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Hop (*Humulus lupulus* L.) Essential Oils and Xanthohumol Derived from Extraction Process Using Solvents of Different Polarity

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Abstract: This study evaluates the content of essential oils (EOs) and prenylated flavonoid Xanthohumol (XN) in extracts of Slovenian hops, cultivar Aurora, obtained by using fluids of different polarity. It is a continuation of our previous work, investigating the extraction of bitter acids from hops. Extraction was conducted semi-continuously, using sub- and supercritical fluids of different polarity, i.e., carbon dioxide (CO_2) and propane as non-polar and dimethyl ether (DME) as the polar solvent. The experiments explored a temperature range between 20 °C and 80 °C and pressures ranging from 50 bar to 150 bar. The content of XN in extracts was analysed using high-performance liquid chromatography and experiments demonstrated the largest concentration of XN was obtained using DME. In order to analyse the EO components in extracts, connected with a distinct odour, the steam distillation of extracts was performed and GC analysis was employed. Hop oil derived from CO_2 extracts at specific conditions, had the highest relative concentration of linalool, β-caryophyllene and α-humulene, and oil derived from propane extracts had the highest content of all other five selected components (myrcene, geraniol, farnesene, α-selinene and δ-cadinene). The relative content of the investigated EO components in DME extracts was similar to that in propane extracts.

Keywords: hop extract; hop essential oils; flavonoids; carbon dioxide; propane; dimethyl ether

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1. Introduction

Humulus lupulus L., more commonly referred to as hops, has a long history. The first description as "lupus salictarius" dates back to the first century B.C. in the book "Naturalis Historia" [1]. Hops were first used for medicinal purposes. Nowadays, hops and their extracts have turned the focus of scientists globally towards exploring the biological effects when used in conventional medicine. New developments point towards the plant as being a valuable source of material in the areas of nutraceuticals, dietary supplements and other health-promoting products. The hop plant is a perennial, herbaceous climbing plant from the *Cannabaceae* family that regrows each spring from the rhizomes of its underground rootstock. It is widely cultivated in Europe, West Asia and North America [2,3]. The plant is a slender climbing vine that produces stems annually and grows in excess of 6–7 m per season. It is dioecious, i.e., the plant has separate genders. Male flowers are not cultivated as they do not develop lupulin glands, whereas female plants do. Wind dispersal aids the natural pollination of hops [4,5]. Lupulin glands are found in the hop inflorescence produced by cone-shaped catkins (Figure 1) of female hop plants and are rich in EOs and resins. These glands are known to contain numerous unique bioactive secondary metabolites, consisting of prenylated flavonoids (xanthohumol and desmethylxanthohumol), bitter

acids, phenolic compounds and EOs [6]. Substances determined in lupulin are often called resins and EOs. Hops are generally known as an ingredient in beer. The first records of using hops in the brewing of beer appeared in the late 11th century [7]. Hop resin content provides the bitterness and EOs the aroma and flavour. The most abundant components in the soft resins are hop acids, formed of two groups of compounds, alpha-acids (humulones) and beta-acids (lupulones). In the brewing process, these act as preservative and bittering agents [8].

Figure 1. Hop cones of the female hop plant (*Humulus lupulus* L.) cv. Aurora. Photo credit: Katja Bizaj—Hop field in Slovenia in pre harvest season of year 2021.

In the 11th century, a book assigned to the physician Mesue reported the first applications of hops as a medicinal plant with their anti-inflammatory properties [7]. Hop was primarily used for the treatment of sleep and anxiety disorders. It was also employed as an agent to enhance gut function and the improvement of pharmaceutical performance because of its antimicrobial and antifungal characteristics [9]. Oxidative stress is an important factor contributing to the development of chronic diseases, and polyphenols extracted from enriched plants are widely used as a source of natural antioxidants [10]. Over the years it has been reported about a wide range of further effects, both *in vivo* and *in vitro*, including the treatment of diabetes mellitus, anti-carcinogenesis, and cardiovascular diseases [11]. Hop extracts are a valuable source of bioactive (antibacterial, antifungal, cardioprotective, antioxidant, anti-inflammatory and antiviral effects, etc.) compounds [12,13]. The most abundant prenylated flavonoid existing in hops, xanthohumol (XN) (Figure 2), is secreted within the hop resin (lupulin) by glandular trichomes on the adaxial surfaces of cone bracts and displays numerous beneficial bioactivities, in particular, including antioxidant activities [14,15].

