# Comparative methods applied for the determination of total iron from beer samples

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**Abstract** Iron has an important role on the prevention of anemia, which is an important public health problem. Results from studies, conducted in humans and animals, have shown that iron ions ( $Fe^{2+}$  and  $Fe^{3+}$ ) have cariostatic properties. Alone or in combination with other ions, like fluoride and copper, iron has a great effect on the reduction of the cariogenic potential of the sugar. The iron content of beer should be as low as possible. Under normal conditions, the iron content of fermented beer is below 0.2 ppm. If it is higher in the finished beer, a pickup of iron after fermentation is indicated. Iron is said to enter more readily in solution in a beer highly saturated with CO<sub>2</sub> gas. Highly oxidized beers also dissolve more iron. High amount of iron can contribute to color increase due to an interaction with wort and/or beer tannins and hop constituents.

The aim of this work was the implementation and optimizations of some UV-VIS molecular absorption spectrometric methods for determination of total iron from commercial beer samples; the results were compared with those obtained by flame atomic absorption spectrometry (FAAS) using a spectrometer Shimadzu AA 6200. Also, in this study, was compared the efficiency of different digestion procedures (dry ash procedure and digestion procedure with nitric acid) on beer samples. Prior to analysis, the beer samples were degassed. The most appropriate method for the determination of iron in beer was found FAAS and the concentrations obtained were in the range of 0.33 - 1.59 mg/L. For determination of the relative accuracy of the applied methods for iron analysis the "t" test was performed.

Keywords: iron, beer, UV-VIS spectrometry, FAAS

#### 1. Introduction

The investigation of iron species in beer has chemical interest, because the Fe(II)/Fe(III) ions play an important part in the activation of O<sub>2</sub> and the initiation of beer aging and staling processes [1].

Iron has an important role on the prevention of anemia, which is an important public health problem. Results from studies, conducted in humans and animals, have shown that iron ions (Fe<sup>2+</sup> and Fe<sup>3+</sup>) have cariostatic properties. Alone or in combination with other ions, like fluoride and copper, iron has a great effect on the reduction of the cariogenic potential of the sugar.

The iron content of beer should be as low as possible. Under normal conditions, the iron content of fermented beer is below 0.2 ppm. If it is higher in the finished beer, a pick up of iron after fermentation is indicated. Iron is said to enter more readily in solution in a beer highly saturated with  $CO_2$  gas. Highly oxidized beers also dissolve more iron. High

amount of iron can contribute to color increase due to an interaction with wort and/or beer tannins and hop constituents [2-7].

For the first time beer was brewed in ancient Egypt, from grains of wheat and then from rice. Gradually penetrate into Greece and then in the Roman Empire, its preparation being done by primitive methods [8].

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Communities) are numerous determinations, especially for determining the physical properties: color, refractive index, calories content, total acidity, foam as well as chemical properties: iron, copper, calcium, carbon dioxide, sulfur dioxide, alcohol, protein, starch, enzymes [9-11].

## 2. Experimental

# Reagents and solutions

The working solutions were prepared by diluting the stock solutions to appropriate volumes. All reagents were of analytical-reagent grade and all solutions were prepared using deionised water.

#### Sample preparation

Beer samples studied in this paper were collected from the local market: alcohol-free beer two samples of blond and two samples of brown beer (SB1, SBR1, SB2 and SBR2). Determination of iron in the investigated samples was done in beer sample after a mineralization step with nitric acid and from dissolved ash.

#### Sample analysis

After bringing the samples in a measurable form, the obtained solutions were analyzed using FAAS and UV-VIS molecular absorption spectrometric methods to determine the iron concentration. Two sets of measurements were made: analysis of samples after the mineralization process and ash analysis.

#### FAAS

Iron was determined by FAAS in air/acetylene flame using an aqueous standard calibration curve. Analyses were made in triplicate and the mean values are reported.

A Shimadzu atomic absorption spectrometer (Model AA 6200) equipped with air-acetylene flame was used for the determination of iron in beers. The device is based on monofascicol system and is equipped with computerized display and data processing and could be read the concentration value, directly. Acetylene of 99.99% purity at a flow rate of 1.8-2.0 L/min was utilized as a fuel gas and also as a carrier gas for introducing aerosols. Concentrations of iron were measured using monoelement hollow cathode lamp.

