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A new kind of antioxidant test

A new kind of antioxidant test has been developed. The test is based on the indigo carmine decomposition in the presence of an organic matter, radical initiator and copper ions in the reaction mixture. The decomposition can be inhibited by other added compounds followed by different absorbance decrease which is considered as a stage of antioxidant activity. Antioxidant capacity of phenolic compounds, aminoacids, ethanol, ascorbic acid and EDTA was measured during copper-cata-lysed-maltose decomposition in the presence of peroxodisulfate as a radical initiator.

BC 36 Beer

(Descriptors: Antioxidants, prooxidants, radical compounds, indigo carmine, peroxodisulfate.

Deskriptoren: Antioxidantien, Prooxidantien, Radikale, Indigocarmin, Peroxodisulfat).

1 Introduction

The ageing of beer is supposed to be associated with radical degradation of certain beer constituents. Free radicals are also responsible for producing aldehydes as well as for other degradation products, which can be determined by various physical or chemical methods.

The only method to be able to detect radicals directly is ESR spin trapping which is usually used for predicting beer flavour stability. Beer contains antioxidants protecting important beer compounds by terminating these free radical reactions. During beer ageing antioxidants become destroyed and radicals increase which can be recognised through ESR signals. This is the so called “lag period” concept which is widely used for beer ageing prediction (1).

Antioxidant efficiency has often been estimated by reduction power measurement (2). Another conception of antioxidant and pro-oxidant testing is based on radica1 initiator addition which is followed by degradation products determination (3). The thermally initiated decomposition of the $K_2S_2O_8$ radical initiator represents a very powerful source of reactive free radicals which can be effectively used for initiating various processes in wine and beer. Antioxidant or pro-oxidant testings are also possible (4).

So far there has been only little knowledge about natural free radical sources in beer. Fenton’s reaction is usually supposed to be the main source of radicals provided that transition metal ions and hydrogen peroxide are present. Thermal carbohydrate degrada-tion has also been supposed to be a starter of free radica1 generation and amplification (3).

Furalaldehyde, reductones and hydroxymethylfurfural result from thermal sugar degradation or radical sugar decomposition (3). Aminoacids can also undergo the radical degradation giving a rich cocktail of many various compounds (5, 6).

Organic dye destruction might also be useful to monitor free radical generation in the presence or absence of radical initiators (7). Strong reductones can promote dyes over-oxidation or over-reduction followed by their destruction which is catalysed by transition metals with redox capacity.

These reactions might be used for antioxidant or pro-oxidant activity determination. The organic matter is destroyed by the radical initiator in the presence of transition metal which is indicated by indigo carmine decomposition. The stage of decomposition is changed in the presence of various compounds which inhibit this reaction (Indigo Carmine Decomposition Test – ICDT).

2 Experimental procedures

2.1 Chemicals

All chemicals were purchased from Sigma Aldrich except indigo carmine, pyrogallol and pyrocatechol (Merck). The deionized water conductivity was less than 0.2 µS.

Antioxidant test ICDT

- ☐ Pipette appropriate samples volume (usually 1 ml) of tested compounds + 1 ml phosphate buffer (c = 1/15 mol.1^{-1}) into 13 x 160 mm test tubes. Refill up to 9.7 ml with deionized water.
- ☐ Add 100 µl of indigo carmine (INDC) (c = 1000 mg.1^{-1}) + 100 µl cupric chloride dihydrate (c = 0.0268 g.1^{-1}) and mix.
- ☐ Add 100 µl of potassium peroxodisulfate (c = 10 g.1^{-1}) and mix thoroughly at starting time. Final solution contains sample, INDC (c = 10 mg.1^{-1}), Cu^{2+} (c = 0.1 mg.1^{-1}) and $K_2S_2O_8$ (c = 0.1 g.1^{-1}) allow it to react at room temperature.
- ☐ Measure absorbance at 610 nm after 10 min or determine bleaching time visually.

3 Results and discussion

Various kinds of potential antioxidants and pro-oxidants were tested at pH = 5 which is close to that of typica1 brewing substrates (Table 1).

All organic compounds tested were able to support radical over-oxidation of indigo carmine in the presence of radical initiator. The strongest effect was observed with maltose which could be partially inhibited by other compounds addition (Table 1).

