A-73

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Variation of bitter substances from hop pellets during storage

Chunfeng Liu[1,2] Yunyun Zhou[1,2] Yongxian Li[1,2] Xiangsheng Yin[3] Qi Li*[1,2]

1 Key Laboratory of Industrial Biotechnology, Ministry of Education, Jiangnan University, Jiangsu Province, P. R. of China; 2 Lab of Brewing Science and Technology, Jiangnan University, Wuxi, 214122, Jiangsu Province, P. R. of China. 3 Cargill Malt, McGinty Road West, MS 135, Wayzata, MN 55391 USA

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Targets

The method of detecting iso-α-acid, α-acid, and β-acid simultaneously by HPLC technology was established in this study. Through analyzing the change of iso-α-acid, α-acid, and β-acid during storage process, a new evaluation index for hop freshness degree was obtained. This new index was used to evaluate the freshness of aging hops caused by different temperature, which displayed a good prospect of application.

Detection method of hop bitter substances

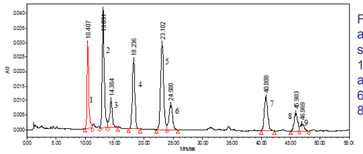

Fig. 1 Chromatogram of α-acid ,β-acid and iso-α-acid in Hop extracts standard samples.
1. iso-co-α-acid 2. iso-α-acid 3. iso-ad-α-acid 4.cohumulone 5.humulone 6.adhumulone 7.colupulone 8.lupulone 9.adlupulone

Table 1 Results of spike recovery

Hop samples	index	Background values(mg/L)	Addition (mg/L)	Detection value(mg/L)	Recovery(%)	RSD (%)
Tsingdao Flower	iao-α-acid	0	10	10.5	105.2	2.75
			100	90.6	90.6	2.96
			200	181.6	90.8	2.34
Marco Polo	iso-α-acid	0	10	10.9	109.5	2.89
			100	89.9	89.9	2.34
			200	183.1	91.5	2.15
Tsingdao Flower	α-acid	334.8	20	380.9	107.4	1.54
			200	565.7	105.8	1.87
			400	766.3	104.3	1.98
Marco Polo	α-acid	673.2	20	759.2	109.5	1.79
			200	948.7	108.6	2.01
			400	1146.4	106.8	1.95
Tsingdao Flower	β-acid	245.8	20	269.1	101.2	1.96
			200	434.8	97.5	2.13
			400	625.6	96.9	2.09
Marco Polo	β-acid	189.6	20	214.4	102.3	1.95
			200	395.1	101.4	2.34
			400	575.7	97.6	2.02

Qualitative analysis of aged hops by HPLC-MS

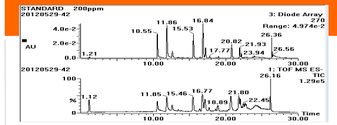

Fig. 2 RP-HPLC (a) and total negative ion current chromatography (b) of α-acids, β-aicds and iso-α-aicds standard samples

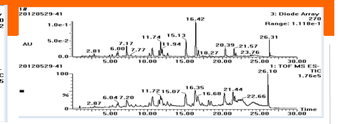

Fig. 3 HPLC (a) and total negative ion current chromatography (b) of ageing hops extract

Table 4 Data of hops extract

Peak	Retention time (min)	Wave length (nm)	mass-to-charge ratio(m/z)	Compounds
1	10.6	270	347	iso-co-α-acid
2	11.7	270	361	iso-α-acid
3	12.6	270	361	iso-ad-α-acid
4	15.1	315	347	cohumulone
5	16.3	315	361	humulone
6	16.7	315	361	adhumulone
7	20.4	315	399	colupulone
8	21.3	315	413	lupulone
9	21.6	315	413	adlupulone

Separation of bitter substances was achieved using a EC 250/4 Nucleosil 100-5 C18 column (250 mm×4.6 mm). The mobile phase were aqueous solution (containing 0.1% phosphoric acid and 0.2 mM EDTA·2Na; solvent A) and acetonitrile (solvent B) at the flow rate of 1 ml/min with gradient elution program for 50 min. The method is with high accuracy and low detection limit, and the average recovery of α-acids, β-acids and iso-α-acids is 95.0-105.0%.Nine main analogues of the α-acids, β-acids and iso-α-acids were separated simultaneously, fast, and effectively.