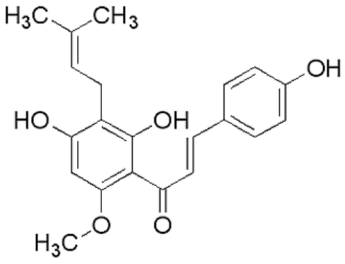

Figure 2. Chemical structure of xanthohumol.

Hop oil is one of the most complex EOs derived from plants. The oil is a mixture of at least 1000 compounds, which include alcohols, terpenes, organic acids and phenolics [16]. Hop essential oil (HEO) accounts for up to 4.0% of the dried hop weight [17] and hydrocarbons represent between 50 and 80% of the total oil content. The main terpenes found are the monoterpene β-myrcene and the sesquiterpenes β-caryophyllene and α-humulene [18]. The second group are oxygen-containing compounds representing around 30% of the total oil [19–21] and the third group, the thioesters, sulphides and other sulphur-containing compounds, are found only in traces [20–24]. Those compounds can be divided into groups based on the type of aroma they provide, such as citrus, herbal, floral, fruity or a typical hoppy aroma. However, the greatest contribution of aroma is attributed to the monoterpene alcohols and oxidation products of mono- and sesquiterpenes [25,26]. HEO has been used in traditional medicine for centuries because of the healing effects of those terpenes and terpenoids [7,11,27]. Analgesic, anticancer, sedative and anti-inflammatory effects are only some of the most important health benefits of HEO. Typically, total hop oils are isolated from hops in two steps. The first step is liquid or supercritical extraction, which produces pure resin or oil-rich hop extract. In the second processing step, HEO can be obtained by steam distillation or molecular distillation of the hop extract under high vacuum [28]. Further determination of total hop oils into hop essences can be achieved using chromatographic methods [29].

Many papers have discussed the sub- and supercritical fluid extraction of hops and the usage of various gases as extracting solvents. The efficiency of the process is frequently reported to rely on the solvent used. Conventional solvents have good selectivity, a relatively low boiling point and a low enthalpy of vaporization, facilitating the low energy removal of the solvent at the end of the process. However, cost reduction, combined with restrictions placed against organic solvents has reduced the number of available solvents for use. Carbon dioxide has proved to be a highly efficient primary solvent for extraction as a result of its deep penetration into biological materials and its high solvent power [21]. Depending on the conditions and technologies used, a variety of final product compositions can be obtained [30]. CO_2 has been used as the primary solvent for over 90% of supercritical fluid extraction (SFE) studies [21]. Supercritical CO_2 is an effective solvent for the extraction of non-polar compounds, which include hop soft resins, oil and aroma components. Additionally, CO_2 has a large quadrupole moment, which enables it to dissolve some moderately polar compounds such as polyphenols [31,32]. To extract biologically active prenylated flavonoids such as XN, a co-solvent is needed. Compressed propane has been proposed as a viable non-polar solvent in several studies, which demonstrates good extraction yields and a high antioxidant activity of extracts [33–38].

However, based on the authors' knowledge, no study has investigated the extraction process of hops using DME and sulfur hexafluoride (SF_6) as solvents. As such, Bizaj et al. investigated sub- and supercritical extraction of Slovenian hops, Aurora variety, by using sub- and supercritical fluids of different polarities [39]. DME was used as a polar solvent, and CO_2, propane and SF_6 were employed as non-polar solvents. Differences in the extracted concentrations of α- and β-acids obtained from hop pellets were observed. The study further determined mass transport coefficients, crucial for the design and scale-up of the extraction process with mathematical modelling presented in detail. DME at 60 °C and 150 bar yielded the highest extraction yield (25.6%). DME also demonstrated tuneability

with temperature and pressure both having a remarkable effect on the extraction yield and max. concentration of both α-acids and β-acids. The highest extracted concentration of α-acids was 9.6% (40 °C, 100 bar) and for β-acids, 4.5% (60 °C, 50 bar). In the study, propane proved to be the second-best solvent based on extraction yield (18.7% under operating conditions 60 °C and 150 bar) with concentrations of both α- and β-acids also being high (8.7% of α-acids and 4.3% of β-acids). With CO_2, the maximum yield (12.2%) was reached at 40 °C and 150 bar with a max. concentration of α-acids of 7.9%. SF_6 proved to be a very poor solvent for extracting hop resins, having a maximum extraction yield of only 0.9% at 60 °C and 150 bar.

In the present work, the content of hop prenylflavonoid (XN) and EOs have been determined in hop extracts obtained by the extraction of Slovenian hop cv. Aurora using CO_2, propane and DME, while extracts derived with SF_6 as a solvent were not analysed further because of low extraction yield.

