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Glucose – a Reducing Sugar? Reducing Properties of Sugars in Beverages and Food

The properties of reducing sugars are interesting for the shelf life of beverages, particularly beer, and for human nutrition. For the brewing process the different reducing potentials and the mode of action of fermentable sugars are vitally important, especially during wort boiling where the reactions of sugars are accelerated. Additionally, several breweries use non-fermentable sugars in the brewing process to imbue the beer with unique flavour, body and mouthfeel.
An optimised method to ascertain the reduction potential of sugars against Fe^{3+} at low pH was developed in this work. Sugars behave differently at low pH compared to the generally known behaviour described by Fehling when using NaOH. At low pH conditions, the formation of the open chain aldehyde structure of glucose is inhibited. Fructose has a higher ability to generate the open structure, resulting in stronger reducing properties. The results show at pH 4.3 the strongest reduction potential results from isomaltulose (Palatinose™), followed by fructose, Vitalose® and maltotriose. The higher reduction potential of the "non-reducing" sucrose compared to glucose can be explained by the invert sugar's acid hydrolysation. Additional investigations give further evidence about the behaviour of fermentable sugars during the brewing process. Thereby is beside the described mode of action of glucose, fructose and sucrose, the detected stronger reduction potential of maltotriose versus maltose remarkable.
The optimised Chapon method can be used to support the investigation of the complex reaction mechanism of the different sugars in beverages like juice, wine and beer as well as during the brewing process and during storage.

Descriptors: reduction potential, reducing sugars, Chapon, Fehling, beverages, beer

1 Introduction

The properties and the mechanism of functional carbohydrate reactions in food and beverages at a low pH environment such as jam, juice, wine, beer, honey, etc. are becoming increasingly interesting. Especially the properties of reducing sugars and their individual reduction potentials are important to determine the shelf life of beverages and also their role in human nutrition. For the brewing process the different reducing potentials and the mode of action of fermentable sugars are vitally important, especially during wort boiling where the reactions of sugars are accelerated. Furthermore several breweries use non-fermentable sugars to increase the beer palate fullness (e.g. Palatinose™, Vitalose®). Besides the direct addition of sugars to the final beer, it is a standard procedure to add this kind of sugars at the end of the wort boiling process prior to fermentation. Previous investigation [6] showed that the addition of non-fermentable sugars leads to a higher extract after fermentation and in correlation to the osmotic pressure to a higher SO_2-content, which improves the beer palate fullness and oxidative beer stability. Based on these results [6] it was interesting to investigate the reduction properties of these non-fermentable sugars in comparison to fermentable sugars.

The reducing sugars are generally described as any sugar that, in basic solution, has an aldehyde or a ketone group which allows the sugar to act as a reducing agent. In the last decades, the reduction potential of sugars has been determined using different scientifically accepted methods (e.g. [5, 7, 8, 11]), which are based on the reduction potential of sugars against transition metal ions with specific oxidation states in basic solutions. One of these traditional methods is the Fehling method [5], which uses copper ions (Cu^{2+}) in a basic potassium sodium tartrate solution for the determination of the reduction potential. The tartrate ions in the solution are used as complexing agents to keep the copper ions in solution and to avoid the precipitation of cupric hydroxide. The test based on the reactivity of aldehydes in the reduction of the copper ion Cu^{2+} to Cu^{+} whereby the aldehydes are oxidised to carboxylic acids. The tartrate ion is unable to complex the cuprous ion Cu^{+} and the formation of yellow Cu (I) hydroxide can be observed. The following dehydration causes a red to brown precipitation of Cu_2O in the solution. The test is commonly used for reducing sugars but not specific for aldehydes like the aldose glucose. Also a α-hydroxyketon like fructose gives a positive result with the Fehling test, because fructose can be transformed to glucose or mannose and other products with reducing capacity under alkaline conditions [4].

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The method according to *Chapon et al.* [2] to ascertain the reduction potential of beverages differs from the Fehling method. The method describes the reduction potential of beverages against Fe^{3+} by the formation of a red complex between Fe^{2+} and 2,2'-bipyridyl at low pH values. The described method is known to be inapplicable for the determination of the reduction potential of sugars [7].

Our research work has confirmed that the proposed analytical parameters for the method according to Chapon are unqualified for the detection of the reducing properties of sugars against Fe^{3+}. Nevertheless by varying the analytical parameters like temperature, concentration and time, our investigations showed that the functional principle of the basic reaction mechanism, the reduction of Fe^{3+} is able to differentiate the reduction potential of sugars within low pH range. Based on these results, it was possible to develop an optimised method for the determination of the different reduction potential of sugars against metallic ions in the pH range between 2.8 and 4.5 in food and beverages (e.g. jam, juice, wine, beer, honey, etc.). The method can also be used to gain information on the behaviour of sugars during storage. The detected differences between the reduction properties of sugars, in different pH ranges, are surprising. For instance, the generally known reducing sugar, glucose [4, 5, 12], is losing its reducing properties at low pH and at the same time an increase of the reduction potential of sucrose, a well known non-reducing sugar could be observed [1, 4, 5]. Regarding low and high pH, the changes in the reducing properties of different sugars, such as fructose and isomaltulose (Palatinose™) can be explained easily. On the other hand specific sugars like maltotriose, with more similar glucose parts within the molecular chain, appear to behave more complicated.

