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Optimization of ITEX/GC-MS method for beer wort volatile compounds characterisation

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Abstract

In order to characterize the Magnum hop variety volatile profile in beer wort, we determined the composition of volatile compounds during the main fermentation process of beer wort using the ITEX/GC-MS technique. An Shimadzu GC/MS-QP 2010 equipment was used for samples analysis. For a better extraction of volatile compounds by ITEX-GC-MS of the testing vial and to avoid the saturation of the detector, parameters like quantity of sample, injection volume, incubation temperature, incubation time, split ratio, scan range, start time, total flow, column temperature, column flow, were optimized. The major compounds founded in wort beer samples, during the primary fermentation, were derived from a vast array of compounds that arise from a number of sources. In this way, we founded different categories of secondary products: higher alcohols, esters, organic acids and aldehydes. The chemical constituents of the beer wort were separated and identified using the GC-MS NIST libraries.

Keywords: volatile compounds, wort beer, ITEX /GC-MS, method optimization

1. Introduction

Hop cultivation began in Bavaria (Hallertau) and Bohemia (Saaz) in the 8th and 9th centuries, and because of their superior anti-bacterial properties, at some time later hops became the standard flavouring and bittering agent for beer all over Europe and the world. Today there are many varieties of hops and a number of hop products are used either alone or in combination to produce an enormous range of different hop flavours [1].

Hops are rich in flavour compounds, which give beer its typical flavour. However, the majority of these compounds are largely evaporated during boiling. Linalool is often used as an indicator for the presence of hop aroma compounds.

Before wort is converted to beer by brewer's yeast, it undergoes several process steps that influence the quality of the end-product.

These process steps include mashing, lautering, boiling, trub separation and cooling. The boiling process serves many purposes, from which conversion of hop bitter substances might be the most known. However, it is equally important to control the levels of flavour constituents in wort, because they can have a major impact on the final beer flavour and the flavour stability of the beer. Due to the high temperature while boiling, the rate of several chemical reactions increases markedly [2].

During the alcoholic fermentation of wort, in addition to the major products of ethanol and carbon dioxide, the yeast *Saccharomyces cerevisiae*, excretes a wide range of flavour compounds including higher alcohols (also called fusel oils), esters, carbonyls, sulphur compounds, organic and fatty acids, and a number of miscellaneous compounds. The production of these compounds

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relates to the overall metabolic balance of the yeast culture. These flavour compounds have a significant effect on the flavour of beer, wine and other alcoholic beverages [3].

Several extraction–concentration methods have been employed for the analysis of volatile compounds in beer, such as liquid–liquid extraction, simultaneous extraction and distillation, solid-phase extraction, supercritical fluid extraction. Most of these methods produce extracts with flavour compounds that are representative of the liquid matrix and not of the headspace. The most widely used headspace sampling technique for volatile isolation is, however, static, dynamic headspace analysis or purge and trap technique [4].

However, no literature can be found about method optimisation for the analysis of the entire wort volatile fraction, which differs greatly from the volatile fraction of beer. In wort no competition or interference may be expected from abundant volatiles, masking the presence of trace compounds [2].

Therefore the aim of this study was to optimise a ITEX/GC-MS method for the analysis of Magnum hop variety volatile profile in beer wort during the main fermentation process.

2. Materials and Method

Samples. Malt pilsner wort (12°P) prepared with 100% barley malt and hopped with Magnum hop variety (10.0-12.6% α-acids and 5.0–7.0% β-acids), from Sighişoara, region from Transylvania, Romania, originated from Germany, manufactured in the laboratory scale production (2L), was taken in this study. For the experiment, a bottom-fermenting industrial lager brewing Saccharomyces cerevisiae yeast strain was used.

Pilsner malt grinded by a laboratory mill in grist, with fine granulation for a maximum effective power.

