CHARACTERISATION OF SLOVENIAN HOP (*Humulus lupulus* L.) VARIETIES BY ANALYSIS OF ESSENTIAL OIL

Andreja ČERENAK[8], Martin PAVLOVIČ[9], Monika OSET LUSKAR[10], Iztok Jože KOŠIR[11]

UDC / UDK 633.791:665.52:543.635(045)
original scientific article / izvirni znanstveni članek
received / prispelo: 10. 10. 2011
accepted / sprejeto: 29. 11. 2011

Abstract

The Slovenian hop is known in Slovenia and internationally for its fresh, hoppy aroma. The aim of this research was to characterize our four hop varieties - Savinjski golding, Aurora, Dana, 31/299 and breeding line A6/58 based on compounds of essential oils which give a distinctive type of odour. This preliminary study revealed that all the varieties included have a similar type of odour.

Key words: hop, essential oil, characterization, odour

KARAKTERIZACIJA SLOVENSKIH SORT HMELJA (*Humulus lupulus* L.) Z ANALIZO ETERIČNEGA OLJA

Izvleček

Slovenski hmelj je poznan v Sloveniji kot tudi mednarodno po sveži hmeljski aromi. Cilj raziskave je bilo okarakterizirati štiri slovenske sorte hmelja - Savinjski golding, Aurora, Dana, 31/299 in križanca A6/58 na osnovi komponent eteričnega olja, ki dajejo hmelju različen vonj. Preliminarna študija je pokazala, da imajo vse vključene sorte hmelja soroden tip vonja.

Ključne besede: hmelj, eterično olje, karakterizacija, vonj

[8] PhD, Slovenian Institute of Hop Research and Brewing / Inštitut za hmeljarstvo in pivovarstvo Slovenija, Cesta Žalskega tabora 2, 3310 Žalec, Slovenija, e-mail: andreja.cerenak@ihps.si
[9] PhD, the same address, e-mail: martin.pavlovic@ihps.si
[10] B.Sc., the same address, e-mail: monika.oset-luskar@ihps.si
[11] PhD, the same address, e-mail: iztok.kosir@ihps.si

1 INTRODUCTION

Hops, cones of the hop plant (*Humulus lupulus* L.), are used in brewing beer to add bitterness and aroma. The quality of the final product – beer – depends on the hop variety used in the process. Those breweries that still use traditionally aromatic hops tend to buy hops of a known variety and origin. Traditional classification of hop cultivars divides them into three groups: (I) high alpha-acid, (II) intermediate alpha-acid and (III) aroma (noble) varieties.

In order to remain competitive, hop breeders must respond to the ever-changing needs of the brewing community by providing suitable new varieties. Slovenia traditionally produces its own European aroma hops, comprising approximately 3% of world hop production.

Dry hop contains 0.5-2.0% of essential oil. Hop essential oils consist of a large number of different components and although their composition is influenced by the environment, it is a good indicator of the genetic variability among different hop accessions (Kovačevič and Kač, 2011). The chemical composition of hop oil is conventionally described as a mixture of hydrocarbon compounds, oxygenated compounds and sulphur-containing compounds (Sharpe and Laws, 1980).

In contrast to the resins, the essential oil of hops has received little attention. There is not much information available about the effect of hop oil on beer flavour, although a great deal is known about the chemical composition of the oil (Sharpe and Laws, 1980). Moreover most of the data are dispersed widely through the literature. Quite a lot of research work using essential oil analysis was done to identify the hop varieties (Kenny, 1990; Kralj et al., 1991; Kovačevič and Kač, 2001; Kovačevič and Kač, 2002; Shellie et al., 2009).

In this study, specific essential oil components from three new hop aroma genotypes , i.e. two new cultivars Dana and 31/299 (with proposed name Styrian gold) and a breeding line A6/58, were compared with traditional Slovene varieties Savinjski golding and Aurora. The comparison of compounds was done based on analysis suggested by Whittock and Koutoulis (2011) of Australian aroma varieties.

2 METHODS

All hop samples were picked at the time of their technological maturity (end of August / beginning of September) on the experimental farm of the Slovenian Institute of Hop Research and Brewing in Žalec, and from the fields of two hop

farmers in Prekopa and Turiška vas during the harvest season of 2010 and 2011, respectively.

The hop fields of all varieties were cultivated with good agronomic practises at each location. Altogether, six samples of each variety were included in the research.

