Kaunas University of Technology

Faculty of Chemical Technology

Processing of Hops (*Humulus lupulus* L.) into Valuable Functional Components by Optimizing Supercritical Fluid and Pressurized Liquid Extraction Processes

Master's Final Degree Project

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Summary

Hops (*Humulus lupulus* L.) are valued in the brewing industry for providing bitterness, aroma and taste to beers and in pharmacognosy for their antioxidant, antimicrobial, sedative and estrogenic effects. Hop extracts obtained through sustainable green processes contain valuable bioactives such as bitter acids, essential oils and polyphenols. Emerging novel applications of hop extracts are fuelled by the research on the ever-widening range of beneficial health effects of hop phytochemicals. This work aimed to develop effective supercritical carbon dioxide extraction (SFE-CO_2) and pressurized liquid extraction with ethanol (PLE-EtOH) for the production of non-polar and polar extracts from dual-purpose *Ella* hops with high yield, strong antioxidant potential and high content of selected phytochemicals.

After evaluation of commercial one-stage SFE-CO_2 process at 10-15 MPa and 40°C of ground *Ella* hop pellets, response surface methodology (RSM) with central composite design (CCD) was applied to optimize the SFE-CO_2 process at 25-45 MPa for high yield and in vitro oxygen radical absorbance capacity ($TEAC_{ORAC}$ assay). Through optimized SFE-CO_2 (37 MPa, 43 °C, 80 min) conditions ~3-fold higher extraction yield, antioxidant and bitter acid recovery was achieved under significantly shorter extraction time compared to one-stage SFE-CO_2 at 10-15 MPa and 40 °C. The spectrophotometrical analysis of carotenoids and chlorophylls showed a negligible amount (<0.04%). The major volatiles identified through SPME-GC×GC-TOF-MS analysis, β-pinene, β-myrcene, β-humulene, α-humulene, α-selinene and methyl-4-decanoate attributed fruity, herbal, spicy and woody odour to the extracts.

The impact of SFE-CO_2 parameters on the bitter acid content was evaluated and optimized by RSM-CCD for maximum recovery. The optimal conditions (36 MPa, 40 °C, 90 min) produced a similar yield and higher total bitter acid content as the recovery of α-acids increased compared to the previous SFE-CO_2 extract (37 MPa, 43 °C, 80 min).

The antimicrobial effect of the SFE-CO_2 extract (36 MPa, 40 °C, 90 min) was determined through agar well diffusion assays and confirmed against a strain of *S. aureus* and *E. coli.* The effect of the extract was also tested on the viability of skin cancer, healthy keratinocyte and fibroblast cells.

Under the biorefinery concept, the residue of bitter acid optimized SFE-CO_2 (HR) was further utilized for the extraction of polar compounds. PLE-EtOH process was optimized by RSM-CCD for high yield, antioxidant activity (measured by TPC and ORAC assays) and recovery of xanthohumol, a bioactive prenylflavonoid (analysed by HPLC-DAD). Optimal conditions were separated, maximized yield coupled with strong antioxidant potential (85 °C, 18 min), and maximized xanthohumol content (40 °C, 15 min). The PLE-EtOH extracts proved to have antimicrobial effects against a *S. aureus* strain in an agar well diffusion assay.

Nóra Emilia Nagybákay. Apynių (*Humulus lupulus* L.) perdirbimas į vertingus funkcionaliuosius komponentus optimizuojant ekstrakcijos virškriziniais ir aukšto slėgio tirpikliais procesus. Magistro baigiamasis projektas / vadovė Assoc. prof. dr. Vaida Kitrytė; Kauno technologijos universitetas, Chemijos Technologijos Fakultetas.

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Santrauka

Apyniai (*Humulus lupulus* L.) aludarystėje yra labai vertinami, nes alui suteikia kartumą, specifinį kvapą ir skonį, o farmacijoje dėl jų antioksidacinio, antimikrobinio, raminamojo ir estrogeninio poveikio. Taikant įvairius ekstrakcijos metodus apynių ekstraktuose galima sukoncentruoti nemažai vertingų bioaktyvių junginių, tokių kaip karčiosios rūgštys, eteriniai aliejai ir polifenoliniai junginiai. Papildomai, moksliniai tyrimai apie apynių fitocheminių komponentų teigiamą poveikį sveikatai leidžia pagrįstai tikėtis, jog tokie ekstraktai turėtų plačias pritaikymo galimybes įvairiose maisto, farmacijos ir kosmetikos pramonių srityse. Šio darbo tikslas buvo sukurti efektyvius apynių virškrizinės CO_2 ekstrakcijos (SKE-CO_2) ir ekstrakcijos etanoliu aukštame slėgyje (ETPS-EtOH) pocesus, siekiant pagaminti nepolinius ir polinius aromatinių/karčiųjų *Ella* apynių ekstraktus, pasižyminčius didele išeiga, aukšta *in vitro* antioksidacine geba ir dideliu tikslinių fitocheminių medžiagų kiekiu.

Apynių SKE-CO_2 procesas buvo optimizuotas taikant CCD-RSM, siekiant gauti didžiausią ekstrakto išeigą bei deguonies radikalų absorbcijos gebą *in vitro*. Taikant optimalias SKE-CO_2 sąlygas (37 MPa, 43 °C, 80 min) per žymiai laiką buvo gauta 3 kartus didesnė išeiga, *in vitro* antioksidacinis aktyvumas bei α- ir β-karčiųjų rūgščių kiekis lyginant su tradiciškai pramoninei apynių ekstraktų gamybai taikomu vienos pakopos SKE-CO_2 10-15 MPa slėgyje bei 40 °C temperatūroje. Pigmentų karotinoidų ir chlorofilų kiekis ekstraktuose buvo <0,04%. Pagrindiniai identifikuoti lakieji aromato junginiai nustayti SPME-GC×GC-TOF-MS metodu buvo β-pinenas, β-mircenas, β-humulenas, α-humulenas, α-selinenas ir metil-4-decenoatas, kurie ekstraktams suteikė vaisių, žolelių, aštrų bei medienos kvapus. Papildomai vertinant SKE-CO_2 parametrų įtaką karčiųjų rūgščių kiekybinei ir kokybinei sudėčiai nustatyta, kad ekstrahuojant apynius šiek tiek žemesniame slėgyje bei temperatūroje (36 MPa, 40 °C, 90 min) galima išskirti didžiausią α-ir β-karčiųjų rūgščių kiekį. Nustatyta, kad šis SKE-CO_2 ekstraktas slopina *S. aureus* ir *E. Coli* patogeninių bakterijų augimą bei turi įtakos odos vėžio bei sveikų ląstelių (keratinocitų ir fibroblastų) gyvybingumui.

Poliniams junginiams išskirti iš apynių liekanos po SKE-CO_2, buvo optimizuotas ETPS-EtOH procesas siekiant gauti didžiausią ekstrakto išeigą, *in vitro* antioksidacinį aktyvumą bei bioaktyvaus prenilflavonoido ksantohumolio kiekį ekstrakte. Remiantis gautais rezultatais, buvo nustatytos šios optimalios sąlygos: (1) 85 °C ir 18 min didžiausiai išeigai ir *in vitro* antioksidaciniu aktyvumu pasiekti; (2) 40 °C ir 15 min didžiausiam ksantohumolio kiekiui ekstrakte gauti. Nustatyta, kad be aukštos antioksidacinės gebos šie ETPS-EtOH ekstraktai pasižymėjo ir antimikrobiniu aktyvumu prieš *S. aureus* padermę.

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List of abbreviations

Assoc. prof. – associate professor;

Lect. – lecturer;

Prof. – professor.

8-PN – 8-prenyl-naringenin;

ABTS+ – 2.2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid);

CO_2 – carbon dioxide;

E – extract;

EtOH – ethanol;

GAE – gallic acid equivalent;

H2O – water;

HP – hop pellets;

HR – hop residue after SFE-CO_2;

IXN – isoxanthohumol;

MIC – minimal inhibitory concentration;

ORAC – oxygen radical absorbance;

PLE – pressurized liquid extraction;

RSM – response surface methodology;

SFE-CO_2 – supercritical fluid extraction with carbon dioxide;

SPME – solid-phase microextraction;

GC – gas chromatography;

TOF – time of flight;

MS – mass spectrometry;

t – tonne;

TE – Trolox equivalent;

TEAC – Trolox equivalent antioxidant capacity;

TPC – total phenolic content;

XN – xanthohumol

Introduction

Hops (*Humulus lupulus* L.), family Cannabaceae, are perennial climbing plants harvested for their resinous cones (flowers). Current hop pelletization technologies allow for reserving 90% of cone material (T-90) without significant change in their chemical composition. Today, more than 90% of hops produced are used in the brewing sector to provide organoleptic characteristics such as bitterness, aroma, and taste to beers [1]. Hop products are known for their sedative, antioxidant, and antimicrobial properties [2]. In recent years, diverse health-improving effects such as anti-inflammatory, anticarcinogenic, antitumor, antidiabetic and neuroprotective activities were also ascribed to hop phytochemicals [3–5]. In light of these discoveries, hop products (especially extracts) with valuable organoleptic and bioactive components such as essential oils, bitter acids, and prenylflavonoids are in demand by the food, nutraceutical, agricultural, pharmaceutical and cosmetical industries.

Supercritical carbon dioxide extraction (SFE-CO_2) is a commercially widespread green technique used by the hop extract industry. This process is considered sustainable, safe, not hazardous for the environment, the CO_2 gas is readily eliminated (and may be recirculated) from the extracts. At an industrial scale, SFE-CO_2 is typically performed at relatively low pressures (up to 15 MPa) as a one stage process to recover jointly the essential oils and a fraction of bitter acids or as a two-stage process to obtain them in separate fractions from hops or their pellets [6–9]. However, the commercial SFE-CO_2 extractions are characterized by prolonged extraction times and the produced extracts are tailored for the brewing processes [9–11]. Optimization for single or multiresponse desireability function with chemometric tools such as design of experiments (DOE) based on response-surface methodology (RSM) coupled with three-level full factorial, central composite (CCD), or Box-Behnken designs could increase the effectiveness of the extraction processes. In line with the biorefinery concept, the hop residue after SFE-CO_2 can be further utilized by extracting the valuable polar components (e.g. polyphenols) that remain in the plant material, through similarly optimized pressurized liquid extraction (PLE) technology [12]. Therefore, a multi-step valorization of the hop material could be achieved, which is lacking in the industry now.

The multi-step extraction processes following the biorefinery concept can be based on dual-purpose classified hop varieties such as *Ella*, *Columbus, Galaxy*, etc. which are rich in both essential oils and bitter acids. Our chosen material is *Ella* (previously named St*Ella*), a popular dual-variety from Australia, characterized by relatively high bitter acid content (13-19%) and hoppy, floral notes thanks to its essential oils. The single variety *H. lupulus* extracts with different bioactive compound compositions and therefore selective properties can find novel applications in the functional food, nutraceutical, pharmaceutical, and cosmetic industries [2]. Consequently, optimizations of the extraction technologies for various purposes are a relevant subject and could make a significant effect on the utilization of hop extracts in the futu re.

Therefore this research was aimed to develop effective SFE-CO_2 and PLE-EtOH processes for the production of non-polar and polar extracts from dual-purpose *Ella* hops with high yield, strong antioxidant potential and high content of selected phytochemicals. The set goals for the fulfillment of these aims were the following:

1. To optimize SFE-CO_2 process parameters using RSM-CCD to produce hop extract with high yield, strong antioxidant potential and maximum recovery of α-and β-bitter bitter acids.

