Direct detection of beer photodegradation in commercial bottles and introduction of a new Light-Struck Flavour Susceptibility Index

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Abstract

Exposure of beer to light results in the formation of undesirable flavours or complete spoilage. Photo-damaged beer has a specific, so-called skunky or light-struck flavour (LSF). The compound responsible for LSF is 3-methylbut-2-ene-1-thiol (MBT). Riboflavin (RF) plays a key role in the formation of MBT. It absorbs light in the blue part of the spectrum and transfers excitation energy to isohumulones. This process is accompanied by the decomposition of RF, which causes a decrease in the absorbance of the sample at 450 nm. The decomposition is directly related to the formation of LSF. In this study, the decrease in absorbance associated with the defined illumination of model and real beer directly in commercial bottles was measured. The decrease in absorbance correlated with the decrease in RF concentration and the formation of LSF detected by the sensory panel. The Light-Struck Flavour Susceptibility Index was introduced as a rate of the beer susceptibility to light degradation and the formation of LSF.

Keywords: beer; light-struck flavour; photodegradation; 3-methyl-2-butene-1-thiol; riboflavin

1 Introduction

It is well known that exposure of beer to light leads to the development of undesirable flavours or even complete spoilage of the beverage. As early as 1875, the German professor Lintner described the effects of light radiation on the quality and taste of beer. He called the unpleasant taste that appears in beer after leaving it in the light, as the so-called light-struck flavour (LSF), often also referred to as the skunk flavour (Lintner, 1875).

3-Methyl-2-butene-1-thiol (MBT) is believed to be the main compound responsible for the light-struck flavour. Its formation is associated with non-enzymatic reactions in which isohumulones react with sulphur components of amino acids and their derivatives. The current and generally accepted mechanism for the formation of light-struck flavour is based on the absorption of light radiation and the subsequent transfer of excita-tion energy. Thus, the first possibility is the excitation of riboflavin (RF) into a singlet state after the absorption of visible light with wavelengths 350–500 nm. This excited singlet state of RF subsequently leads to a triplet state caused by intersystem conversion (Templar et al., 1995). Then energy of the excited RF triplet state is transferred to the isohumulones to form their triplet excited state. The energy transfer from RF to isohumulones is probably accompanied by degradation of RF to photoproducts. Also, isohumulones can be excited by direct exposure to ultraviolet radiation. In the excited state, the isohumu-lones are cleaved by the so-called Norrish Type I cleavage resulting in 4 methylpent-3-enoyl radical. This radical is then decarbonylated, leading to the radical precursor of MBT, which is bound to a suitable source of sulphur to form MBT (De Keukeleire et al., 2008; Heyerick, 2001).

Nonetheless, only visible light with a wavelength longer than 350 nm penetrates into glass and plastic containers, while UV light is absorbed in the wall. Since isohumulones do not absorb visible light, the light damage in glass vessels occurs only due to excitation of RF.

Sakuma et al. (1991) demonstrated that the increase in MBT content after the exposure of model solutions to light is linearly dependent on RF concentration. Once the excited RF interacts with isohumulones, it breaks down into photoproducts that change the optical properties of the sample (Pozdrik et al., 2006). Gabriel and Sigler (2018) proved that the changes in the optical properties of beer due to light degradation can be observed directly in commercial glass or plastic vessels.

The optical damage of beer is currently assessed only in the case of a complaint due to undesirable flavours and odours. Neither the beer itself nor the beer in final packaging is tested for susceptibility to optical damage. For objective and comparable testing, it is necessary to define light exposure of beer and then to evaluate the degree of optical damage by a suitable method. The degree of optical damage of beer is usually determined by the sensory evaluation performed by a qualified sensory panel of experts (Irwin et al., 1993). Nevertheless, sensory analysis is time consuming and requires both trained professionals with proven special skills (sensory tests) and appropriate premises, i.e. well-equipped sensory laboratory or at least a suitable tasting room. In addition, it is very difficult to perform a sufficiently fast routine assessment of multiple samples, because the olfactory sensors in the nose are quickly saturated at higher LSF intensities and their regeneration is slow. Further, the intensity of LSF must be determined quickly after pouring the beer into the glass, because the odour escapes relatively quickly. For these reasons, it is practically impossible to implement sensory evaluation as a part of a routine operational inspection.