2 Materials and methods

Acetic acid ACS reagent ≥ 99.7 %, CH_3CO_2H, M = 60.05 g/mol
Sigma-Aldrich Chemie GmbH, 82024 Taufkirchen, Germany, www.sigmaaldrich.com; CAS 64-19-7

Ammonium iron (III) sulphate dodecahydrate p.a. ≥ 99 %, $(NH_4)Fe(SO_4)_2 \times 12\ H_2O$, M = 482.19 g/mol
Merck KGaA, 64271 Darmstadt, Germany, www.merck.de; CAS 7664-93-9

Copper (II) sulfate pentahydrate p.a. ≥ 99 %, $CuSO_4 \times 5\ H_2O$, M = 249.68 g/mol
Merck KGaA, 64271 Darmstadt, Germany, www.merck.de; CAS 7758-99-8

D-(+)-maltose monohydrate Type II ≥ 95 %, $C_{12}H_{22}O_{11} \times H_2O$, M = 360.31 g/mol
Sigma-Aldrich Chemie GmbH, 82024 Taufkirchen, Germany, www.sigmaaldrich.com; CAS 6363-53-7

Fructose ≥ 99 %, $C_6H_{12}O_6$, M = 180.16 g/mol
Südzucker AG, 68165 Mannheim, Germany, www.suedzucker.de; CAS 57-48-7

Glucose monohydrate ≥ 99 %, $C_6H_{12}O_6 \times H_2O$, M = 198.17 g/mol
AGRANA Fruit Germany GmbH, 78467 Konstanz, Germany, www.agrana.de; CAS 14431-43-7

Maltohexaose ≥ 95 %, $C_{36}H_{62}O_{31}$, M = 990.86 g/mol
ABCR GmbH & Co. KG, 76187 Karlsruhe, Germany, www.abcr.de; CAS 34620-77-4

Maltopentaose ≥ 95 %, $C_{30}H_{52}O_{26}$, M = 828.72 g/mol
Sigma-Aldrich Chemie GmbH, 82024 Taufkirchen, Germany, www.sigmaaldrich.com; CAS 34620-76-3

Maltotetraose (HPLC) ≥ 96.1 %, 6.3 % water, $C_{24}H_{42}O_{21}$, M = 666.59 g/mol
Sigma-Aldrich Chemie GmbH, 82024 Taufkirchen, Germany, www.sigmaaldrich.com; CAS 34612-38-9

Maltotriose (HPAE/PAD) ≥ 95 %, $C_{18}H_{32}O_{16}$, M = 504.44 g/mol
Sigma-Aldrich Chemie GmbH, 82024 Taufkirchen, Germany, www.sigmaaldrich.com; CAS 1109-28-0

Maltotriose pure ≥ 98 %, $C_{18}H_{32}O_{16}$, M = 504.44 g/mol
SERVA Electrophoresis GmbH, 69115 Heidelberg, Germany, www.serva.de; CAS 1109-28-0

Maltotriose BioChemica ≥ 97 %, $C_{18}H_{32}O_{16}$, M = 504.44 g/mol
AppliChem GmbH, 64291 Darmstadt, Germany, www.applichem.com; CAS 1109-28-0

Palatinose™ (isomaltulose) ≥ 98 %, $C_{12}H_{22}O_{11}$, M = 342.30 g/mol
Südzucker AG, 68165 Mannheim, Germany, www.suedzucker.de; CAS 13718-94-0

Phosphoric acid puriss. p.a. ≥ 85 %, H_3PO_4, M = 82.03 g/mol
Sigma-Aldrich Chemie GmbH, 82024 Taufkirchen, Germany, www.sigmaaldrich.com; CAS 7664-38-2

Polydextrose ≥ 99 %, $(C_6H_{10}O_5)_n$
Südzucker AG, 68165 Mannheim, Germany, www.suedzucker.de

Potassium phosphate monobasic ACS reagent ≥ 99 %, KH_2PO_4, M = 136.09 g/mol
Sigma-Aldrich Chemie GmbH, 82024 Taufkirchen, Germany, www.sigmaaldrich.com; CAS 7778-77-0

