Bulletin UASVM Agriculture, 67(2)/2010
Print ISSN 1843-5246; Electronic ISSN 1843-5386

The Varietal Classification of Hops Products by Chemometrics Method

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Abstract: The varietal clasification of hop products according with Romanian and European regulations is done in several groups according with their use in the brewing industry as aroma and bitter hops. The clasification is done according with some chemical parameters, but these do not garantee that the product is a pure variety or is a blanding. Using chemometric method as cluster analysis, clasification according with chemical description of hop products the clasification can be predicted more precisely.

Keywords: chemometrics, hop resins, α acids, β acids, hop characterization, cluster analysis.

INTRODUCTION

The hop plant (*Humulus lupulus* L.) is widely cultivated throughout the temperate zones of the world for its female inflorescences (commonly referred to as 'cones'), which are used in the brewing industry to add bitterness, aroma and flavour to beer.

The number of registered hop varieties increases all the time. The assortment of cultivated hops conforms to changes in brewing technology and to economics of production process (Krofta, 2003). Market varieties of hops are classified to the groups according to their use in the brewing industry parallelly with increasing knowledge of the composition of hop resins and other secondary metabolites (Forster and Schmidt 1993). An up-to-date classification scheme was worked out by Vent and Vent (1999). According to this scheme hop varieties are sorted into four groups – fine aroma, aroma, bitter (dual-purpose) and high-alpha ones.

The chemical composition of hops, for the same variety is influenced by soil characteristics, climatic characteristics of the culture, plant protection treatments applied. Hop flowers are produced in various products such as pellets, hop extracts and volatile oils, mainly used in the brewing industry. The international hops trade liberalization forced most growers of hop to process cones to ensure conservability of active principles, reducing the volumes of transport, in order to standardize dosing easier and ensure consistency of quality of the finished beer. The chemical composition of products depends on the hop variety, processing technology adopted and the performance of processing equipment Production and marketing of hops in Romania is regulated by Law no. 627/2002. According to this law the hops harvested and processed in Romania have to undergone to certification procedure before to be sold. Designation of origin certificate can be issued only in areas recognized for hops harvested production or preparation and the hop varieties have to belong to the Community Catalog and/or official list of varieties of Romania, with quality standards. Certificate of

origin of hops is an important tool for traceability of beer. First and most important link is the farmer that is required to improve the quality of hops by monitoring quality parameters on the flow of processing and identification of criteria to variety authentication. Romanian legislation provides for minimum quality hop criteria. Increased competitiveness of hop production in Romania in comparison with the European Community can be achieved only by proof quality. Romanian manufacturer must certify that the goods in terms of quality are at least equivalent to the minimum trading limits adopted for like products harvested within the European Union.

The aim of the study was to evaluate the chemical composition of four romanian hop cultivars and clasification by cluster analysis.

MATERIALS AND METHODS

The study of the chemical composition was carried on for four varieties of hop: Magnum (MG), Brewers Gold(BG), Hüller Bitterer(HB) and Perle(PR), cultivated in pedo-climatic areas from Transilavenia, in the Saschiz and Seleuş farms from Mureş. The cultivars from Romania are used exclusively for the production of beer. The hop flower (cone) is harvested, dried for the conservation of the active principles, grinded and pressed in granules, named pellets. The hop pellets are the raw material for the biotechnology of beer.

For the traceability study of the biological active substances, on the technological flow of processing the hop pellets, were used hop flower samples at their technological maturity (PR-F; HB-F; BG-F; MG-F), dried hop samples from the drying installations from Saschiz and Seleuş farms (PR-U; HB-U; BG-U; MG-U), and also pellets type 90 obtained in the pelletisation installations, from Seleuş farms(PR-P; HB-P; BG-P; MG-P).

The most important substances from the cones are without doubt the ones that give bitterness to the beer. „The bitter substances" from hop is an original name of the resins of hop and this technology is used for the extraction of some chemical compounds that are not defined as resins but can be isolated from these. The Nomenclature Hop Committee recommends the classification of hops resins:

- Group A: unspecific fraction
- Group B: specific compounds and mixtures of specific compounds.