The degree of sensory damage can be also assessed on the basis of the MBT content analysis (Templar et al., 1995). But, analytical procedures for MBT detection are very complex, expensive and time-consuming and can only be performed with state-of-the-art instrumentation and qualified personnel (Sakuma et al., 1991). Hence, the MBT analysis in beer is also not very useful for routine brewery practice.

Until now, it was not possible to assess the degree of the optical damage of beer directly in closed packages without opening them. However, an opened package means that the beer comes into contact with oxygen from the air, causing irreversible changes. Dissolved oxygen has been shown to suppress LFS formation (Irwin et al., 1993). Therefore, the packaging can no longer be resealed and the beer further investigated, for example subjected to continued exposure.

The aim of this work was to show that the light damage of beer can be optically monitored directly in a closed commercial bottle. At the same time the Light-Struck Flavour Susceptibility Index (**LSFI**) was introduced as a susceptibility rate of beer to light degradation and the formation of light-struck flavour at the defined light intensity.

2 Materials and methods

Model beer preparation

Model beer consisted of 4 mM citrate/phosphate buffer (pH = 4.5) containing 5 µg/l RF, 25 mg/l cysteine, 50 ml isohumulones and 5% ethanol was prepared as follows immediately before each experiment. 0.6 g of tripotassium citrate (p.a., Sigma-Aldrich) was dissolved in 900 ml of deionized water and the pH was adjusted to 4.5 with citric acid (99%, Sigma-Aldrich). Deionised water was prepared using an Aqual 35 instrument, while the conductivity was less than 0.2 µS/cm. 25 mg of L-cysteine (Bioultra, Sigma-Aldrich) and 5 µg/l of RF (for biochemistry, Sigma-Aldrich) were added to the buffer solution along with 50 ml of 96% ethanol (Sigma-Aldrich). The solution was stirred for 20 min at 100 rpm. Further, 50 µl of aqueous isohumulones solution (30% w/v of their potassium salts; purchased from Research Institute of Brewing and Malting) was added. The volume of the buffer solution was replenished to 1 l with deionized water and the solution was purged with carbon dioxide (Linde, Inc.) for 30 min. The concentrations of RF, isohumulones and L-cysteine hydrochloride in the experiments ranged from 1–10 µg/l, 0–100 µg/l, 0–100 µg/l, respectively.

Commercial beer samples

Pilsner-type beer samples in 0.5 l Nord-Rhein Westfalen green bottles (NRW) were provided by local Czech brewery and packed in paper box protecting samples from light.

Absorbance determination using Colorturb instrument

A prototype of the COLORTURB instrument, developed at the Department of Chemical Physics and Optics of Charles University in cooperation with the company 1-Cube s.r.o., was used to measure optical parameters. The apparatus measures samples in cuvettes and/or directly in commercial bottles (Figure 1). The sample volume can vary from 0.1 l for cuvettes to 2 l for PET bottles (Gabriel and Sigler, 2018). To minimize the effect of the bottle´s wall inhomogeneity on the measurement results, the sample is placed in a chamber filled with an immersion liquid (distilled water) and undergoes a defined rotation during the measurement. The RGB LED light source installed in the device

emits light in three wavelength bands independently. Thus, the Colorturb apparatus gives three absorbance values that are measured using light sources on three different spectral bands AbsRed (630 nm), AbsGreen (518 nm) and AbsBlue (466 nm), together with turbidity values. Nevertheless, turbidity values were not been used in this work.

Illumination adapter

A special adapter was developed for a defined and homogeneous illumination of bottled beer samples. The adapter consisted of a plastic tube, the inner surface of which was fitted with a strip of blue LEDs emitting the wavelength of 450 ± 30 nm corresponding to the maximum absorbance of RF. The LEDs were installed along the entire tube length and the intensity of the LEDs could be changed using a controlled current source. The bottle containing the sample was placed inside the tube. The luminosity of LEDs was measured with a Voltcraft luxmeter and the illuminance reached values between 7000 lx at the edge to 8200 lx in the middle of the cylinder (tube). To minimize heating of the illuminated sample, fans were installed at the bottom of the adapter to blow air into the tube.