The grist obtained, was mixed with water at 40°C in 1:4 ratio, followed the brewing process. After filtration, the sugar solution, in brewers' jargon called 'wort', was boiled 60 minutes with the addition of hops (Hallertau Magnum). Was used a few grams of hops (10g α acids/hL wort), a major ingredient with crucial impact on well-defined beer features. After cooling and removal of spent hops, the liquid, known as 'hopped wort' is pumped into fermentation vessels and yeast is added under aeration for growth. During the anaerobic phase, yeast cells convert sugars to ethanol and carbon dioxide. 'Bottom fermentation' was conducted: the temperature during primary fermentation process was 8-10°C, for 5 days.

ITEX analysis. Samples of 2.5/5/8 ml of beer wort primary fermented was weighted into a 10 ml glass vial. For a better extraction of volatile compounds by ITEX/GC-MS of the testing vial and to avoid the saturation of the detector, parameters like: quantity of sample, injection volume, incubation temperature, incubation time, number of strokes, split ratio, start time, scan range, total flow, column temperature and column flow, were optimized. The ITEX methods tested, are presented in Table 1.

GC-MS analysis. Wort sample was taked at the end of primary fermentation process - after 120hrs of fermentation, and subsequently frozen at−25°C, to minimize the loss of very volatile compounds.

The trub was removed by filtration after defrosting, in order to remove all possible precipitates before preparing the wort samples. For this analysis, the wort sample, hopped with Magnum hop variety and taked after 120hrs of the primary fermentation, was called WMGf120.

The volatile compounds of the primary fermented beer wort sample, were quantified by ITEX/GC/MS technique. The analyses were carried out on a Shimadzu GC-MS QP-2010 model gas chromatograph–mass spectrometer equipped with an AOC-5000 autosampler (CombiPAL). Separation was achieved on a capillary column: 50m x 0.32mm x 0.25µm, Phenomenex, USA. Helium was used as carrier gas. GC temperature programe: from 70°C to 200°C at 4°C/min and to 270°C (2 min) at 20°C/min. Injector temperature: 250.0°C. Injection volume: 500µL, 1000 respectively, Split ratio: 1:25, 1:50, 1:100 respectively, depending on the applied ITEX method . Detector: MS, ion source temperature: 250.0°C. Interface temperature: 250.0°C. The identification of separated compounds was made based on the comparison of the obtained mass spectra with the ones from the mass spectra libraries, NIST27 and NIST147.

Table 1. ITEX extraction methods and the optimised parameters for WMGf 120 beer wort sample

Method abreviation	Quantity of sample analysed (ml)	Quantity of sample injected (µl)	Incubation Tempera-ture (^{0}C)	Incubation time (min)	Number of strokes	Split ratio	Start time (min)	Scan range (m/z)	Total flow (ml/ min)	Column Tempera-ture (°C)	Column flow (ml/min)
mMB1	2.5	500	80	15	30	25	4.00	40	47.2	35	1.70
mMB2	2.5	500	80	15	30	50	4.00	40	89.7	35	1.70
mMB3	5	500	60	20	20	25	4.00	29	46.2	40	1.66
mMB4	5	1000	80	15	20	100	4.00	29	174.7	35	1.70
mMB5	5	1000	80	15	30	25	5.5	29	47.3	35	1.70
mMB6	8	1000	60	20	20	25	4.00	29	44.0	50	1.58
mMB7	5	500	60	20	20	25	4.00	40	46.2	40	1.66
mMB8	5	1000	80	15	20	25	5.5	40	47.3	35	1.70
mMB9	2.5	500	60	20	30	20	4.00	40	29.9	60	1.28

3. Results and Discussion

The separeted constituents of the fermented beer wort hopped with Magnum hop variety were identified by comparing the obtained mass spectra with those from GC-MS libraries NIST147 and NIST127. These compounds separated and identified by all nine ITEX methods and their concentration (%), are presented in Table 2.

The major compounds found by the most ITEX methods applied, in WMGf120 beer wort sample, hopped with Magnum hop variety, were: 1-Butanol 3-methyl, 1-Butanol 3-methyl acetate, Butanoic acid ethyl ester and Hexanoic acid ethyl ester.