Unspecific fraction analysis

The analytical methods are international recommended procedures from Analytica EBC (Tab. 1).

Tab. 1

Methods used for the analysis of the unspecific fractions (group A)

Aim of the study	Used method	Parameters
Conductometric hop value	The conductometric titration of methanolic extract (Analytica 7.5)	alpha acids LCV, %
Total resins	The extraction in methanol and diethyl ether (Analytica 7.5)	Total resins RT, %
Soft resins	The extraction in methanol and diethyl ether, followed by the fragmentation in hexane (Analytica 7.5)	Soft resins RM, % Hard resins RD=RT-RM β fractions=RM-LCV
Hop moisture	Termogravimetry (Analytica 7.2)	S.U.= 100-u, %

Specific fractions analysis

The analysis of the specific fractions of the hop samples were made by RP HPLC, as is describe in Analytica EBC method 7.8., modified in LICSA – USAMV Cluj-Napoca Laboratory. It were identificated and quantificated α- acids, β- acids and iso-α-acids. The method was developed and validated for the bitter acids analysis from hop.The method of validation and analysis procedure of the biological samples was made according to the RENAR and LICSA Laboratory procedures from USAMV Cluj-Napoca validation guides.

Tab. 2

The method used for the analysis of specific fractions (group B)

Method:	HPLC chromatography (method 7.8 Analytica EBC modified)
Standards	ICE-2 for α and β bitter acids; ICS-I2 for iso-α-bitter acids
Instrument	HPLC Shimadzu
Column	Nucleosil 5C18, 5μm
Mobile Phase	Methanol: phosphoric acid:water=750:240:10 v/v/v
Elution Program	Concentration Gradient
Flow	1 ml/min
Volume	10 μl
Column Temperature	35^{o}C
Detection	UV at 270 nm for iso-α-acids and 314 nm for α and β bitter acids

RESULTS AND DISCUSSION

Certificate of origin for hops sold in our country and the European Community, according to regulations is based on variety identification, place of origin of culture and vintage year. At this point variety identification is made by affidavit of the producer, without a strong argument supported by a quality certificate of authenticity. Romanian SR 13482:2003 standard provides technical requirements for organoleptic and physico-chemical class of hop cones, without specific identification methodology for variety. The importance of hops for the brewing industry and other industries is reconsidered in terms of biologically active compounds. The brewers will choose variety of hops on a specific fingerprint of markers of quality and authenticity. In beer industry, hops is no longer used for a long time as a flower. Products used today are hop pellets, hop extracts, preizomerized or isomerized products. In these conditions to maintain varietal purity is sometimes difficult even for advanced processing units. In these circumstances for correct information to the recipient we must identified specific tools for traceability the variety of product from harvest to processing products.

In this study has been identified HPLC-UV chromatographic fingerprint of α and β acids and nonspecific resin fraction from Magnum, Brewers Gold, and Pearls Hüller Bitterer cultivars. These compounds have been proposed as markers of quality of Romanian hops. To define these markers as markers of authenticity has been developed a statistical method for classification. According with the results of the chemical composition of hops it was design specific profile of each variety examined. The results are presented in Tab. 3-4-5-6.

Tab. 3

The caracterisation of Magnum cultivar

MAGNUM CULTIVAR						
CHARACTERISTICS	MG-F		MG-U		MG-P	
	% m/m	±DS	% m/m	±DS	% m/m	±DS
COHUMULONA	2,15	0,49	1,75	0,57	1,45	0,20
N+ADHUMULONA	10,42	0,33	7,60	0,02	5,38	0,18
COLUPULONA	2,57	0,72	2,20	0,02	2,05	0,04
N+ADLUPULONA	4,13	0,09	2,98	0,04	2,62	0,04
LCV	13,44	0,06	10,79	0,22	9,24	0,22
RASINI TOTALE	27,88	0,27	27,77	0,24	27,37	0,24
RASINI MOI	25,24	0,15	21,07	0,21	19,01	0,19
RASINI DURE	2,64	0,12	6,70	0,03	8,36	0,05
FRACTIUNEA BETA	11,80	0,09	10,28	0,01	9,77	0,03
UMIDITATEA	7,46	0,02	8,58	0,04	9,20	0,06
Σ Acizi alfa	12,58	0,81	9,35	0,59	6,83	0,38
Σ Acizi beta	6,70	0,81	5,18	0,05	4,67	0,08
Σ Acizi alfa / Σ Acizi beta	1,88		1,80		1,46	
%Cohumulona / Σ Acizi alfa	17,12		18,71		21,27	
%Colupulona / Σ Acizi beta	38,40		42,42		43,99	

