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Evaluation of Volatile Compounds from Hüller Bitterer Variety Grown in Romania by Chemometric Methods

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Abstract

The composition of volatile oil from Hüller Bitterer variety grown in Romania, was presented in our previous study. In this study chemometrics methods were applied for highlighting the statistical correlations existing between the genotype (variety), chemotype (composition), and phenotype (phenophase of cone development) in accordance with essential oils investigated. The essential oils content of hop cultivar was significantly dependent on the phenophases of the cones and types of samples (cones and pellets). Using the cluster analysis there have been highlighted major and minor volatile compounds present in hop variety. Principal component analysis provided the possibility of monitoring the accumulation of essential oil during the agricultural year, and at the same time, the differentiation of the phenophases of variety.

Keywords: hop cones, essential oil, *Humulus lupulus*, cluster analysis, principal component analysis

1. Introduction

Over centuries, hop (*Humulus lupulus* L.) is used as an essential ingredient in the manufacturing of beer since its components add the typical bitter taste and contribute to the attractive aroma of the final beverage [7]. Beer is one of the world's most popular beverage products. Beer is all natural ingredients, so moderate consumption contributes to a healthy daily diet [15].

Inflorescences contain glandular structures (lupulin gland) which secrete a powder, lupulina, rich in secondary metabolites: hop resins, polyphenolic compounds, essential oils and other related [2].

The iso- α -acids originating from hop resins are predominantly responsible for bitterness, whereas a number of compounds in the essential oil are responsible for imparting hoppy odor and aroma to beer [6]. Because the composition of hop oil contributes to the aroma of beer, the essential oil profile of hop samples contains valuable information for brewers [5]. The composition varies depending on: intrinsic and extrinsic factors during growth, processing conditions, and the extraction method used to isolate the essential oil [6].

Methods for characterization of hop varieties include analyses of bitter acids and essential oils, followed by comparison of the chemical composition, particularly

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chromatographic profiles [9]. Gas chromatography and mass spectrometry was successfully employed for identification and quantification of volatile compounds from different matrices [1, 3, 4, 8, 31, 39].

Extensive studies on the characteristics and composition of hops were conducted in Romania, in Hop Culture Research Center and Medicinal Plants of the University of Agricultural Science and Veterinary Medicine Cluj-Napoca [10, 11, 12, 13, 14, 15, 16, 27, 32, 33, 34, 35] and applications of herbal hop compounds [19, 20, 22-24, 25, 26]. The volatile oil from some Romanian hops varieties was characterized regarding the composition of volatile oil to emphasize t21e differences between the different varieties of hops cultivated in our country [37].

The volatile compounds extracted and separated from Hüller Bitterer variety using the ITEX/GC-MS technique, was presented in our previous study [26]. Using this technique, 60 volatiles were separated from hop cultivars, with 50 of them being identified. In-tube extraction (ITEX) technique requires no or minimal sample preparation, allowing a simple, efficient and rapid enrichment of volatile or semi-volatile compounds during the headspace extraction [18,28,30] and can be coupled with GC-MS for further separation and identification of compounds. ITEX-GC/MS method is suitable for the determination of volatile compounds from hop samples and the results obtained by this method can be used for discrimination of hop varieties by chemometrics analysis [21].

The objective of the present investigation was to characterize the Hüller Bitterer variety grown in Romania by volatile compounds content using statistical analysis.

2. Materials and methods

2.1. Plant material

Samples from Hüller Bitterer (HB), classified as aroma (low α-acid), were collected in 2011 year crops. The hop cultivar was cultivated in the climatic areas of Transylvania, in the Seleuş farms from Mureş County. The female hop inflorescence (cones) were picked during three phenophases of development (end of August/beginning of September). In phenophase I (F I), the cones are small-scale, measuring between 2-2,5 cm. This phenophase coincide with the period of shaping of the cone and the lupulin gland. In the phenophase II (F II) cones have medium size measuring approximately 3cm and the lupulin glands are fully formed being in full ripeness. In the last phenophase (F III), cones have the technological maturity to be harvested measuring 4 cm. The cones were dried for 48 hours, in a cool dark place for the conservation of the active principles. The pellets (P) samples were obtained from the pelletization station. All hop samples were labeled and stored until the analysis at -20ºC.

2.2.Essential oil extraction and ITEX/GC-MS analysis

The parameters of ITEX/GC-MS technique, as well as the volatile composition of Hüller Bitterer variety, was described in our previous study [26].

2.3. Statistical analysis

For the characterisation of the hop variety, the obtained chromatographic matrix (Salanță et al., 2015) was subjected to cluster analysis (CA) with the Euclidean distances and principal component analysis (PCA) with cross-validation. Chemometric analyses were performed using Matlab (version 7.2.0232/2006) and Unscrambler X software Version 10.1 (CAMO Software AS, Oslo, Norway).

3. Results and discussion

Using ITEX/GC-MS technique, 60 volatiles were separated from hop cultivars (fig.1), with 50 of them being identified.