2. To compare efficiency of optimized SFE-CO_2 to the commercial one-stage SFE-CO_2 process of hop extraction industry.
3. To compare in vitro antioxidant activity and phytochemical composition (α- and β acids, volatile constituents and pigment content) of selected SFE-CO_2 extracts.
4. To evaluate antimicrobial properties and determine the effect on the viability of cancerous and healthy skin cells of the selected SFE-CO_2 hop extracts.
5. To optimize the PLE-EtOH process parameters using CCD-RSM for high yield, in vitro antioxidant activity and recovery of polar prenylflavonoids from the hop SFE-CO_2 residue.
6. To evaluate the phytochemical composition (prenylflavonoid- and pigment content), antioxidant- and antimicrobial properties of various hop PLE-EtOH extracts.

1. Literature review

1.1. Morphology, varieties and production of hops

Hops are dioecious, perennial climbing plants from the family of Cannabaceae, the only other representative is Cannabis (hemp and marijuana). Humulus lupulus L. is the only species of its genus that produces resinous cones (flowers), utilized at an idustrial scale [1]. These cones contain the lupulin glands which secrete the essential oils and other resinous compounds, polyphenols that are of interest to the food, pharmaceutical and cosmetic industry.

Hops are classified for their characteristics used in brewing: bitter, aroma, or dual purpose. Bitter hops have higher α-acid content (~8.6 – 23.0 %) than the aroma hops (~3.8 – 5.1 %) which are used for their characteristic volatile compounds (fruity, pineapple, citrus, etc.) [13]. Dual-purpose hops have a relatively high level of both aroma components and bitter acids [14]. Widely known aroma varieties are *Hallertau*, *Spalt* and *Mount Hood*, bitter are *Admiral*, *Yakima Cluster*, and *Zeus*, and dual-purpose are *Cascade*, *Hüller Bitterer*, and *Galaxy*. *Ella* is a dual-type originating from Australia. On the market, the % of α-acid content of hops is a parameter for the price, none the less the increasing value of aroma-type hops is changing the rules of the market. The annual hop production amounted to 80 to 100 thousand tonnes, which equals 8 to 10 thousand t α-acid according to the European Commission. Production has been growing and by 2019 it increased to over 120 thousand t, recent years saw a surplus in some regions e.g. Europe [15]. Hops are dried and usually immediately pulverized or pelletized after harvest to minimize the loss of bitter and aromatic substances due to the oxidative degradation caused by storage temperature, presence of oxygen and, passing of time. To this end, dried hop products are packaged in aluminum foil packets under an inert atmosphere and stored in cold storage (temperature 0–4 °C) or even in frozen form. The two major types of hop pellets are the T-90 and T-45, retaining 90 or 45 % of the material and have indistinguishable or half of the main chemical components (bitter acids, essential oils, polyphenols), respectively [16]. Hops can be further processed by supercritical carbon dioxide extraction. Thus, extraction and isolation of valuable compounds allow for more precise application methods while the form of the product and its required storage conditions are both economically and technologically advantageous for the industries [17].

1.2. Application

Hops have been used as a healing plant for over two millennia, it has been recommended against sleeplessness, sores and ulcers, external poultice for inflamed bowels, chest and throat-colds. Also for constipation, as part of cough mixtures, given as a tea for headache and bladder inflammation, as an infusion for period pain, as a warm application for ear and toothaches, as a filled pillow against rheumatism and many others [18]. Throughout the 11th to 16th-century hop cones were added to beer in Europe, which had been flavored with other herbs before (e.g. wormwood), its purpose was to act as a preservative, the bitter taste was an additional characteristic. The young shoots of the hop plants in the spring were made into salads, the wax from tendrils provided a reddish-brown dye, the fibers were used in textiles, the hard stalk for basket waving, and the leaves and spent hops were given to animals [19]. The Food and Drug Administration of USA decleared “essential oils, oleoresins (solvent-free), and natural extractives (including distillates) of *Humulus lupulus* L. […] GRAS for human consumption” ([21CFR182.20]). Today more than 90% of produced hops are used in the brewing sector. It contributes to beer by providing characteristic aroma, bitterness, flavor stability, mouthfeel, foam, lacing and inhibits the growth of spoiling microorganisms [20]. Hops are applied by various methods e.g. ‘first wort hopping, late-boil addition, dry hopping, etc., the timing of

addition and type of hop product greatly influence the beverage [1]. Every variety has its characteristic volatile profile and key odorant compounds, based on the absence/presence and different ratios of these compounds distinct flavor can be realized in beers [21]. Other beverages sold include sparkling Hoptea (made by Hoplark, Inc.) and dried mixes of herbs for tea concture [22]. In Europe, hops supplements are found in the form of powders, liquid extracts (ethanol extract drug extract ratio/dry extract ratio 1:1; sweet wine extract 1:10), tinctures (ethanol extract 1:5), and dry extracts (50% methanol extract DER 4 to 5:1) [23]. The German Commission E has given its approval for using hops in cases of anxiety, restlessness, and insomnia. Products for the treatment of insomnia and other sleep disorders are made up of hop cones or in a mixture of herbs with similar sedative effects e.g. *Valeriana officinalis* [24], but it has to be noted that hops might interact with and decrease or increase the effect of sedative drugs. Herbal remedies containing hops for aiding sleep can be also found in the form of capsuled products, content can range from 30 to even 445 mg of hop in available products.

Before the arrival of hop picking machines in the early 20th century, the cones were harvested by hand, besides increased tiredness at this time, women hop-pickers complained about menstrual disturbances. Studies showed a strong estrogenic effect of hop products, the potent hop phytoestrogen responsible, 8-prenylnaringenin was identified in 1999 by Milligan et al. [25].

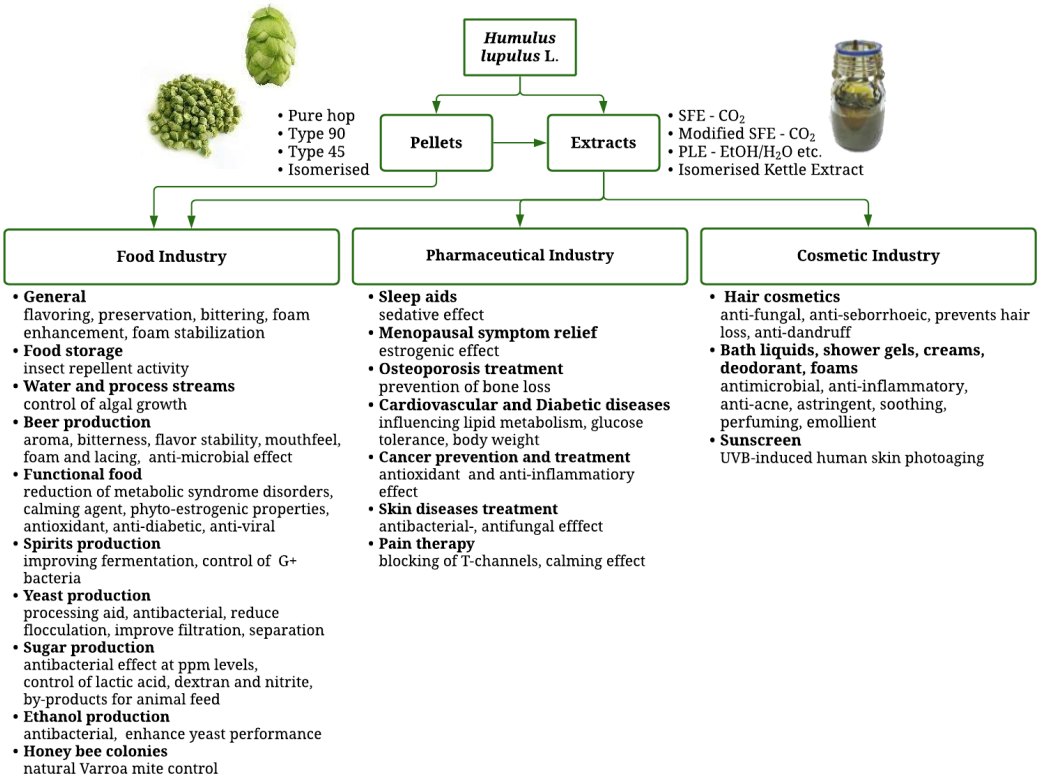


Figure 1. Industrial and lab-scale application of hop products in the food, pharmaceutical and cosmetic industry and corresponding effects

Today it is used for menopausal symptoms, hormonal imbalances in the form of dietary supplements [26]. Based on the method and its conditions standardized extracts were produced, which can be applied in the food industry (see Figure 1), e.g. improving fermentation of spirits [27, 28], processing aid for yeast and sugar production [29], antibacterial for ethanol production [30, 31], as nutraceutical functional food ingredients [32] as algae prevention in water systems and process streams [33], as a biochemical miticide for Varroa mite control in beehives in the form of potassium salts of hops β

acids [34, 35]. The byproducts after harvesting strobiles as a potential lead absorption material in contaminated water systems were applied [36]. *Humulus lupulus* L. extract as an ingredient in cosmetics can be classified as antimicrobial, anti-inflammatory, antioxidant, anti-acne, antiperspirant, astringent, emollient, skin conditioning, soothing, tonic and perfuming. SpecialChem, the material selection platform offers 24 cosmetic ingredient products containing hop extracts on its own or in combination with other active substances [37]. Generally, the extract is incorporated into shower gels, creams and masks. In hair cosmetics it provides anti-fungal and antiseborrheic effects to reduce the brittleness of hair, prevent hair loss and dandruff (see Figure 1) [38].

1.3. Phytochemical composition

The average chemical composition of dried hop cones consists of 15-30% total resins, 0.5-3 % essential oil, 15 % proteins, 2 % monosaccharides, 0.1 % and trace-amounts of waxes, steroids, 8 % ash, 10 % moisture, 43% of cellulose and other. T-90 pellets reflect the chemical profile of the native hop[39]. The resins dissolve in diethyl ether and cold methanol. If hot methanol would be used, it would disperse the present waxes, which would normally crystallize in cold methanol [1]. The soft resins are soluble in hexane, the hard resins are not [40]. Major classes of hop phytochemicals are presented in Figure 2.

1.3.1. Soft resin – bitter acids

Soft resins are grouped into α-acids and β-fraction (i.e. β-acids and uncharacterized soft resins). In methanol, the α-acids with lead(II) acetate make an insoluble lead salt, while the β-fraction does not. The tertiary alcohol function of α-acids could be involved in the formation of the salt structure [41]. The main α-bitter acidsare made up of 35-70% humulone, 20–65% cohumulone and 10–15% adhumulone. Present to a lesser extent is posthumulone, prehumulone and adprehumulone. The difference between them is in the acyl side chain of the molecule[40]. Their isomerization to iso-α-bitter acids is the main reaction that happens optimally at 100-130°C and pH 8-10 during wort boiling in beer production. Each α-acid transforms into two iso-α-acids, either in cis or trans forms. These isomers become more soluble in water (beer) in contrast to the parent molecules which are insoluble in water [20]. The β-acids show poor solubility in water and do not isomerize during wort boiling, only trace amounts are transferred into beer, but they are potential bitter taste precursors, just like α-bitter acids [42]. The uncharacterized soft resins are the compounds that are left precipitation of the α-acids and crystallization of the β-acids [43]. The quantities of α- and β-acids and their homologs vary greatly between hop varieties and also depend on climate and cultivation conditions, like growing region and year of harvest. The total resins range from 10 to 30 % in dry weight of hop cones and T-90 pellets [1].