There were distinctive differences between pyrogallol (1,2,3-trihydroxybenzene) and pyrocatechol (1,2-dihydroxybenzene)

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Table 1 ICDT value for various compounds tested (c = compounds concentration in the final reaction mixture)

Compound	c (g.l^{-1})	A_{610}	Mixture	c (g.l^{-1})	A_{610}
deionized w.	–	0.275	maltose	10	0.000
maltose	10	0.000	+	–	–
ethanol	40	0.005	ethanol	40	0.005
ascorbic acid	0.1	0.231	ascorbic acid	0.1	0.028
pyrogallol	0.1	0.260	pyrogallol	0.1	0.034
pyrocatechol	0.1	0.291	pyrocatechol	0.1	0.232
phenylalanine	0.1	0.272	phenylalanine	0.1	0.189
methionine	0.1	0.000	methionine	0.1	0.000
EDTA	0.001	0.272	EDTA	0.001	0.270

as well as between methionine and phenylalanine in inhibition activity during the indigo carmine decomposition induced by maltose.

Methionine was also partially efficient as an inhibitor of a radical reaction giving a little longer bleaching time in comparison to maltose itself despite of zero absorbance after 10 min-reaction. The better distinguishing of the inhibition effect can also be reached by peroxodisulfate concentration lowering up to 10 mg.l^{-1} in the reaction mixture.

The most efficient compound inhibiting INDC decomposition was EDTA which confirmed the reaction induced by transition metals.

ICDT reaction strongly depended on pH as well as on transition metal presence (fig. 1). The catalytic effect of Cu^{2}+ Cu^{+}, Fe^{2+} or even Cu has also been confirmed. We suppose organic matter oxidation to be a source of other radicals taking part in this reaction. Reducing compounds could ensure oxygen transfer to reaction starting oxygen radicals generation in the presence of transition metals.

Although indigo carmine represents a sensitive goal for radical attack other organic molecules – being even more efficient in radicals trapping – could occur.

We have also been able to realise indigo carmine decomposition in beer, brewing intermediates or maltose in the presence of transition metals and air oxygen without radical initiator at slightly higher temperature 45 ºC within two days. Methylene blue or methylene red could also be reduced by wort or beer in the absence of air witnessing reductone formation even at low pH of the beer. Such a complicated system can accept oxygen in the presence reducing substances generating radicals and presenting oscillating reactions (8).

4 Conclusion

- An organic matter oxidation by oxygen in the presence of the redox-active transition metals catalysing this reaction can be the source of oxygen free radicals.
- Organic dyes can serve as indicators of radical oxidation as well as of radical reduction.
- Different colour forms of dyes can be reversibly transferred into each other which can be followed by radical over-oxidation or over-reduction.
- Model system of maltose, copper ions, indigo carmine and a radical initiator e.g. peroxodisulfate is suitable for antioxidant and pro-oxidant testing.

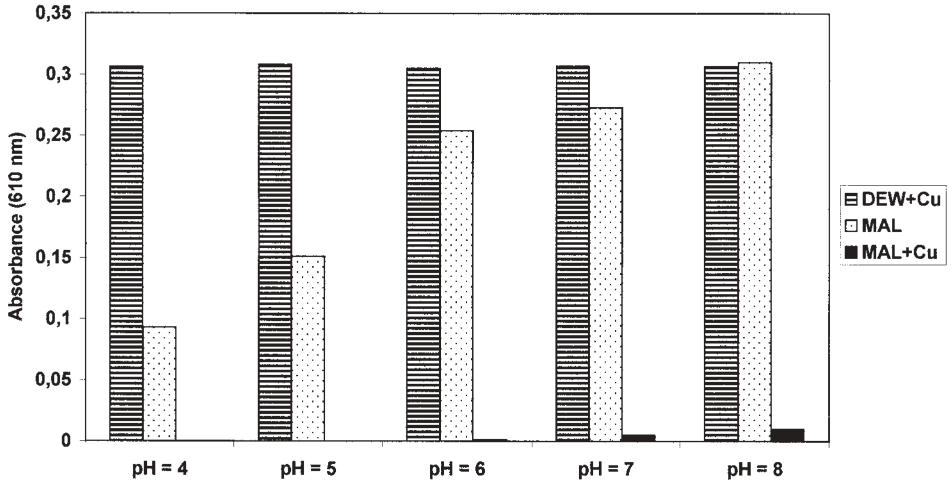

Fig. 1 The absorbance after 10 min reaction of INDC (c = 10 mg.l^{-1}) and $K_2S_2O_8$ (c = 0.1 g.l^{-1}) in deionized water with Cu^{++} (c = 0.1 mg.l.$^{-1}$) (DEW+Cu), maltose (c = 10 g.l^{-1}) with (MAL+Cu) and without Cu^{++} (MAL)

