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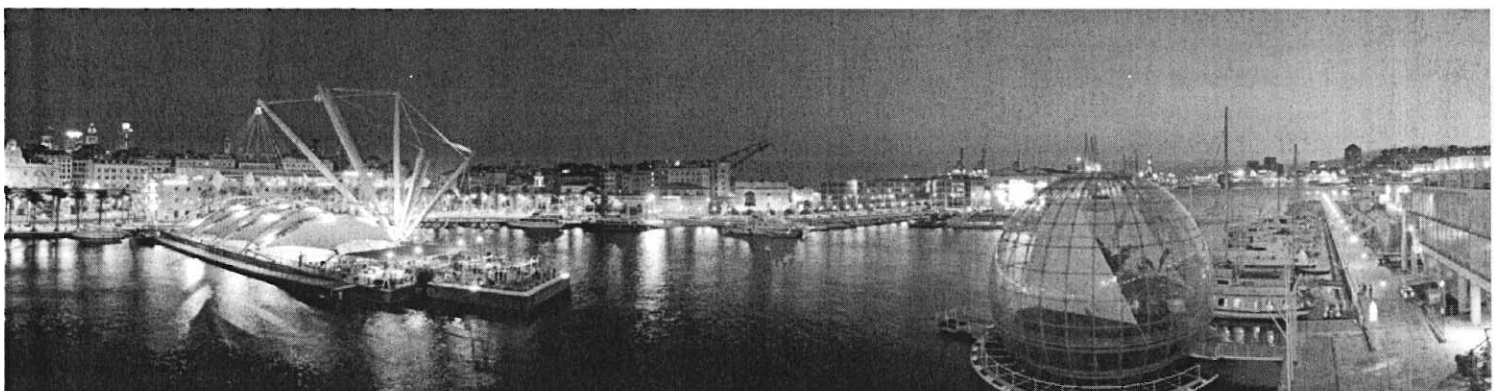
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## Classification of international hop genotypes by near infrared spectroscopy

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### Abstract

Nowadays, food authentication is considered of primary importance for the production process, also for the beer industry. The female inflorescences (cones) of hop plant (*Humulus lupulus* L.) are the most important raw material in the brewing industry because they influence the organoleptic properties of beer (bitterness, flavour, and aroma). This is due to the hop secondary metabolite profile, which consists of three broad chemical groups: hop-acids ( $\alpha$ - and  $\beta$ -acids), essential oils, and polyphenols (Benitez et al 1997). Differences in secondary metabolite composition depend on genotype and on growing environment. Therefore, different cultivars grown in different environments can have distinct bittering potentials and flavour profiles (McAdam et al 2013). For this reason, it is of crucial importance for brewers to be sure about the hop variety, geographical origin and year of production, since every type of beer has its own hop recipe. The hop assessment is currently carried out applying traditional destructive techniques, which require a considerable amount of samples, and are time-consuming (Machado et al 2018). Therefore, the aim of the present study was to find a rapid and reliable methodology to identify hop cultivars, using near infrared (NIR) spectroscopy, with multivariate analysis. To this purpose, a first analysis was achieved on NIR spectra of 98 samples of Cascade cultivar grown in different regions of Italy (Lombardy, Tuscany, Emilia Romagna, Latium, Abruzzo, Basilicata, and Sicily). Then, a second step was carried out on a set of 161 NIR spectra from seven regions and six cultivars (Cascade, Chinook, Columbus, Comet, Fuggle, and Nugget). Linear discriminant analysis allowed us to correctly classify the 100 % and 92 % of the samples, respectively. Our results demonstrated that the NIR spectroscopy can be a promising tool for a rapid and non-destructive characterization of hop cones according to the variety and geographical origin.

### Main references:

- J. L. Benitez, A. Forster, D. De Keukeleire, M. Moir, F.R. Sharpe, L. C. Verhagen, K. T. Westwood (1997): *Hops and hop products*. Carl-Verlag, Germany.
- E. L. McAdam, J. S. Freeman, S. P. Whittock, E. J. Buck, J. Jakse, A. Cerenak, and R. E. Vaillancourt, *BMC genomics*, **14**(1), 360. (2013)
- Jr. J. C. Machado, M. A. Faria, I. M. Ferreira, R. N. Páscoa and J. A. Lopes, *Talanta*, **180**, 69-75 (2018).



