Characterisation of Chilean hop (*Humulus lupulus* L.) ecotype Ranco in the Los Ríos Region

Caracterización del ecotipo de lúpulo (*Humulus lupulus* L.) chileno Ranco en la Región de Los Ríos

Behn, A.[a*], Jerez, A.[b*], Villatoro, J.M.[b], Celedón, M.[d], Lüer, C.[a], Kausel, G.[c]

[a] Institute of Plant Production and Protection, Facultad de Ciencias Agrarias y Alimentarias, Universidad Austral de Chile, Casilla 567, Valdivia, Chile.

[b] Institute of Pharmacy, Faculty of Sciences, Universidad Austral de Chile, Casilla 567, Valdivia, Chile.

[c] Institute of Biochemistry and Microbiology, Faculty of Sciences, Universidad Austral de Chile, Casilla 567, Valdivia, Chile.

[d] Lúpulos Chile SpA., Fundo Rofuco s/n, Casilla 174, Paillaco, Chile.

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**Corresponding author:*
Anita Behn; Alejandro Jerez
E-mail address:
anita.behn@uach.cl
alejandrojerez@uach.cl

ABSTRACT

In recent years, southern Chile has experienced an increase in hop production mainly due to the boom of craft breweries. In the Los Ríos Region, several local hop ecotypes have been collected during the past ten years, with the Ranco ecotype being the most distributed and cultivated in the region. Therefore, it is urgent to provide producers and consumers with information that allows evaluating the potential contribution of hops in terms of differentiating the local production of craft beer and hop subproducts. The Ranco ecotype has a club shape with medium-sized dark green leaves, long side shoots in the upper part of the plant and anthocyanin pigmented stem. The cones are medium sized, concentrated in the upper third of the plant, revealing mid to late maturity. From an agronomic point of view, the plant shows vigorous and sturdy growth but is susceptible to downy mildew (*Pseudopernospora humuli*) and has low tolerance to mites (*Tetranychus urticae)*. The average yield prospection of this ecotype ranges between 1,000 and 1,350 kg ha^{-1} in the Los Ríos Region and the cones are characterised by a predominant herbal and woody aroma. Although the hop cones analysed in this study presented a low percentage of bitter acids, significant amounts of polyphenols were detected in different parts of the plant which suggests a potential use in the food and cosmetic industry as well as the possible development of new regional beer varieties. In this study, the morphological and chemical characteristics of this novel hop ecotype are presented for the first time to contribute to the sustainable development of the brewing industry and added-value hop products in the Los Ríos Region.

RESUMEN

El aumento de la producción de lúpulo se debe principalmente al auge de las cervecerías artesanales. En la Región de Los Ríos, se han recolectado varios ecotipos locales de lúpulo en los últimos diez años, el ecotipo Ranco es hasta ahora el más distribuido y cultivado en la región. Por ello, es urgente brindar información a productores y consumidores para evaluar el potencial aporte diferenciando la producción local de cerveza artesanal y subproductos de lúpulo. El ecotipo Ranco es una planta en forma de garrote, hojas de tamaño mediano de color verde oscuro, brotes laterales largos en la parte superior de la planta y un tallo con pigmentación antociánica. Los conos son de tamaño mediano, concentrados en el tercio superior de la planta, revelando una madurez media a tardía. Desde el punto de vista agronómico, la planta muestra un crecimiento vigoroso y robusto, pero es susceptible al mildiú (*Pseudopernospora humuli*) y con baja tolerancia a los ácaros (*Tetranychus urticae*). El ecotipo Ranco exhibe una prospección de rendimiento en promedio entre 1.000 y 1.350 kg/ha en la Región de Los Ríos, con conos con aroma predominantemente herbáceo y leñoso. Los conos de lúpulo analizados en este estudio presentaron un bajo porcentaje de ácidos amargos, pero en diferentes partes de la planta se detectaron cantidades importantes de polifenoles, lo que sugiere un uso potencial en la industria alimentaria y cosmética, así como en el desarrollo de nuevas variedades regionales de cerveza. Las características morfológicas y químicas presentadas por primera vez de este novedoso ecotipo de lúpulo, aportan conocimientos útiles para fortalecer el desarrollo sustentable de las industrias cerveceras y de productos en base a lúpulo con valor agregado en la Región de Los Ríos.

Palabras clave: *Humulus lupulus*, lúpulo, ecotipo chileno, alfa ácidos, polifenoles

INTRODUCTION

Hop (*Humulus lupulus* L.), is considered an aromatic plant cultivated for industrial purposes, mainly to produce beer (Leskovar, 1978). Hop provides a bitter taste, aroma and foam stability in beer (Seefelder, 1999). Also, the chemical diversity of its compounds exhibits antibacterial and antifungal effects (Karabín *et al.,* 2016). In addition, hop cones are rich in pharmacologically important compounds such as polyphenols, flavanones, chalcones and derivatives of phloroglucinol with high medicinal value that can be used to control sleep disorders, activate the gastric function or as a mild sedative (Zanoli and Zavatti, 2008). Furthermore, clinical studies revealed a therapeutic potential to reduce Alzheimer's disease and control oestrogen deficiency after menopause, being also effective as an antidepressant and anti-stress product (Chadwick *et al.,* 2007; Kyrou *et al.,* 2017; Huang *et al.,* 2018). Germany and the US are the largest producers worldwide, whereas other hop producing countries are Spain, Argentina, Canada, China, Japan, South Africa, New Zealand, and Australia (BarthHass, 2021). The total worldwide production reached 122,003 tons, with a total productive area of 62,366 ha and an average yield of 1.95 tons/ha (BarthHass, 2021). According to estimates, about 99.5% of world production is destined for the brewing industry and the remaining percentage is used in the pharma industry (Seefelder, 1999). However, the current increasing demand for naturally derived compounds with medicinal effects implies developing innovative products based on hop or including hop as an ingredient.