HPLC analysis

The HPLC Ultimate 3000 instrument (Dionex) connected to a multi-channel fluorescence detector was used for HPLC analysis. Chromatographic separation was achieved on an Ascentis® Express C18 (250 mm × 3 mm × 5 µm) column equipped with a C18 guard column, at a temperature of 30 °C and using the gradient elution. Mobile phase A consisted of 0.05 M phosphate buffer (K_2HPO_4, KH_2PO_4, pH 7; Lach-Ner, Czech Republic), while mobile phase B was methanol (Sigma-Aldrich). The elution gradient mode was carried out as follows: 0 min/97% B; 5–15 min linear decrease to 30% B;15–25 min/30% B; 25–30 min rapid increase to 97% B; 30–35 min 97% B. The total separation time was 35 min, flow rate 0.3 ml/min and injection volume 5 µl. The excitation wavelength was 440 nm and RF was detected at an emission wavelength of 520 nm (Hucker et al., 2011).

Sensory analysis of light-struck flavour

Sensory analysis took place in the sensory laboratory of the Research Institute of Brewing and Malting equipped
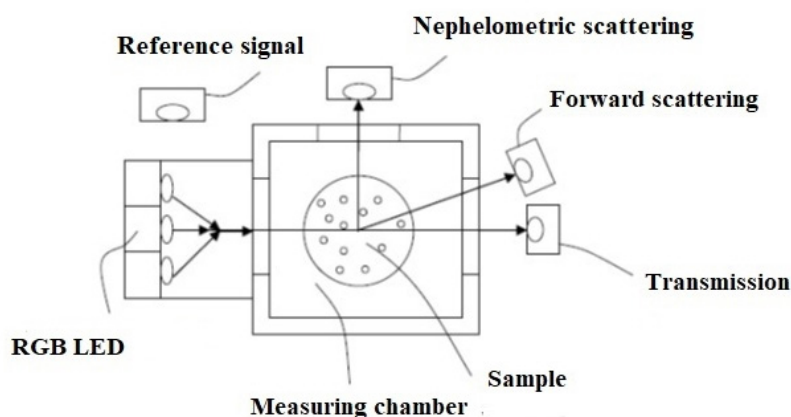

Figure 1 *Scheme of Colorturb instrument*

with special boxes with ventilation and lighting. The panel of sensory experts was composed of at least 6 evaluators who were trained in beer sensory analysis in compliance with ISO 8586:2015 and ISO 11132:2012. The sensory experts evaluated the intensity of the light-struck flavour in studied samples on the scale of 0–5, where 5 is the highest intensity. Only the odour of the beer samples was assessed, without mouth-tasting, directly in the original bottles and immediately after opening the bottle. The bottles with samples were indicated by a random combination of letters and the assessors accessed samples separately. The bottles were covered with a plastic cup between each assessment to prevent greater leakage of aromatic substances. The basic statistic parameters such as average and standard deviation were calculated from obtained data.

3 Results and discussion

Light degradation of model beers

Model beers composed only of selected basic compounds showed how these substances react to the light exposure without interference of many other beer components. That is, a set of model beers with the same composition was exposed to light for different times in the illumination adapter. Before and after illumination model beer samples were placed in Colorturb, where the absorbance at 466 nm (AbsBlue) was measured. Then the bottle was opened to measure the absorption spectra also on a spectrophotometer and to analyse the RF concentration by HPLC. At the same time, the sensory analysis served to evaluate the presence and intensity of LSF flavour. The results are summarized in Table 1. The difference be-

tween the absorbance values measured on the Colorturb before and after the sample illumination gives the parameter ΔAbsBlue. Figure 2 demonstrates a comparison of the absorption spectra of the samples depending on the illumination time. The absorption spectrum of the sample without illumination corresponded to the spectrum of pure RF, because other components of the model beers do not absorb in the visible region of the spectrum.

Sensory resolution of long-lighted and the most light-damaged samples decreased due to the low saturation threshold of LSF off-flavour. The highly trained sensory panel was able to reliably distinguish between unilluminated and poorly illuminated samples.