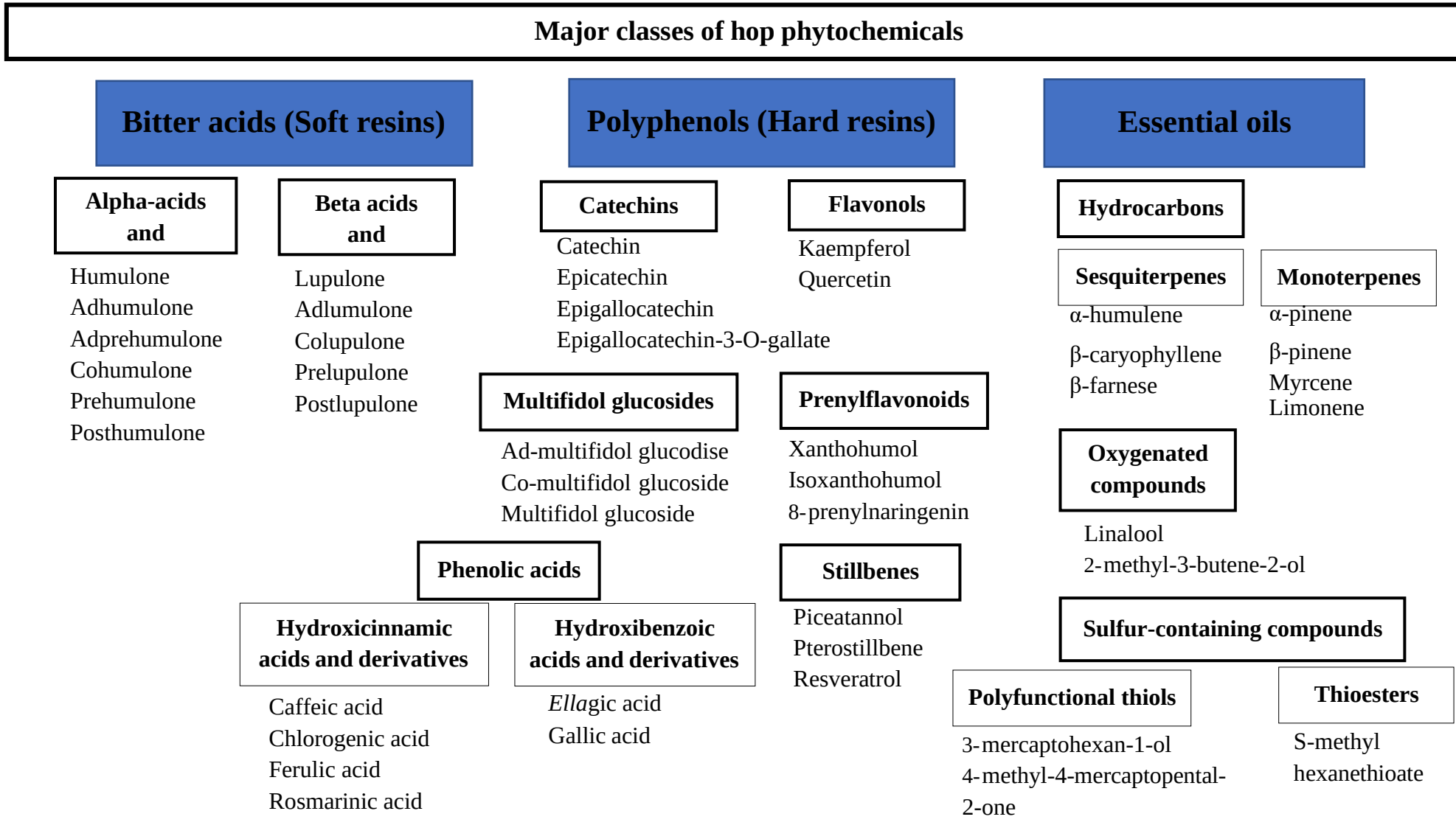


Figure 2. Major classes of hop phytochemicals and representative compounds [3]

1.3.2. Hard resin – polyphenols

Hard resin is defined by its solubility in methanol or diethyl ether, and insolubility in hexane or low boiling paraffinic hydrocarbons [43]. The hard resin fraction in fresh hops consists largely of prenylflavonoids (less polar polyphenols) and not of components related to soft resins. The total hard resin fraction increases through oxidative aging, by the degradation products derived from the bitter acids. Contrary to this, the sum of prenylflavonoids remains unchanged compared to their content in fresh hops [24]. Except for the prenylflavonoids secreted by the lupulin glands, the polyphenols are present in the strig and bract [1]. Hop cones contain 3 to 6% polyphenols [2]. Their levels are not diminished by T-90 type palletization, but the humulone (bitter acid) enriched T-40 type is lacking in phenolic compounds. Hop polyphenols may be grouped into flavonols, flavan-3-ols, phenolic carboxylic acids (derivatives of benzoic acid and cinnamic acid), and other phenolic compounds (prenylflavonoids, stillbenes etc.). The main hop flavonols are quercetin, kaempferol, and myricetin; most of them as glycosides. Hop flavan-3-ols are catechin, (−)-epicatechin, and (+)-gallocatechin, they can form dimers, trimers, and oligomers (proanthocyanidins) [1]. Examples of phenolic carboxylic acids are gallic, protocatechuic, 4-hydroxybenzoic, vanillic, p-coumaric, caffeic, ferulic, and sinapic acids among others.

Prenylflavonoids are secondary metabolites found in hops, their role is the protection against pathogens, harmful insects and environmental stress. The general structure of flavonoids consists of the core flavane (2-phenyl-benzo-gamma-pyrane). The prenyl group is attached to the flavane nucleus, which is responsible for the compound's non-polar characteristics [44]. Based on their chemical structure they can be further divided into prenylated chalcones: xanthohumol (XN), desmethylxanthohumol (DMX), or prenylflavanones: 6-prenylnaringenin (6-PN) and 8-prenylnaringenin (8-PN) [26]. Chalcones, on account of their free hydroxyl group, can isomerize to flavanones, found in hop cones at very low concentrations. The isomerization product of XN is isoxanthohumol (IXN) [44], and of DMX is 6- and 8-PN [45]. The content of XN ranges from 0.1 to 1% of the dry cone or T-90 hop pellets, dependent on hop variety [46]. Other flavonoids from 10 to 100-fold lower abundance are also present, but some have strong bioactive effects such as 8-PN, the most potent known plant-derived phytoestrogen [25]. Following the biorefinery concept, the waste material left after extraction with SFE-CO_2 and the spent hops the by-products of the brewing process can be further capitalized. This residue is high in valuable flavonoids such as XN and other non-polar compounds. XN is the major flavonoid, that has been the subject of various research due to its specific bioactivity, detailed later in this work [47].

1.3.3. Essential oil

The content of essential oil (EO) in dry hop cones ranges from 0.5 to 3.0%. The hop EO can be grouped into 3 fractions: hydrocarbons (monoterpenes, sesquiterpenes, aliphatic hydrocarbons), oxygenated compounds (terpene alcohols, sesquiterpene alcohols, other oxygenated compounds), and sulfur-containing compounds (e.g. thioesters, sulfides). The hydrocarbon fraction representing 50 to 80 % of EO is characterized by the major monoterpenes: myrcene, α-pinene, β-pinene, limonene; and the sesquiterpenes: α- humulene, β-farnesene, β-caryophyllene, α-selinene, β-selinene and γ-muurolene. Myrcene content can be as high as 52% [48]. The fraction of oxygenated compounds increase throughout ripening, product manifacturing, and storage even up to 30 %. It consists of terpenic alcohols (e.g. linalool), aldehydes, ketones, epoxides, acids, and esters. Sulfor-containing constituents are found at low levels (<1 %) in hop EO. Trasformation of some compounds can take

place after metabolization i.e. to α-terpineol and linalool, with anti-inflammatory and sedative properties, respectively [49].

1.4. Bioactivity of hop phytochemicals

1.4.1. Antimicrobial effect

Hop compounds studied showed antibacterial, antifungal, antiviral and antiparasitic effects, the applied materials were essential oil, hop cone and leaves extract (see Table 1), as well as isolated or pure constituents. The application of these properties is diverse, from use as a food additive, antimicrobial packaging, natural preservative/active antimicrobial agent in pharmaceutical and cosmetic products to modulation of the gut microbiome for the treatment of gastrointestinal diseases.

Table 1. Antimicrobial activity of hop extracts (based on review by Bocquet et al. [50])

Hop extract	Bacteria	Fungi	Virus
Essential oil	Gram(+): *Bacillus subtilis*; *Enterococcus faecalis*; *Listeria monocytogenes*; *Staphylococcus aureus* Gram(-): *Escherichia coli*; *KlebsiElla oxytoca*; *KlebsiElla pneumoniae*; *Proteus mirabilis*; *Proteus vulgaris*; *Pseudomonas aeruginosa*; *SalmonElla typhimurium*; *Yersinia enterocolitica*	*Candida albicans*; *Trichophyton mentagrophytes*	n.d.
Cones extract	Gram+: *Bacillus subtilis*; *Enterococcus faecalis*; *Staphylococcus aureus; Staphylococcus epidermidis Corynebacterium xerosis* Gram-: *Escherichia coli*	*Candida albicans*; *Trichophyton mentagrophytes*	Bovine Viral Diarrhea Virus (BVDV); Cytomegalovirus (CMV); Hepatitis B (HBV); Human immunodeficiency virus (HIV); Herpes simplex virus (HSV); Human influenza virus; Human rhinovirus; Human respiratory syncytial virus (RSV); Yellow fever virus
Leaves extract	Gram+: *Staphylococcus aureus* Gram-: *Escherichia coli*	n.d.	n.d.

nd: no data

The antimicrobial activity exhibited by hop bitter acids was associated with the incorporation of a prenyl group into the bacterial cell plasma membrane [51]. They act as a mobile-carrier ionophore causing disruptions in the cell processes, which lead to starvation and cell death [2]. Bitter acids are weak acids, their antibacterial effect decreases with lowering of pH. The longer the acyl side chain and the higher the count of prenyl groups, the more hydrophobic is the compound, eliciting a stronger antimicrobial effect [41]. Studies reported higher antimicrobial activity for β-acids compared to α-acids [52]. An antibiotic cream was formulated based on the commercial product Neosporin® with lupulone as a substitution for bacitracin, the inhibition zones were found to be similar [53]. SFE-CO_2 hop extract (50% α- and 22% β-acid) inhibited in vitro *Corynebacterium xerosis* and *Staphylococcus epidermidis*, for this reason, caused body odor reduction in vivo by a hops⁄zinc ricinoleate containing deodorant product [54]. The incorporation of antimicrobial agents into coatings can have a great influence on food quality and safety [55]. Supercritical CO_2 hop extract was incorporated into chitosan-based films successfully [56], encapsulation of lupulin and xanthohumol (XN) into nanochitosan proved to have better bioavailability in vitro. Furthermore, the antimicrobial effect increased in synergism with chitosan and chitosan seemed to mask the bitter taste, which can be a

limiting factor in bitter acid utilization. As in the case of polymeric biofilms developed using soy protein isolate, oxidized potato starch, carboxymethyl cellulose or gelatin with incorporated ethanol-based hop extract that enhanced their antibacterial and antioxidative properties [57]. Our increasing awareness of the role of the human microbiome in certain widespread diseases such as obesity in the developed world can help us understand and mitigate, even prevent them. Since bitter acids display many and sometimes selective inhibition of microorganisms, the application of hop extract in the treatment of chronic metabolic and intestinal diseases may provide a novel method [58]. More research is warranted on the use of hop extracts in connection with the living human microflora.