2. Materials and Methods

2.1. Materials

For this research, the hop cultivar Aurora was used. Aurora is a diploid hybrid between Northern Brewer and a TG seedling of unknown origin. It has an intense hoppy aroma as well as an intensive green colour. Hop cones were harvested and picked in a selected hop garden planted in Lower Savinja Valley in Slovenia at their optimal maturities between 23rd and 30th of August, air dried at 60 °C, homogenised and compressed into pellets. These were consequently purged with inert gas N_2 and sealed in laminated polythene/metallised polyester bags. Pellets were stored away from sunlight at temperature between +1 °C to +4 °C to inhibit excessive oxidation. The content of XN in raw material (pellets) was 0.53% (*w*/*w*).

CO_2 (>99.5% purity) was obtained from Messer Slovenija d.o.o. (Ruše, Slovenia). Propane (>99.5% purity) and DME (>99.5% purity) were obtained from Linde plin d.o.o. (Celje, Slovenia). All chemicals used for HPLC and GC analysis were purchased from Sigma-Aldrich (Darmstadt, Germany).

2.2. Equipment and Experimental Procedures

2.2.1. Methods for Characterization of Material

The pellets were stored in refrigerated storage for a month and then ground in domestic grinder. To obtain homogeneous hop particles for further experiments the sieve analysis was performed to separate the fractions. The median particle size of grinded hop pellets was 1300 µm, determined from particle size distribution curves (frequency and cumulative arithmetic). Using standard Analytica—EBC method 7.2 (Analytica—EBC 2007) [40], the moisture content of raw material and extracts was determined. The moisture content was taken under consideration in all calculations, and therefore yields and concentrations are expressed on dry basis.

2.2.2. Sub- and Supercritical Fluid Extraction (SFE)

Extractions were conducted using a semi-continuous flow apparatus using CO_2, propane and DME as solvents. The extractions with CO_2 and propane were performed at temperatures 20 °C, 40 °C, 60 °C and 80 °C and extractions with DME at 40 °C, 60 °C and 80 °C, while the pressure range was from 100 bar to 150 bar for CO_2 and 50 bar to 150 bar for propane and DME. The apparatus and experimental procedure is described in previous work [39]. Extracts obtained were stored in dark at temperature between +1 °C to +4 °C for further analysis.

Equation (1) was used to calculate the extraction yield.

$$Yield_{(Ext.)} = \frac{m_{\ extract}}{m_{of\ matrix}} * 100\% \quad (1)$$

2.2.3. Analysis of Xanthohumol in Extract by HPLC

According to Analytica—EBC 7.7 method [41], high-performance liquid chromatography (HPLC) was employed to determine prenylated flavonoid xanthohumol in extracts, utilising liquid chromatograph Agilent 1200 Series (Agilent, Palo Alto, CA USA). In total, 0.5 g of hop extract were added to 80 mL of the mixture of diethyl ether/methanol and 0.1M aqueous hydrochloric acid in a ratio of 10/50/20 ($v/v/v$). Extracts were filtered through the Chromafil Xtra PET- 45/25 (Macherey-Nagel, Düren, Germany) disposable syringe filter, and 10 µL injection loop on HPLC injector was used. The separation was achieved on Nucleodur 5–100 C18, 125 × 4 mm HPLC analysis column (Macherey-Nagel, Düren, Germany). The isocratic mobile phase comprised of distilled water, methanol (J.T.Baker, Phillipsburg, NJ USA) and 85% aqueous solution of orthophosphoric acid (MERCK, Darmstadt, Germany) in a ratio of 775/210/9 ($v/v/v$) and the detection was carried out with diode array detector (DAD) set at 370 nm. The quantification was performed utilising the external standard (HHV, Mainburg, Germany). All solvents were obtained from Sigma-Aldrich (Darmstadt, Germany) and were of analytical grade purity.