A set of measurements was performed on model beer samples to monitor the rate of RF degradation depending on the concentration of RF (1–10 μg/l),

Table 1 *Effect of illumination time on parameters of model beers*

Light exposure (min)	ΔAbsBlue	RF (mg/l)	LCH (mg/l)	LSF
0	0.000	4.92	0.00	0.0
2	-0.112	3.90	0.64	2.3
6	-0.415	1.55	2.12	3.1
10	-0.463	1.14	2.38	3.3

ΔAbsBlue – the difference in the absorbance before and after illumination; RF – riboflavin; LCH – lumichrome; LSF – light-struck flavour intensity on the scale 0–5, where 5 is the highest intensity

Figure 2 illustrates significant decrease in the absorption peak at 450 nm with increasing illumination time. The decrease in absorbance at a wavelength of 450 nm corresponds to the decrease in absorbance of AbsBlue measured on a Colorturb (Table 1). Alterations in the absorption spectra are associated with changes in the composition of the model beers during illumination time. The RF content decreased as a result of its breakdown into photoproducts under the light exposure, and at the same time the value of AbsBlue also decreased. On the contrary, the LSF aftertaste increased. The decrease in AbsBlue value correlated well with the decrease in RF concentration and the LSF off-flavour formation.

Furthermore, HPLC confirmed that lumichrome (LCH) was predominantly formed as the main product of RF degradation by light. The LCH spectrum showed no absorption maximum at 450 nm but displayed an absorption maximum at 350 nm, see Figure 2. The light degradation of RF to LCH could explain the decrease in the RF absorption maximum at 450 nm and the shift of the absorption maximum at 370 nm to lower wavelengths.
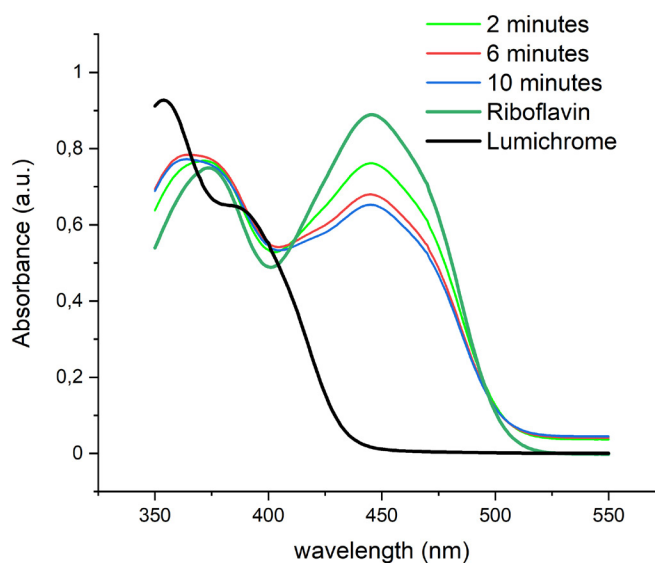

Figure 2 *Changes in the absorption spectrum of model beer depending on illumination time*

cysteine (0–100 μg/l) and isohumulones (0–100 μg/l). It was proved that the ΔAbsBlue magnitude was directly proportional to RF and isohumulone concentration. However, the concentration of cysteine as a sulphur source did not affect the rate of RF degradation (results not shown).

The decrease in AbsBlue under the light exposure corresponded to the formation of LSF and MBT. It was concluded that this is a suitable marker of light degradation in the beer sample.

Light degradation of commercial beer samples in green bottles

Samples of Pilsner-type beer bottled in 0.5 l green bottles were used to monitor light degradation directly in commercial packaging. Green bottles were chosen because they partially transmit blue light in the spectral region of 420–480 nm corresponding to the absorption maximum of RF and thus allow light degradation of the sample. The optical path of the beam in the bottle was determined to be 5.0 ± 0.1 cm for the type of bottles used (Gabriel and Sigler, 2018). This parameter was obtained by comparing the absorbance values measured on the Colorturb and on a spectrophotometer using a 1 cm cuvette.

The average value of the wall absorbance in the selected bottles was determined by measuring a set of bottles filled with distilled water on Coloturb. The absorbance value achieved in blue light (AbsBlue) was 0.64 ± 0.04. It should be noted that light passed through the bottle wall twice when the AbsBlue was measured.

$$Transmitance\,[\%] = 100 * 10^{\left(-\frac{AbsBlue}{2}\right)} \qquad (1)$$

Thus, it can be calculated (equation 1) that only 44% to 50% of the incident light with the wavelength of 450 nm penetrated the bottle. The light transmittance of these bottle types varied in the order of percentage units.

Commercial beer samples were taken directly from the bottling line and kept in the dark. During the measurement, the samples were placed in the illumination adapter for a defined time and exposed to defined illu-mination. During illumination, samples were transferred to the Colorturb every 10 minutes to measure AbsBlue absorbance.