The moisture and essential-oils content was determined using standard Analytica-EBC methods 7.2 and 7.10 (Analytica-EBC 2007). The composition of the essential oils was determined according to standard Analytica-EBC method 7.12 by gas chromatography (GC) on Agilent GC chromatograph series 6890, equipped with a flame ionization–detector (FID).

3 RESULTS AND CONCLUSION

The compounds that were qualified using GC-mass spectrometry (GC-MS) included esters (one thioester), ketones and alcohols (monoterpine and acyclic sesquiterpene alcohols) in the oxygenated group, and mono- and di-terpenes, cyclic monoterpenes, sesquiterpenes and byclic sesquiterpenes. The oxygenated compounds present in hop essential oil tend to provide fruity and floral aromas (Murray et al, 1987). Esters and ketones are identified with fruity odours, while the alcohols tend to be identified with floral odours. Terpenes (myrcene, humulene, farnesene, and caryophyllene) and their oxides (humulene epoxides I and II) are associated with citrus (limonene), herbal and spicy/woody odours. The relative composition of essential oil data for 19 compounds (87-91 % of the total essential oil) identified as having fruity, floral, citrus, herbal and spicy/woody aromas are presented in Table 1.

The amount of essential oil relative to alpha acids (μL/g alpha-acid) is an important metric when determining the dosing of aroma hops. Dana has an alpha-acid content of 13.5 % on average and 3.15 mL of essential oils on average. However, essential oil levels relative to the alpha-acid in Dana are 233 μL/g alpha-acid. Therefore, with careful dosing, it should be possible to use high alpha-acid flavour hops (such as Dana) without unduly affecting the bitterness profiles of the beer being produced.

Table 1: Summary of 19 essential oil compounds, connected with a distinct odour in four Slovenian grown varieties and one breeding line, expressed as an average of all the samples included. All data are in relative %.

Preglednica 1: Povzetek 19 component eteričnega olja, povezanih s specifičnim v onjem, pri 4 slovenskih sortah in enem križancu, izraženem v povprečju vseh vključenih vzorcev. Vsi podatki so v rel. %.

Genotype	**Savinjski golding**	**Aurora**	**Dana**	**31/299**	**A6/58**
Essential oil components					
Fruity					
isobutil isobutyrate	0,01	0,00	0,01	0,01	0,00
2-nonanone	0,17	0,38	0,22	0,24	0,20
methyl nonanoate	0,00	0,00	0,00	0,00	0,00
2-undecanone	0,39	1,34	0,49	0,29	0,31
methyl decadienoate	0,61	0,48	0,27	0,79	0,35
Sum of fruity	***1,18***	***2,20***	***0,98***	***1,32***	***0,86***
Floral					
linalool	0,60	0,75	0,63	0,30	0,72
geraniol	0,09	0,19	0,15	0,32	0,07
Sum of floral	***0,69***	***0,94***	***0,77***	***0,62***	***0,79***
Citrus					
limonene	***0,16***	***0,19***	***0,20***	***0,22***	***0,20***
Herbal					
beta pinene	0,50	0,51	0,53	0,80	0,74
beta selinene	0,22	0,12	0,10	0,16	0,26
alpha selinene	0,65	1,11	0,58	0,51	0,58
gamma cadinene	1,30	0,90	1,03	1,13	0,86
delta cadinene	0,12	0,09	0,10	0,31	0,19
humulene epoxide I	0,53	0,24	0,13	1,04	0,20
humulene epoxide II	0,19	0,56	0,52	0,09	0,25
Sum of herbal	***3,51***	***3,52***	***3,00***	***4,03***	***3,09***
Spicy/Woody					
myrcene	41,61	51,36	56,76	44,88	52,05
caryophyllene	8,67	5,76	6,59	9,03	6,26
beta farnesene	5,23	5,98	6,47	5,30	15,62
humulene	26,40	17,78	16,54	21,69	12,21
Sum of spicy/woody	***81,91***	***80,87***	***86,36***	***80,90***	***86,13***
% oil accounted for	***87,45***	***87,72***	***91,31***	***87,09***	***91,07***

Linalool, considered a positive indicator of hop quality, is included as a compound with floral aroma, but it is not clear that levels of free linalool in the essential oil of hops have a direct relationship with levels of linalool in beer. There is evidence that free linalool in beer is degraded rapidly, and that glycosidically-bound linalool is responsible for most of the detectable linalool in beer (Kollmannsberger et al., 2006). Whether levels of free linalool in the essential oil of a particular hop are related to the levels of glycosidically-bound linalool in any variety is not understood. With regard to levels of free linalool in the varieties included in this study, the lowest levels were observed in the 31/299 and the highest in the Aurora (Table 1).