## Classificazione geografica e varietale di luppoli da germoplasma internazionale mediante spettroscopia nel vicino infrarosso

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### Riassunto

Oggigiorno l'autenticazione degli alimenti è considerata di primaria importanza nei processi produttivi, anche per l'industria della birra. Le infiorescenze femminili (coni) della pianta del luppolo (*Humulus lupulus* L.) sono la materia prima più importante per i birrifici poiché influenzano le proprietà organolettiche della birra (amaro, sapore, aroma). Ciò è dovuto al profilo fitochimico dei metaboliti secondari del luppolo, caratterizzato da tre gruppi di composti chimici: acidi ( $\alpha$ - e  $\beta$ -acidi), oli essenziali e polifenoli (Benitez et al 1997). Le differenze nella composizione dei metaboliti secondari dipendono dal genotipo e dall'ambiente di coltivazione. Pertanto, cultivar differenti coltivate in ambienti diversi possono presentare specifici potenziali amaricanti e aromatizzanti (McAdam et al 2013). Per questo motivo, è di fondamentale importanza per i birrai essere sicuri della varietà del luppolo, dell'origine geografica e dell'anno di produzione, poiché ogni tipo di birra ha una propria ricetta. La valutazione del luppolo viene attualmente effettuata applicando tecniche tradizionali distruttive che richiedono una quantità elevata di campioni e molto tempo per le analisi (Machado et al 2018). Pertanto, lo scopo del presente studio è di proporre una metodologia rapida e affidabile per identificare le cultivar di luppolo, utilizzando la spettroscopia NIR, con analisi multivariata. A tal fine è stata realizzata una prima analisi su spettri NIR di 98 campioni della cultivar Cascade coltivata in 7 regioni italiane (Lombardia, Toscana, Emilia Romagna, Lazio, Abruzzo, Basilicata, Sicilia). In seguito, una seconda analisi è stata effettuata su 161 spettri NIR da 7 regioni e 6 cultivar di luppolo (Cascade, Chinook, Columbus, Comet, Fuggle, Nugget). L'analisi discriminante lineare ha permesso di classificare correttamente il 100% e il 92% dei campioni, rispettivamente. I nostri risultati hanno dimostrato che la spettroscopia NIR può essere uno strumento promettente per la caratterizzazione rapida e non distruttiva dei coni di luppolo in base alla varietà e all'origine geografica.

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- J. L. Benitez, A. Forster, D. De Keukeleire, M. Moir, F.R. Sharpe, L. C. Verhagen, K. T. Westwood (1997): *Hops and hop products*. Carl-Verlag, Germany.
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## INTRODUCTION

Hop (*Humulus lupulus* L.) is a dioecious perennial, climbing plant native of Europe, Asia, and North America. The female strobiles (cones) represent the most interesting parts of the plant from a technological point of view, as they are one of the essential ingredients of brewing industry. Over the last few decades, Italy has been assisting to the rise and the establishment of the craft beer industry, which gained great success and credibility, becoming one of the most significant phenomena of the Italian agri-food sector. In order to address the growing internal demand of raw materials, several farmers have begun cultivating international hop varieties in different Italian regions. However, hop quality depends largely on its variety and on growth environmental conditions.

### AIMS

In light of these considerations, the present study investigates for the first time on the potential of vibrational spectroscopy coupled with chemometrics to assess the geographical and varietal origin of different international hop genotypes grown in Italy.

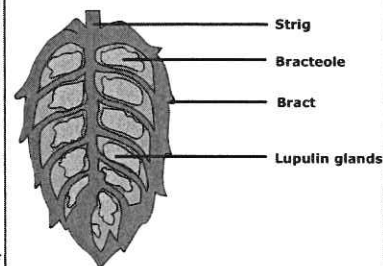


Figure 1: Cross sections of hop cone

## MATERIALS & METHODS

For the varietal determination, NIR spectra of 161 hop samples from seven regions and six cultivars (Fig.2) were acquired. Then, NIR spectra of 98 samples of Cascade hop genotype, grown in different Italian regions (Fig. 3) were acquired to assess the geographical origin. All spectra were collected using an iS 50 Nicolet smart FT-NIR integrating sphere (Thermo Fisher Scientific Inc., USA), operating in transmittance mode. Wavelengths from 12000 to 4000  $\text{cm}^{-1}$  were acquired with a resolution of 4  $\text{cm}^{-1}$ , applying an interval between spectra data points of 0.482  $\text{cm}^{-1}$ .