Figure 3 shows the decrease in the value of AbsBlue (blue LED) measured on the Colorturb depending on the illumination time. The AbsBlue decrease can be approximated by a straight line in the initial phase of the light exposure, i.e. 0–50 min. The equation of a straight line was:

$$AbsBlue = 2{,}077 - 0{,}0011 * Time\,[min.] \qquad (2)$$

where the linear coefficient quantifies the discoloration rate of the sample.

The effect of illumination time on beer parameters was determined using Colorturb. The ΔAbsBlue absorbance value was calculated as the difference between the sample absorbance before and after illumination. Then sensory and HPLC analysis followed, while the results are summarized in Figure 4. As the illumination time increases, the discoloration of the sample increases (ΔAbsBlue), LSF intensity increases, the RF content decreases and LCH content increases. RF content decrease correlates well with ΔAbsBlue decrease and LSF intensity increase.

To verify the previous results, a similar experiment was repeated for several batches of the same type of beer filled in the same type of green bottles. The RF concentration in beer samples ranged from 250 to 390 µg/l before light exposure.

Figure 5 confirms that the decrease in RF content correlates well with the AbsBlue discoloration and with the formation of LSF intensity. Again, the sensory panel was able to distinguish more reliably unlit and poorly lit samples because of the low sensory saturation threshold of LSF. Sensory resolution decreased with the length of light exposure and with the degree of sample light-damage.

Although it is problematic to compare the intensity of the LSF aftertaste on different days, it can be stated that the presence of LSF was detected in all sensory sessions as well as in all samples with a decrease in ΔAbsBlue greater than 0.06.

The presented results confirmed that the decrease of absorbance at 450 nm (AbsBlue) after illumination is a suitable marker of light-induced degrada-
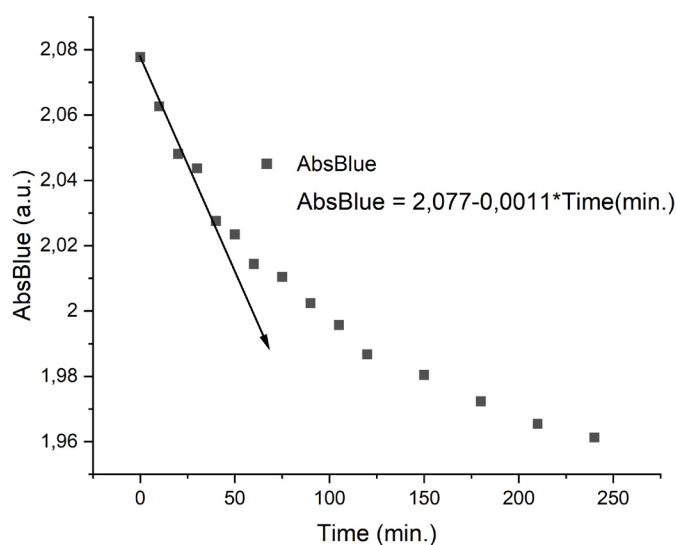

Figure 3 *Dependence of AbsBlue absorbance (blue LED) measured on Colorturb on illumination time*

tion processes as well as the formation of MBT and LSF, both in model samples and in real beer. The rate of decrease in AbsBlue absorbance (Figure 5) under defined illumination is proportional to the rate of sample degradation and LSF aftertaste formation.

The Light-Struck Flavour Susceptibility Index

Based on the presented results, we introduced the Light-Struck Flavour Susceptibility (LSFS) index as a tool for evaluation of beer susceptibility to degradation by light and the LSF formation. The LSFS index is defined as the rate of LSF aftertaste formation with the corresponding rate of AbsBlue decrease under defined illumination. For the sample in Figure 3, the LSFS index is given by the linear coefficient of the line interpolated by AbsBlue values measured between 0 and 50 minutes of illumination (LSFS = -0,0011 / min). If we illuminate samples in the same illuminator, the LSFS index will allow us to compare the resistance of different beers and packaging to light degradation. The validity of the LSFS index will be further verified on different beers and packaging.