Hop is a perennial, dioecious, diploid 2x = 2n = 20 + XX in females and 2x = 2n = 20 + XY in males, with apical dominance and a climbing habit (Winge, 1929; Neve, 1991). Its taproot has great exploratory capacity, reaching 1.5 m in-depth and more than 2 m laterally (Teuber, 2001). It generates an underground crown of semi-woody rhizomes from which the aerial part grows out annually, depending on the daylight condition. This plant has annual regrowth and useful life of 12 to 15 years. The aerial vegetative structure reaches up to 7 m high through dextrous growth; in male plants, male flowers are grouped in pollen-producing panicles, while in female plants the condensed inflorescences are commonly called cones (Neve, 1991; Teuber, 2001). Only the female plants are useful for beer production because they provide specific secondary metabolites found in lupulin, a yellowish substance produced through the secretory glands in the bracts of cones (Kavalier *et al.,* 2011). In these plant structures, the polar and volatile phenolic terpene metabolites are synthesised, being the volatile ones of interest for the aromas (Kishimoto *et al.,* 2006). The lupulin produced in glands of the female flowers is composed mainly of resins (α-acids, β-acids) and essential oils (Eri *et al.,* 2000; Oladokun *et al.,* 2016).

The α-acids (humulones) and β-acids (lupulones) represent a great portion of the dry constituents of hop cones, and they are associated with the flavour and bitterness of beer, besides playing an important role in plant properties such as antimicrobial potential (Sanz *et al.,* 2019; Arruda *et al.,* 2021). The main α-acids are cohumulone, humulone and adhumulone which are isomerised to iso-α-acids under high-temperature conditions. Isohumulol, the compound derived from hop that is also present in beer, presents diverse benefits for human health (Yajima *et al.,* 2004). Iso -α-acids are considered the main agents of beer bitterness, exhibiting antibacterial activity, mainly against Gram-positive bacteria (Kramer *et al.,* 2015; Steenackers *et al.,* 2015; Arruda *et al.,* 2021). Furthermore, the main β-acids are colupulone, lupulone and adlupulone which constitute approximately 10% of the hop dry weight and play a potential role in the characteristic flavour of the plant and antimicrobial activity (Larson *et al.,* 1996; Formato *et al.,* 2013; Arruda *et al.,* 2021).

Globally, hop companies are focused on developing new varieties with unusual aromas, called FlavourHops, looking for ecotypes and unknown varieties with enhanced contents and compositions of aromatic compounds that might be the result of different environmental conditions (Mongelli *et al.,* 2016). Originally, hop was grown in Southeast Europe and Asia Minor, reaching Chile after a turbulent history during the 19th century introduced by German settlers (Bernedo, 1999; Del Valle *et al.,* 2003). In Chile, around 20 ecotypes have been collected so far, recognising one of them with an aroma that is unique in the world (Vargas, 2016). The characterisation of hop ecotypes identified in the Los Ríos Region has begun only recently. The ecotypes of hops collected in this area might originate from old commercial varieties that adapted to geographical isolation, being exposed to unique climate and soil conditions for about 170 years (Eibel *et al.,* 2015). Considering the importance of the environment and the GxE interactions, the characterisation of hop will enable the differentiation of novel aromas that could potentiate the local production in the Los Ríos Region (Lüer, 2019). Furthermore, all beer raw materials could be produced in the Los Ríos Region with a steadily increasing hop production, offering a great potential to differentiate the craft beer production and the raw material industry. This study aims to present the phenotypic and chemical data of the Ranco ecotype, the most cultivated hop ecotype in the Los Ríos Region. The information generated from this study contributes to promoting the sustainable development of craft brewing and the pharmaceutical and cosmetic industries in southern Chile.

MATERIAL AND METHODS

Plant material and morphological characterisation

The plant material used corresponds to the hop ecotype called Ranco. The hop plants were screened and collected at the end of March 2020 and 2021 in Hueimén, borough of Lago Ranco, and Valdivia, borough of Valdivia, both located in the Los Ríos Region. The analysed material corresponded to cones, leaves and stems of a representative sample of three plants from each location.

Morphological characterisation was performed according to code HUMUL_LUP, UPOV (International Union for the Protection of New Varieties of Plants). For the description of new plant varieties, the key provided by UPOV is applied internationally. This description standard permits the comparison of characters between varieties. The descriptions of each character of an observed structure are represented by a number (UPOV, 2006). The aromatic profile for characterization was taken from www.lupuloshueimen.cl.