Potassium sodium tartrate tetrahydrate p.a. ≥ 99 %, $C_4H_4KNaO_6 \times 4\ H_2O$, M = 249.68 g/mol
Merck KGaA, 64271 Darmstadt, Germany, www.merck.de; CAS 6381-59-5

Sodium acetate anhydrous p.a. ≥ 99 %, CH_3COONa, M = 82.03 g/mol
Merck KGaA, 64271 Darmstadt, Germany, www.merck.de; CAS 127-09-3

Sucrose ≥ 99.7 %, $C_{12}H_{22}O_{11}$, M = 342.30 g/mol
Südzucker AG, 68165 Mannheim, Germany, www.suedzucker.de; CAS 57-50-1

Sulfuric Acid p.a. 95-97 %, H_2SO_4, M = 98.08 g/mol
Sigma-Aldrich Chemie GmbH, 82024 Taufkirchen, Germany, www.sigmaaldrich.com; CAS 7664-93-9

Vitalose® ≥ 98 %, contains mainly trehalulose, artificial sweeteners
Südzucker AG, 68165 Mannheim, Germany, www.suedzucker.de; Reg.-No.: 3867637

2,2′-Bipyridyl puriss. p.a. ≥ 99 %, $C_{10}H_8N_2$, M = 156.18 g/mol
Sigma-Aldrich Chemie GmbH, 82024 Taufkirchen, Germany, www.sigmaaldrich.com; CAS 366-18-7

Chapon method

The Chapon method (Chapon et al. [2]) describes the reduction potential of beverages and reducing substances in solutions against Fe^{3+}. The method's principle relies on the reduction of Fe^{3+} to Fe^{2+} which reacts with 2,2'-bipyridyl to form a red coloured complex with its absorbance at 510 nm. The progress of that reaction is shown in figure 1.

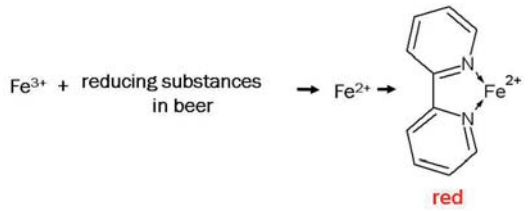

Fig. 1 Formation of 2,2'-bipyridyl iron complex

Preparation of solutions and measurement conditions

Solution A was prepared by dissolving 150 mg $NH_4Fe(III)(SO_4)_2 \times 12\ H_2O$ in approx. 25 mL bi-distilled water and 0.2 mL concentrated H_2SO_4. After the salt was completely dissolved, the volume was made up to 50 mL by adding bi-distilled water.

Solution B was prepared by dissolving 50 mg 2,2'-bipyridyl in approx. 45 mL bi-distilled water and 4 mL 0.1 N H_2SO_4. After the salt was completely dissolved, the volume was made up to 50 mL by adding bi-distilled water.

Both solutions are mixed shortly before starting the measurement.

The sugars were dissolved in phosphate buffer (1 M, pH 4.5) and the measurement was carried out for 300 s at 20 °C.

Optimised method to ascertain reducing sugars at low pH:

The sugars were dissolved in acetate buffer (1 M, pH 4.5) and the measurement was carried out for 1 h at 60 °C.

Fehling method

The Fehling test is used to differentiate between water soluble aldehyde and ketone functional groups. It can also be used as a test for reducing sugars. It is prepared initially in two separate solutions, known as Fehling's A and Fehling's B. Fehling's A is a blue aqueous solution of copper (II) sulphate pentahydrate crystals while Fehling's B is a clear solution of aqueous potassium sodium tartrate tetrahydrate. Equal volumes of the two solutions are mixed to get the final Fehling's solution, which has a deep blue colour.

Preparation of solutions

Fehling A: 7 g copper (II) sulphate pentahydrate dissolved in 100 mL bi-distilled water.

Fehling B: 35 g potassium sodium tartrate tetrahydrate and 10 g sodium hydroxide dissolved in 100 mL bi-distilled water.

In each case 5 g of sugars were dissolved in 100 mL bi-distilled water and 4 mL Fehling reagent were added. This solution was boiled for 10 minutes.

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3 Results and discussion

In the first pre-trial, the method according to Chapon et al. [2], was applied for the determination of the reduction potential of glucose, fructose, sucrose, Vitalose®, polydextrose and Palatinose™ (isomaltulose) during a reaction time of 300 s (20 °C, pH 4.5).

The results in figure 2 confirm the results of *Moll* [7] that the analytical parameters of the used method are unqualified for the detection of the reducing properties of sugars against Fe^{3+}. Even increasing the reaction time to 30 min yielded no detectable differences between the sugars in respect to the reducing potential.