The characteristics of iron calibration curves are: concentration range from 0.020 to 4.000 mg/L and the correlation coefficient was 0.9976.

#### **UV-VIS molecular absorption spectrometry**

#### a) with 1,10 phenantroline.

In a 50mL flask 10mL analyzed sample is introduced then 2mL of 20% sodium sulphite solution, 1 mL hydrochloric acid 25%, 2mL hydroquinone 2.5% and 1mL 1,10 phenantroline 0.5% were added and allowed to stand for 15 minutes. 10mL of 20% ammonium acetate solution were added, make up with distilled water and mix. The absorbance was read at 528nm using the Camspec M330 spectrometer.

#### b) with sulfosalicilic acid.

In a volumetric 50mL flask 10mL analyzed sample were introduced then 10mL buffer and 1mL sulfosalicilic acid were added, make up with distilled water and mixed. The absorbance was read at 466nm using the Camspec M330 spectrometer.

#### *c*) with potassium tiocianate

In a 50mL flask, 10mL of sample is introduced, 1 mL HCl 25%, 5 drops of hydrogen peroxide, 1 mL KSCN; the mixture was stired and finnaly brought to volume with distilled water. The absorbance was read at 474 nm using the Camspec M330 spectrometer.

#### Standard addition method

It have been prepared two solutions A and B, solution A contains only unknown sample and solution B unknown sample and a measured volume of standard solution of Fe (II) [12].

#### Solution A:

- in a 50mL volumetric flask was added 2mL sample, 5 mL sodium acetate, 5 mL of hydroxylamine hydrochloride, then shaked for mixing; 5 minutes wait, then add 5mL 1.10 phenanthroline; 10 minutes wait to stabilize color, dilute to volume with distilled water;

- the solution absorbance was readed at 510nm to DR 2000 spectrometer versus a reference solution.

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#### Solution B:

- in another flask was added 2mL sample solution, 2mL standard solution Fe (II) and then the other reagents used at solution A were added in the same order;

- the solution absorbance was readed at 510nm versus a reference solution.

For determination of the relative accuracy of the applied methods for iron analysis the t test was performed.

# 3. Results and Discussions

3.1. FAAS

In **table 1** are presented the average values of Fe concentrations determined in beer samples by FAAS.

 Table 1. The content of Fe in beer samples by
 FAAS

	Concentrations (mg/L)			
Beer samples	Ash	Mineralisation		
SBR1	$0.4937 \pm 0.0020$	$1.5927 \pm 0.0036$		
SB1	$0.4290 \pm 0.0026$	$1.0690 \pm 0.0024$		
alcohol-free	$0.5705 \pm 0.0018$	$0.7270 \pm 0.0035$		
SB2	$0.3327 \pm 0.0039$	$0.7970 \pm 0.0018$		
SBR2	$0.4347 \pm 0.0033$	$1.0307 \pm 0.0016$		

Alcázar and his colleagues have determined the iron content of 32 samples of beer: blond (0.162 to 1.065 mg/L), brown (from 0.096 to 0.432 mg/L) and low alcohol (0.081 to 0.378 mg/L) by ICP-AES [13]. Comparing the values obtained from samples of beer studied in this paper (see Table 1.) with those obtained by Alcázar it can be concluded that the closest iron concentrations are those determined from ash of brown and low alcohol beers.

Bellido-Milla and coworkers have determined the iron content (0.06 to 0.55 mg/L) in 25 samples of beer by atomic absorption spectrometry [11]. Iron concentrations determined from ash or after mineralization of samples of beer are in concordance with those from literature, only in brown and low alcoholic beer Ursus greater differences have occurred.

Comparing with literature data it was observed that more precise determination is obtained when the measurements are performed in ashes solution. 3.2. Molecular absorption spectrometry with 1,10 phenantroline

Divalent iron formed a colored complex with 1.10 phenanthroline named ferrous 1.10 phenanthroline. The color intensity of complex formed is measured.

The absorption spectrum of the compound formed by iron with 1.10 phenanthroline is presented in **Fig. 1**. where the maximum of absorbance is at 528nm.