At the end of the cultivation period, three representative plants of the ecotype were obtained, which were processed by determining direct measurements such as leaf area, dry weight, and the number of cones (Barrera and Melgarejo, 2010). There was no access to root measurements because hop is a perennial plant and is used in the following year's production. The structures were separated by stems, bracts, leaves and cones. The plant material was taken to the Seed Laboratory of the Faculty of Agricultural and Alimentary Sciences, Universidad Austral de Chile, and was dried for 48 h in forced air ovens at 50 °C. The cones for chemical analyses were dried for 6 hrs at 60 °C. Subsequently, the dry plant material was subjected to quantification tests of analytes of interest and estimation of the total polyphenols at the Laboratory of Analytical Instrumentation of the Institute of Pharmacy, Faculty of Sciences, Universidad Austral de Chile.

Environments

The morphological data and the samples for chemical characterisation were taken from two locations, Hueimén (Lúpulos Hueimén) and Valdivia (Cervecería Kunstmann), with those companies kindly providing the aerial part of the whole hop plants.

Lúpulos Hueimén is located near to Lago Ranco, Los Ríos Region, at latitudes 40° 23' 14.3" S, 72° 27' 60.0" W. Their commercial production is established on soils that correspond to the Ranco Series, i.e., Andisol type with a loamy-silty texture. These volcanic soils are deep, and they are positioned on lake terraces with slightly undulating topography with a slope of 2 to 5%. The material corresponds to gravels and sands of different degrees of cementation of fluvioglacial origin. The Series was described in Ignao, therefore it coincides with the town of Hueimén (CIREN, 2003). The town of Lago Ranco has a warm marine climate that is described as Petrohue agro-climate (Santibáñez *et al.,* 2017). Hueimén, is located at an altitude of 446 m above sea level, while the Lago Ranco locality is 89 m above sea level. The average annual temperature corresponds to 9.7 °C, with a maximum of 21.2 °C in January and a minimum of 2.5 °C in July (Santibáñez *et al.,* 2017).

The experimental hop orchard of the Kunstmann Brewing Company is in Valdivia at the latitudes 39° 50' 16.1" S, 73° 16' 48.2" W and is mainly used for touristic and experimental purposes. The soil corresponds to the Valdivia series of the Andisol type, with a loamy-silty texture, soils moderately deep and developed from volcanic ash, corresponding to a tuff (deposit of highly compacted volcanic materials) called Cancagua. The topography is gently undulating with a 5-8% slope and good drainage (CIREN, 2003). The Valdivia agro-climate, located on a coastal strip between parallels 39° and 42°, presents cool summers and mild winters rated as a humid hydric regime with an annual relative humidity of 78% (Santibáñez *et al.,* 2017). Its average annual temperature corresponds to 11.9 °C, with a maximum of 24.5 °C in January and a minimum of 4.3 °C in July, with a frost-free period from October to April. Valdivia is 17 m above sea level and has the same climate type as the agroclimatic zone of Rupanco, defined as warm temperate mesothermal climate with per humid humidity regime (Santibáñez *et al.,* 2017).

Chemical characterisation

Hydromethanolic hop extracts

Two hundred and fifty milligrams of dried cones, leaves, stems, and cones pellets were weighed in a 250 mL glass beaker, then 20 mL of an acidic hydromethanolic solution were added (90:10 methanol: water acidified with orthophosphoric acid to pH 2.5) and the mixture was shaken for 1 h on an orbital shaker at 250 rpm. Subsequently, the extracted solution was filtered through a syringe filter Whatman® Uniflo® (25 mm, 0.45 µm) to a 25 mL volumetric flask and brought to volume with the extraction solvent.

HPLC Determination of α- and β-acids in hop cones

The analyses were conducted immediately after the delivery of the samples at the Laboratory of Analytical Instrumentation of the Institute of Pharmacy of the Faculty of Sciences, Universidad Austral de Chile. The

hydromethanolic extracts of hop cones were analysed using an HPLC method adapted from the American Society of Brewing Chemistry (method Hops-14) for the determination of α- and β- bitter acids and their analogues (American Society of Brewing Chemists, 2010). The analyses considered the cones of 3 plants of the Ranco Ecotype and the analyses were performed in triplicates for each case.

The extracts were filtered through a 0.45 µm nylon membrane. The HPLC system was equipped with a quaternary pump system and DAD detector (Shimadzu, Japan). A reverse-phase column C18 (100-5-C18 4.6 × 250 mm) (Kromasil, Sweden) was used. The separation was run at 30 °C with a 1.5 mL/min flow rate. The isocratic elution consisted of mobile phase A, composed of acidified methanol (0.1% v/v orthophosphoric acid) and mobile phase B HPLC water (acidified with orthophosphoric acid to pH 2.5) in 88:12 proportion. The injection volume was 20 µL and the wavelength used for detection was 327 nm. A mixture of α-acids and β-acids was used as an external standard (ICE-4, International Calibration Standard) containing 10.98% cohumulone, 31.60% N + adhumulone, 13.02% colupulone, 13.52% N + adlupulone, 42.58% total α-acids, and 26.54% total ß-acids (Versuchsstation Schweizerische Brauereien, Switzerland). The contents of α- and β-acids were calculated using standard calibration curves. The analytical run time was set at 16 min (Figure 1).

Determination of the Total Phenolic Content

Total phenolic content was measured by the Folin-Ciocalteu assay (Kähkönen *et al.,* 1999). In summary, an aliquot of 100 µL of the extract was added to a 10 mL volumetric flask. Then, 900 µL of distilled water and 4 mL of a Folin-Ciocalteau 1:10 solution was added. After three minutes, 5 mL of a 7.5% sodium carbonate were added and finally completed with water. The volumetric flask was put in the dark for 60 min, and the quantification was performed in a Thermo Evolution 201 spectrophotometer at 740 nm. A gallic acid calibration curve with five concentration points (5-150 ppm) was used to express the results as g of gallic acid/kg of dry sample (g GAE/kg DM) in all the hydro-methanolic hop extracts.