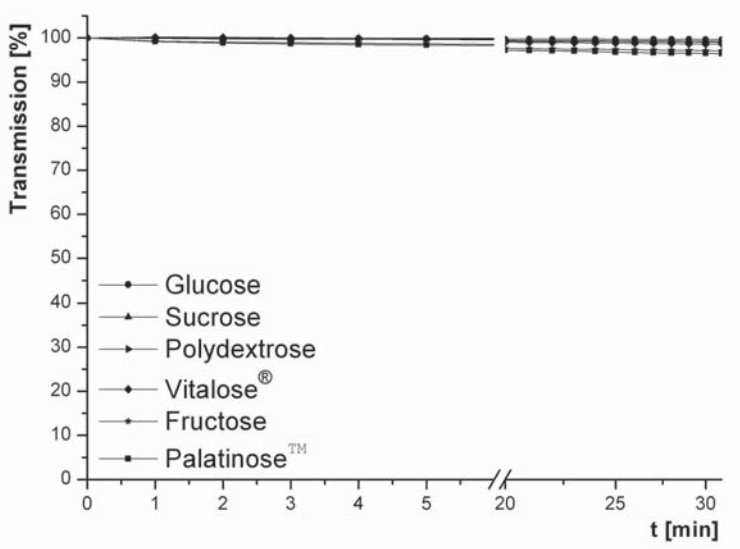

Fig. 2 Investigation concerning the reduction potential according to Chapon [2] of Mono- & Di-Sugars 0.1 M in phosphate buffer (1 M, pH 4.5) at 20 °C/5 min

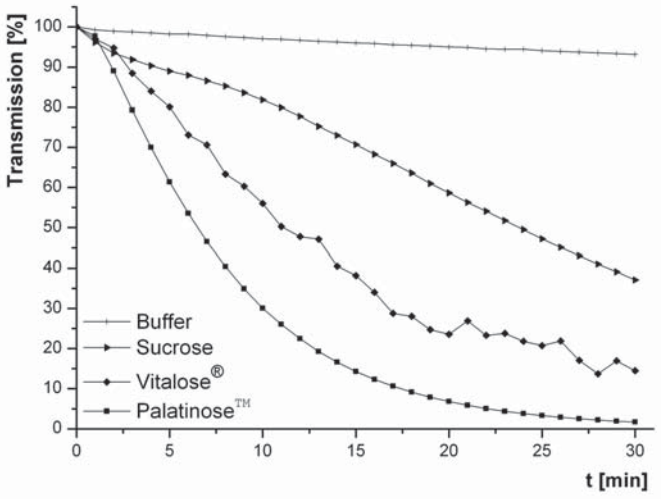

Fig. 3 Determination of the reduction potential acco ding to Chapon [2] of Mono- & Di-Sugars 0.1 M in phosphate buffer (1 M, pH 4.5) at 80 °C /30 min

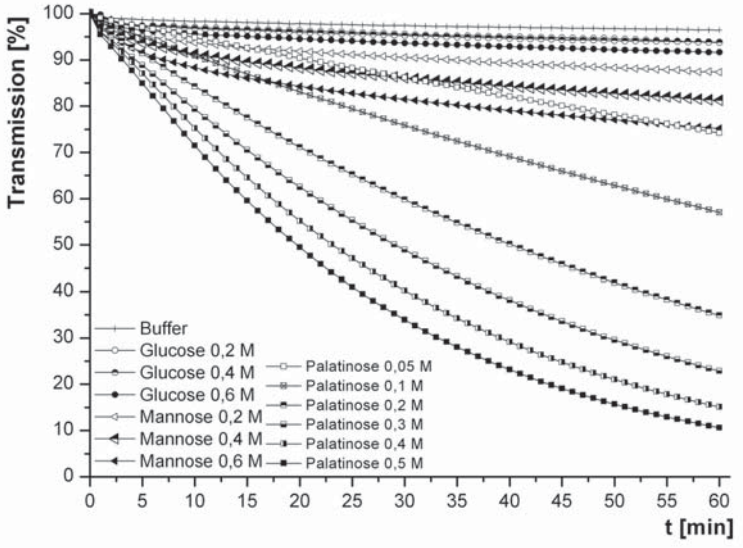

Fig. 4 Investigation with varied concentrations of different sugars to find out a reasonable sugar concentration for the optimised Chapon method (60 °C/60 min)

In a second set of investigations, the influence of temperature on the reaction rate was studied.

A reaction temperature of 80 °C led to an acceleration of the reaction processes including the reduction of Fe^{3+} by the sugar solutions. The results in figure 3 clearly demonstrate that under the chosen conditions the isomaltulose (Palatinose™) shows the strongest reduction potential against Fe^{3+}, followed by Vitalose® and sucrose.

As a result of these pre-trials, the Chapon method was optimised by setting the temperature to 60 °C and the reaction time to 1 h. For a clear differentiation of the sugar characteristics it was also necessary to use an acetate buffer at pH 4.5 instead of phosphate buffer to avoid possible haze formation under the given conditions.