The antimicrobial effect of certain hop polyphenols against bacteria, fungi, and protozoan parasites is connected to their hydrophobicity, their ability to accumulate after entering the cell. Furthermore, phenolics influence bacterial cell surface hydrophobicity, the assembly of adhesins in the cell wall dependent on sortase activity, this way the adhesion step for biofilm development could be disrupted [59]. Alvesalo et al. (2006) demonstrated inhibition by phenolic compounds of gram-negative *Chlamydia pneumoniae*, responsible for some respiratory diseases such as pneunomia. Quercetin and myricetin displayed strong inhibitory effect [59]. The phenolics gallic, caffeic and ferulic acids were effective against *S. aureus*, *L. monocytogenes* and *Pseudomonas aeruginosa* [60]. Gallic acid also inhibited *E. coli* and its biofilm formation [61]. Prenylflavonoids display inhibition on mainly gram-positive bacteria Staphylococcus and Streptococcus. Hop extracts increased sensitivity to linezolid, a bacteriostatic antibiotic, effective against e. g. infections caused by multidrug-resistant *enterococci* and methillic resistant *S. aureus* (MRSA) [62]. Additionally, synergism was observed with ciprofloxacin and tobramycin. Polymyxin is not active on gram-positive organisms by itself however exhibited an inhibitory effect in combination with XN [53]. Research on the effectiveness of MRSA inhibition by ethanol extracts of hops (90% ethanol) showed positive results [47, 63, 64], with antibiotic rifampicin the effect was eight times more active in combination with XN. Nosocomial infectious antibiotic-associated diarrhea caused by *Clostridium difficile* is not easily treatable, XN showed inhibiting effects comparable to antibiotics for resistant bacteria [65]. Hop extract (total polyphenols 7.1 ± 0.35 μg/mg of extract) inhibited Influenza A virus replication prior, during and even after infection, and reduced infectivity of tested strains (PR8, NWS, pH1N1 and ULSTER), implying that the inhibitory effect is additive [66]. These results indicate that flavonoids from spent hops and other polyphenols can be lead compounds in natural pharmaceutical products [47].

The antimicrobial activity is lower for essential oil extracts without the presence of bitter acids and hard resins [2]. Nuutinen (2018) reviewed the antimicrobial activities of terpenes found in Humulus lupulus EO [4]. Myrcene showed an inhibitory effect against gram-negative *Escherichia coli* and *Proteus vulgaris* bacteria [67]. While β-Pinene was effective against *S. aureus*, *Ec. faecalis*, *E. coli* and *P. vulgaris*, additionally, medium effect against *Candida albicans* was also observed [67]. Humulene inhibited *Enterococcus faecalis*, *E. coli*, *Pseudomonas aeruginosa* and *SalmonElla sp*, similarly to β-Caryophyllene [67].

1.4.2. Antioxidant effect

Plants are well-known sources of natural antioxidant compounds, which can help treat and prevent various oxidative stress-related diseases (tumors, diabetes, Alzheimer's, Parkinson's, etc.). Besides the very active polyphenols (e.g. xanthohumol), other hop constituents, bitter acids and essential oil terpenes (linalool, β-pinene, γ-terpinene, β-farnese) also portray antioxidant potential [2].

The antioxidant effect of bitter acid compounds was studied in the form of extracts, enriched extracts and purified components. Humulone and lupulone exhibited inhibition of lipid peroxidation, all results indicated higher activity than that of R-tocopherol and ascorbic acid in 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity (DPPH-RSA) assay [68]. RSA was not the same for bitter acids. From the strongest to the weakest: α- acids, β-acids, dihydro-iso-α-acids, hexahydro-iso-α-acids, and tetrahydro-iso-α-acids (IC_{50}; 0.21, 0.96, 1.36, 1.40, 1.78 mg/mL, respectively). For lipid peroxidation inhibitory activity (LIA): α-acids, β-acids, iso-α-acids, tetrahydro-iso-α-acids, dihydro-iso-α-acids, hexahydro-iso-α-acids [69]. Prevalent links were found between strong *in vitro* oxygen radical scavenging capacity and high soft resin, mainly humulones, content in various hop extracts [70–72].

Generally, polyphenols exhibit different types of antioxidant effects, scavenging of reactive oxygen, singlet oxygen and nitrogen species (hydroxyl, peroxyl, superoxide, hydrogen peroxide). They also suppress enzymes such as NADPH oxidase, cyclooxygenases, xanthine oxidase and lipoxygenases that are responsible for the generation of reactive oxygen species [2]. Their antioxidant potential is connected to the position of hydroxyl groups and their number, and the additional OH-groups on carbons in the 4th position may have an enhancement effect [73]. In combination with total flavonoid content (TFC), in vitro oxygen radical absorbance capacity (ORAC) assay, DPPH, ABTS and ferric reducing ability of plasma (FRAP) assay, the antioxidant capacity can be more accurately characterized, especially for comparison with existing literature [74, 75]. Gerhauser [76] reported that in ORAC assay the most active compounds were cinnamic, caffeic, and ferulic acids, gallocatechin, xanthohumol, and myricetin from phenolic compounds.

Results of antioxidant assays (e. g. TBARS, DPPH) of terpene compounds of essential oil are contradicting, properties range from neutral to strong activity and even pro-oxidant for the same compound (linalool) [2]. β-caryophyllene prevented lipid oxidation more effectively than antihyperlipidemic drug Probucol or α-tocopherol [77], while in another study it showed results closer to IC_{50} of ascorbic acid by DPPH and FRAP [78].

1.4.3. Anti-inflammatory and anticarcinogenic activities

The anti-inflammatory properties of hop bitter acids and their derivatives (especially iso-α-acids) affect different diseases through inhibition of COX-2 driven PGE_2 production, - the transcriptional activity of NF/κB, AP-1 and CREB, -kinases (responsible for inflammatory signals), also interacting with pro-inflammatory cytokines such as interleukin ((IL)-10) [5, 69]. Bitter acids can influence the detoxification enzymes in control of cytochrome P-450 enzymes that activate various procarcinogens, colupulone exerted such effect in vivo in mice [79]. Angiogenesis inhibition was exerted by humulene and lupulone in different ways. Inhibition of proliferation was reported for lupulone (colonic carcinoma, human leukemia U937 cells) [80], colupulone and hexahydrocolupulone (human breast cancer) [81].

Hop polyphenols exert various anticarcinogenic activities [82]. The prenylflavonoids are powerful selective inhibitors of cytochrome P450 enzyme family [83]. Polyphenols can inhibit enzymes such as matrix metalloproteinase, vascular endothelial growth factor, in effect hindering angiogenesis (the formation of new vessels). Quercetin kaempherol, *Ella*gic acid, myricetin were eliciting these kind of effects [2]. The enzymes quinone reductase, glutathione S-transferase and UDP-glucuronosyl transferase are activated by quercetin, myricetin, kaempferol and xanthohumol and thus influence detoxification of carcinogens [82]. 6-PN and 8-PN (50 - 100 μmol/L) also interferes with enzymes,

in vitro, a strong inhibition of cellular histone deacetylases activity was reported in melanoma cellsm the cell viability decreased and the growth inhibited [84]. Flavonoids can have an effect on aromatase enzyme, which transforms androgens to estrogens in the breast, and thereby controls estradiol levels in the blood [85]. Consequently, inhibiting the aromatase enzyme with dietary supplements in obese menopausal women may be an effective prevention of cancer [86]. 8-PN mimics 17-β-estradiol (10–20,000-fold lower potency) and through that suppresses critical symptoms and reduces the risk of estrogen carcinogenesis [87]. For example, XN, IX, and 8-PN reduced breast cancer cells proliferation and caused apoptosis [85]. 8-PN also showed significant antigenotoxic activity on models of main stages of colon tumor formation [88]. Seliger et al. (2019) concluded that regular intake of a sufficient variety of plant-derived polyphenols could decrease the level of cardiotoxic DAUNol, a less effective and cardiotoxic metabolite of conventional chemotherapy drugs (DAUN). In an in vitro study, 8-PN strongly inhibited activation of NF-κB, the gene expression regulator of genes that produce proteins in connection with inflammation-inducing factors [90]. The inhibition of NF-κB activation is the chemo preventative target also demonstrated by the flavonoids kaempferol, quercetin, naringenin, and anthocyanin [91].

Myrcene is one of the main terpenes found in hop essential oil in many varieties. When the anti-mutagenic activity of myrcene was studied, it inhibited some forms of cytochrome P-450 isoenzymes [24]. Myrcene also affected UVB-induced human skin photoaging, suggesting the possible utilization in UV-filter sunscreen products (see Figure 1) [92]. The sesquiterpene, humulene showed anti-inflammatory effects and reduced NF-kB activation, furthermore caused apoptosis in colorectal cancer cells [4]. While caryophyllene and caryophyllene oxide generated apoptosis through the NF-κB pathway as well in lymphoma and neuroblastoma cells [93].

The terpene alcohol abundant in hop EO, linalool acts against the proliferation of cervical, skin, stomach, bone, breast and, liver cancer cells [101]. Furthermore, it activated the production of cytokines responsible for the regulation of tumor formation [100]. As well as selectively reduced the number of melanoma cells and prooxidant activity in tumor tissues in comparison with antioxidant activity in liver tissue [4] and reversed doxorubicin resistance in human breast cancer cells [96]. Additionally, significantly enhanced cytotoxicity and apoptosis in combination with nanoparticles against ovarian carcinoma-type cancer cells [97].

1.4.4. Estrogenic effects

8-PN shows distinct estrogenic activity based on the position of the prenyl group at C8 [98]. The estrogenic activities can be asserted in two ways: (i) binding to specific estrogen receptors and (ii) acting as an enzyme effector. Based on the first action they can be called naturally occurring selective estrogen receptor modulators [99]. 8-PN is an agonist of estradiol receptors (ER), for ERα the activity of 8-PN is 70-fold lower than 17-β-estradiol,. 8-PN shows around twice the affinity for ERα than ERβ, while XN has no affinity for the ERs [100]. 8-PN strongly binds to a subtype of ERα, differing from other estrogenic isoflavones, which react more with the ERβ [101]. 8-PN augments progesterone receptor mRNAs levels comparable to 17-β-estradiol [102].

The estrogenic activity of 8-PN makes it effective as a substance used in the treatment of menopause, for the reason that it relieves the common symptoms of hot flashes [103]. Treatment could help with the difficulties presented by the lack of estrogen in the body [104]. Estrogenic properties and the high potency which exceeds the studied phytoestrogens (eg. genistein and daidzein from soy or clover), make 8-PN and thus hop supplement/extracts a feasible alternative to Hormone Replacement Therapy

(HRT), even effective in the prevention and treatment of osteoporosis and control of climacteric symptoms [26]. Complementary and alternative therapeutic approaches of HRT are at the forefront of research, and in demand by patients [105, 106].

8 PN also showed positive outcomes for menopause accompanying osteoporosis based on its binding to ERα in bone tissue [107]. Regular intake of estrogenic flavonoids is a potential habit that could help prevent osteoporosis and contribute to bone health. Osteoporosis, a decrease in bone mass and density happen when the bone formation and bone resorption are not regulated equally [26]. Prenylflavonoids may influence gene expression in bone cells by repressing osteoclast expression It is assumed that prenylflavonoids can modulate gene expression in bone cells, enhance expression in osteoblasts responsible for the formation of bone tissue and suppress the expression of bone cells that resorbs bone tissue. 8-PN was analyzed as a possible preventative substance against osteoporosis in vitro and in vivo with positive results [108]. Osteogenic differentiation is also affected in vitro by 6-PN, XN, and IXN through other mechanisms [22].

1.4.5. Sedative effects

Hops were noted for their sedative and relaxing effect centuries ago. Schiller et al. [109] identified the α-acids as responsible for these effects, however, β-bitter acids and EO also have some effect. The studied hop extracts decreased the spontaneous locomotor activity and body temperature, while the ketamine-induced sleeping time increased. The α-acids modulated GABA- induced responses, this was reported in other studies as well for hop extracts[110], and XN [111]. Humulone and 6-PN were exerting the greatest modulatory effects at low μmol concentrations through $GABA_A$ receptors [112]. Cohumulone and adhumulone showed less effect and 6-PN was the most effective positive allosteric modulator out of prenylflavonoids XN, 8-PN and IXN [113]. Zanoli *et al.* [114] reported a similar effect on sleeping time and behavior despair by a hop SFE-CO_2 extract and an α-acid fraction.