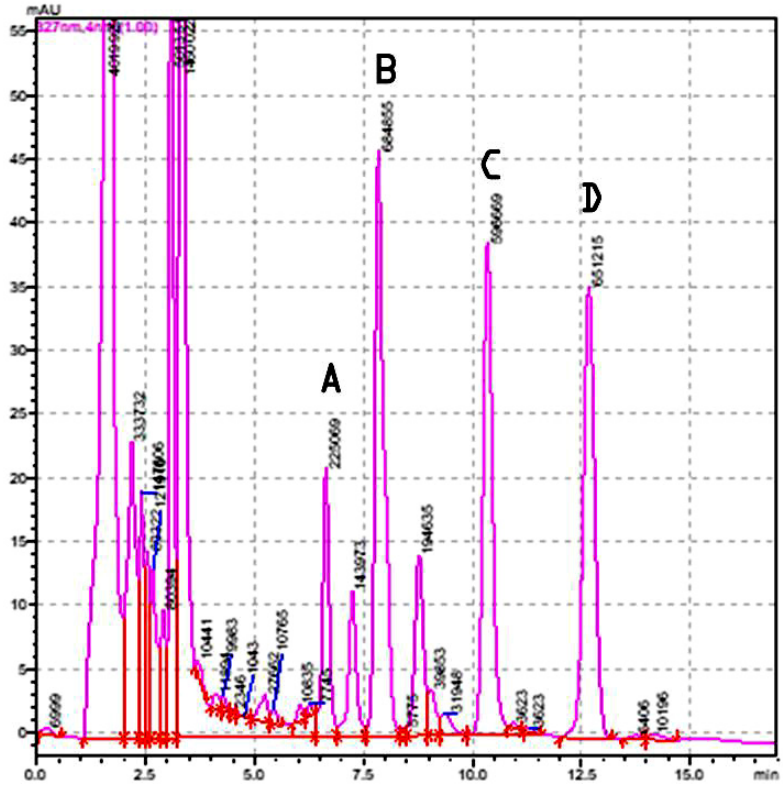

Figure 1. Chromatogram of the analytical method applied to Ranco ecotype hop cones samples, where **A** is cohumulone, **B** n+adhumulone, **C** colupulone and **D** n+adlupulone.

Figura 1. Cromatograma del método analítico aplicado a muestras de conos de lúpulo del ecotipo Ranco, donde **A** es cohumulona, **B** n+adhumulona, **C** colupulona y **D** n+adlupulona.

RESULTS AND DISCUSSION

The present results contribute to the morphological and chemical description of Ranco, the novel hop ecotype collected in the Los Ríos Region. Local hop growers are planting Ranco ecotype, as it is a robust plant and is easy to multiplicate, therefore, it is relevant to present a preliminary description of the ecotype despite the limitations of the experiment such as replicates in locations. Hop is exposed to an important environmental influence on plant development, and significant GxE interaction was revealed (Lüer, 2019). The plants were only evaluated in the localities of Hueimén and Valdivia because the morphological characterisation must be performed on adult plants. The description method was based on the UPOV criteria since most of the hop varieties were described using these parameters in order to build a baseline to collect further information on the possible ancestor.

The crop of hop was first documented in the valleys of Southeast Europe and Asia Minor, spreading during the 12th and 13th centuries between monasteries of Central Europe that produced beer at the time of fasting (Leskovar, 1978). Later, in the 14th century, it was cultivated in the Netherlands to finally reach England where it was recognised as a harmful pest and was forbidden from 1524 on for many years. Hop was first introduced to the United States in 1629 with a prosperous outcome. Currently, its cultivation has spread throughout the world between 35° and 55° latitudes in the northern and southern hemispheres (Leskovar, 1978). The hops widely grown in Europe and North America probably reached southern Chile with the German settlers in 1851, more specifically at the request of Karl Anwandter who settled in the city of Valdivia where he founded a brewery (Bernedo, 1999). Although the first cultivating attempts failed in the province of Santiago, the crop was introduced successfully in Valdivia in 1884 (Teuber, 2001; Cououmdjian, 2004). The Anwandter Brewing Company grew hops for its own industry, but other Chilean beer producers preferred imported products (Couyoumdjian, 2004). In 1937, 50 ha of hop was being cultivated in Chile but, because of the late frosts, the low demand from brewers, and the shortage of labourers required for the handling and harvesting, the business was doomed to failure (Teuber, 2001). Between 1966 to 1969, the Compañía de Cervecerías Unidas (CCU), established field experiments evaluating the three planting areas of Paine, San Carlos, and Osorno but without promising results (CORFO, 1982). In 1980, hop cultivation was re-evaluated experimentally by Intec-CORFO, with trials carried out in Talca, Chillán, Temuco, Valdivia, and Osorno. Currently, a boom in the craft brewing industry has reopened the interest in producing this crop (Kausel and Behn, 2016). In 2015, the company Lúpulos del Ranco inaugurated its first commercial crop (Vargas, 2016). At present, three commercial productions are in the Los Ríos Region, while two other companies are in the Araucanía Region northward.