As it can be noticed, from the eleven varied parameters (quantity of sample, injection volume, incubation temperature, incubation time, number of strokes, split ratio, scan range, start time, total flow, column temperature, column flow,), the quantity of sample, incubation temperature, incubation time, column temperature, and column flow had the major influence on the volatile compounds extraction, from the primary fermented beer wort.

The ITEX methods most successful on the identification of a large number of volatiles compounds in beer wort sample, at the end of primary fermentation process, were: mMB3, mMB7, mMB8, and mMB1.

For example, by mMB3 and mMb7 methods (5 ml sample, 60°C, 20 minutes of incubation, 40°C column temperature and 1.66 ml/min column flow) a number of 32 volatile compounds (mMB3 method) and 31 volatile compounds (mMB7) method were separated from the beer wort sample while using the mMB8 and mMB1 methods (2.5 ml sample, 80°C, 15 minutes of incubation, 35°C column temperature and 1.70 ml/min column flow), the number of separated compounds was 30 and 29 respectively.

If the amount of sample and the incubation temperature is lower (2.5 ml sample, 60°C, 20 minutes of incubation), with a higher column temperature (60°C) (mMB9 method) the number of separated compounds decrease to 15.

In the same case with a higher amount of sample, higher incubation temperature (5 ml sample, 80°C, 15 minutes of incubation), and also higher split ratio (1:100), total flow (174.7 ml/min), the separated compounds decreases more, to 13 (mMB4 method).

In the most methods applied at 4.80 min retention time has been identified the saturation of the detector, for 1- Butanol 3-methyl volatile compound. Methods that did not detect this saturation were; mMB2, mMB4, mMB5, mMB9. The obtained chromatograms by mMB2, mMB4, mMB5, mMB9 method, from the WMGf120 primary fermented beer wort sample are presented in Figure 1.

One of the explanations for this behavior may be that by increasing in the same time the quantity of sample, and the incubation temperature, or by increasing the column temperature, split ratio, total flow, the vial is enriched in compounds that are present in a larger concentration in the primary fermented beer wort sample and that inducing a saturation of the detector at 4.80 min retention time, for 1- Butanol 3-methyl volatile compound.