The essential oil component linalool was also reported to have anxiolytic, sedative and hypnotic, effects in mice, while no effect on motor performance was observed [115]. The suggested mode of action is through the $GABA_A$ receptor modulation similarly to the other hop constituents mentioned before [112].

1.4.6. Metabolic disturbances and vascular diseases

Iso-α-acids positively alter lipid metabolism, glucose tolerance, and body weight. These derivatives effectively lowered serum triglycerides and raised serum HDL-cholesterol, and reduced insulin resistance [116]. Also, significant improvement was observed in cognitive decline induced by a high-fat diet in mice [117]. Iso-α-acids may be utilized to prevent obesity-related to food consumption, but because of their bitterness, their acceptability by subjects can be negatively influenced. To solve this problem, an extract rich in oxidized hop bitter acids, with less bitterness, safely reduced body fat over a period of 12 weeks. Especially the abdominal visceral fat of healthy humans that are overweight was affected in a placebo-controlled study of 200 subjects [118]. The effect of beers with different bitter acid content (α-, β-, and iso-α-acids) on gastric acid secretion was studied [119]. Reportedly, this action is in correlation to their bitter acid levels (α-acids < iso-α-acids< β-acids). The authors attributed the effect to the bitter taste [40] and proposed that so-called ‘stomach-friendly beer’ could be made.

Phenolic compounds show positive effects in diabetes-related diseases, on lipid metabolism, thus influencing obesity. Hop flavonoids have blood-sugar and cholesterol-lowering effects contributing

to their positive effect on obesity. XN reportedly plays part in the inhibition of diacylglycerol acyltransferase, the secretion of apolipoprotein B, a major representative of the cholesterol LDL, and α-glucosidase that controls the level of blood glucose [120–122]. 8-PN treatments reduced triglyceride, cholesterol and alkaline phosphatase presence, prevented body weight gain and increased insulin sensitivity, glucose tolerance in mice, suppressing lipogenesis. Suggesting that diet rich in 8-PN could help cure diabetic-associated metabolic disturbances [123].

8-PN can inhibit human platelet aggregation and have anti-adhesive effects. It acts through the inhibiting of several proteins necessary for platelet activation and aggregation. 8-PN supplementation may be used to help prevent and treat vascular diseases e.g. atherosclerosis, myocardial infarction, coronary artery disease, or thrombosis [124].

1.5. Extraction techniques for the isolation of bioactive hop phytochemicals

1.5.1. Conventional and intensified extraction techniques

Target compounds of extraction techniques in hops are the bitter acids for their bittering and bioactivity (e.g. antimicrobial effect), the polyphenols, especially the prenylflavonoids for their many potential health effects (e.g. chemoprevention, estrogenic activity) and the essential oils for their aroma properties [2]. According to the nature of these compounds, different methods are applicable. The extraction conditions, particularly, the choice of solvent is critical for reaching high purity and maximum recovery. The variety of hop, climate and, cultivation conditions, like harvest region and year all influence the levels of desirable substances in the plant material [1].

Initially, bitter acids were extracted by solid-liquid extraction with organic solvents like hexane, methylene chloride, trichloroethylene and alcohols (methanol, ethanol, propanol, butanol) [40]. These extracts also contain β-acids, polyphenols, fats, waxes and chlorophyll. The α-acids were the most sought-after fraction for beer production, other resins and β-acids were precipitated (aqueous alkali metal hydroxide was added to the polar solvent extract solution, the formed product could be later utilized in the brewing process) [125]. For extracts rich in polyphenols polar solvents are desirable, previous studies were conducted with ethanol, methanol, acetone, ethyl acetate, propanol and, dimethyl formaldehyde, etc. [126]. Generally, the polyphenols are said to be extracted more efficiently when water is present, however, it is dependent mainly on the applied extraction technique and the solvent-water ratio [3]. A hop tannin extract from spent hops (residue after utilization in the brewing process) extracted with 90% ethanol-water was made with the help of centrifugation into pure resin extract as a sticky dark green fraction and hop tannin extract as yellow water fraction. This can also be used in the brewing industry to reduce the perception of sunstruck flavor, precipitate haze active proteins and accelerate lautering [127].

Essential oils are conventionally extracted by steam distillation [128], hydrodistillation [129], soxhlet extraction (for reference purposes). Distillation methods can cause elevated levels of thermal degradation products and have the disadvantage of longer extraction time and lower yield [3]. Similar volatile concentrates can be obtained by solid-liquid extraction with hexane or ethanol, and when liquid CO_2 is applied it excludes hard resins and polyphenolic materials in the extract [130]. Soxhlet and solid-liquid extraction were performed by alternative bio-based green solvent 2-methyloxolane, the study results indicated higher hop aroma yield with faster kinetic and concluded 2-methyloxolane to be a potential bio-based extraction solvent [131]. Methyl tert-butyl ether is a suitable solvent for quantitative lipid recovery from hops, Soxhlet extracts contained a high percentage of polar compounds (47-67%), the most abundant were bitter acids (25-43%) dependent on hop variety [132].

Microwave-assisted extraction (MAE), microwave-assisted hydrodistillation (MAH), pulsed electric field (PEF), ultrasound-assisted extraction (UAE), pressurized liquid extraction (PLE), pressurized hot water extraction (PHWE), pressurized fluid extraction: e. g. supercritical fluid extraction (SFE) are successful intensification strategies for the extraction of various hop components [133]. Diverse extraction conditions are applied to produce standardized extracts with specialized beneficial effects [24]. The extraction should be selective, cost-effective, not hazardous for the environment, therefore non-toxic solvents are required such as ethanol or supercritical CO_2 [134]. Deep eutectic solvents (DES) have been also proposed as a 'green' alternative to industrially used organic solvents [135].

1.5.2. Supercritical CO_2 Extraction

Supercritical CO_2 extraction technology has reached significant industry levels over the last decades, its key advantages over conventional methods are fast extraction, improved kinetics, no filtration, no undesirable solvent traces, fractionation possibilities, high extract quality, and environmental sustainability. Economically advantageous as collection and reuse of CO_2 are possible in large-scale operations [136]. Its technical complexity, large initial investment and the many condition parameters to be optimized require adequate human and monetary resources to be profitable. It is a widely used technique for producing hop materials used mainly in beer production. Other large-scale operations are decaffeination of coffee/tea, extraction of cannabidiol (CBD) oil from Cannabis L. and aroma/flavor constituents from plants (berries, herbs) [14, 137–140].

The CO_2 used can be in a liquid, subcritical or supercritical state. There is no distinction between the liquid and gaseous phases in the supercritical state (>30 °C and >7.4 MPa). CO_2 has higher variable solubility, better mass transfer rates, and selectivity than other solvents used in conventional methods [141]. It behaves as a non-polar solvent, extracting non-polar and slightly polar compounds. The effectiveness of SFE-CO_2 in terms of cumulative yields and selectivity towards specific hop constituents can be achieved by modifying P, T, and τ; other factors, such as particle size, co-solvent addition may also be important [142]. An increase in T helps with the solute of compounds, however, the fluid density will reduce from certain T levels, and cause a decrease in extraction efficiency, knowledge of this teeter effect is important for optimization of the process. Extraction conditions between 7 to 80°C and 1 to even 40 MPa can yield a wide range of extract compositions. Within the subcritical range of 1–1.2 MPa and 7–8 °C the essential oils are favored [141], higher pressure 8-15 MPa can increase yield [143, 144]. In another study, a CO_2 density of 500 kg/m^3 (50 °C, 11.15 MPa) was reported as optimal considering selective and quantitative extraction of hop EO for aroma variety, Saaz [11]. While Zekovic et al. (2007) used 790 kg/m^3 gas in a two-stage separation extraction from Magnum cultivar. Pressure 12-20 MPa facilitates extraction of bitter acids with an increasing α-acid/β-acid ratio [145]. Bitter acid composition is highly influenced by the variety of hop, optimization of supercritical CO_2 extraction is necessary. Total yield increases at 20 to 40 MPa, presence of fats, waxes and chlorophyll will influence extract properties; up to 100 MPa increasing levels of polyphenols, tannins are extracted [146].

The extraction process can be completed in one stage at continuous pressure and temperature, or in two and more stages (see Table 2), often called density programming because pressure and temperature have a direct influence on the density of CO_2 and thus its solvating power [141]. The first step at 8 MPa, 50°C, 2.5 h extracts the most volatile hop oil constituents. Then the hop residue is extracted at 11 MPa, 50°C, 2.5 h for isolation of the hop sesquiterpenoid fraction [147].

Table 2. Examples of SFE-CO_2 conditions, yield and composition of extract

Mode	Hop variety	Type	Extraction conditions				Yield (%)	Main constituents	Ref.
			p, MPa	T, °C	τ, min	CO_2 density, kg/m^3			
SFE-CO_2 two stage	*Magnum*	bittering					13.35 7.54	EO BA, α-acids (41% in E)	[14]
	Hallertau Tradition	aroma	15 30	40 40	150 150	790 915	6.18 2.46	-	
	Spalt Selekt	aroma					9.09 2.31	-	
	Saaz	aroma	8-9 11	50 50	125	290 500	-	EO (floral) EO (spicy, sesquieterpenoid)	[10]
	Magnum	bittering	28-30 85-90	50 75-90	-	>856 >967	-	EO and BA XN	[148, 149]
	Marynka	dual	100	50	-	1082	-	XN (6.49 % in E)	[151]
SFE-CO_2 one stage	*Marynka*	dual	30	50	-	870	-	α-Acids (41%) ; β-Acids (19,5% in E) XN (0,15% in E)	[151]
	Marynka	dual	25	50	-	834	11.4	-	[133]
	Marynka	dual,	8.5	50	10	252	-	EO (optimal recovery at trap 5°C)	[152]
	Saaz	aroma	8-10	30-60	-	200-600	-	-	[153]
	Nugget	dual	20	40	150	840	-	Oleoresin yield (0.01% in HP); α-acids (0.04% in E); α-acid/ β-acid ratio 1,71;	[154]
	Wye Northdown	dual	20	40	180	840	10.75	α-acids (25% in E), β-acids (10% in E)	[155]
	Hallertau Mittelfrüh	aroma	20	55	-	754	7.1	-	[144]
	Magnum	bittering	85	80	-	988	-	XN (1.23% in E) ISX (0.22% in E)	[148]
	Mantiqueira	aroma	25	80	110	687	7.6	-	[143]
SFE-CO_2 + EtOH	*Mantiqueira*	aroma	25	80	30	687	10.5	- (solvent:material 2:1)	[143]
	Qingdao flower	bittering	25	50	-	834	-	Flavonoids (0.078% in E) (solvent:material 50%, EtOH conc. 80%)	[150]

SFE-CO_2: supercritical carbon dioxide extraction; EtOH: ethanol; EO: essential oil; BA: bitter acids; E: extract; HP: hop pellets; conc.: concentration

US patent from 2018 describes oil-enriched extract obtained at 15 MPa (1-8 h; 31-80 °C) de-oiling with molecular distillation offers highly specialized composition (6-40% oil), while spent hops in the second stage at 25 MPa produces variety-specific bitter acid extract (15-80% α-acids) [145].