4 Conclusion

The light degradation of beer is associated with the decomposition of riboflavin and with a change in the optical properties of the sample such as decrease of absorbance at 450 nm. It has been proven that the main factors that affect light-induced processes in beer are the colour of the bottle, i.e. the attenuation degree of the light passing through the bottle wall, riboflavin concentration and isohumulone content. Furthermore, it is also expected that the overall beer composition, especially the content of substances capable of interacting with excited RF and preventing the transfer of excitation energy to isohumulones, may influence the degree of beer damage.
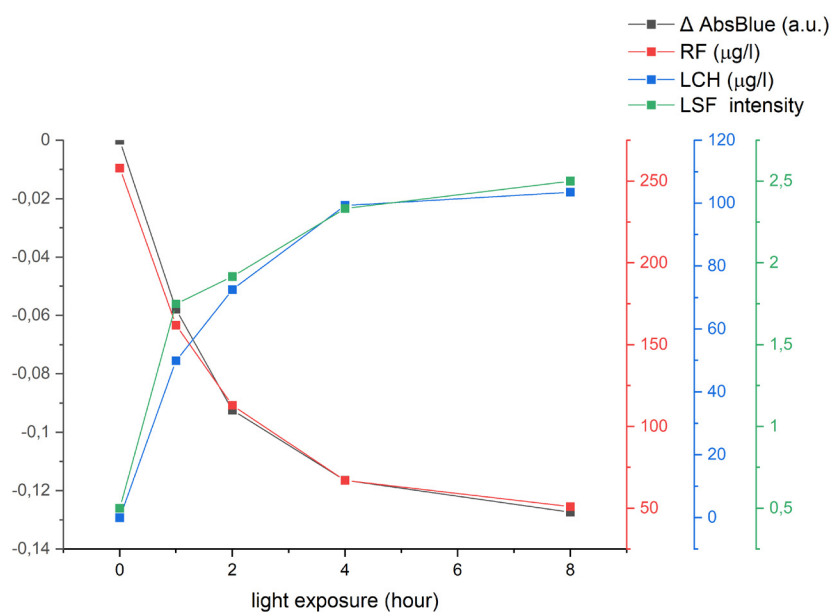

Figure 4 *Effect of the light exposure time on commercial beer parameters. ΔAbsBlue - the difference in the absorbance; RF – riboflavin; LCH – lumichrome; LSF – light struck flavour intensity*
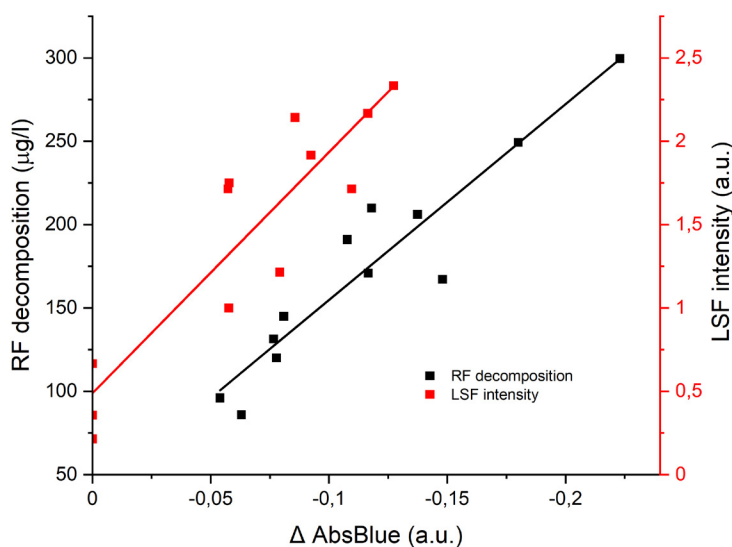

Figure 5 *Correlation of riboflavin degradation and LSF intensity increase with decrease in AbsBlue*

The Colorturb apparatus enabled the detection of even small changes in absorbance at 450 nm directly in commercial bottles. The detected changes in absorbance caused by light degradation correlated with the formation of LSF off-flavour and the decrease in riboflavin concentration. Although the changes in absorbance were small, the sensitivity of optical detection was comparable to the sensitivity of sensory analysis.

Optical detection of absorbance changes in conjunction with an illuminator adapter can be used to monitor the susceptibility of beer to light degradation and the formation of LSF off-flavour directly in a commercial bottle. Another possible use is an investigation of the effect which storage and transport conditions might have on light degradation of final products.

5   Acknowledgement

6   References

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