Tab. 4

The caracterisation of Brewers Gold cultivar

BREWERS GOLD CULTIVAR						
CHARACTERISTICS	MG-F		MG-U		MG-P	
	% m/m	±DS	% m/m	±DS	% m/m	±DS
COHUMULONA	2,40	0,17	2,18	0,12	1,92	0,15
N+ADHUMULONA	6,04	0,24	4,51	0,14	3,69	0,02
COLUPULONA	3,15	0,12	2,64	0,09	2,53	0,02
N+ADLUPULONA	2,39	0,09	1,59	0,05	1,45	0,01
LCV	8,50	0,12	7,22	0,11	6,99	0,20
RASINI TOTALE	21,83	0,24	21,32	0,19	21,08	0,29
RASINI MOI	19,30	0,18	16,69	0,22	16,22	0,18
RASINI DURE	2,53	0,06	4,63	0,03	4,86	0,10
FRACTIUNEA BETA	10,79	0,05	9,47	0,11	9,23	0,01
UMIDITATEA	9,11	0,01	8,76	0,03	8,54	0,05
Σ Acizi alfa	8,44	0,40	6,68	0,26	5,61	0,17
Σ Acizi beta	5,55	0,21	4,22	0,14	3,98	0,03
Σ Acizi alfa / Σ Acizi beta	1,52		1,58		1,41	
%Cohumulona / Σ Acizi alfa	28,45		32,59		34,25	
%Colupulona / Σ Acizi beta	56,85		62,41		63,56	

Tab. 5

The caracterisation of Huller Bitterer cultivar

HULLER BITTERER CULTIVAR						
CHARACTERISTICS	MG-F		MG-U		MG-P	
	% m/m	±DS	% m/m	±DS	% m/m	±DS
COHUMULONA	2,08	0,06	2,13	0,15	1,56	0,09
N+ADHUMULONA	6,67	0,06	5,99	0,01	4,14	0,03
COLUPULONA	2,50	0,03	2,57	0,01	1,90	0,02
N+ADLUPULONA	3,10	0,03	2,86	0,01	2,27	0,01
LCV	9,84	0,01	8,03	0,11	6,07	0,01
RASINI TOTALE	22,98	0,35	22,95	0,34	22,25	0,42
RASINI MOI	20,79	0,22	18,81	0,26	15,59	0,36
RASINI DURE	2,19	0,14	4,14	0,08	6,66	0,06
FRACTIUNEA BETA	10,95	0,20	10,78	0,15	9,52	0,36
UMIDITATEA	8,52	0,05	9,52	0,02	8,21	0,05
Σ Acizi alfa	8,75	0,12	8,13	0,17	5,70	0,12
Σ Acizi beta	5,60	0,05	5,43	0,02	4,17	0,03
Σ Acizi alfa / Σ Acizi beta	1,56		1,50		1,37	
%Cohumulona / Σ Acizi alfa	23,78		26,25		27,32	
%Colupulona / Σ Acizi beta	44,72		47,37		45,50	

