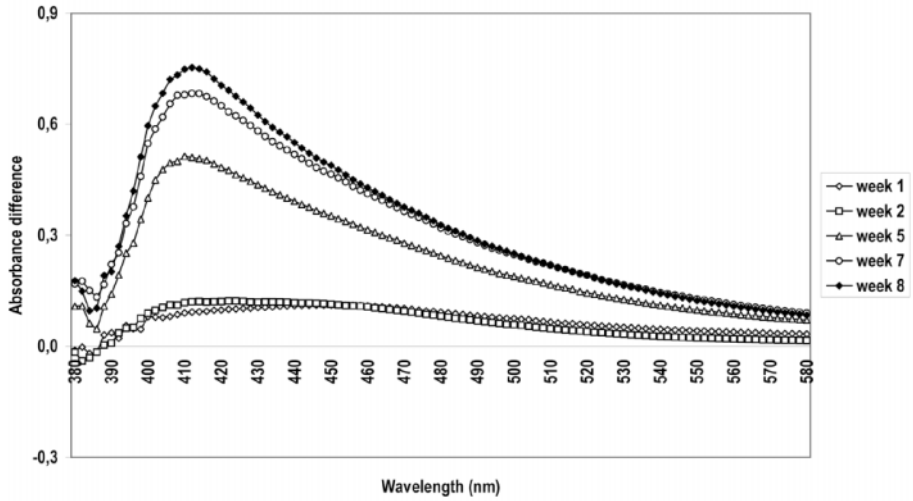

Fig. 5 Differential spectra of the bottled beer stored 1 – 8 weeks at 45 °C

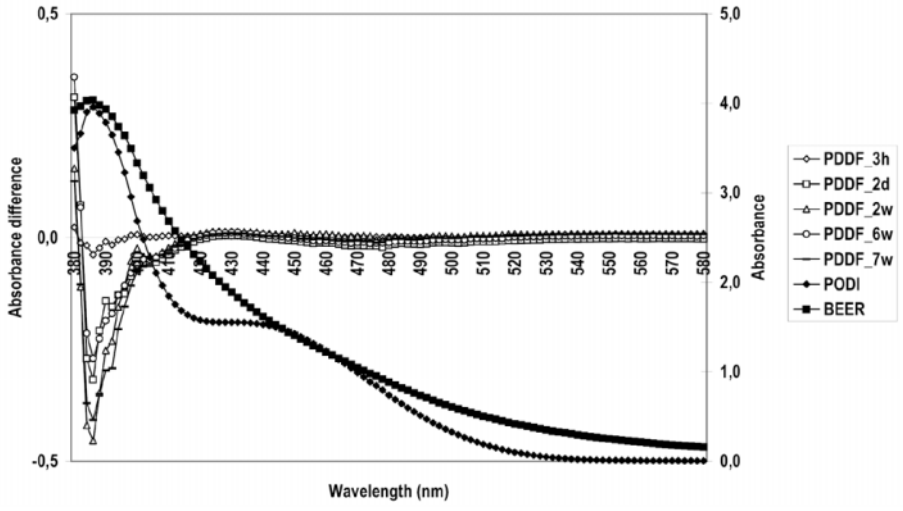

Fig. 6 Absorption spectra of potassium dichromate (0.2 g/l) (PODI) and beer (BEER) and differential spectra of potassium dichromate (PDDF) stored at 20 °C for 3 h – 7 w

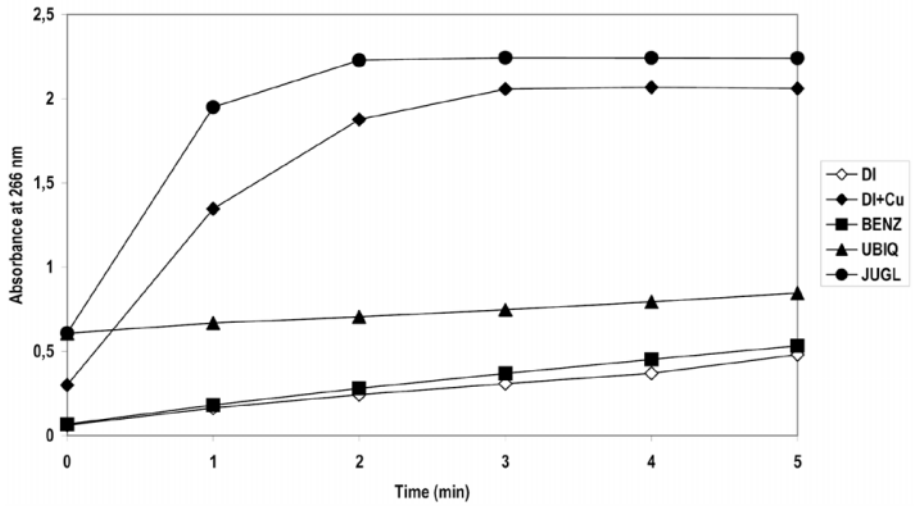

Fig. 7 Reoxidation of the photobleached methylene blue (10 mg/l) in ascorbic acid solution (0.01%) in aerated (A) deionised water (DI) with Cu^{2+} (0.1 mg/l), p-benzoquinone, ubiquinone 0 and juglone (10 mg/l) addition

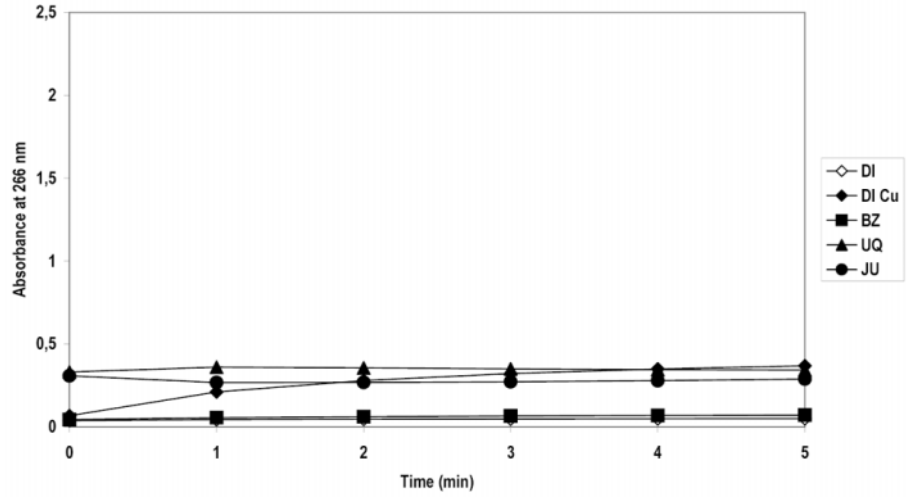

Fig. 8 Reoxidation of the photobleached methylene blue (10 mg/l) in ascorbic acid solution (0.01%) in deaerated (D) deionised water (DI) with Cu^{2+} (0.1 mg/l), p-benzoquinone, ubiquinone 0 and juglone (10 mg/l) addition