The following pre-trial was carried out to find a reasonable sugar concentration for the analytical method and to figure out a range of linear correlation between reduction potential and sugar concentration. For the investigation different sugar concentrations up to 0.6 M were used for glucose, mannose and the sugar with the strongest reduction potential in the previous trials, isomaltulose (Palatinose™).

Considering the influence of the used buffer solution on the transmission during the measurement in the evaluation, the results in figure 4 and 5 demonstrate a linear correlation between the increasing sugar concentrations and the slope of the measured reduction potential up to an approximately 40 % lower transmission after the accelerated aging (1 h, 60 °C). In this range the higher reduction potential of mannose against glucose is noticeable whereby both sugars possess a clear linear correlation to the measured reduction potential up to the highest sugar concentration of 0.6 M. Contradictory the strong reduction potential of isomaltulose (Palatinose™) leads to a non linear correlation with a sugar concentration > 0.1 M and a significant strong distortion with a concentration of > 0.2 M.

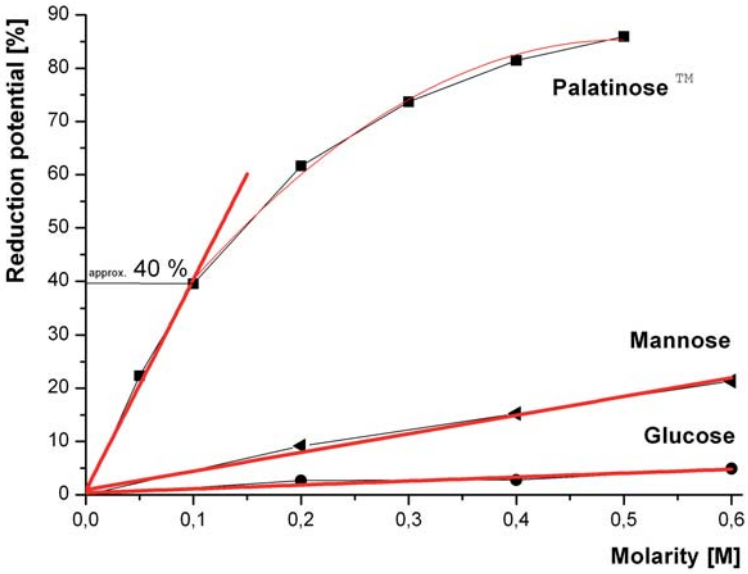

Fig. 5 Correlation between reduction potential and sugar concentration

For these kinds of sugars the maximal acceptable and reasonable sugar concentration is given by 0.2 M under the described analytical conditions. The strong distortion of the reduction potential with a higher sugar concentration makes the comparison of the different sugar characteristics very complicated; consequently we used for further investigations a specified sugar concentration of ≤ 0.2 M to generate the maximum differences in the reduction potential in an approximately linear range.

The first general application of the optimised Chapon method was to ascertain the reduction potential of six different sugars and to verify the influence of the buffer solution on the detected transmission over the time.

The results in figure 6 demonstrate the strongest reduction potential of the sugar solutions in the order of isomaltulose (Palatinose™)

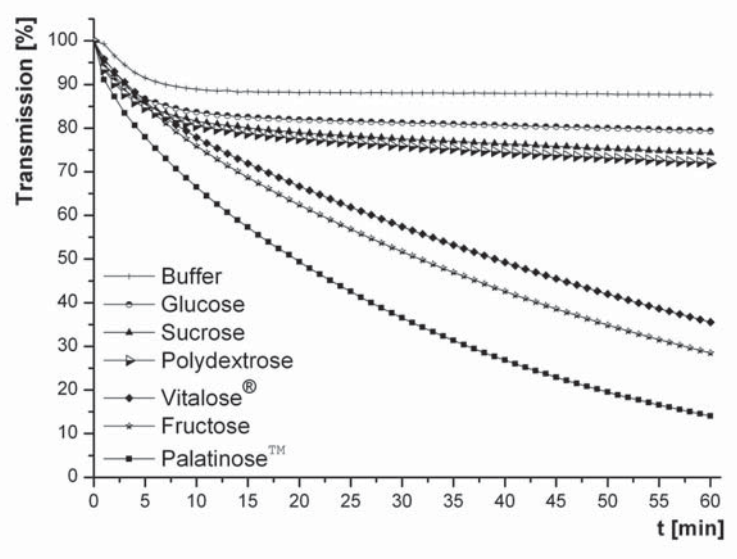

Fig. 6 Optimised method according to Chapon [2] at 60 °C /60 min

Mono- & Di-Sugars 0.2 M in acetate buffer (1 M, pH 4.5)

(Polydextrose = 2 x mass Sucrose)

> fructose > Vitalose® before the other sugars follow with a clear distance. It is notable that also polydextrose and sucrose have a higher reduction potential against Fe^{3+} than glucose. The significant loss of the reducing properties of the generally known "reducing sugar" glucose at low pH can be explained by a change in the equilibrium between the cyclic hemiacetal form, without a free aldehyde group, and the open chain aldehyde structure. At low pH, the formation of the open chain aldehyde structure of glucose is inhibited.