After the targeted extraction of essential oils and bitter acids, the hop material still contains valuable phenolic compounds (e.g. flavonoids) that remain in the material on account of their polar nature [156]. Extract rich in XN were prepared from spent hops at 100 MPa, 50°C with a two-stage separation (6.49 % xanthohumol) from *Marynka* [151] or at a very high temperature of 80°C at 85 MPa (1.23% of xanthohumol and 0.22% of isoxanthohumol) from *Magnum* [148]. Tyśkiewicz et al. (2018) reviewed the SFE conditions studied in the extraction of phenolic compounds from plant sources and concluded that the use of carbon dioxide modified by polar solvent may increase the effectiveness of the extraction. The methods include modification by addition of ethanol (T ~ 20-80°C, p ~ 8-60 MPa), methanol (T~35-100°C, P ~ 10-65 MPa), water (T~30-60°C, p ~ 10-35 MPa), aqueous ethanol (T ~ 35-80°C, p ~ 22-39 MPa). Other possible modifiers are ethyl acetate, ethyl lactate and hexane, modification can be even done in two or more stages with different modifiers. Guo-qing et al. (2005) reached 0.79% yield maximum of flavonoids at the optimal conditions of 50 °C, 25 MPa from a range of 40-60°C and 20-30 MPa, with the ratio of solvent to material (50%), ethanol concentration (80%) from *Qingdao big flower* [150]. Ethyl-acetate increased yield and antioxidant activity of SFE-CO_2 extract compared to pure CO_2 [143]. The highest phenolic content does not correspond necessarily to the highest yield, therefore optimization is a relevant topic.

1.5.3. Pressurized liquid extraction

Pressurized liquid extraction (PLE), also known as pressurized fluid extraction, enhanced solvent extraction or accelerated solvent extraction is a green technique, based on the minimum amount of food-grade solvent (especially water [157]) used for selective extraction of bioactive components, without change in their bioactivity or chemical structure. Solvents are heated above their atmospheric boiling point, resulting in enhanced solubility and mass transfer properties [12]. Factors affecting PLE: nature of the analyte, positioning and bonding of the analyte, particle size, water content, solubility or diffusion-controlled extraction, and dynamic or static mode. The main parameters of extraction are the type of solvent, pressure, temperature and time (number of cycles). Enhancers are drying-, dispersing agents, also called ‘inert matrix’ or other additives [158]. Advantages, other than the possible use of different polarity solvents, include the control of extraction parameters during a fully automated process. Selectivity can be improved e.g. by pre-treated material, and PLE in combination with other extraction methods (MAE, PEF, SFE, etc.) to achieve a more effective produce cycle.

PLE has been applied for a wide range of compound groups from plant materials, such as phenolic compounds, polyphenols, carotenoids, oils and lipids, essential oils, etc. Čulík et al. [159] developed a PLE method for bitter acids of hops as a labor- and cost-saving alternative to EBC 7.7 extraction method (used for official bitter acid determination in hop products), the optimized extraction conditions were 15 MPa, 80°C, 15 min, solvent: methanol-diethylether (1:1), inert matrix: sea sand (50-70 μm). Compared to SFE-CO_2 and steam distillation, the recovery efficiency of hop EO with PLE was lower, when two stages were applied: first extraction 11MPa, 50°C, 5 min, solvent: hexane (45 mL); second extraction at 11 MPa, 50°C, time 5 min, solvent: dichlorometane (45 mL) [160]. Pressurized hot water extraction (PHWE) conditions for highly isoxanthohumol enriched hop extract (150°C, 10.3 MPa, 6x5 min cycles) were compared to and was more selective for IXN than PLE technologies with subcritical ethanol and with three solvents (hexane, ethanol and water)

consecutively. A sequence of solvents should extract in a first step (hexane) the lowest polarity compounds (terpenes, waxes and bitter acids), for further extractions with more polar solvents, ethanol or water are suitable for flavonoids under PLE conditions [161].

Following the biorefinery concept, after the extraction of bitter acids and hop oils with SFE-CO_2, there have been studies about further utilization of the hop residue by UAE with ethanol or methanol. After filtration, the phenolic extract can be used for the production of purified xanthohumol through several steps [9]. The SFE-CO_2 residue was also extracted with ethanol based on good manufacturing practices and the extract was standardized as an estrogenic dietary supplement [162].

Another refining cycle is proposed, when the polar-compound-rich residue of SFE-CO_2 is further treated by PLE processes utilizing polar solvents. In this way, the first cycle provides a non-polar SFE-CO_2 extract containing essential oils and bitter acids, while the second cycle produces a polar PLE extract rich in hop phenolic compounds. To the best of the author's knowledge, optimization of SFE-CO_2 utilizing hop pellets and of subsequent PLE-EtOH from the SFE-CO_2 residue by multi-response desirability functions based on RSM-CCD has not been published yet.

2. Materials and methods

2.1. Plant material

The extracted material for SFE-CO_2 was dual-purpose hop cv. *Ella* T-90 pellets (further abbreviated as *Ella* hops), containing 7.1% moisture, minimum 13.4% α-bitter acids and 1.40% essential oil, were obtained from the Baltic Brewing Supplies OÜ (Tallinn, Estonia). Before the extraction experiments, pellets were ground by an ultra-centrifugal mill ZM 200 (Retsch, Haan, Germany) using a 0.5 mm hole size sieve. Before the PLE experiments, 1150.00 g of ground hop pellets (0.5 mm) were placed in a 10 L cylindrical extractor of SFT-110 extraction system (Supercritical Fluid Technologies, Newark, DE, USA) and extracted by SFE-CO_2 for 30 min in static mode, then dynamic mode at 36 MPa, 40 °C for 90 min to remove bitter-acid containing lipophilic fraction. .Hop residue after SFE-CO_2 (further abbreviated as HR) was stored at ambient temperature in sealed glass container prior to the further analysis.

2.2. Chemicals

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, 97%) and n-alkane standard solution C_7-C_{30} (1000 μg/mL each component in hexane) were purchased from Sigma-Aldrich Chemie (Taufkirchen, Germany); 2-(3-hydroxy-6-oxo-xanthen-9-yl)benzoic acid (Fluorescein (FL) and 2,2'-azobis-2-methyl-propanimidamide dihydrochloride (AAPH) were from Fluka Analytical (Bornem, Belgium); NaCl, KCl, KH_2PO_4 and $K_2S_2O_8$ from Lach-Ner (Brno, Czech Republic); Na_2HPO_4 from Merck KGaA (Darmstadt, Germany); carbon dioxide and nitrogen gases (99.9%) from Gaschema (Jonava, Lithuania). International Calibration Extract 4 (ICE-4), containing α- and β-acids (10.98% of cohumulone; 31.60% of humulone+adhumulone; 13.02% colupulone; 13.52% lupulone+adlupulone), was obtained from Labor Veritas AG (Zürich, Switzerland). Analytical standards of xanthohumol (≥98%), isoxantholumol (≥98%) and (±)-8-prenylnaringenin (≥98%) were obtained from Cayman Chemicals. Divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibers and 20 mL SPME-vials were purchased from Supelco (Bellefonte, PA, USA). All solvents were of analytical and HPLC-grade.

2.3. Extraction of *Ella* hop by high pressure intensified extraction technologies

2.3.1. Supercritical fuild extraction with carbon dioxide

Ella hop SFE-CO_2 extracts were obtained under different experimental SFE-CO_2 conditions in the SFT-110 extraction system (Supercritical Fluid Technologies, Newark, DE, USA) using 20.000 ± 0.002 g of ground hop pellets (0.5 mm), placed in a 50 mL cylindrical extractor (38 mm inner diameter, 136 mm length) between two layers of the cotton wool to avoid particle carry-over to the system. To simulate currently used processes, the impact of the dynamic extraction (continuous flow of supercritical CO_2) time (15-300 min; further abbreviated as τ) on the SFE-CO_2 extract yield was determined at the fixed 10, 12.5, and 15 MPa pressure (further abbreviated as P) and 40 °C temperature (further abbreviated as T) combinations. In continuation, SFE-CO_2 was further optimized at the higher pressure levels employing response surface methodology (RSM) using central composite design (CCD): P (25-45 MPa), T (40-60 °C); τ (30-90 min). Several response factors were selected for first optimization experiment: extract yield (RFI) and ORAC (RFII) and for second experiment: cohumulone, mg/g HP (RFI); humulone + adhumulone, mg/g HP (RFII), colupulone, mg/g HP (RFIII), lupulone+adlupulone, mg/HP (RFIV), total α-acids, mg/g HP (RFV); total β-acids, mg/g HP (RFVI), total bitter acids, mg/g HP (RFVII); α / β ratio (RF VIII). The static extraction of 10 min was

conducted prior to each dynamic extraction experiment based on the previously performed studies [163–165]. Constant extraction temperature was maintained by the surrounding heating cover of the extractor. The flow rate of CO_2 was controlled manually by the micro-metering valve and kept at 1.8-2.2 SL/min (standard liters per minute at standard state: P_{CO2} = 100 kPa, T_{CO2} = 20 °C, ρ_{CO2} = 0.0018 g/mL) during all experiments. The extracts were kept under the nitrogen flow for 10 min and stored in opaque bottles at -18 °C before the analysis. The yields of extracts were measured gravimetrically (±0.001 g) and expressed as g/100g hop pellets (further abbreviated as HP). All extraction experiments were performed in duplicate.

2.3.2. Pressurized liquid extraction (PLE) of hop residue after SFE-CO_2

PLE was performed from the HR with ethanol as a solvent in an accelerated solvent extraction apparatus ASE 350 (Dionex Sunnyvale, CA, USA) 7.500± 0.002 g of HR were mixed with 3.500 ± 0.002 g of diatomaceous earth (ratio of 2.14:1) and placed in a 15 mL Dionex stainless steel extraction cell (2.9 cm diameter, with a stainless-steel frit and a cellulose filter at the ends of the cell to avoid solid particles in the collection vial). For the optimization of extraction conditions, PLE-EtOH was performed at various temperatures (T, 40-100 °C) and time (τ,15-45 min; 3 cycles × 5-15 min) combinations. The system pressure (10.3 MPa), pre-heating time (5 min), cell flush volume (100%) and purge time (120 s) with nitrogen to collect the extract in the vial was kept constant for all PLE experiments. Ethanol was evaporated in a Büchi V–850 Rotavapor R–210 (Flawil, Switzerland), PLE-EtOH extracts were kept in a freezer (-20°C) prior to the analysis, yields were determined gravimetrically (±0.001 g) and expressed as g/100 g HR and HP.

2.4. Antioxidant activity assays of hop extract

2.4.1. Oxygen radical absorbance capacity (ORAC) assay

Following the procedure of Prior et al. [166] with some modifications, 25 µl of the SFE-CO_2 (0.03 mg/mL in MeOH) or PLE-EtOH extract (0.15 mg/mL in MeOH) or blank (MeOH) was mixed with 150 µL of fluorescein solution (14 µmol/l) in the 96-well black opaque microplates, preincubated for 15 min at 37 °C, followed by rapid addition of 25 µL of AAPH solution (240 mmol/l) and fluorescence recording at every cycle (1 min × 1.1, a total of 120 cycles) using 485-P excitation and 520-P emission filters. Raw data were exported from the Mars software to Excel 2016 (Microsoft, Roselle, IL), and the area under the fluorescence decay curve (AUC) was calculated as:

$$AUC = 1 + \sum_{i=1}^{i=150} \frac{f_i}{f_0}, \quad (1)$$

f_0 – the initial fluorescence reading at 0 min; f_i – the fluorescence reading at time i.

The results were expressed as mg TE/g E, HR and HP using calibration curves with Trolox (250-1500 µmol/L). Experiments were performed in quadruplicate.