Tab. 6

The caracterisation of Perle cultivar

PERLE CULTIVAR						
CHARACTERISTICS	MG-F		MG-U		MG-P	
	% m/m	±DS	% m/m	±DS	% m/m	±DS
COHUMULONA	2,05	0,15	1,87	0,11	1,31	0,11
N+ADHUMULONA	5,49	0,03	5,30	0,02	4,56	0,80
COLUPULONA	2,53	0,00	2,19	0,42	2,18	0,37
N+ADLUPULONA	2,77	0,00	2,59	0,26	2,50	0,28
LCV	9,25	0,11	7,21	0,15	6,40	0,01
RASINI TOTALE	21,99	0,25	21,52	0,32	21,31	0,29
RASINI MOI	19,77	0,34	17,21	0,27	16,30	0,32
RASINI DURE	2,22	0,09	4,31	0,05	5,01	0,04
FRACTIUNEA BETA	10,52	0,23	10,00	0,12	9,90	0,31
UMIDITATEA	8,52	0,05	8,36	0,03	7,67	0,08
Σ Acizi alfa	7,54	0,18	7,17	0,14	5,87	0,91
Σ Acizi beta	5,30	0,01	4,78	0,68	4,68	0,65
Σ Acizi alfa / Σ Acizi beta	1,42		1,50		1,25	
%Cohumulona / Σ Acizi alfa	27,21		26,04		22,32	
%Colupulona / Σ Acizi beta	47,783		45,854		46,605	

Abreviation: %m/m – content g/100g dry matter; DS- standard deviation; Σ α-acids– sum of cohumulon and n+adhumulon; Σ β- acids – sum of colupulon and n+adlupulon

Data interpretation was done by cluster analysis, including multivariate statistical methods for classifying a set of heterogeneous components in relatively homogeneous groups, according to several criteria. Result analysis aims besides ensuring homogeneity within groups and differentiation as large groups.

For classification of varieties of hops was applied Unweighted Pair Group Method with Arithmatic Mean (UPGMA), based exclusively on markers of quality, without any a priori definition, the result being most significant solution possible. Matlab programming environment 7.2.0232/2006 version, with the statistics toolbox was used to analyze experimental data for descriptive classification (cluster analysis). Results of analysis are classification trees (dendrogram) in Figures 1, 2 and 3.