Yields of XN, which represent the wt% of discharged XN in relation to the initial concentration of XN in raw material, are calculated using Equation (2)

$$Yield_{(XN)} = \frac{m_{(XN)in\ extract}}{m_{(XN)in\ raw\ material}} \times 100\% \quad (2)$$

2.2.4. Analysis of EO Content and Composition by GC

Determination of total EO content in extract was carried out according to Analytica—EBC 7.10 method [42]. Briefly, 25 g of extract was mixed with 1000 mL of deionized water and steam distilled for 3 h. Identification and quantification of components of hop essential oil were carried out according to Analytica—EBC 7.12 method [43]. In total, 0.2 mL of collected oil was diluted with 5 mL of hexane and separated by GC analysis. Hewlett-Packard 5890 series GC system (Hewlett-Packard, Palo Alto, USA) equipped with the flame ionisation detector and HP-1 capillary column (30 m × 0.25 mm, 25 µm, Agilent, Palo Alto, USA) and nitrogen 5.0 as a carrier gas with a flow rate of 0.6 mL min^{-1} was used. In total, 1 µL of solution was injected in the injector at the temperature of 200 °C. The temperature programme was 1 min at 60 °C, 2.5 °C min^{-1} to 190 °C, 1 min at 190 °C, 70 °C min^{-1} to 240 °C and 11 min at 240 °C. The detection was carried out on a flame ionization detector set at 260 °C. For all analysed components the standard compounds, i.e., myrcene, linalool, geraniol, β-caryophyllene, α-humulene, farnesene, α-selinene and δ-cadinene were used for identification and were purchased from Sigma-Aldrich, Darmstadt, Germany. Method was validated regarding the linearity at six points over the expected concentration range, LOQ, accuracy and repeatability (Table 1).

Table 1. Concentration range, correlation coefficients, accuracy, repeatability and LOQ data.

EO Component	*Linearity Range* (g/L)	*Correlation Coefficient* R^2	*LOQ* (g/L)	*Accuracy* (%)	*RDS* (%)
myrcene	2.72–27.23	0.9996	1.85	102.25	2.45
linalool	0.24–1.18	0.9994	0.09	92.71	13.93
geraniol	0.16–0.81	0.9986	0.09	80.16	9.40
β-caryophyllene	0.12–0.60	0.9992	0.05	96.69	9.62
α-humulene	9.36–23.4	0.9946	2.37	100.64	4.85
farnesene	1.14–2.86	0.9954	0.76	114.63	4.64
α-selinene	0.24–0.60	0.9927	0.13	84.14	5.33
δ-cadinene	0.43–0.98	0.9976	0.26	103.25	4.20

3. Results and Discussion

3.1. Content of XN in Hop Extracts

The concentrations of XN in the hop extracts obtained with CO_2, propane and DME are presented in Table 2. The yield of XN calculated using Equation (2) exemplifies the wt% of the extracted XN in relation to the initial concentration of XN in the raw material. Equation (1) was used to represent the overall yield of the extraction process in wt%.

Table 2. Yield of extracted Xanthohumol and its concentration in the extracts expressed in weight % (mean ± SD) prepared by using CO_2, propane and DME. (Limit of quantification, LOQ = 0.1%).

Solvent Used for Extraction	*T* (°C)	*p* (bar)	$Yield^{*}_{(Ext.)}$ (%)	$W^{**}_{(XN)}$ (%)	$Yield^{***}_{(XN)}$ (%)
CO_2	20	100	11.4	0.0	/
	20	150	11.7	0.0	/
	40	100	2.6	0.0	/
	40	150	12.2 ± 0.2 a	0.0	/
	60	150	6.5	0.0	/
	80	150	3.7	0.0	/
propane	20	50	12.6	0.0	/
	20	100	15.3	0.0	/
	20	150	15.3	0.0	/
	40	50	15.6	0.0	/
	40	100	16.9	0.0	/
	40	150	17.8	0.04 ± <0.1	1.35
	60	50	18.4	0.04 ± <0.1	1.38
	60	100	18.6	0.13 ± <0.1	4.57
	60	150	18.7 ± 0.1 b	0.12 ± <0.1	4.21
	80	50	14.3	0.0	/
	80	100	16.1	0.01 ± <0.1	0.30
	80	150	16.9	0.01 ± <0.1	0.32
DME	40	50	24.9	1.96 ± 0.1	92.11
	40	100	22.9	1.92 ± 0.1	82.83
	40	150	25.3	1.72 ± 0.1	82.12
	60	50	23.9	1.82 ± 0.1	81.96
	60	100	24.9	1.65 ± 0.1	77.56
	60	150	25.6 ± 0.5 c	1.78 ± 0.1	85.96
	80	50	24.7	1.20 ± 0.1	56.0
	80	100	24.0	1.36 ± 0.1	61.46
	80	150	23.5	1.48 ± 0.1	65.62

* Extraction yield expressed in wt%, a, b, c—based on triplicate experiments (mean ± standard deviation). ** Concentration of xanthohumol in extract expressed in wt% (mean ± standard deviation). *** Yield of xanthohumol isolated regarding initial concentration of xanthohumol in raw material expressed in wt%.