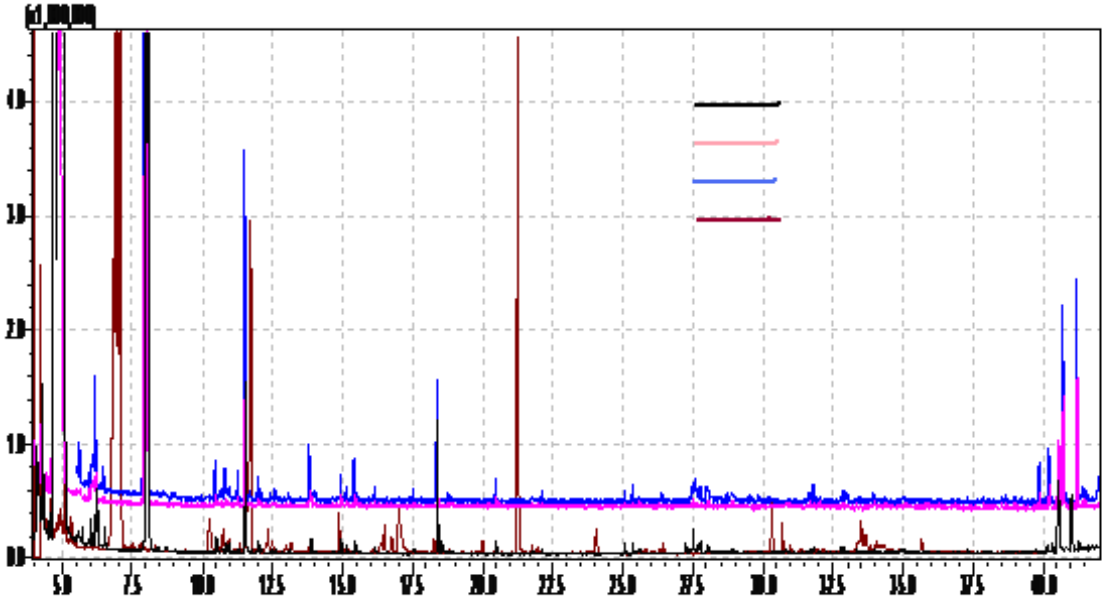

Figure 1. The ITEX/GC-MS chromatograms of WMGf120 Magnum beer wort sample at the end of primary fermentation process, by mMB2, mMB4, mMB5, mMB9 ITEX methods, that did not detect the saturation of the detector

Of the methods showed no saturation of the detector, the best was mMB5, because thereby have identified several compounds - 22 volatiles compounds, than 17 volatiles compounds – by mMB2 method, 15 volatiles compounds – by mMB9 method and 13 volatiles compounds – by mMB4 method. By this method also different compounds from Magnum hop variety in primary fermented beer wort which were not separated by mMB2, mMB4, mMB9 methods, were identified: β-Mircene; D-Limonene; Propanoic acid 2-methyl,2-methylbuthyl ester, Cariophyllene, and α-Cariophyllene.

Therefore for a better extraction of volatile compounds from Magnum hop variety in the primary fermented beer wort, for a better profile of beer wort volatile compounds, and to avoid the saturation of the detector by ITEX-GC-MS, technique, most suitable parameters have been: quantity of sample: 5 ml, incubation temperature: 80°C, incubation time: 15 min , split ratio: 1:25, start time: 5.5 min, injection volume: 1000µl, column temperature: 35°C, and column flow: 1.70.
The major compounds found by mMB5 method in primary fermented beer wort, hopped with Magnum hop variety, were 1 Butanol 3-methyl acetate (63.68%), Hexanoic acid ethyl ester (8.05%), Octanoic acid ethyl ester (3.24), Butanoic acid ethyl ester (1.72%). Also the identification of volatile compounds from Magnum hops variety, was performed: as β–myrcene (0,56%), β- and α - cariophyllene (0,25% respectively 0,28), D-Limonene (0,27 %) and Propanoic acid 2 methyl 2methylbuthyl ester (0,21%).

4. Conclusion

A ITEX/GC-MS method for the Magnum hop variety volatile compounds profile characterization in primary fermented beer wort, was optimized. The optimized parameters were the quantity of sample, injection volume, incubation temperature, incubation time, split ratio, scan range, start time, total flow, column temperature, column flow. The best results obtained were by mMB5 method applied. Thus by this method, the greatest influence on the volatile compounds extraction, from primary fermented beer wort sample, was attributed to correlation between the parameters of sample quantity , incubation temperature, incubation time, split ratio and start time. Increasing the quantity of sample , incubation temperature and decreasing the incubation time, by 1:25 split ratio and 5.5 min start time, has as

result, a slight decrease of the extracted and separated compounds number, but in the same time without the saturation of the detector, the main Magnum hop volatile compounds from the fermented beer wort were identified. By increasing the incubation temperature and decreasing the sample quantity or vice versa and by a increase of split ratio and column temperature, although they separated several compounds, does not have as result a better extraction of volatile compounds from Magnum hop variety in beer wort sample. In our opinion, the ITEX method optimal parameters were: 80°C incubation temperature, 15 min incubation time, 5 ml sample quantity, 1:25 split ratio, 35°column temperature and 5.5 min start time - for 1-Butanol 3-methyl volatile compound elimination.

As perspective, the optimized ITEX /GC-MS method will be used for the analysis of volatile compounds from beer wort samples hopped with different hop varieties in order to discriminate the hop varieties markers based on beer volatile composition.