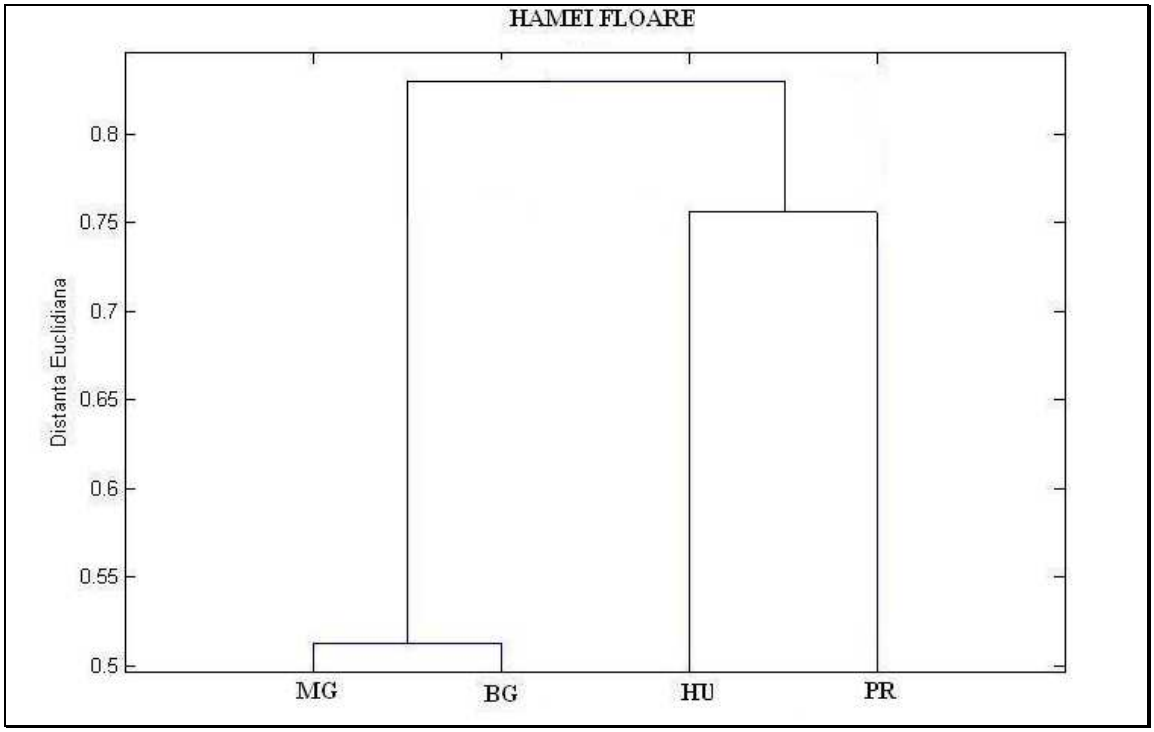

Fig.1 Dendogram for hops cultivar Magnum, Brewers Gold, Hüller Bitterer and Perle for whole cones clasification by Cluster Analysis

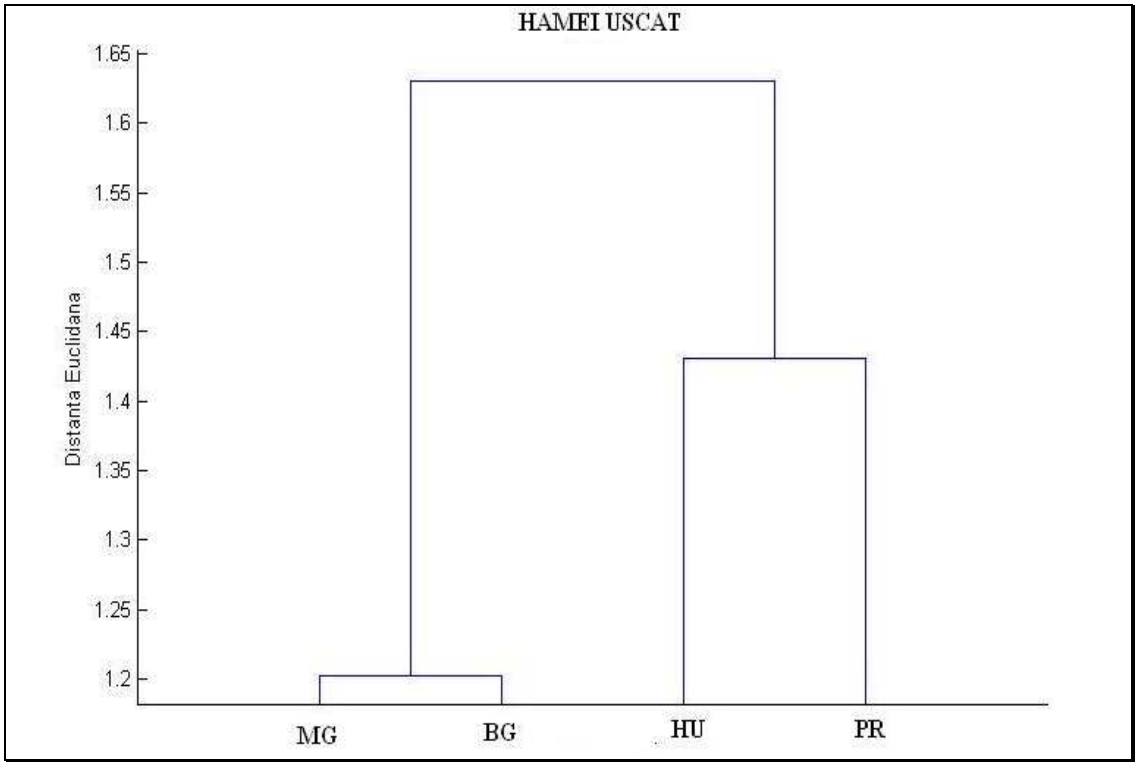

Fig.2 Dendogram for hops cultivar Magnum, Brewers Gold, Hüller Bitterer and Perle for dry cones clasification by Cluster Analysis