Morphological characterisation of hop ecotype Ranco

Fully developed adult plants of about three years on the field were screened according to the settings in the official UPOV tables for varieties, the commonly used method to describe hops worldwide. The morphological screening of the ecotype Ranco was compared in two locations, Hueimén and Valdivia (Table 1). Furthermore, the scores were compared with the description of the hop cv. Hallertauer Mittelfrüh made by Lutz and Kneidl (2009). Since the old German variety was probably introduced by German settlers and could be one of the ancestors of Chilean ecotypes, including Ranco.

According to the shoot's anthocyanin pigmentation, the ecotype scored 7 in Hueimén and 5 in Valdivia which means a medium to strong anthocyanin pigmentation, being comparable with a score 6 as described in Hallertauer Mittelfrüh (Lutz and Kneidl, 2009). Even though the scoring slightly differed, both hops showed pigmented stems with an intensity that might marginally vary depending on environmental factors, as corroborated by observations recorded in the two locations Hueimén and Valdivia.

The classification for the size of the blades character was carried out according to the length of the blade; small leaves (3) measured between 1.7 to 6.7 cm, medium leaves (5) from 6.8 to 14 cm and large leaves from 14.1 to 25.5 cm (Engelhard *et al.,* 2011). The ecotype Ranco presented a score of 5 in both locations, with leaves ranging between 12.1 and 14 cm long. According to Lutz and Kneidl (2009), in the case of Hallertauer Mittelfrüh the rating corresponds to 5 and 7, ranging from medium to large leaves. Environmental conditions and fertilisation might influence this characteristic which suggests that the data coincide with this characteristic. Larger leaves imply a greater assimilation area, influencing the crop yield but not necessarily the quality of the cones (Engelhard *et al.,* 2011).

The leaf colour character of the Ranco ecotype varied between dark green and very dark green, depending on the location, with a high mode of 7 and 9. The variety Hallertauer Mittelfrüh is described with 5, medium green colour (Lutz and Kneidl, 2009). This character is not comparable between plants grown under different conditions; the score does not coincide with the values obtained from Ranco ecotype, and the difference could also be explained by the soil, where the amount of organic matter is mainly related to the nutrients in the soil (N, P, K, Fe).

Regarding the plant's shape, the ecotype scored 5 in both locations, which corresponds to a club shape. On

Table 1. Morphological characterisation of the Ranco ecotype in 2 locations of the de Los Ríos Region.
Tabla 1. Caracterización morfológica del ecotipo Ranco en 2 localidades de la Región de Los Ríos.

UPOV Characters		*Valdivia*	*Hueimén*
1	Main shoot: anthocyanin colouration	5	7
2	Leaf: size of blade	5	5
3	Leaf: colour of upper side of blade	7	9
8	Plant: shape	5	5
10	Side shoot from middle third of plant: length	3	5; 7
11	Side shoot from upper third of plant: length	7	7
12	Side shoot from middle third of plant: density of foliage	3	5
17	Cone: size	5	3
18	Cone: shape	4	5
19	Cone: degree of opening of bracts	2	2
20	Cone: intensity of green color	5	5
21	Bract: size	3	3
22	Bract: ratio width/length	5	3
23	Bract: length of apex	5	5

the other hand, Lutz and Kneidl (2009), described Hallertauer Mittelfrüh as cylindric to club shape (4), close to Ranco ecotype score. It has been observed that the plants shape is a very stable character over the environments when the plant reaches its full size.

For the descriptor "length of the side shoot from the middle third of plant" (UPOV), Ranco ecotype showed scores of 3 and 5 or 7 in the locations of Valdivia and Hueimén, respectively. This indicates a significant influence of the environment on this character, even under the same planting conditions. In the upper third of the plant, all plants showed scorings of 7, long length of the sides in both locations. Hallertauer Mittelfrüh scored 2 and 5, respectively, indicating shorter side shoots in the third and upper parts of the plant than the Ranco ecotype. Screening of the foliage density showed less density in Valdivia (3) and medium in Hueimén (5), a characteristic probably influenced by conditions and components of the soil. Hallertauer Mittelfrüh obtained 6 in these scorings.

About the characters of the cones, the size was classified into small (up to 3 cm), medium (3 to 6 cm) and large (bigger than 6 cm). This classification was determined according to the sizes observed in different hop varieties since UPOV does not define the classification in cm. Therefore, the results are not comparable. Ranco was classified as a medium size cone (5) hop and Hallertauer Mittelfrüh presents cones of medium (5) and large size (7) (Lutz and Kneidl, 2009).

Cone shape was classified as a wide oval shape in Valdivia (4) and globose form in Hueimén (5). The shape of the cones is assumed to be medium ovate in Hallertauer Mittelfrüh (3). Therefore, there is no coincidence with the shape of the cones between the variety and the ecotype.

The degree of opening of the bracts can be classified as slightly open (2) in both locations and closed in Hallertauer Mittelfrüh (1) (Lutz and Kneidl, 2009). However, this character depends on the moment of scoring because the bracts on the cones start as closed and begin to open slightly, then after harvest maturity some hops have open bracts.

The intensity of the green colour of the cones was classified as 5 for the Ranco ecotype in both locations. In this ecotype, the cones presented an intense colour, equivalent to Hallertauer Mittelfrüh (5). The size of the bracts in the Ranco ecotype was classified as small with a score of 3 whereas in Hallertauer Mittelfrüh it is medium to large (6) (Lutz and Kneidl, 2009).