**Fig. 1.** The absorption spectrum of the compound formed by iron with 1.10 phenanthroline

The plotted calibration curve is shown in **Fig. 2**; it can be noticed a good linearity and the correlation coefficient 0.99960.



Fig. 2. Calibration curve for determination of iron with 1,10 phenantroline ( $\lambda = 528$ nm) on the working range 0.199 to 5.991 mg/L

Although the calibration curve shows a good linearity and the method was recommended by the Romanian Institute for Standardization [14]. The

iron concentrations in beer samples measured by the spectrometric method with 1,10 phenantroline were below the detection limit of the method that means is not enough sensitive.

3.3. Molecular absorption spectrometry with sulfosalicilic acid

Sulfosalicilic acid reacts with Fe (III) form at pH = 5.00, in the presence of acetic acid buffer solution of ammonium acetate, a complex red with blend ratio of 1:2.

The absorption spectrum of compound formed by iron with sulfosalicilic acid is given in **Fig. 3**.



Fig. 3. The absorption spectrum of the compound formed by iron with sulfosalicilic acid.

The plotted calibration curve is shown in **Fig. 4**; it can be noticed a good linearity and the correlation coefficient 0.99984.



Fig. 4. Calibration curve for determination of iron with sulfosalicilic acid ( $\lambda = 466$ nm) on the working range 0.234 – 5.859 mg/L.

In **Table 2** the average values of Fe concentrations in beer samples using

spectrophotometric method with sulfosalicilic acid are presented.

Table	2.	The	content	of	Fe	in	beer	samples	using
spectro	oph	otom	etric me	tho	d w	vith	sulfo	salicilic a	icid.

	Concentrations (mg/L)		
Beer samples	Ash	Mineralisation	
SBR1	20.5	44.89	
SB1	4.18	4.86	
alcohol-free	2.66	4.94	
SB2	1.16	3.28	
SBR2	5.12	26.91	

It is observed higher concentrations of iron for brown beer samples, but this can be attributed to the color interference of these samples.

Therefore the determination has been performed in disolved ashes and for the mineralized sample has achieved lower values; but compared with literature data or with FAAS results are higher. Following the results it can be concluded that this method is not suitable for determining iron in beer or other investigations are necessary (likely to be interference).

# 3.3. Molecular absorption spectrometry with potasium tiocianate

Divalent iron oxidized to trivalent iron, forms a colored complex with KSCN, whose intensity is measured spectrophotometrically.

In **Fig. 5** the absorption spectrum of the compound formed by iron KSCN is presented.



Fig. 5. The absorption spectrum of the compound formed by iron with KSCN

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The plotted calibration curve is shown in **Fig. 6**; it can be noticed that the curbe is linear and has the correlation coefficient 0.99927.



Fig. 6. Calibration curve for determination of iron with KSCN ( $\lambda = 474$ nm) on the working range 0.199 - 3.495 mg/L

In **Table 3** the average values of Fe concentrations in beer samples using spectrophotometric method with KSCN are presented.

 Table 3. The content of Fe in beer samples using spectrophotometric method with KSCN.

	Concentrations (mg/L)		
Beer samples	Ash	Mineralisation	
SBR1	3.08	31.35	
SB1	0.32	1.76	
alcohol-free	0.12	1.69	
SB2	0.47	1.58	
SBR2	0.56	9.64	

It can be noted that iron concentration values are significantly higher following its determination from a mineralized sample of beer. The iron concentrations founded from ash are comparable as order of magnitude with those obtained by FAAS. It is more accurate to determine iron in samples of beer after their mineralization due to the potential interferences with beer color, especially for brown beer samples.

#### 3.4. Standard addition method

1.10 phenanthroline reagent is mainly used for analysis of trace iron. Iron can be in two oxidation states II and III. Oxidation state adjustment is achieved with hydroxylamine hydrochloride.

In the **Table 4** comparative average values of Fe concentrations in beer samples (after mineralization or dry-ashing followed by the ash dissolution) using the standard addition method are presented.

 Table 4. The content of Fe in beer samples using standard addition method.