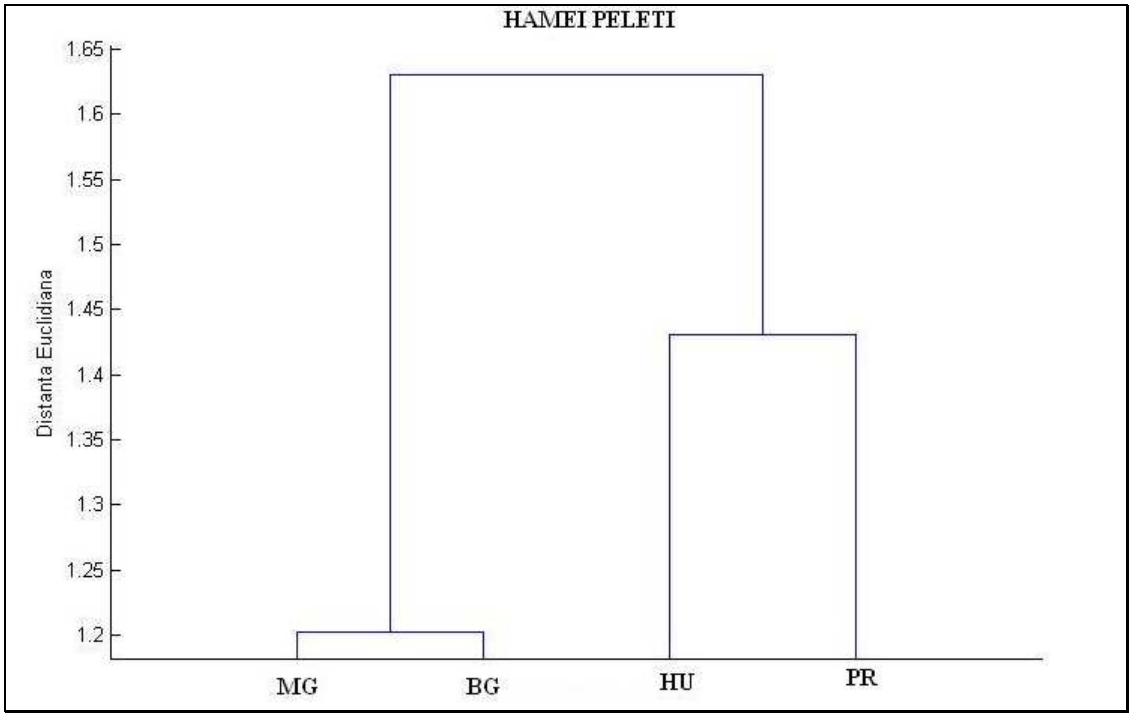

Fig.3 Dendogram for hops cultivar Magnum, Brewers Gold, Hüller Bitterer and Perle for hops pellets clasification by Cluster Analysis

CONCLUSIONS

From dendogram shown in Fig. 1 we obtain a classification of whole hop flower in three classes.

Class I: contains two groups of hops:

• group a. Perle and Hüller Bitterer

• group b. Brewers Gold and Magnum

This classification coincides with the classification of varieties made in theoretical analysis, varieties that bitter and aromatic varieties. between the two classes there is a considerable Euclidean distance of 0.7 units making classification distinguishing these two groups.

Class II: contains three groups of hops:

• group a. Perle

• group b. Bitterer Hüller

• group c. Magnum and Brewers Gold

Within Class II there is a distinction between aroma varieties, that can be said that the variety Hüller Bitterer has dual character, being intermediate between aroma and bitter varieties.

Class III contains four group of hop:

• group a. Perle

• group b Hüller Bitterer

• group c. Brewers Gold

• group d. Magnum

Class III consists of four groups, therefore, closely Euclidean spaced condiderabile so, that can make a clear distinction of varieties (for example, between varieties Perle and Magnum is a Euclidean distance of 0.85 which indicates that these two varieties are significant differences in terms of quality markers). For validation and statistical analysis the method were performed for dry hops and pellet hops, also. As shown in Figure 2 and 3, the method keeps the classification within classes and groups for hops flower and hops pellets. A significant difference is observed between whole hops and hops flower processed (dry and pellets), by increasing the Euclidean distance between varieties, implying alteration of the markers of quality during processing. The validated method of classifying can be used as a tool to trace and authenticate the flow of processing varieties of hops.

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