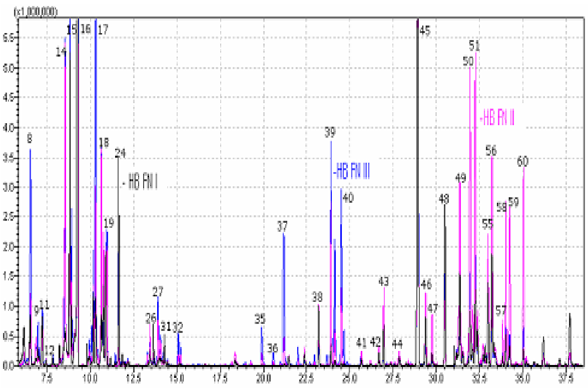

Figure 1. The overlapping chromatograms of Hüller Bitterer hop cones during phenophases

The most important volatile compounds found in Hüller Bitterer hop variety belonging to the monoterpenes and sesquiterpenes classes are represented by: β-myrcene, β-caryophyllene and α-humulene [25,26].

The dendrograms obtained for the major volatile compounds identified and quantified in the experimental year 2011 (fig.2 A-D), reveal as a major compound β-myrcene for samples of dried cones and hop pellets, and β-caryophyllene for samples of volatile oil extracted from dried cones and pellets; at the opposite pole is linalool for all samples. Due to its abundance, myrcene is important for the odor of fresh hop essential oil. Linalool and geraniol have been determined to be important odorants contributing to the floral character of hop essential oil and beer [6].

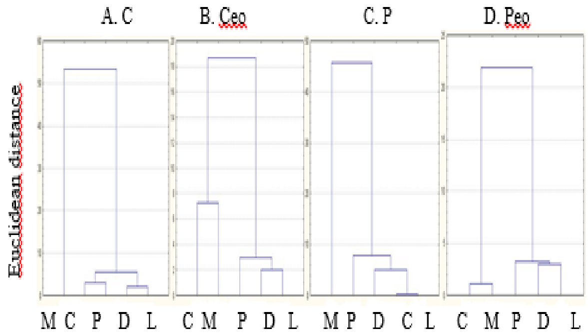

Figure 2. Dendrograms of major volatile compounds identified in samples of Hüller Bitterer variety (M-β myrcene, P-β pinene, D-limonene, C- β-caryophyllene, L-linalool)

Note: „C"-hop cones; „Ceo"-essential oils from hop cones; „P"- pellets; „Peo"- essential oils from pellets

In case of minor components (fig.3, fig.4) were identified four groups of volatile compounds, with Euclidean distance between the groups relative higher. Predominant compounds are represented by 2-penthanol propanonate, 2-methyl-3-methylbutyl propanoate, γ-elemene, β-*trans*-ocimene and β-phelandrene, while α-cubebene and ylangene belong to the category of those identified in a minimum quantity.

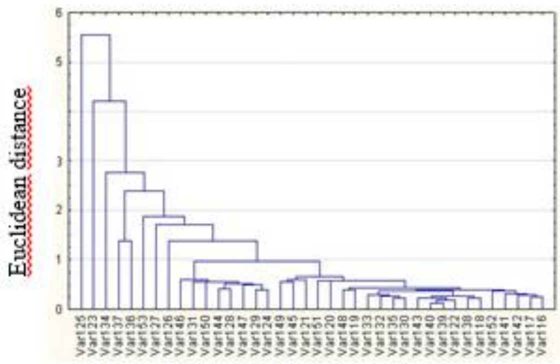

Figure 3. Dendrogram of minor compounds found in Hüller Bitterer hop variety-cones samples

Note: methyl heptanoate (var 125), 2-methyl-methylbutyl-propanoate (var 123), n.i.-not identified (var 134), methyl nerolate (var 137), 4-methyl -decanoate (var136), γ-elemene (var 153), beta-*trans*-ocimene (var 127), β-phelandrene (var 126), β-selinene (var 146), methyl -octanoate (var 131), γ -cadinene (var 150), n.i. (var 144), β-*cis*-ocimene (var 128), γ-selinene (var 147), 6- methyl-heptanoate (var 129), 2-methyl-3-methylbutyl propanoate (var 124), delta-cadinene (var 149), γ-murolene (var 145), α-pinene (var 121), β-guaiene (var 151), 1,3-nonadiena (var 120), α-amorfene (var 148), methyl hexanoat (var 119), n.i. (var 133), methyl -nonanoate (var 132), 4-methyl decanoate (var 135), 3-carene (var 130), aromadendrene (var 143), ylangene (var 140), α-cubebene (var 139), 2-pentanol propanoat (var 122), methyl -decanoate (var 138), 3- methyl-1-butanoate (var 118), n.i. (var 152), copaene (var 141), n.i. (var 142), 2-methylpropyl propanoate (var 117), 2-hexenale (var 116)