Concentrations (mg/L)				
Ash Mineralisation		Mineralisation		
	acid pH	neutral pH		
9.162	3.855	4.893		
1.378	1.730	6.584		
1.478	2.200	4.790		
1.071	1.800	4.776		
3.922	3.593	3.799		
	Ash 9.162 1.378 1.478 1.071 3.922	Concentrations           Ash         Mineralisation acid pH           9.162         3.855           1.378         1.730           1.478         2.200           1.071         1.800           3.922         3.593		

In **Table 4** is noted that iron concentration values determined from the mineralized sample are higher than those obtained by FAAS. So it can be said that the standard addition method (which eliminates interference) could be directly applied with good results to the determination of iron in beer samples.

For determination of the relative accuracy of the applied methods for iron analysis the "t" test was performed. T test in pairs can be used for samples with different concentrations, which are analyzed by two analytical methods and differences in each pair are compared. Null hypothesis to be tested indicates that there are significant differences between pairs of results.

Applying the "t" test it can be concluded that method with acid sulfosalicilic and method with potassium tiocianate applied in the mineralized samples, have similar degrees of accuracy, because the obtained critical values of "t" (1.59) is lower than the critical value of "t" for 4 degrees of freedom (n-1), which is 2.78.

Also, it can be concluded that FAAS, molecular absorption spectroscopy in UV-VIS with KSCN and molecular absorption spectroscopy in UV-VIS with sulfosalicilic acid can be applied with the same degree of precision (because "t" values were 1.68; 1.95 and 0.32).

Therefore the studied methods can be applied with the same degree of accuracy to both samples: mineralized and dissolved ash.

### 4. Conclusions

The aim of this work was the implementation and optimizations of some UV-VIS molecular absorption spectrometric methods for determination of total iron from commercial beer samples; the results were compared with those obtained by flame atomic absorption spectrometry using a spectrometer Shimadzu AA 6200. Also, in this study, was compared the efficiency of different digestion procedures (dry ash procedure and digestion procedure with nitric acid) on beer samples.

The most appropriate method for the determination of iron in beer was found to be flame atomic absorption spectrometry (FAAS).

Also iron can be determined by standard addition method using UV-VIS molecular absorption spectrometry with KSCN and with sulfosalicilic acid. The results of the last two methods are comparable with those obtained by FAAS and with those reported in the literature with the same degree of precision, as the "t" test in pairs have showed.

The spectrometric method with 1.10 phenantroline is not recommended for the concentration levels of iron in beer samples.

#### 5. References

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[1]. P. Pohl and B. Prusisz, Journal of Food Composition and Analysis, **23**, 86–94 (2010) [2]. I. Matsushige and E. de Oliveira, Food Chemistry, **47**, 205-207 (1993)

[3]. M. Thiemi Kato, S. H. de Carvalho Sales-Peres and M. A. R. Buzalaf, Archives of Oral Biology, **52**, 1109-1111 (2007)

[4]. B. Kodyat, S. Kosen and S. de Pee, Nutrition Research, **18**, 1953-1963 (1998)

[5]. P. Ampan, S. Lapanantnoppakhun, P. Sooksamiti, J. Jakmunee, S. Kradtap Hartwell, S. Jayasvati, G. D. Christian, and K. Grudpan, Talanta, **58**, 1327-1334 (2002)

[6]. R. M. Halcrow, Some comments on beer analysis, <u>http://www.probrewer.com/</u>

[7]. Beer culture, The history of beer, http://www.beer-and-health.com

[8]. I. Savu, Bere, Cvas, Cidru – Practic, Editura Gramar, București, 8-15 (1993)

[9]. B. Vanderhaegen, F. Delvaux, L. Daenen, H. Verachtert and F. R. Delvaux, Food Chemistry,**103**, 404–412 (2007)

[10]. B. Vanderhaegen, H. Neven, H. Verachtert andG. Derdelinckx, Food Chemistry, 95, 357–381(2006)

[11]. A. Oñate-Jaén, D. Bellido-Milla and M.P. Hernández-Artiga, Food Chemistry, **97**, 361–369 (2006)

[12]. E. Chirila and S. Birghila, Instrumental method of analysis. Apllications (in Romanian), Ovidius University Press, 78-88, (2002)

[13]. A. Alcázar, F. Pablos, M. J. Martín and A. G. González, Talanta, **57**, 45 – 52, (2002)

[14].\*\*\* Romanian Institute for Standardization, Ethyl alcohol and alcoholic beverages, Iron determination (in Romanian), STAS 184/19-90, (1990).

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