Contradictory to this, at low pH, fructose has a higher ability to generate the open chain structure resulting in much stronger reducing properties. The increasing reduction potential of the "non-reducing sugar" sucrose at low pH can be explained by the invert sugar's acid hydrolysed formation and the appearance of the strong reduction potential of fructose. In summary, it is obvious that the reduction potential of the generally known "non-reducing sugar" sucrose in food and beverages with low pH is higher than that of glucose, which is generally known to be a "reducing-sugar".

To guarantee that the reaction conditions at 60 °C contribute to an acceleration of the reaction and that the high temperature does not initiate the reaction itself, additional experiments at longer reaction times and lower temperatures (40 °C, 37 °C, 30 °C and 20 °C) were carried out.

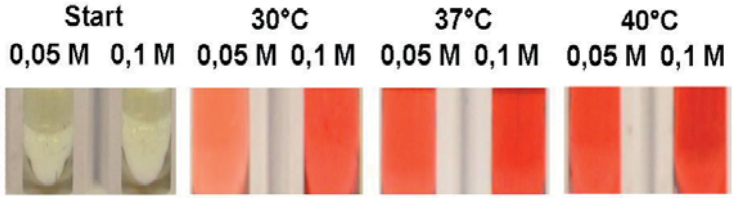

Fig.7 Storage experiment with Palatinose™ for 2 days at different temperatures

The results in figure 7 show the reduction of Fe^{3+} after a storage time of 2 days at 30, 37, 40 °C by isomaltulose (Palatinose™) in two different concentrations (0.05, 0.1 M).

The temperature dependent increase in red colour of all solutions, caused by the formation of Fe^{2+}-2,2'-bipyridyl complex during storage, clearly show the acceleration of the reducing processes. On the other hand the increase of red colour at 30 °C demonstrates that the normally used temperature of 60 °C in the optimised Chapon method is not an initiator for these kinds of reactions. In fact the reactions also taking place at low temperatures but with a lower reaction rate.

To simulate "normal" storage conditions of food and beverages, an additional storage experiment was carried out where the reduction behaviour of glucose, fructose and isomaltulose (Palatinose™) over 4 days at 20 °C was determined.

Fig. 8 Storage experiment with different sugars for 4 days at 20 °C

The results in figure 8 clearly show that fructose and isomaltulose (Palatinose™) have a significantly higher reduction potential at pH 4.3 as compared to glucose.

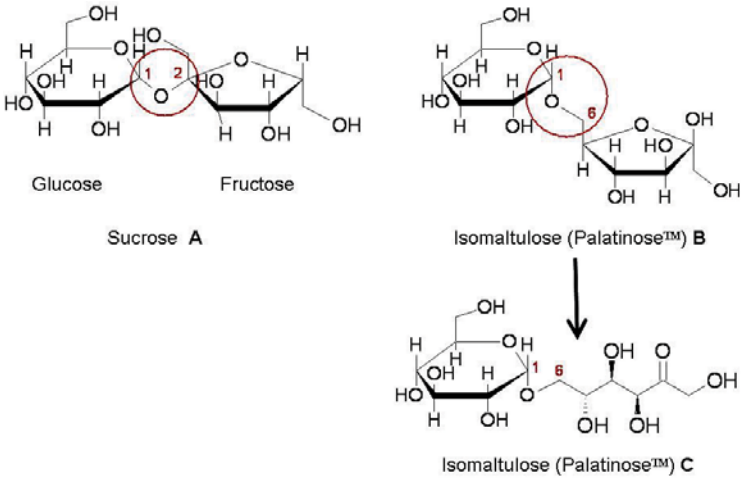

Fig. 9 Structure of sucrose and isomaltulose (Palatinose™)

The explanation for the high reduction potential of isomaltulose (Palatinose™) in comparison to sucrose is given by the different glycosidic linkage and the strong reducing properties of fructose. The 1,2 glycosidic linkage in sucrose (Fig. 9A) inhibits the formation of the open structure. On the other hand, the 1,6 glycosidic linkage in isomaltulose (Palatinose™, Fig. 9B) permits the opening of the ring structure as shown in Figure 9C and leads to a higher reduction potential against Fe^{3+}. The higher availability of the open

ring structure in the equilibrium of isomaltulose (Palatinose™) accelerates the reaction and leads to a higher reduction potential against Fe^{3+}.