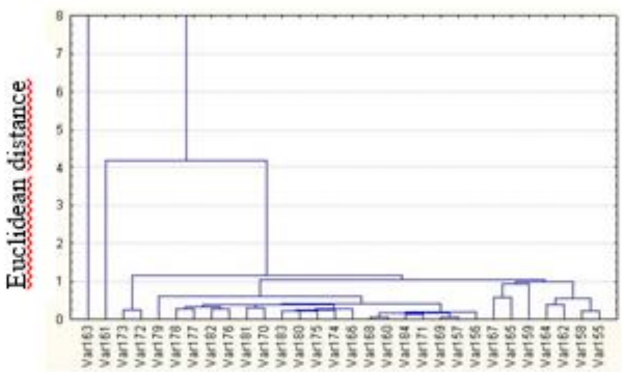

Figure 4. Dendrogram of minor compounds found in Hüller Bitterer variety- pellets samples

Note: 2-methyl-3-methylbutyl-propanoate (var 163), 2-penthanol propanoate (var 161), copaene (var 173), ylangene (var 172), α-guaiene (var 179), β-selinene (var 178), γ -murolene (var 177), delta-cadinene (var 182), germacren D (var176), γ-cadinene (var 181), 3-carene (var 170), β-guaiene (var 183), α-amorfene (var 180), α-humulene (var 175), aromadendrene (var 174), β-*trans*-ocimene (var 166), γ -terpinene (var 168), tiophivalic acid (var 160), γ-elemene (var 184), metyl-octanoate (var 171), metyl 6-metyl heptanoate (var 169), metyl hexanoate (var 157), 3-metyl-1-butanoate (var 156), β-*cis*-ocimene (var 167), β-phelandrene (165), α-pinene (var 159), metyl heptanoate (var 164), 2-metyl-3-methylbutyl propanoate (var 162), 1,3-nonadiene (var 158), 2-metylpropil propanoate (var 155)

Using cluster analysis there have been highlighted both major and minor volatile compounds present in hop variety and their evolution in the samples analyzed (cones, pellets and volatile oils). The dendrograms of the major compounds have the same classification in the case of all types of samples, resulting in a group of similarity. The major compounds found in all samples are represented by: β-myrcene, β-caryophyllene and β-pinene.

By analyzing the dendrograms of minor compounds from cones and pellets samples it can be observed a difference between the samples of dry hop cones and pellets, because of the increasing Euclidean distance, which implies changing qualitative markers during the processing of hops and aggressive drying in specialized installations.

The first two principal components explained 85% of the variance of data, showing a good discrimination between phenophases, as presented in the PCA bi-plots (fig. 5). The composition of the samples of volatile cones is well differentiated from the volatile composition of the essential oil extracted by hydro distillation. Also, it can be seen a similitude between the phenophase III and pellets sample.

In order to visualise more clearly the importance of each variable, the correlation loadings plot (fig. 6) was computed. The compounds from the inner ellipse indicate 50% of the explained variance, whereas those found in the outer ellipse (e.g. 6-1,3-*trans*-nonadiene, 13 - β-myrcene, 18 - limonene, 19 – β - phelandrene, 21 – beta – *cis* - ocimene, 38 - ylangene, 39 - copaene, 4 - caryophyllene) indicate 100% of the explained variance.

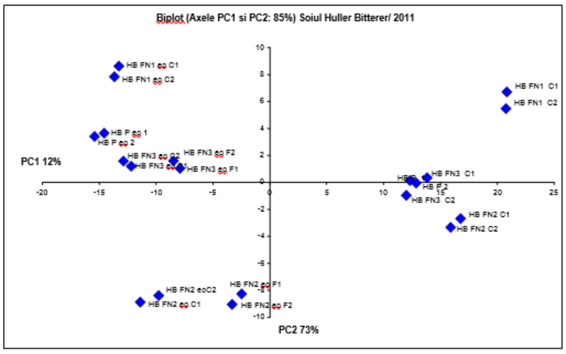

Figure 5. Principal components analysis bi-plots of volatile compounds

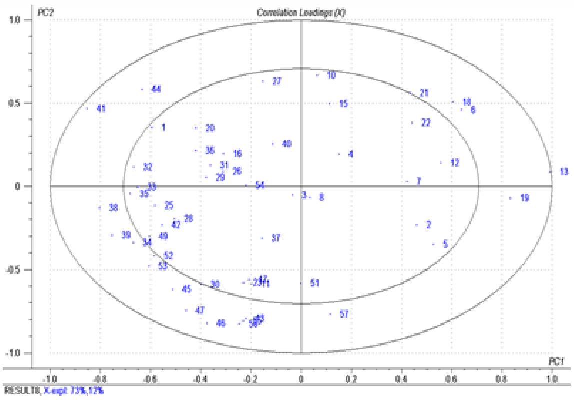

Figure 6. Correlation loading bi-plot for the hop cultivar analysed. The numbering of the compounds refers to Table 1 presented in previous study (Salanţă et al., 2015)

By using PCA analysis it was noted that the volatile profile varies according to the type of analyzed sample (cones, pellets or essential oil) leading to a good differentiation between the phenophases and the type of the sample studied, our result can be useful in the optimal harvesting period.

Conclusions

Chemometric analysis is a useful tool for interpretation of the results, therefore the Principal Components Analysis (PCA) provided the possibility of monitoring the accumulation of essential oil during the agricultural year, and at the same time, the differentiation of the phenophases of variety. Also, statistical processing of data provided essential information for the growers of hops on the influence of the pelletization process on the content of essential oil.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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