2.4.2. Total phenolic content (TPC) by Folin-Ciocalteu's assay

Folin-Ciocalteu' Folin-Ciocalteu' assay was carried out by the procedure of Singleton, Orthofer and Lamuela-Raventós (1999), with some modifications. For the analysis, 150 µl of the SFE-CO2 extracts (0.3 mg/mL in MeOH) or PLE-EtOH (0.3 mg/mL in MeOH) or blank (MeOH) were mixed with 750 µl of Folin-Ciocalteu's reagent (2M), previously diluted with distilled water (1:9, v/v), and after 3 min of reaction, 600 µl of Na_2CO_3 solution (75 g/L), left in dark for 2 hours and absorbance measured

at 760 nm. The results were expressed as mg GAE/g E, HR and HP using dose-response curves for gallic acid (0-80 g/mL). Experiments were performed in quadruplicate.

2.4.3. Determination of ABTS•+ scavenging assay

Following the protocol of Re et al.[167] firstly, phosphate buffer saline (PBS) solution (75 mmol/l; pH 7.4) was prepared by dissolving 8.18 g NaCl, 0.27 g KH2PO4, 1.42 g Na2HPO4 and 0.15 g KCl in 1 L of ultra-pure water. The $ABTS^{\bullet+}$ solution was prepared by mixing 50 mL of ABTS (2 mmol/l PBS) with 200 µl $K_2S_2O_8$ (80 mmol/l) and allowing the mixture to stand in the dark at room temperature for 16 h prior to use. The working solution were prepared by diluting the $ABTS^{\bullet+}$ solution with PBS to obtain the absorbance of AU 0.700±0.010 at 734 nm. To a 3000 µl working $ABTS^{\bullet+}$ solution 50 µl of extract (0.25 mg/mL in EtOH) or blank (EtOH) were added, for 2 hours mixtures were kept in the dark, then the absorbance measured at 734 nm. The results were expressed as mg TE/g E, HR and HP using dose-response curves for Trolox (250-1250 µmol/L). Experiments were performed in quadruplicate.

2.5. Phytochemical composition

2.5.1. Determination of α-and β-acid composition by UPLC-MSn analysis

For the quantitative and qualitative determination of hop bitter acids, 10 ± 0.001 mg of SFE-CO_2 extracts were dissolved in MeOH and further diluted to a final concentration of 10 µg/mL and filtered through a polyamide filter into vials before UPLC-MSn analysis. The analysis was performed using an Acquity UPLC H-class system (Waters, Milford, MA, USA) combined with a Waters XEVO TQ-S mass spectrometer (Waters, Milford, MA, USA). The Acquity UPLC was equipped with a binary solvent delivery system, an autosampler with a column thermostat, and a data station running the MassLynx 4.0 aquisition and data processing software. An Acquity BEH C18 column (1.7 µm, 100 × 2.1 mm, i.d.) was used to separate compounds. The mobile phase was initially composed of 50% eluent A (0.3% of formic acid in water) and 50% B (0.3% of formic acid in acetonitrile), followed by a linear increase of B from 50% to 100% in 7 min, then holding at 100% B for 1 min and finally equilibrating the column to initial conditions (50% of B) for 4 min. The eluent flow rate was 0.4 mL/min. The effluent was introduced directly into the mass spectrometer equipped with an ESI source. Compounds were monitored by their characteristic fragment ions: 349.16 → 225.08 for cohumulone and 363.16 → 239.02 for humulone in the positive ionization mode; 399.29 → 287.12 for colupulone and 413.29 → 301.13 for lupulone in the negative ionization mode. The capillary voltage was maintained at 3 kV, desolvation temperature – 350 °C, desolvation gas flow – 750 L/h, cone gas flow – 150 L/h, nebulizer pressure – 6 bar. Nitrogen was used as the desolvation and nebulizing gas. Argon was introduced into the collisional cell at a rate of 0,15 mL/min as the collision gas. The external calibration curve for α-and β-acid quantification was designed using ICE-4 standard at concentrations ranging from 2.5 to 30 µg/mL; results were expressed as mg/g E and HP. Experiments were performed in triplicate.

2.5.2. Determination of the total chlorophyll and carotenoid content

As previously described by Lichtenthaler and Buschmann [168], the total content of the selected pigments (chlorophylls and carotenoids) was determined spectrophotometrically, measuring the absorbance of SFE-CO_2 hop extracts (10 mg/mL acetone) or PLE-EtOH hop extracts (2.5 mg/mL EtOH) at 662 nm, 645 nm, and 470 nm wavelengths. The concentrations of chlorophyll A, chlorophyll

B, total chlorophyll, and total carotenoid content (μg/mL of extract) were calculated using the following equations and further expressed as μg/g extract E, HR and HP or μg/g of hop pellets (measurements performed in quadruplicate):

$$C_{Chloropyll\ A} = 11.24 \times Abs_{662} - 2.04 \times Abs_{645} \quad (2)$$

$$C_{Chloropyll\ A} = 20.13 \times Abs_{645} - 4.19 \times Abs_{662} \quad (3)$$

$$C_{Total\ carotenoids} = (1000 \times Abs_{470} - 1.90 \times C_{Chlorophyll\ A} - 63.14 \times C_{Chlorophyll\ B})/214 \quad (4)$$

2.5.3. Determination of volatile compound composition by SPME-GC×GC-TOF-MS analysis

In order to determine the volatile compound composition, 0.100 ± 0.001 g of SFE-CO_2 extracts were placed in a 20 mL SPME-vials and subjected to the solid-phase microextraction (SPME) with a DVB/CAR/PDMS fiber at the following conditions: temperature 40 ℃, equilibration time 15 min, extraction time 30 min. The analysis of SPME-derived samples was conducted on a comprehensive gas chromatography-time-of-flight mass spectrometry (GC×GC-TOF-MS) LECO Pegasus 4D system, consisting of an Agilent 7890A GC system, a Gerstel multipurpose sampler MPS (Gerstel GmbH, Mulheim an der Ruhr, Germany) coupled with a high-speed TOF-MS detector (LECO, St. Joseph, MI, USA) and a four-jet cryogenic modulator (Zoex, Houston, TX). The chromatographic system was made up of a primary column BPX-5 (30 m, 0.25 mm i.d., 0.25 μm film thickness) (SGE Analytical Science, Australia) linked with a secondary column, BPX-50 (2.0 m, 0.10 mm i.d., 0.1 μm film thickness). Working conditions were: desorption time 5 min; oven temperature started at 40 °C (hold 1 min) and ramped to 250 °C at 7 °C/min rate (hold 1 min); modulator offset temperature 15 °C; transfer line to MSD 250 °C; the GC injector port temperature set at 150 °C then ramped to 250 °C at 720 °C/min; carrier gas (He) 1 mL/min; split ratio 1:20; TOF-MS acquisition rate 10 spectra/s, mass range 30-550 m/z units; detector voltage 1550 V; ion source temperature 250 °C. Data from the GC×GC-TOFMS system was collected by ChromaTOF software v.4.22 (LECO) after a solvent peak delay of 360 s. Volatile compounds were identified by comparing their mass spectra with the Adams, NIST, MainLib, and Replib mass spectral libraries (acceptable matches: signal-to-noise ratio >50 and similarity >750). The linear retention indexes (LRI) were calculated using the retention times of C_7-C_{30} n-alkanes series and further compared with previously published data in the literature [169–175], when available.

$$Linear\ Retention\ Index\ (\%) = 100 \times \left(n + \frac{Rt(s) - Rt(n)}{Rt(n+1) - Rt(n)} \right) \quad (5)$$

Rt(s) - retention time of compound
Rt(n) - retention time of alcane eluting before compound
Rt(n+1) - retention time of alcane eluting after compound
n - number of carbons in alcane eluting before compound

The results were expressed as GC peak area arbitrary units×10^7 (further abbreviated as AU) and percentage (%) of the total GC peak area. Experiments were performed in triplicate.

2.5.4. Determination of prenylflavonoid content by HPLC-DAD analysis

Detection and quantification of the selected prenylflavonoids xanthohumol, isoxanthohumol and 8-prenylnaringenin was performed as described by Dhooghe et al. with slight modifications [176] in a Thermo Scientific Ultimate 3000 HPLC system coupled to a diode array detector and an automatic injector (Thermo Fisher Scientific, Waltham, MA). Chromatographic separation was achieved with a Phenomenex Luna 5μ C18(2) 100Å 150×4.60 mm column (Phenomenex, Aschaffenburg,

Germany) equipped with a security guard column, thermostated at 30 ℃. Solvent A was Milli Q water and solvent B 0.25% formic acid in acetonitrile with a constant flow rate of 1.0 mL/min and an injection volume of 10 µl. The gradient for the analysis was 0-3 min (20% B); 3-30 min (20-75% B), 30-35 min (75% B), 35-38 min (75-20% B) and 38-40 min (20% B). Chromatograms were recorded at 370 nm for the determination of xanthohumol, and at 290 nm for the determination of isoxanthohumol and 8-prenylnaringenin, and analyzed using Chromeleon 7 software. The external calibration curves were designed using standard compound at concentrations ranging from 20 to 100 µg/mL; results were expressed as mg/g E, HR and HP. Experiments were performed in triplicate.

2.6. Antimicrobial activity measured by agar well diffusion assay

The agar well diffusion method was used for antimicrobial activity determination, modified from the method by Ložienė et al. [177]. Culturing of microorganisms was performed on a slant agar (Plate Count Agar (PCA) (REF 610040, Liofilchem, Italy) at 37 °C for *Escherichia coli* ATTC 8739, *E.coli* NTC 12900 and *Staphylococcus aureus* ATCC 25923 bacteria and a slant agar from DRBC agar base (REF 610237, Liofilchem, Italy) at 25 °C for *Candida albicans* ATCC 90028 yeast. The bacterial and yeast cell suspension was prepared from 18 h culture adjusted to inoculation of 1×10^6 colony-forming units per mL (CFU/mL). 200 mL of sterile Plate Count Agar was inoculated with 2 mL of bacterial cell suspensions, for yeast 200 mL of DRBC agar base was inoculated with 2 mL of cell suspension;15 mL of inoculated agar was pipetted into the sterile Petri plates. After solidification, 10 mm diameter wells were punched in the agar and filled with 25 µl of hop PLE-EtOH extract solutions, prepared in ethanol at 10, 25 and 50 mg/mL concentrations. Solvent (ethanol) were used as a control. The plates with bacteria were incubated at 37°C (*E. coli* ATTC 8739, *E.coli* NTC 12900, *S. aureus* ATCC 25923), the yeast (*C. albicans* ATCC 90028) at 30°C for 24 h and the antimicrobial effect was assessed by measuing the diameter of clear (inhibition) zones surrounding the wells (diameter of inhibition zone + diameter of the well). An average inhibition zone was calculated from 3 replicates.

2.7. Assessment of keratinocyte, fibroplast and skin cancer cell viability[1]

Healthy immortalized human keratinocyte HaCaT (CLS Cell Lines Service, 300493), healthy fibroblast (HDFa) and skin cancer (A-375) cell lines, purchased from the Cell Lines Service GmbH (Germany), were selected to evaluate the cytotoxic properties of *Ella* hop SFE-CO_2 extract, obtained under the optimized extraction conditions. Cells were seeded in culture flasks containing DMEM (Gibco Dulbecco's Modified Eagle Medium, Life Technologies (Thermo Fisher Scientific, Waltham, MA, USA)). with 10% of fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin and further incubated at 37°C with 5% CO_2 and saturated humidity. Culture transfer was performed once a week, and the medium was renewed twice a week. Cell viability was assessed by measuring their ability to metabolize MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) as previously described by Van Meerloo et al. [178]. Briefly, cells were seeded in a 96-well plate (2×10^4 cells/well) and incubated for 24 hours at 37 °C with 5% CO_2, followed by the addition of the *Ella* hop SFE-CO_2 extract (5 mg/mL in EtOH/H_2O, 30% v/v) at the different volumes (1-150. The control cultures were incubated with the culture medium only without the extract tested. After the 24-h treatment, the medium was removed, and the cells were washed twice with 100 µL of HBSS (Hanks' balanced salt) solution, followed by the addition of 180 µL HBSS and 20 µL MTT (5 mg/ml). The

[1] Extracts were provided for Lithuanian University of Health Sciences, analyzed by the research group of Prof. Dr. Kristina Ramanauskienė

cells were incubated for 2 h at 37 °C with 5% CO_2, the solution was removed, leaving the formazan crystals at the bottoms of the wells. To solubilize the crystals, 100 µL of DMSO (dimethyl sulfoxide) was added to each well, and the level of absorbance was read with a Tecan Multiscan microplate reader at the wavelength of 520 nm. Experiments were performed in triplicate. The cell viability (%) was calculated using the following equation:

$$Cell\ viability\ (\%) = \frac{ABS_{Sample}}{ABS_{Control}} \times 100 \quad (6)$$

ABS_{Sample}: absorbance of extract-treated cells treated after elimination of $EtOH/H_2O$ (30% v/v) impact; $ABS_{control}$: absorbance of control cells.

