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Determination of iso-alpha-acids, alpha- and beta-acids in isomerised hop pellets by HPLC

Submitted by an behalf of the EBC Analysis Committee

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1 Introduction

For the production of isomerised hop pellets (iso-pellets) hop powder is admixed with a catalyst (e.g. MgO). After pelletisation and packaging under inert gas atmosphere this mixture is heated to approx. 50 °C for 8-12 days. During this period alpha-acids are converted into iso-alpha-acids whereas beta-acids are not affected. Small residues of non-isomerised alpha-acids are present in iso-pellets and have to be considered for the application of this hop product in the brewhouse. Thus a method for the simultaneous determination of iso-alpha-, alpha- and beta-acids is necessary. The EBC Analysis Committee decided to test a combination of the methods EBC 7.7 (for sample preparation) and EBC 7.8 (for HPLC analysis). In the summer of 2003 a collaborative trial was carried out using five samples of isomerised hop pellets. Six members of EBC Analysis Committee and two members of AHA (Arbeitsgruppe Hopfenanalyse) participated in this collaborative trial.

2 Experimental

The organisation of the collaborative trial and the statistical treatment of the data were performed according to the international standard ISO 5725.

The five samples were circulated to eight participants. The participating laboratories were asked to determine the iso-alpha-acids, alpha- and beta-acids contents in each sample, in duplicate, and to record the result to two decimal places, according to the instructions given in the methods EBC 7.7 and EBC 7.8. The method EBC 7.7 was used for sample preparation. Two minor modifications were made. Firstly methanol, diethyl ether and hydrochloric acid were added to the hop sample at the same time and the whole mixture was then shaken for 40 minutes. Secondly the organic phase was diluted 1: 50 after phase separation. The method EBC 7.8 was used for HPLC determination. As an alternative for the HPLC column "Nucleosil" (currently recommended in the method EBC 7.8) Macherey-Nagel has recently developed a new column (Nucleodur 5-] 00 C18 ec, 125 x 4). This new column is highly suitable for the simultaneous determination of iso-alpha-, alpha- and beta-acids. However, the parameters given in the current version of the method EBC 7.8 for the "Nucleosil" column have to be modified. The following HPLC parameters were recommended:

□ Column: Nucleodur 5-100 C 18 ec, 125 x 4 (Macherey-

Nagel article no. 760001.40);

- Flow rate: 1 ml/min;
- Temperature: 35 °C;
- Detection: 270 nm / 314 nm;
- Eluent A: methanol;
- Eluent B: 750 ml methanol / 240 ml water / 10 ml ortho-phosphoric acid.
- Programme: 0 – 9 min 100 % B 270 nm
- 9 – 17 min 100% B 314 nm
- 17 – 25 min 65% B 314 nm
- 25 – 30 min 100 % B 314 nm

All participants used the new HPLC column "Nucleodur" according to these parameters. The international calibration standards ICS-II and ICE 2 were used for external calibration. The eight laboratories reported results on all five samples.

3 Results

The raw data returned by the participating laboratories are shown in Table 1. Graphical consistency testing using Mandel's h and k statistics were used in the statistical evaluation of the data to identify stragglers or outliers. The results of the statistical treatment are also indicated in Table 1. Based on the results of the statistical treatment, it was decided to omit the outliers from the calculation of the precision data.

The precision data using the rest of the analytical results are summarised in Tables 2-4. Repeatability r_{95} and reproducibility R_{95} depend on the mean of iso-alpha-acids in the range of 2,7 - 12,5 % m/m. For alpha- and beta-acids in isomerised pellets the precision figures do not depend on the mean.

Table 2 Summa of the precision data for iso-alpha-acids

	A	B	C	D	E
n	7	7	8	8	7
s_r^2	0,0033	0,0087	0,0240	0,0242	0,0245
S_L^2	0,0063	0,0109	0,1945	0,4743	0,4486
S_R^2	0,0096	0,0196	0,2185	0,4985	0,4731
m	2,69	6,1 6	1 0,04	11,92	12,47
r	0,16 0,26	0,43	0,44	0,44	
R	0,27 0,39	1,3 I	1,98	1,93	

r_{95} 0,030 m + 0,085

R_{95} 0,144 m

Table 1 Original data of the collaborative trial (unit % m/m)