	CASCADE	CHINOOK	COLUMBUS	COMET	FUGGLE	NUGGET
<b>Origin</b>	USA	USA	USA	USA	United Kingdom	USA
<b>Profile</b>	Aroma	Dual purpose	Bittering	Bittering	Aroma	Bittering
<b>Alpha acid (%)</b>	5.5-9.0	11.5-15.0	14.5-17.5	8.0-10.5	3.0-5.6	13.5-16.0
<b>Beta acid (%)</b>	6.0-7.5	3.0-4.0	4.5-6.0	4.0-5.0	2.0-3.0	4.4-5.5
<b>Total oil (mL/100g)</b>	0.8-2.5	1.0-2.5	2.5-4.5	1.2-2.0	0.7-1.4	1.0-3.0

Figure 2: Varietal characteristics of investigated hop genotypes (<https://ychhops.com>)



Figure 3: Cultivation zones

Linear Discriminant Analysis (LDA) was applied to spectral data for classification purpose. LDA performance was evaluated by comparing the number of correctly categorized objects to the number of all objects, usually in percent.

## RESULTS

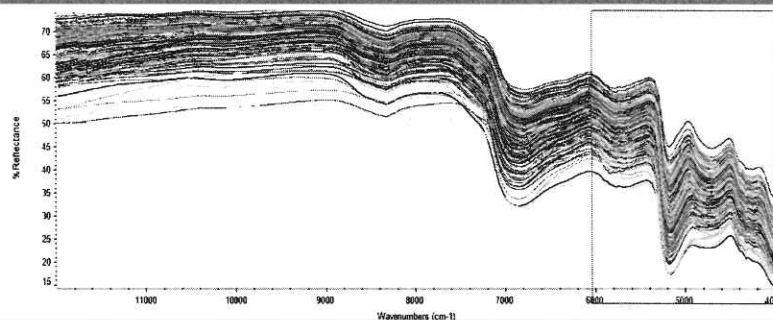


Figure 4: Samples NIR spectra and spectral regions used for LDA predictions

- ❖ LDA best prediction results were obtained using raw spectral data in the 6.000–4.000  $\text{cm}^{-1}$  range (Fig.4).
- ❖ LDA provided a satisfactory classification of hop samples according to their botanical origin (Fig. 5). LDA function 1 (F1), function 2 (F2), and function 3 (F3) accounted for 52.3, 37.6 and 7.2% of the total variance, respectively.
- ❖ LDA analysis, carried out on a set of 98 NIR spectra of Cascade cultivar grown in seven different Italian regions, provided a good classification of hop samples according to their geographical origin (fig. 6). LDA function 1 (F1), function 2 (F2), and function 3 (F3) accounted for 50.2, 35.8 and 13.5% of the variance, respectively.
- ❖ LDA developed model correctly classified the 100% and 92% of the samples, in relation to their geographical and varietal origin, respectively.

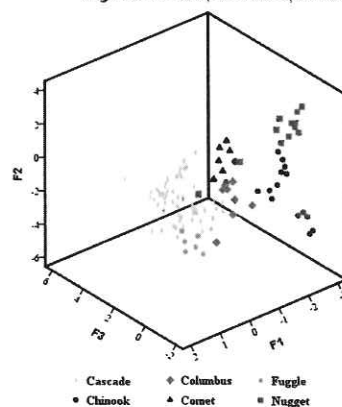


Figure 5: LDA: score plot and varietal classification results

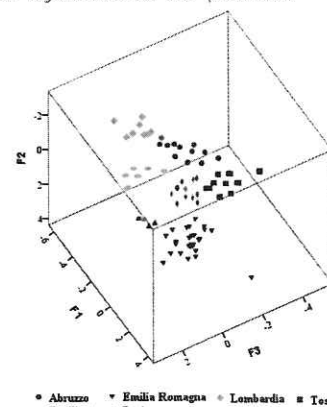


Figure 6: LDA: score plot and geographical classification results

## CONCLUSIONS

Our results demonstrated that NIR spectroscopy can be a promising tool for a rapid and non-destructive characterization of hop cones according to their varietal and geographical origin. However, further investigations are needed to strengthen the model.

## Acknowledgements

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