Bitter substances in hop pellets during storage

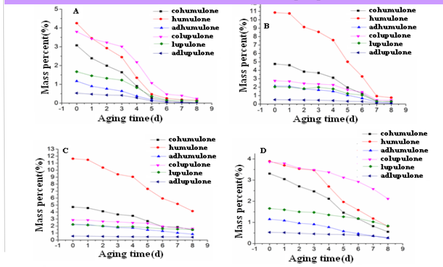

Fig.4 Variation of main homologues of α-acid and β-acid of hops during forced ageing process (50℃). A-Tsingdao flower; B-Marco Polo, C-Columbus, D-Mixed pellets.

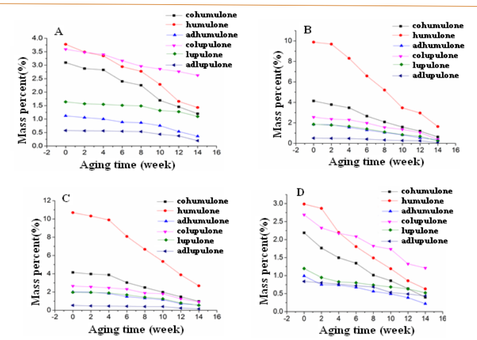

Fig. 5 Variation of main homologues of α-acid and β-acid in hops during forced ageing process (30℃). A-Tsingdao flower; B- Marco Polo, C-Columbus, D-Mixed pellets.

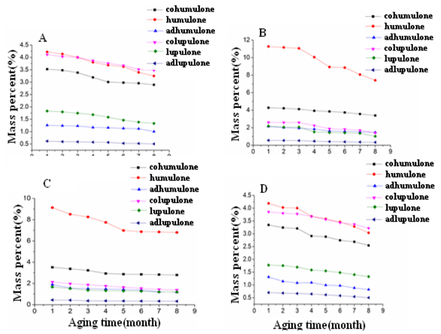

Fig. 6 Variation of the main homologues of α-acid and β-acid of hops during hop storage(-4℃). A-Tsingdao flower; B-Marco Polo, C-Columbus, D-Mixed pellets.

The content of α-aicds in Tsingdao Flower, Marco Polo, Columbus and Mixed Pellets decreased by 8.05%, 12.24%, 12.02% and 5.12% respectively after aging for 6 days at 50 ℃, decreased by 3.80%, 9.99%, 8.35% and 4.86% after aging for 12 weeks at 30 ℃, and decreased by 1.42%, 5.41%, 3.64% and 2.44% after storage for 8 months at -4 ℃ (Fig.4-6). These differences probably could be contributed to the distribution of hop components, such as the ratio of α-aicd and β-acid, the ratio of humulone and adhumulone and the content of cohumulone.

Hop storage index & degredation degree

The variation of hop freshness was well described by HFI (Hop Freshness Index, $HFI=M_{humulone} \times M_{\beta\text{-acid}} / M_{\alpha\text{-acid}}$). The indicator is from aicds content of hops, compared HSI value and the hop degradation degree, it shows the HFI can evaluate different varieties of hops more accurate. The indicator is used to evaluate the hop ageing caused by different temperature and has a good prospect of application.

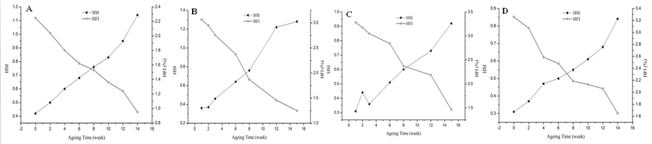

Fig.7 Variation of HSI and HFI of hops during forced ageing process under 30℃. A-Tsingdao flower; B- Marco Polo, C-Columbus, D-Mixed pellets.

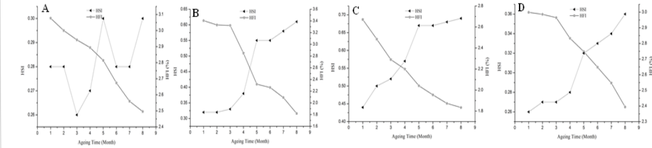

Fig.8 Variation of HSI and HFI of hops during storage under -4℃. A-Tsingdao flower; B-Marco Polo, C-Columbus, D-Mixed pellets.

Conclusion

► A convenient and accurate method was proposed for the determination of α-acids, β-acids and iso-α-acids simultaneously in hops using RP-HPLC.

►The results showed that the content of α-aicds and β-acids decreased constantly during storage, wherein the variation of humulone content was the most obvious. The degradation rate of the different varieties of hops was very different.

► Compared to HSI value and the hop degradation degree, it shows the HFI can evaluate different varieties of hops more accurately.