Table 2. The composition of WMGf120 beer wort sample during primary fermentation process determined by ITEX/GC-MS technique using different extraction methods

No.	Compound	Retention Time (min)	Concentration (% from total peaks area)								
			mMB 1	mMB 2	mMB 3	mMB 4	mMB 5	mMB 6	mMB 7	mMB 8	mMB 9
(0)	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
1.	2,3 Pentanedione	4.201	8.62	1.76	1.23	0.58	-	-	-	-	-
2.	Propanoic acid, ethyl ester	4.647		-	0.34	1.04	-	-	-	-	-
3.	1-Butanol, 3-methyl-	4.721	17.27	67.95	-	73.81	-	-	47.07	-	-
4.	1-Butanol, 2-methyl	4.982		-	-	4.46	-	-	-	-	-
5.	Acetic acid, 2-methylpropyl ester	5.702	0.33	-	0.24	-	0.69	2.08	0.56	1.20	0.55
6.	Triethyl borate-Boric acid	6.049	0.43	0.14	-	0.11	0.20	-	-	1.22	-
7.	Butanoic acid, ethyl ester	6.250	2.06	0.68	-	0.50	1.72	7.01	1.91	7.60	2.10
8.	Propanoic acid, 2-methyl-, 2-methylpropyl ester	7.247	-	-	-	-	-	-	0.08	-	-
9.	1-Butanol, 3-methyl-, acetate	7.969	48.2	12.52	20.73	12.22	63.68	28.32	10.14	16.46	66.41
10.	2-Pentanol propanoate	8.230	-	-	-	-	-	-	0.19	-	-
11.	Propanoic acid, pentyl ester	9.330	-	-	-	-	-	0.36	-	-	-
12.	Benzaldehyde	10.490	0.61	0.13	0.32	0.09	1.11	0.78	0.54	1.32	0.73
13.	Acetic acid, heptyl ester	10.713		-	-	-	-	-	0.38	-	-
14	Phenol	10.847	0.32	0.06	0.22	0.05	0.63	0.72	-	0.61	0.43
15.	β-Mircene	11.257		-	7.88	-	0.56	0.49	0.54	0.41	-
16.	Hexanoic acid, ethyl ester	11.532	6.06	1.28	2.69	1.55	8.05	23.10	9.70	18.41	9.71
17.	Octanal	11.729	0.09	-	-	-	-	0.15	0.08	-	-
18.	1-Butanol, 2-methyl-, propanoate	11.754	-	-	0.12	-	-	-	-	-	-
19.	Acetic acid, hexyl ester	11.975	0.30	0.07	-	0.13	0.40	1.17	0.66	1.00	0.34
20.	Propanoic acid, 2-methyl-, 2-methylbutyl ester	12.071	-	-	0.29	-	0.21	0.79	0.32	0.32	-
21.	Acetic acid, decyl ester	12.432	-	-	-	-	-	-	0.46	-	-
22.	D-Limonene	12.596	-	-	0.12	-	0.27	-	0.09	0.56	-
23..	Acetic acid, heptyl ester	12.656	-	-	0.19	-	-	-	-	-	-
24.	Benzeneacetaldehyde - Acetaldehyde, phenyl	13.092	0.25	-	-	-	-	-	0.07	-	-
25	Heptanoic acid, ethyl ester	13.277	-	-	0.12	-	-	0.13	0.15	0.24	-
26.	Acetophenone	13.840	0.55	0.10	0.51	0.02	1.41	1.75	0.84	1.83	1.04
27.	Methyl 6-methyl heptanoate	14.157	-	-	0.13	-	-	-	-	-	-
28.	β-Pinene -6,6-dimethyl-2-methylene-	14.439	-	-	0.70	-	-	-	-	-	-

Table 2. (continued)