XN is a flavonoid of the chalcone group. It is a non-polar compound that is only partially soluble in hot water but highly soluble in alcohol or alcohol–water mixtures. On the other hand, it is insoluble in non-polar solvents such as hexane. In the literature, the extraction process using non-polar CO_2 is described as inept for XN extraction [44–47], unless an organic co-solvent is added. However, it has been found that CO_2 can be a good solvent for XN at pressures above 500 bar and temperatures above 60 °C [48]. The results of the present work (Table 2) agree with the previous findings and show that, although CO_2 generally acts as a selective solvent for the extraction of non-polar compounds, it does not dissolve XN in the temperature and pressure ranges investigated. The fraction of hops extracted by CO_2, under sub- and supercritical conditions at densities ranging from 427.2 kg/m^3 to 904.0 kg/m^3, contained hop bitter acids (α-acids and β-acids) [39] and hop oil, and no XN was found. In the case of propane, the maximal concentration of XN in the extract was obtained at conditions that also gave the maximal yield of extraction (60 °C and 100 bar).

The highest yields of XN were obtained with DME, which were 20 times greater than for extracts obtained using propane. These results were obtained with DME at 40 °C and 50 bar. Increasing the pressure to 150 bar, the yield of XN decreased. Conversely, at 80 °C, the yield of extraction decreased but the yields increased with increasing pressure. When comparing the results, the highest yield of XN (92.1%) was obtained by extraction with DME at 40 °C and 50 bar where the extraction yield was also high (24.9%). The results show that XN is highly soluble in DME, making it possible to extract almost all XN from the raw material.

3.2. Identification and Evaluation of the Selected Essential Oil Components in Extracts

Figure 3 gives a typical chromatogram, and the relative amounts (rel.%) of hop oil components in hop extracts obtained with CO_2, propane and DME are presented in Table 3. These identified components were myrcene, linalool, geraniol, β-caryophyllene, α-humulene, farnesene, α-selinene and δ-cadinene. Compounds in HEO can be divided into groups, based on the type of aroma they provide. The aromas presented in this paper are floral, herbal and spicy-woody or a typical hoppy aroma. The greatest contribution to the aroma is attributed to the oxidation products of mono- and sesquiterpenes [25,26], and their contributions to the woody or spicy characteristics have been reported [49,50]. The most aroma-contributing compound and quality indicator of hops is linalool with a floral odour, and it is the major constituent of the alcohol fraction, followed by geraniol [51]. Both are noted as highly flavour active and contribute to the aroma in terms of floral and citrus odours. Linalool is a hydration product of myrcene and, during fermentation, geraniol can also be transformed to some extent into linalool [52]. Oxidation degradation products of sesquiterpenes are generally sesquiterpene alcohols, and the oxidation products of α-humulene and β-caryophyllene are the epoxides found in hops [23].