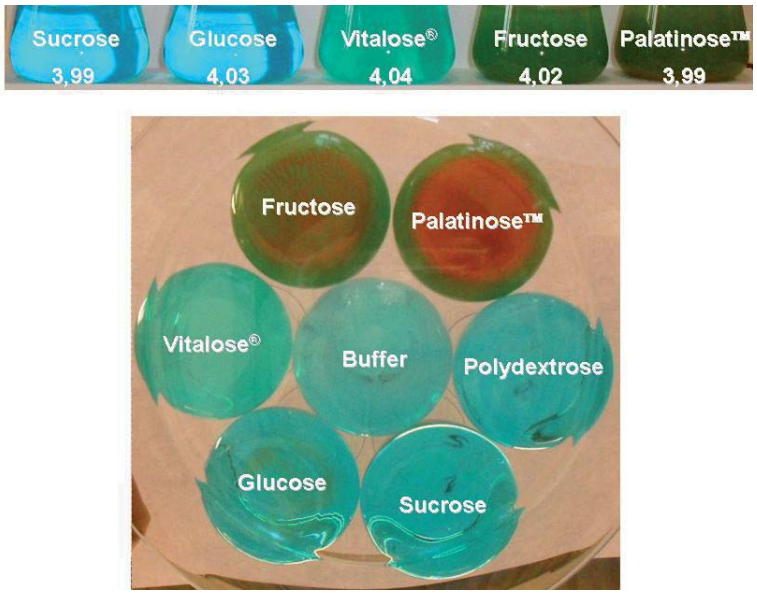

Fig. 10 Ascertainment of the reduction potential of different sugars according to Fehling at low pH (3.99–4.04)

Additional investigations using the described reaction mechanism according to *Fehling* [5] and *Evans et al.* [3] for the ascertainment of the reduction potential by reduction of Cu^{2+} and formation of CuOH, Cu_2O, showed similar results for the reduction potential of the measured sugars in the described pH-range (Fig. 10).

The strong reduction potential of isomaltulose (Palatinose™) results in a fast formation of yellow CuOH and red Cu_2O whereas the blue coloured Cu^{2+}-solution is changing into dark green and the Cu_2O is concentrated at the bottom of the flask. Vitalose® forms CuOH in the same time period and is responsible for a light green colouration. In contrast, the sugars glucose and sucrose cause no change in the colour of the blue Cu^{2+}-solution. These results confirm the investigations by Evans et al. [3] who found that fructose in comparison to glucose causes an accelerated formation of CuOH and Cu_2O at low pH and demonstrates the missing reduction potential of glucose in the low pH range of beverages and food. The comparable effects of the sugars by reducing Cu^{2+} and Fe^{3+} approve the functional principle of the optimised Chapon method.

A typical application of the old Chapon method (Chapon et al. [2]) is the determination of the reduction potential in wort and beer. Based on this, the application of the optimised Chapon method to analyse the reduction potential of fermentable sugars was obvious.

The results in figure 11 show that the lowest reduction potential of the fermentable sugars at pH 4.3 results from glucose and sucrose. The reduction potential increases slightly with maltose. A significantly higher potential can be observed from maltotriose.

The strong reduction properties of fructose in comparison to other sugars can be explained by the higher ability to generate the open chain structure at low pH, whereas the mechanism, as

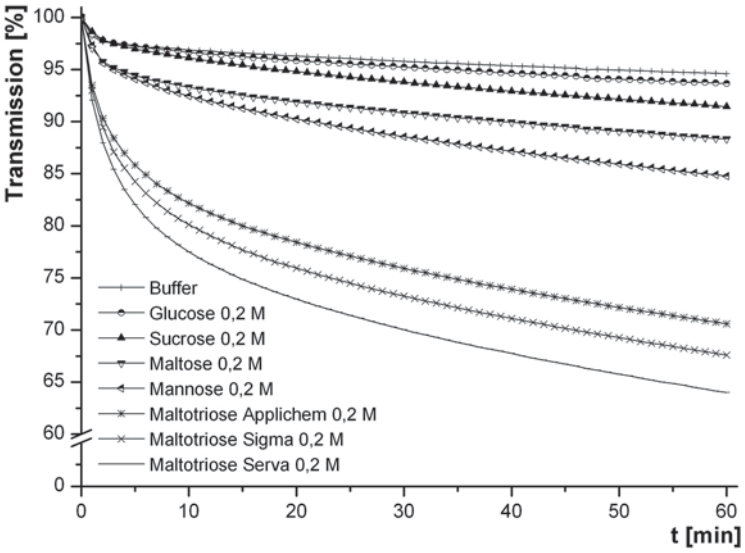

Fig. 11 Aditional investigations of fermentable sugars – especially maltotriose samples of different manufacturers (Sigma Aldrich, Serva, Applichem)

maltotriose possesses a higher reduction potential than maltose, is not fully obvious.