For the width/length ratio of the bracts, the Ranco ecotype presented a mode of 5, but some scoring also corresponds to a smaller ratio (3). Lutz and Kneidl (2009), described Hallertauer Mittelfrüh with a small width/length ratio of the bracts (3), which coincides with some notations of the Ranco ecotype even though Ranco showed a bigger ratio than Hallertauer Mittelfrüh for this character.

Finally, for the apex length character, the Ranco ecotypes presented bract apices of medium size (5) and

also short (1) but in less amount. Hallertauer Mittelfrüh has a long bract apex (7) (Lutz and Kneidl, 2009), therefore, in this case there is no coincidence of what has been described.

The comparison of the ecotype in Hueimén and Valdivia shows coincidence in 8 out of the 14 characters evaluated. However, they differ in cone size and shape and score equally in the bracts. Eibel *et al*., (2015), compared three different Chilean ecotypes morphologically and reported that the ecotypes only differed in shape and size of the cones, while the leaf characteristics did not present statistically significant differences (data obtained from Chilean ecotypes located in different areas). For the remaining observed characters, there was no source of comparison. Therefore, according to these phenotypic data and compared to the variety Hallertauer Mittelfrüh, it is difficult to infer the degree of kinship between the variety and the ecotype, with the more remarkable coincidence being the anthocyanin pigmented stems. Otherwise, the congruence in scorings of the Ranco ecotype in both locations showed the same tendency so that the ecotype could be morphologically differentiated from hop varieties. These results revealed a more significant influence of the genotype rather than the environment (Beatson and Brewer, 1994).

Comparing the degree days necessary for flowering, the ecotype presented a total of 1035.8 degree-days, showing a greater accumulation of degree-days for flowering than the 867.9 degree-days required by Hallertauer Mittelfrüh (Rossini *et al.,* 2016). Furthermore, Hallertauer Mittelfrüh is considered an early ripening variety, compared to ecotype Ranco, which possessed a medium to late maturity.

To determine yield prospecting of the Ranco ecotype, a planting density of 2,211 plants per ha was assumed with a planting frame of 3 m between row and 1.5 m on row (Magadán *et al.,* 2011). The foliar area developed in Hueimén was greater than in Valdivia, but cone size and weight did not show major differences when comparing the results of the two locations (Table 2). In commercial plantations of the Ranco ecotype, and depending on the harvest and post-harvest conditions, yields of 1,000 to 1,350 kg/ha were commonly reached in the region. Among the agronomic aspects, it was found to be susceptible to *Pseudopernospora humuli* and shows low tolerance to *Tetranychus urticae* (data not shown).

This study gives an indication of the behaviour of the ecotypes in different locations, showing the impact of the environment even within the same region. The relevance of the morphological data is given by the size of the leaves and the size and shape of the cone which provide information about the potential yield per plant. The plant shape provides information, among others, about the need to control fungal diseases such as *Pseudoperonospora humuli.* Since the plant is club-shaped, it lacks an adequate amount of foliage at the base to prevent the rapid ascent of the fungus from the soil or weeds, being an ecological solution to reduce the applications of chemicals for disease control. On the other hand, in a more cylindrical plant the side and cone are more homogeneous, presenting less competition and larger cones. The length of the side shoots also helps with nutrient distribution and product homogeneity, allowing light through the leaves. Furthermore, the morphology of the bracts, size, shape, degree of opening, and width/length ratio contribute to the protection of lupulin within the cone.

The Los Ríos Region has a privileged climate for growing hops with high yields and optimum development of the hop components. Van Holle *et al.* (2021), observed that not only the variety as well the *terroir* may have a significant impact on hop's biochemical and brewing characteristics and consequently, on the resulting beer flavour profile (Van Holle *et al.,* 2021).

The aromatic profile of the Ranco ecotype is predominantly herbal and woody with stone fruit aroma, as described by Lúpulos Hueimén (Figure 3). It has been tested to produce pilsner beer with dry hopping, obtaining positive feedback from the sensory panel participants (data not shown).

Chemical characterisation

Bitter acids

The α-acids (humulones) and β-acids (lupulones) represent a significant portion of the dry constituents

Table 2. Description of the foliar area, number of cones, weight, and size of cones of one plant (2019). (Referential information without statistical validity).

Tabla 2. Descripción del área foliar, número de conos, peso y tamaño de los conos de una planta (2019). (Información referencial sin validez estadística).

Location	Foliar area (cm^2)	Number of cones	Cone weight (g)	Cone size (cm)
Valdivia	15163.45	1222	0.17	2.2
Hueimén	22654.65	3404	0.12	2.0

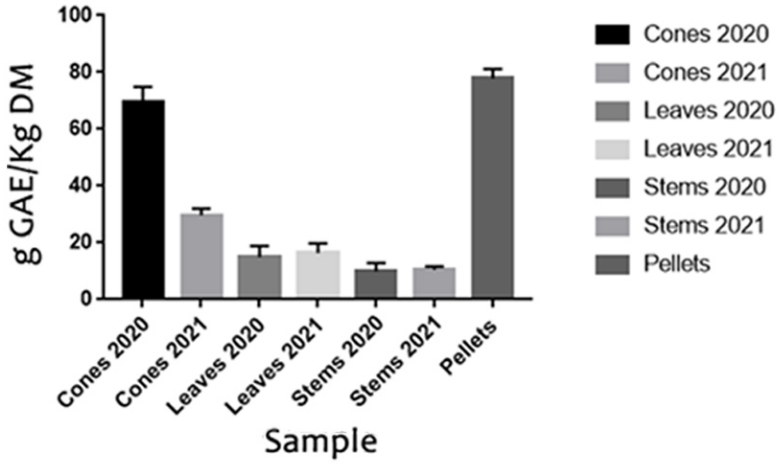

Figure 2. Total polyphenols (g GAE/kg DM) in hops samples for the harvest in 2020 and 2021.