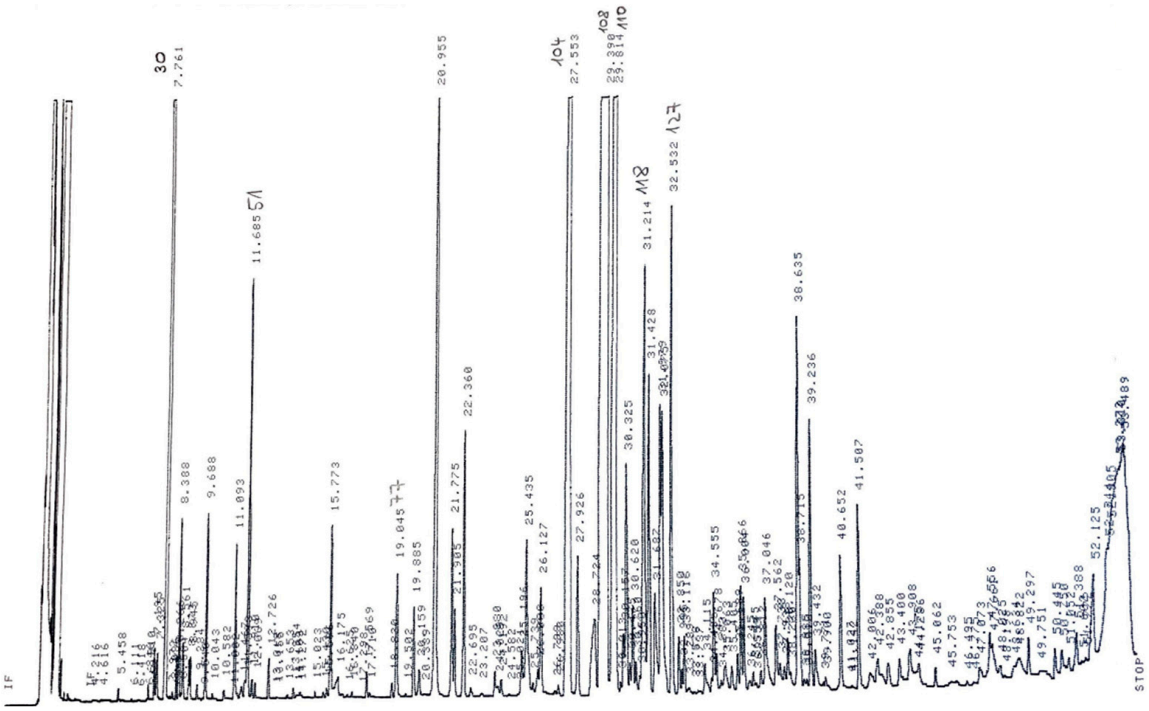

Figure 3. GC analysis chromatogram of Aurora variety HEOs obtained with propane as solvent at 40 °C and 50 bar. (Peak values: 30—myrcene, 51—linalool, 77—geraniol, 104—β-caryophyllene, 108—α-humulene, 110—farnesene, 118—α-selinene, 127—δ-cadinene.)

Table 3. Evaluation of hop oil marker components, connected with a distinct odour, present in the oil in extracts derived with different solvents in combination with different pressures and temperatures, expressed in relative %.