(0)	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
29.	Octanoic acid, methyl ester	14.885	-	-	0.16	-	-	-	-	-	-
30.	Linalyl isobutyrate	14.958	0.83	0.13	-	-	-	-	-	-	-
31.	Nonanal	15.143	0.30	0.06	0.70	-	0.28	0.73	0.38	0.97	0.18
32.	Phenylethyl Alcohol	15.424	0.83	-	0.48	-	1.14	2.29	0.81	1.28	1.10
33.	Decanal	16.720	-	-	-	-	-	0.31			
34.	Decane, 5,6-dipropyl	16.784	-	-	-	-	-	-	0.30	-	-
35.	Acetic acid, octyl ester	16.803	-	-	-	-	-	0.27		0.54	-
36.	Benzoic acid, ethyl ester	17.510	0.15		0.04		0.32	0.30	0.12	0.48	0.13
37.	2-Undecanone	17.774	-	-	0.35	-	-	-	-	-	-
38.	4-Decenoic acid, methyl ester	17.969	-	-	1.12	-	-	-	-	-	-
39.	2,6-Octadienoic acid, 3,7-dimethyl-, methyl ester	18.188	-	-	0.66	-	-	-	-	-	-
40.	Octanoic acid, ethyl ester	18.367	5.63	1.01	1.06	-	3.24	12.95	16.79	24.07	12.95
41.	Dodecanoic acid, 1-methylethyl ester	18.477	-	-	-	-	-	-	0.17	-	-
42.	Octane,-Octyl ether	19.058	-	-	-	-	-	-	0.11	-	-
43.	Copaene	19.212	-	-	0.73	-	-	-	-	-	-
44.	Acetic acid, 2-phenylethyl ester	20.479	0.60	0.10	0.25	-	0.34	1.39	0.42	1.11	0.68
45.	Eudesma-4(14),11-diene, 7-Isopropenyl-4a-methyl	20.898	-	-	1.64	-	-	-	-	-	-
46.	Azulene, 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl	21.540	-	-	0.62	-	-	-	-	-	-
47.	Dodecane	22.406	-	-	-	-	-	0.53	-	-	-
48.	Ethyl 9-decenoate	25.066	0.59	0.08		-	-	-	1.50	2.88	-
49.	Decanoic acid, ethyl ester	25.349	0.38	0.08	0.18	-	0.24	1.32	0.89	1.72	0.70
50.	Tridecane	25.574	0.10	-	-	-	-	-	-	-	-
51.	Caryophyllene	26.402	-	-	4.98	-	0.25	-	-	-	-
52.	α-Caryophyllene	27.581	-	-	6.96	-	0.68	-	-	-	-
53.	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)	27.651	0.17	-	-	-	-	-	-	0.45	-
54.	2,5-Cyclohexadiene-1,4-dione, 2,5-diphenyl		-	-	0.09	0.63	-	-	-	-	-
55.	Nonane, 3-methyl-5-propyl-3-Methyl-5-propylnonane	27.661	-	-	-	-	-	0.51	-	-	-
56.	1-Dodecanol	28.011	-	-	-	-	0.15	0.29	-	1.47	-
57.	Heptadecane, 2,6,10,15-tetramethyl	28.884	0.24	-	-	-	-	-	0.16	-	-
58.	Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester	31.649	-	-	-	-	-	-	-	0.67	-
59.	Dodecanoic acid, ethyl ester Lauric acid, ethyl ester	31.803	0.24	-	-	-	0.57	-	-	1.03	-
60.	Pentadecane	32.035	-	-	-	-	-	-	-	0.28	-
61.	Hexanoic acid, 2-phenylethyl ester	33.306	-	-	-	-	-	-	-	0.31	-
62.	Tetradecane	32.045	0.15	-	-	-	-	-	-	-	-
63.	Undecane, 6-ethyl-	35.666	0.24	-	-	-	-	-	-	-	-
64.	Octanoic acid, 2-phenylethyl ester	38.990	-	-	-	-	-	-	-	0.41	-
65.	Ethyl 9-hexadecenoate	41.004	1.84	0.34	-	-	-	-	0.22	2.78	1.21
66.	Hexadecanoic acid, ethyl ester	41.283	0.19	-	-	-	-	-	0.38	0.45	-

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