Figura 2. Polifenoles totales (g GAE/kg MS) en muestras de lúpulo para la cosecha de 2020 y 2021.

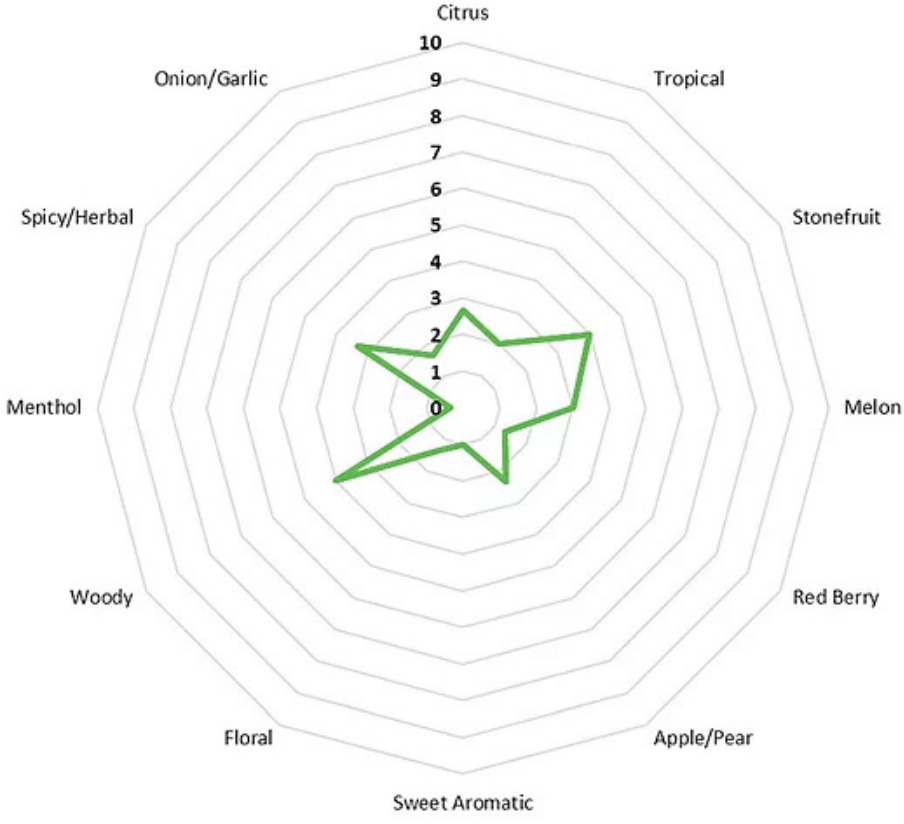

Figure 3. Aromatic description of Ranco ecotype (as shown in www.lupuloshueimen.cl, with permission from Lúpulos Hueimén).

Figura 3. Descripción aromática del ecotipo Ranco (como se muestra en www.lupuloshueimen.cl, con permiso de Lúpulos Hueimén).

of hop cones, being associated with flavour and bitterness of beer, besides playing an important role in plant properties, including antimicrobial potential (Sanz *et al.,* 2019; Arruda *et al.,* 2021). The chemical composition for bitter acids of hop cones was measured on harvested samples collected during the seasons 2020 and 2021 in Hueimén, analysing parameters as α-acids, β-acids, ratio α/β and cohumulone (% relation). The content of α-bitter acids in 2020 mainly ranged between 0.5-1.0% w/w, being lower than β-bitter acids. On the other hand, the content of α-bitter acids in the year 2021 ranged mainly between 0.8-1.2% w/w and was lower than β-bitter acids. Furthermore, the ratio of α/β bitter acids was lower than 1.0 in both years (2020 and 2021). Cohumulone ratio was predominantly in the range 22-27% relative to total α-acids. To carry out the analysis, three plants were considered in each season (Table 3).

The content of α- and β-acids for the Ranco ecotype was lower than that existing in commercial varieties; however, the amount contained was not too far from that of the Saaz variety from the Úštěk area of the Czech Republic in 2018 (Mikyška and Jurková, 2019), where the values of α- and β -acids averaged values of 2.00% and 4.02% w/w, respectively. Likewise, the α/β ratio presented a value of 0.48. At the same time, the cohumulone (% relative to α-acid) reached a value of 23.7 in the Saaz variety for the mentioned area, both values being similar to those existing for the Ranco ecotype.

Results from the Ranco ecotype in Hueimén during previous years showed scores of 2.6% α-acids, 4.8% β-acids, an α/β ratio of 0.542, and 26.1% cohumulone relative to the total acids content. The reasons for the lower values range for the seasons 2020 and 2021 are probably the extensive oxidation that bitter acids undergo when exposed to the environment and the zero-order kinetics of degradation that these acids undergo, even at low temperatures (Skinner *et al.,* 1977).