Essential Oil Components Regarding Aroma Group			*Spicy-Woody*	*Floral*	*Floral*	*Spicy-Woody*	*Spicy-Woody*	*Spicy-Woody*	*Herbal*	*Herbal*
Oil Marker Components			***myrcene* * (rel.%)**	***linalool* * (rel.%)**	***geraniol* * (rel.%)**	***β-caryophyllene* * (rel.%)**	***α-humulene* * (rel.%)**	***farnesene* * (rel.%)**	***α-selinene* * (rel.%)**	***δ-cadinene* * (rel.%)**
Solvent Used for Extraction	***T* (°C)**	***p* (bar)**								
CO_2	20	100	4.04 ± 0.3	0.70 ± <0.1	0.39 ± <0.1	12.46 ± 0.5	33.47 ± 0.7	4.71 ± 0.1	0.95 ± <0.1	2.45 ± 0.1
	20	150	2.50 ± 0.2	0.77 ± <0.1	0.42 ± <0.1	12.39 ± 0.5	43.74 ± 0.9	3.96 ± 0.1	0.95 ± <0.1	2.36 ± 0.1
	40	100	0.10 ± <0.1	0.89 ± <0.1	0.42 ± <0.1	7.47 ± 0.3	28.88 ± 0.6	1.67 ± <0.1	0.87 ± <0.1	1.95 ± 0.1
	40	150	2.57 ± 0.2	0.79 ± <0.1	0.37 ± <0.1	10.28 ± 0.4	37.14 ± 0.8	3.49 ± 0.1	0.92 ± <0.1	2.37 ± 0.1
	60	150	0.66 ± <0.1	2.60 ± 0.1	0.19 ± <0.1	13.49 ± 0.6	46.30 ± 1.0	4.89 ± 0.1	0.98 ± <0.1	2.55 ± 0.1
	80	150	0.04 ± <0.1	0.40 ± <0.1	0.48 ± <0.1	7.60 ± 0.3	31.82 ± 0.7	1.31 ± <0.1	1.01 ± <0.1	2.38 ± 0.1
propane	20	50	13.60 ± 0.9	0.81 ± <0.1	0.66 ± <0.1	11.55 ± 0.5	34.37 ± 0.7	11.73 ± 0.3	2.17 ± 0.1	1.79 ± 0.1
	20	100	17.59 ± 1.2	1.12 ± <0.1	0.44 ± <0.1	11.19 ± 0.5	31.19 ± 0.7	10.76 ± 0.3	1.70 ± 0.1	1.43 ± 0.1
	20	150	12.19 ± 0.8	0.66 ± <0.1	0.36 ± <0.1	10.69 ± 0.4	32.05 ± 0.7	11.15 ± 0.3	2.06 ± 0.1	1.68 ± 0.1
	40	50	9.25 ± 0.6	0.95 ± <0.1	0.44 ± <0.1	10.67 ± 0.4	31.10 ± 0.7	10.62 ± 0.3	1.92 ± 0.1	1.56 ± 0.1
	40	100	14.26 ± 0.9	0.67 ± <0.1	0.37 ± <0.1	9.50 ± 0.4	28.74 ± 0.6	9.65 ± 0.3	1.94 ± 0.1	1.53 ± 0.1
	40	150	3.24 ± 0.2	0.89 ± <0.1	0.45 ± <0.1	11.56 ± 0.5	36.79 ± 0.8	12.44 ± 0.3	2.78 ± 0.1	2.13 ± 0.1
	60	50	8.12 ± 0.5	0.87 ± <0.1	0.51 ± <0.1	11.08 ± 0.5	33.73 ± 0.7	11.68 ± 0.3	2.36 ± 0.1	1.86 ± 0.1
	60	100	5.18 ± 0.3	1.12 ± <0.1	0.51 ± <0.1	10.09 ± 0.4	30.24 ± 0.6	10.20 ± 0.3	2.10 ± 0.1	1.55 ± 0.1
	60	150	10.66 ± 0.7	0.75 ± <0.1	0.42 ± <0.1	10.00 ± 0.4	30.07 ± 0.6	10.16 ± 0.3	1.99 ± 0.1	1.59 ± 0.1
	80	50	1.53 ± 0.1	0.97 ± <0.1	0.36 ± <0.1	12.48 ± 0.5	44.85 ± 0.9	2.66 ± 0.1	1.07 ± <0.1	2.58 ± 0.1
	80	100	2.46 ± 0.2	1.77 ± 0.1	0.60 ± <0.1	9.60 ± 0.4	33.87 ± 0.7	2.33 ± 0.1	2.00 ± 0.1	1.87 ± 0.1
	80	150	0.52 ± <0.1	1.11 ± <0.1	0.61 ± <0.1	10.50 ± 0.4	38.24 ± 0.8	2.25 ± 0.1	0.92 ± <0.1	2.30 ± 0.1
DME	40	50	6.28 ± 0.4	0.76 ± <0.1	0.40 ± <0.1	9.80 ± 0.4	30.18 ± 0.6	10.39 ± 0.3	2.18 ± 0.1	1.66 ± 0.1
	40	100	9.48 ± 0.6	1.12 ± <0.1	0.47 ± <0.1	9.41 ± 0.4	28.65 ± 0.6	9.51 ± 0.2	2.12 ± 0.1	1.52 ± 0.1
	40	150	3.68 ± 0.2	0.82 ± <0.1	0.44 ± <0.1	11.00 ± 0.5	33.94 ± 0.7	11.26 ± 0.3	2.45 ± 0.1	1.80 ± 0.1
	60	50	14.47 ± 0.9	0.87 ± <0.1	0.48 ± <0.1	8.75 ± 0.4	27.25 ± 0.6	8.95 ± 0.2	2.11 ± 0.1	1.50 ± 0.1
	60	100	15.30 ± 1.0	1.11 ± <0.1	0.52 ± <0.1	8.91 ± 0.4	27.53 ± 0.6	9.03 ± 0.2	2.25 ± 0.1	1.52 ± 0.1
	60	150	16.33 ± 1.1	0.73 ± <0.1	0.41 ± <0.1	9.61 ± 0.4	30.23 ± 0.6	9.78 ± 0.3	2.35 ± 0.1	1.71 ± 0.1
	80	50	0.29 ± <0.1	1.42 ± 0.1	0.46 ± <0.1	7.89 ± 0.3	29.11 ± 0.6	2.02 ± <0.1	0.67 ± <0.1	1.65 ± 0.1
	80	100	1.14 ± 0.1	1.60 ± 0.1	0.59 ± <0.1	8.80 ± 0.4	33.47 ± 0.7	2.29 ± 0.1	0.81 ± <0.1	2.13 ± 0.1
	80	150	1.07 ± 0.1	1.46 ± 0.1	0.50 ± <0.1	6.91 ± 0.3	27.01 ± 0.6	1.94 ± 0.1	2.13 ± 0.1	1.77 ± 0.1

* Relative amount of selected components in HEO expressed in wt% (mean ± standard deviation).