In measuring the levels of geraniol, which gives the hop its floral odour, 31/299 has reached the highest level.

Limonene was the only compound which was determined in the category of citrus odour, but even other compounds, such as linalool, geraniol and farnesene, may contribute in the perception of citrus odour or flavour. Relatively high levels of limonene were observed in all varieties, with the lowest found in Savinjski golding. Comparing the genetically close varieties Savinjski golding and 31/299 (a progeny of Savinjski golding), 31/299 can exceed relatively higher levels.

Comparing to the results from Australian researchers Whitock and Koutoulis (2011), Australian varieties have much higher components of herbal odours (e.g., alpha and beta selinene) than the Slovenian hops included in our research. In general, the results of our experiment show that all the varieties tested offer a small degree of the components giving herbal odour.

The highest level of compounds with fruity odours was found in the samples of Aurora, but without any characteristic difference. In general, the essential oils of all tested genotypes do not have a great deal of the compounds with fruity character. It is interesting that methyl nonanoate was not found in any of samples collected.

However, compounds providing a spicy/woody character make up the highest proportion of the essential oils in our varieties. It is difficult to see much of a pattern in the distribution between varieties of these compounds, due to the dominance over this fraction by myrcene (Table 1). Because of its volatility, myrcene is likely to be present only in beer which has been dry hopped. Relatively high levels of spicy/woody odour compounds were seen in Dana and A6/58, following by the other three varieties tested.

In conclusion, we would point out that the composition of the odour compounds for all the Slovenian varieties tested and disclosed in this article are very similar. They all have a very strong spicy/woody character.

As might be predicted, the Slovenian hop breeding program has developed varieties with a more or less similar odour which gives a very pleasant, hoppy aroma to the beer. In this research, we selected only 19 compounds from the broad spectrum of oil components which combine to give the complete odour and taste to the beer. In the future, in our breeding program, we will look for new, different proportions of essential oil compounds to give distinct aroma and taste to the beer.

Acknowledgement
Supported by the project *DMCSEE* (South East Europe program) and by the project *Hop industry lifelong learning program* (Leonardo da Vinci, Transfer of Innovation program).

4 REFERENCES

Analytica-EBC. 2007. Section 7 – Hops, Methods 7.2, 7.7, 7.10, 7.12; Carl, Getränke-Fachverlag, Nürnberg.

Kenny, S.T. 1990. Identification of U.S.-Grown Hop Cultivars by Hop Acid and Essential Oil Analyses. American Society of Brewing Chemists Journal, 48, pp. 3-8.

Kollmannsberger, H., Biendl, M. and Nitz, S. 2006. Occurence of glycosidically bound flavour compound in hops, hop products and beer. Monatschrift fur Brauwissenschaft, 59, pp. 83–89.

Kovačevič, M. and Kač, M. 2001. Solid-phase microextraction of hop volatiles. Potential use for determination and verification of hop varieties. Journal of Chromatography A, 918, pp. 159–167.

Kovačevič, M.,. and Kač, M. 2002. Determination and verification of hop varieties by analysis of essential oils. Food Chemistry, 77, pp. 489–494.

Kralj, D., Zupanec, J., Vasilj, D., Kralj, S., and Pšeničnik, J. 1991. Variability of essential oils of hops, *Humulus lupulus* L. Journal of the Institute of Brewing, 97, pp. 197–206.

Murray, J.P., Westwood, K., and Daoud, I. 1987. Late hop flavour. 21st Congress of the European Brewing Convention, Madrid, Spain, pp. 321–328.

Sharpe, F.R. and Laws, D.R.J. 1980. The essential oil of hops: A review. Journal of the Institute of Brewing, 87, pp. 96–107.

Shellie, R.A., Poynter, S.D., Li, J., Gathercole, J.L., Koutoulis, A. 2009. Varietal characterisation of hop (*Humulus lupulus* L.) by GC-MS analysis of hop cone extracts. Journal of Separation Science, 32, pp. 3720–3725.

Whitock, S., and Koutoulis, A. 2011. New hop (*Humulus lupulus* L.) aroma varieties from Australia. Proceedings of the Scientific Commission, International Hop Growers' Convention I.H.G.C. Lublin, Poland, pp. 10–13.