To ensure that the detected reduction potential of maltotriose does not result from the impurities from the used samples, additional maltotriose samples of different manufacturers were investigated (Sigma Aldrich, Serva, Applichem).

As shown in figure 11, the direct comparison of different maltotriose samples show minor differences in the reduction potential. However, all maltotriose samples possess a significantly higher reduction potential than maltose, which confirms the higher reduction potential of maltrotriose versus maltose.

4 Conclusion

Our research work proved that the originally proposed analytical parameters for the Chapon method, such as concentration, temperature and time, are unqualified to determine the reduction potential of sugars in the low pH range of food and beverages. However, when varying different parameters, like temperature and concentration, it can be observed that the functional principle and the basic reaction mechanism of the Fe^{3+} reduction can be used to obtain information of the reduction potentials of sugars within low pH areas at different temperatures and storage conditions.

Based on this mechanism and in analogy to commonly used forcing tests in measuring beverages, an optimised Chapon method using a reaction temperature of 60 °C for 1 h was used. The implementation of this method showed that sugars behave differently at low pH than under the described parameters in the Fehling test when using NaOH. At low pH 4.3, the strongest reduction potential was achieved with isomaltulose (Palatinose™), followed by fructose, Vitalose® and maltotriose. The lowest reduction potential could be detected by glucose, sucrose and polydextrose. The results could be proved by the reaction mechanism according to Fehling (Cu^{2+}). The comparable effects of the sugars by reducing Cu^{2+} and

Fe^{3+} approve the functional principle of the optimised Chapon method and confirm the useful application of the method for the determination of the reduction potential of sugars at low pH ranges.

The investigations of this work, also confirm the work of Evans et al. [3], who described that the reduction potential of glucose decrease in comparison to fructose. In this pH range, the formation of the open chain aldehyde structure of glucose is inhibited. As opposed to this, fructose has a higher ability to generate the open chain structure at low pH resulting in much stronger reducing properties.

The explanation for the strong reduction potential of isomaltulose (Palatinose™) in comparison to sucrose is given by the different glycosidic linkages. The 1,2 glycosidic-linkage in sucrose inhibits the formation of the open structure. On the other hand, the 1,6 glycosidic linkage in isomaltulose (Palatinose™) relieves the opening of the fructose ring structure. This leads to a higher reduction potential against Fe^{3+}.

Further detailed investigations also showed that polydextrose and sucrose have a minor but higher reduction potential against Fe^{3+} than glucose. The increasing reduction potential of the "non-reducing sugar" sucrose at low pH can be explained by acid hydrolysation thereby forming fructose which shows a strong reduction potential. With an increased pH, the behaviour of the sugars will change and the acid hydrolysed formation is inhibited and the reduction potential of glucose will increase.

Besides the described mode of action of fermentable sugars, like glucose, fructose and sucrose, the stronger reduction potential of maltotriose versus maltose is remarkable. The observed reduction potential of polydextrose (2 x mass sucrose) contradicts the conclusion that a longer chain of sugars is automatically responsible for a higher reduction potential. It is more likely that the specific number of the sugar units, even or uneven (3, 4, 5, 6), in the chain is responsible for the different behaviour. However, based on the low reduction potential of polydextrose, a sugar with a higher degree of polymerisation shows a decrease in the reducing properties in comparison to maltotriose. This information can be helpful to get further evidence on the behaviour of fermentable sugars during wort boiling in the brewing process. For this kind of investigation in a pH range > 5.2, it should be taken into consideration that the application of the method is more complicated and not easy to realise because a FeOH formation and precipitation is observed under these conditions. Especially, for fermentable sugars the possible reductive activity during mashing and wort boiling is interesting because the consumption during fermentation and the low concentration in the final beer.

In summary, it could be verified that the developed optimised Chapon method is applicable and can be used as a forcing test to demonstrate the behaviour of sugars in beverages during storage and to support the investigation of the complex reaction mechanism of sugars in juice, wine, beer and other beverages.

In the future, the optimised Chapon method may be used for additional investigations at different temperatures and low pH values between 2.5–5.2 to get further evidence on the behaviour of sugars during specific processing steps, where sugar reactions are accelerated; for example during the mashing process or the wort boiling in the brewing process.

Acknowledgments

The authors wish to thank the companies Südzucker AG and BENEO-Palatinit GmbH Mannheim, Germany for the support. Palatinose™ (isomaltulose) and Vitalose® (trehalulose) are products of BENEO-Palatinit GmbH, a member of Südzucker AG.

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Received 6 July 2011, accepted 09 August, 2011