The composition of the volatile mixtures present in extract fractions were influenced by temperature, pressure and the selected solvent applied during the extraction. The main terpenes myrcene, β-caryophyllene and α-humulene represented the major peaks and values as can be observed from Figure 3 and Table 3. However, their relative contents varied for various solvents used in the extraction. Both sesquiterpenes, β-caryophyllene and α-humulene, have a similar structure and are less polar than the monoterpene myrcene. Hence, sesquiterpenes were extracted more easily with CO_2 because they are less polar than propane. Myrcene was more soluble in propane and had the highest relative value of 17.6 rel.% observed at 20 °C and 100 bar. The volatiles identified with CO_2 as a solvent were similar to those reported for the EOs, obtained by F. Van Opsteale et al. [53] and Duarte et al. [54], respectively.

The second greatest content of myrcene was detected in the DME extract obtained at 60 °C and 150 bar (16.3 rel.%), although DME is a more polar and aprotic solvent. Linalool concentration varied from 0.4 rel.% to 2.6 rel.% and the best solvent was proven to be CO_2 at 60 °C and 150 bar (2.6 rel.%), while the concentration in propane and DME was similar (1.8 rel.% and 1.6 rel.%). On the other hand, farnesene content was the highest in the propane extract at 40 °C and 150 bar (12.4 rel.%); in the CO_2 extract, lower concentrations were detected (from 1.3 rel.% to 4.9 rel.%), but, interestingly, in DME at a lower temperature, 40 °C, and the highest pressure, 150 bar, relatively high concentrations were detected (11.3 rel.%). Additionally, values related to α-selinene were the highest in propane-derived oil (2.8 rel.%) and DME at 40 °C and 150 bar (2.5 rel.%). Furthermore, δ-cadinene identified in oil derived with both non-polar solvents had the highest concentrations of around 2.5 rel.%. All 27 hop oil samples presented an interesting terpenic profile. In line with expectations, the major compounds in all samples were myrcene, β-caryophyllene and α-humulene. Linalool, geraniol, farnesene, α-selinene and δ-cadinene were identified in all samples, although in much lower relative content. The temperature, pressure and selected solvent applied during the extraction influenced the composition of volatile compounds present in the fractions of the various extracts. Both non-polar solvents (CO_2 and propane) showed a relatively similar chemical profile of volatiles with a majority of sesquiterpenes, but some deviations must be noted. As such, the extracts of cv. Aurora derived with propane had a better terpenic profile with the highest myrcene content, while CO_2 extracts had better relative values of β-caryophyllene and α-humulene. In the review published by F. Van Opstaele et al. [55] regarding SFE of plant EO, it is explained that at a carbon dioxide density of 0.50 g/mL, hop oxygenated sesquiterpenoids required longer extraction times due to their higher molecular weight and polarity, and were completely extracted after 125 min. Therefore, in order to extract total hop oil with CO_2, 125 min is the minimum extraction time under the applied experimental conditions. The extraction time in the present work was around 90 min (data not shown), thus, this could be the reason for the lower value of myrcene in the CO_2 extract. Hop EOs derived with DME somehow had a lower relative amount than all the investigated components, but the difference was not significant.

4. Conclusions

In this study, extracts of hop cones of Slovenian cultivar Aurora, obtained by sub- and supercritical CO_2, propane and DME extraction processes, were analysed for their flavonoid xanthohumol and volatile compounds content, using HPLC and GC methods, respectively. The differences between 27 different hop extracts were observed using a variety of solvents in the extraction process. The results showed that, in spite of the fact that CO_2 acts as a selective solvent for the extraction of non-polar compounds, it was an ineffective solvent for XN over the experimental temperature (from 20 °C to 80 °C) and pressure (from 100 to 150 bar) range. Conversely, DME acts as a polar solvent and the data show it to be the best solvent for dissolving XN from hops, which, to the best of our knowledge, has not yet been reported in the literature. The highest yield of XN of 92.11% (concentration in extract 1.96 wt%) was observed at 40 °C and 50 bar.

Overall, DME showed high variability in solvent power with temperature and pressure both having a remarkable effect on the extraction yield and also on the max. concentration of both acids (α-acids and β-acids) presented in our previous study [39]. With propane, the max. yield of XN was reached at 60 °C and 100 bar and it was 4.57% (0.13 wt% in extract). The opposite findings were noted for the hop oil content in extracts. Hop oil derived from CO_2 extracts at specific conditions had the highest relative values of linalool, β-caryophyllene and α-humulene, and oil derived with propane had the highest content of the other five investigated components (myrcene, geraniol, farnesene, α-selinene and δ-cadinene). Results also showed that for the extraction of hop essential oils, DME is not that superior—as for the extraction of acids and XN. At operating conditions, this study demonstrated that the relative content of selected essential oil components in DME extracts is similar to that in propane extracts.

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