❑ Reductones can play an important role in oxidation reactions being able to accept oxygen in a reaction mixture and to undergo metal catalysed over-oxidation or over-reduction of the dyes.

5 Summary

Savel, J.: Eine neue Art von Tests für Antioxidationsmittel — Monatsschrift für Brauwissenschaft 54, Nr. 9/10, 206 – 208, 2001

BC 36 Bier

Eine neue Art Test für Antioxidationsmittel wurde entwickelt. Der Test beruht auf dem Abbau von Indigokarmin in Gegenwart eines organischen Materials, eines Initiators für Radikale und von Kupferionen im Reaktionsgemisch. Der Abbau kann gehemmt werden durch Hinzufügen weiterer Verbindungen, gefolgt von einer unterschiedlichen Abnahme des Absorptionsvermögens, welche als Stadium der Antioxidationswirkung gilt. Die Leistungsfähigkeit von Phenolverbindungen, Aminosäuren, Ethanol, Ascorbinsäure und EDTA als Antioxidationsmittel wurde während des mit Kupfer katalysierten Maltoseabbaus in Gegenwart von Peroxodisulfat als Initiator für Radikale gemessen.

Savel, J.: Un nouveau mode de tests pour les produits antioxydants — Monatsschrift für Brauwissenschaft 54, No 9/10, 206 – 208, 2001

BC 36 Bière

Un nouveau mode de tests pour des produits antioxydants a été développé. Le test repose sur la dégradation d'indigocarmine en présence d'un composé organique, d'un indicateur pour les radicaux et d'ions de cuivre pour le mélange réactionnel. La dégradation peut être bloquée par l'addition d'autre composés qui est suivie d'une diminution variable du pouvoir d'absorption qui représente l'état de réaction antioxydante. Le rendement des composés antioxydants : composés phénoliques, acides aminés, éthanol, acide ascorbique et EDTA est mesuré par rapport à la dégradation catalytique du maltose par le cuivre en présence de peroxodisulfate comme indicateur pour les radicaux.

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(Manuskripteingang: 22. 2. 2001)

New International Calibration Standards for HPLC Analysis of Isomerized α-Acids

Since 1998, the ASBC, EBC, IoB (now IGB), and BCOJ have been working together toward the creation and international adoption of a single set of HPLC standards for use in the quantitative determination of isomerized and reduced-isomerized α-acids in hop products and in beer. These international calibration standards (ICS) have now been produced, analyzed, and verified for the benefit of the brewing industry by the International Subcommittee for Isomerized Hop α-Acids Standards.

The purity of each standard was determined using various HPLC procedures, elemental analysis, and other methods. The standards were later reanalyzed, using an isocratic version of EBC Method 7.8, following which the total concentrations of the major isomerized α-acids were declared to be 1) DCHA-Iso, ICS-I1 (iso-α-acids standard): 64.5%; 2) DCHA-Rho, ICS-R1 (rho-iso-α-acids standard): 65.5%; 3) Tetra, ICS-T1 (tetrahydroiso-α-acids standard): 97.9%; 4) DCHA-Hexa, ICS-H1 (hexahydroiso-α-acids standard): 65.7%. Samples of these standards showed excellent stability after eight months at room temperature, although freezer storage is strongly recommended. Their purity will be monitored to ensure continuing suitability for use.

Detailed information pertaining to each standard, plus full instructions for use, is available on request and supplied with each purchase. These standards are now available, each at $60 per vial (including packaging and shipping to all destinations).
Contact ASBC headquarters: asbc@scisoc.org or phone +1 (651) 454-7250. (Note: the same standards are also available, at the equivalent price, from Labor Veritas, Zürich, Switzerland; contact admin@laborveritas.ch; Web site: www.laborveritas.ch).