Polyphenols and other compounds

Polyphenols contain one or more aromatic rings and two or more hydroxyl (OH) groups attached to the aromatic rings. The polyphenols that use hops as an ingredient include simple polyphenols (two or more hydroxy groups on a single aromatic ring) or multiple ring structures. Polyphenols are present in hops and have potential in hop-based products.

Historically, hop cones have been used since ancient times as a remedy for many ailments and as a source of polyphenols and bitter acids. It is very effective in treating metabolic syndrome (MS). In addition, hop flavonoids, particularly xanthohumol (XN), are substances with hypoglycemic, antihyperlipidemic, and anti-obesity activities (Dostálek *et al.,* 2017). Other compounds like terpenes are widely used in industry, perfumery, and traditional medicines. Molecules like these are present in hops. They show low toxicity and high bioavailability and readily cross the skin and the blood-brain barrier. They have an excellent therapeutic index, i.e. they are well tolerated without side effects, and the therapeutic effects are achieved far before the lethal dose. Many terpenes exhibit high selectivity over receptors such as transient receptor potential (TRP) channels and dopaminergic and GABAergic receptors. The additive and synergistic action of terpenes with other drug molecules and the improved penetration of other drugs by either adjuvant or covalent fusion have been already gained. Compounds in hops exhibit antibiotic, anti-inflammatory, anti-antioxidant, anticancer and anti-tumour activities. They show anticancer activity often by pro-apoptotic action but are non-toxic for healthy cells or tissues - they are rather neuro-, hepato-, and nephroprotective (Nuutinen, 2018).

These polyphenolic extracts are attractive ingredients for the cosmetic and pharmaceutical industries due to their beneficial biological properties (Zillich *et al.,* 2015). In cosmetics, hops are used in bath lotions, among others (Cieśliński and Idowski, 2003; Astray *et al.,* 2020). Supercritical CO_2 extracts of hop cones were used to formulate shower gels resulting in enhanced skin-conditioning properties due to their content in bioactive ingredients (Vogt *et al.,* 2014). Moreover, the formulations containing hop cone extract have compounds that treat oil and dandruff in hair. The use of hop extract in hair cosmetics is supported by its antifungal and anti-seborrheic properties that decrease

Table 3. Analysis of bitter acids in hop cones of the Ranco ecotype. Results expressed in % w/w (n = 3 plants).
Tabla 3. Análisis de los ácidos amargos en los conos de lúpulo del ecotipo Ranco. Resultados expresados en % p/p (n = 3 plantas).

			HPLC analysis of bitter acids in hop cones				
Ecotype	Origin	Year	α-acids (% w/w)	β-acids (% w/w)	Ratio α/β	Cohumulone	Cohumulone (% rel.)*
Ranco	Hueimén	2020	0.69	1.26	0.55	0.18	26.22
Ranco	Hueimén	2021	1.05	1.97	0.53	0.24	22.86

(*) % rel. = % relative to total α-acid

brittleness, nourish, provide shine, increase strength, and prevent hair loss (Vogt *et al.,* 2014).

In this study, the total polyphenols of cones, leaves, and stems for the 2020 and 2021 seasons were studied, finding values higher than 60 g GAE/kg DM for the cones of the 2020 season and slightly lower than 10 g GAE/kg DM in stems the year 2021. Thus, the results of cones stood out in 2020 over the same matrix of the previous year. The Figure 2 shows the results obtained for the 2020 and 2021 seasons. Further agronomic investigations are needed to elucidate the variability.

In the 2020 season, the quantification of total polyphenols showed higher values for the Ranco ecotype pellets when compared to the variety pellets for the same season and place, with reported values for the commercial varieties Blend, Cascade and Fuggle of 65.22, 70.95 and 75.43 g GAE/kg DM, respectively.

Finally, it is noteworthy that the chemical composition of hops makes it a plant of interest for the development of various products such as medicines, cosmetics, and food supplements, for use in humans or animals.

Further studies on hop ecotypes collected in southern Chile are needed to complement the data presented in this study to promote and strengthen the sustainable development of related industries.

CONCLUSIONS

The Ranco ecotype was observed morphologically as a plant with a club shape, medium-sized dark green leaves, long side shoots in the upper part of the plant and an anthocyanin pigmented stem. The cones are of medium size, concentrated in the upper third of the plant. It has mid to late maturity, and among the agronomic aspects, it was found to be susceptible to *Pseudopernospora humuli*, showing low tolerance to *Tetranychus urticae*. The prospective yield averaged between 1,000 and 1,350 kg/ha.

The hop cones of the Ranco ecotype analyzed in this study presented low percentages for bitter acids, which might be related to storage conditions that need to be controlled to enable an adequate quality of the product used as raw material. Concerning the aroma, the Ranco ecotype exhibited a predominantly herbal, stone fruit and woody aroma. On the other hand, the different parts of the plant presented significant amounts of polyphenols, similar to commercial hop varieties that could allow their use in the food and cosmetic industries.

The Ranco ecotype is a local and novel hop, with a mild aroma and a moderate concentration of bitter acids, revealing significant contents in polyphenols and abundant development of biomass. All these characteristics suggest that Ranco could provide excellent qualities for the food and cosmetic industries while promoting innovation for new regional beer varieties.

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