	Sample				
	A	B	C	D	E
iso-alpha-acids					
laboratory 1	2,87 / 2,88°	6,15 / 6,28	10,06 / 9,97	12,68 / 12,21*	12,65 / 12,84
laboratory 2	2,66 / 2,60	6,20 / 6,21	9,81 / 9,92	12,19 / 12,22	12,41 / 12,47
laboratory 3	2,74 / 2,61	6,00 / 6,26*	9,78 / 10,09	12,51 / 12,22	12,40 / 12,89
laboratory 4	2,72 / 2,69	6,04 / 6,07	9,63 / 9,79	12,18 / 12,13	12,33 / 12,25
laboratory 5	2,70 / 2,57	6,18 / 6,00	9,26 / 9,14°	10,81 / 10,76°	11,23 / 11,14
laboratory 6	2,64 / 2,59	6,36 / 6,42	10,14 / 10,19	12,38 / 12,33	12,59 / 12,48
laboratory 7	2,75 / 2,68	6,04 / 6,02	9,82 / 10,21*	12,11 / 12,35	9,96 / 9,34°°
laboratory 8	2,71 / 3,3**	6,93 / 7,03°°	10,69 / 10,96°	10,75 / 10,88°	13,55 / 13,36
alpha-acids					
laboratory 1	0,56 / 0,55	0,00 / 0,00	0,38 / 0,39	0,30 / 0,19*	0,16/0,13
laboratory 2	0,55 / 0,52	0,00 / 0,00	0,43 / 0,43	0,30 / 0,28	0,22 / 0,23
laboratory 3	0,53 / 0,52	0,00 / 0,00	0,42 / 0,44	0,24 / 0,30	0,21 / 0,22
laboratory 4	0,52 / 0,48	0,06 / 0,04**	0,41 / 0,44	0,30 / 0,26	0,19/0,16
laboratory 5	0,49 / 0,53	0,00 / 0,00	0,39 / 0,40	0,29 / 0,31	0,12 / 0,14
laboratory 6	0,35 / 0,32°°	0,00 / 0,00	0,24 / 0,26°°	0,14 / 0,08°°	0,00 / 0,00°°
laboratory 7	0,56 / 0,58	0,00 / 0,00	0,46 / 0,49	0,35 / 0,36	0,17 / 0,15
laboratory 8	0,43 / 0,45	0,00 / 0,00	0,37 / 0,48**	0,20 / 0,18	0,21 / 0,21
beta-acids					
laboratory 1	3,25 / 3,24	5,13 / 5,00	3,39 / 3,38	6,38 / 6,21	4,20 / 4,05
laboratory 2	2,82 / 2,82	4,36 / 4,35	3,01 / 2,99	5,57 / 5,57	3,65 / 3,66
laboratory 3	2,87 / 2,83	4,52 / 4,66	3,06 / 3,06	5,97 / 6,03	3,72 / 3,92
laboratory 4	3,11 / 2,77	4,62 / 4,55	3,19 / 3,21	5,92 / 5,89	3,78 / 3,74
laboratory 5	3,11 / 2,53**	4,74 / 4,84	3,10 / 3,15	5,59 / 5,72	3,74 / 3,64
laboratory 6	2,84 / 2,77	4,41 / 4,48	3,04 / 3,04	5,59 / 5,51	2,81 / 2,93
laboratory 7	3,16 / 3,11	4,41 / 4,36	3,16 / 3,29	6,00 / 6,10	0,98 / 0,80°°
laboratory 8	2,72 / 2,80**	5,19 / 5,00	6,57 / 7,04°°	2,81 / 2,64°°	4,04 / 4,05

* Mandel's k statistic straggler; ** Mandel's k statistic outlier; ° Mandel's h statistic straggler; °°Mandel's h statistic outlier

Table 3 Summa of the precision data for alpha-acids

	A	B	C	D	E
n	7	8	6	7	7
s_r^2	0,00036	0,00000	0,00020	0,00130	0,00020
S_L^2	0,00163	0,00000	0,00091	0,00190	0,00128
S_R^2	0,00199	0,00000	0,00111	0,00320	0,00148
m	0,52	0,00	0,42	0,28	0,18
r	0,05	0,00	0,04	0,10	0,04
R	0,13	0,00	0,09	0,16	0,11
r_{95}			0,05		
R_{95}			0,10		

Table 4 Summa of the precision data for beta-acids

	A	B	C	D	E
n	7	8	7	7	7
s_r^2	0,0094	0,0059	0,0015	0,0048	0,0063
S_L^2	0,0293	0,0821	0,0170	0,0758	0,1648
S_R^2	0,03870	0,08800	0,01850	0,08060	0,17110
m	2,94	4,66	3,15	5,86	3,71
r	0,27	0,22	0,11	0,19	0,22
R	0,55	0,83	0,38	0,79	1,16
r_{95}			0,20		
R_{95}			0,74		

4 Conclusion

The EBC Analysis Committee judged both the repeatability and reproducibility values obtained in the collaborative trial to be

acceptable, and approved the inclusion of this method for determination of iso-alpha-acids, alpha- and beta-acids in isomerised hop pellets by HPLC in Analytica-EBC. #