2.8. Experimental design

Response surface methodology (RSM) using central composite design (CCD) was employed to identify optimal SFE-CO_2 conditions by determining the effect of pressure (25-45 MPa), temperature (40-60°C), and time (30-90 min) on SFE-CO_2 extract yield and oxygen radical scavenging capacity, selected as the response factors (RF) in the first optimization experiment. The face-centered CCD design had 8 factorial, 6 axial, and 6 center points (in total, 20 experimental runs). In the second optimization experiment, the effect of P, T and τ was observed on the response factors of cohumulone, humulone+adhumulone, colupulone, lupulone+adlupulone, total α-acids, total β-acids and total bitter acids content, as well as α/β ratio in the same face-centered CCD design. RSM-CCD was also utilized for optimization of PLE-EtOH conditions by determining the effect of temperature (40-100°C) and time (15-45 min) on selected RFs: PLE-EtOH yield, xanthohumol content and antioxidant activity measured by total phenol content and ORAC. A face-centered CCD with 4 factorial, 4 axial and 5 center points totaling 13 experimental runs was established. The CCDs and the randomized order of experiments, models, and the second-order polynomial equations for all RFs were established using the Design-Expert 12 software (Stat–Ease Inc., Minneapolis, MN) as previously described elsewhere by our research group [163–165, 179, 180]. Student test (p-value) at 5% probability level ($p<0.05$), 'lack of fit' coefficient, and the Fisher test value (F-value) were used to define the statistical significance and adequacy of the model and each variable for both RFs.

2.9. Statistical analysis

Mean values and standard deviations were calculated using MS Excel 2016. GraphPad Prism 7.04 software (2017) was used to compare the means that had significant variation ($p < 0.05$) applying unpaired t-test or one-way analysis of the variance (ANOVA), continued with Tukey's posthoc test, and calculation of Pearson correlation coefficients (two-tailed, $p < 0.05$) between selected values.

3. Results and discussion

Dual hops are high-value crops, rich in bitter acids and essential oils, and contain specific polyphenols as well. Selected hop compounds are recognized as antioxidative, anti-inflammatory, antimicrobial, sedative, estrogenic, anticarcinogenic, antitumor, antidiabetic and neuroprotective agents [3–5]. Therefore, besides the traditional application in breweries for their bittering and aroma, or the dry relaxing, sedative tea mixtures, hop products found novel utilization areas in the functional food, neutraceutical, pharmaceutical and cosmetic industries [2]. Sustainable intensified extraction methods such as SFE-CO_2 are popular among manufacturers since the 1970s. To satisfy consumer and industrial expectations the typical commercial SFE-CO_2 requires optimization for the production of specialized SFE-CO_2 extracts based on single or multipurpose desirability functions. In accordance with the biorefinery concept, the polyphenol-rich SFE-CO_2 residue can be further extracted with optimized PLE-EtOH, a relatively safe, non-toxic polar solvent. Such produce cycle was optimized for selected responses in this work, the experimental scheme is depicted in Figure 3 (different colored arrows refer to the main stages of this workflow).

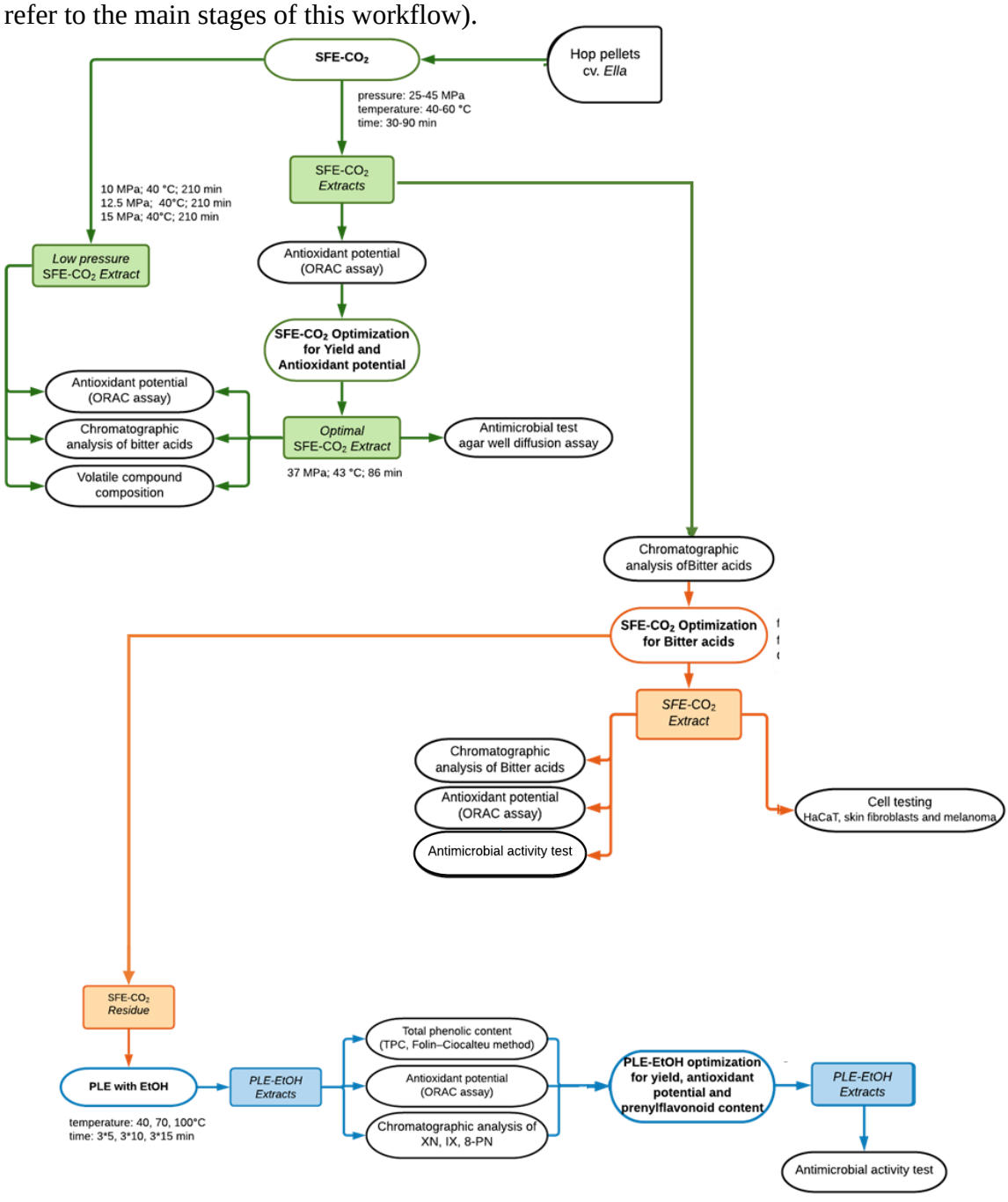


Figure 3. Experimental scheme of the multi-step valorization of *Ella* hop pellets

3.1. Optimization of hop SFE-CO_2 for high yield and antioxidant capacity

3.1.1. Evaluation of SFE-CO_2 hop extracts prepared at 10-15 MPa pressure

The task of this part of the thesis was to assess commonly used process parameters in the hop extraction industry before further SFE-CO_2 optimization experiments. The extraction curves in Appendix 1. depict the cumulative *Ella* hop extract yield as a function of time in a one-stage process at 10-15 MPa and 40 °C, which is most commonly applied at the industrial level [9]. The yields and $TEAC_{ORAC}$ values at the final point of the kinetic experiments (after 300 min) are reported in Table 1. All three extraction curves followed a similar pattern (Appendix 1): ~50% of the final SFE-CO_2 extract yield was obtained after 45 min of extraction, ~80% after 120 min. For 10 MPa, the equilibrium state was reached after 180 min, yielding 9.3 g/100 g of light-yellow extract. For 12.5 and 15 MPa, ~96% of the total extract yields, 19.1 and 22.1 g/100 g, respectively, were recovered after 240 min (see Table 3). Thus, the increase in CO_2 density from 629 kg/m^3 at 10 MPa to 780 kg/m^3at 15 MPa resulted in a remarkable (> 2-fold) increase in yield. Nevertheless, the prolonged τ (>240 min) was required to achieve this aim, which is in agreement with the previous data for SFE-CO_2 of hops at low P range (< 20 MPa) [6, 8, 154]. It could be noted that the shape of *Ella* hop SFE-CO_2 curves (Appendix 1.) is almost similar to the previously reported for other hop varieties exhibiting a rather long, low-yield period at the beginning of extraction [6, 154, 181].

Table 3. Yields and $TEAC_{ORAC}$ of *Ella* hop SFE-CO_2 extracts obtained under the different experimental conditions

Samples	**SFE-CO_2 parameters**			
	SFE-CO_2 I 10 MPa, 40 °C, 300 min	SFE-CO_2 II 12.5 MPa, 40 °C, 300 min	SFE-CO_2 III 15 MPa, 40 °C, 300 min	SFE-CO_2 IV 37 MPa, 43 °C, 80 min
Extract yield, g/100 g HP	9.33 ± 0.46^a	19.11 ± 0.88^b	22.09 ± 0.76^c	26.32 ± 0.46^d
$TEAC_{ORAC}$				
mg TE/g E	1251.79 ± 6.30^a	1281.94 ± 41.22^a	1515.16 ± 26.30^b	1481.17 ± 50.87^b
mg TE/g HP	116.79 ± 0.59^a	244.98 ± 7.88^b	334.70 ± 5.81^c	389.84 ± 13.39^d

SFE-CO_2: supercritical carbon dioxide extraction; TE: Trolox equivalent; HP: hop pellets; E: extract
Significant differences can be seen in the same row indicated by different superscript letters. (one-way ANOVA and Tukey`s test $p < 0.05$)

The antioxidant capacity of lipophilic extracts obtained at 10-15 MPa was assessed using biologically relevant peroxyl radical inhibition-based ORAC assay [182]. As reported in Table 1, the P change from 10 to 15 MPa augmented extract $TEAC_{ORAC}$ by 21% (from 1252 to 1515 mg TE/g). Considering extraction yields, the calculated recovery of TE antioxidants from the pellets was in the range of 117-335 mg TE/g, indicating a nearly 3-fold increase due to the higher P. For applications in functional food, nutraceutical, pharmaceutical, and cosmetic industries, the strong antioxidant potential of SFE-CO_2 extracts is the desired quality characteristic. Thus, the extraction of hop antioxidants at higher yields could be considered a more efficient approach for such purposes, preferably within shorter times to reduce the operational costs of the process.

3.1.2. Evaluation of SFE-CO_2 hop extracts prepared at 25-45 MPa pressure

3.1.2.1. Central composite design and model analysis

In the following steps, the research was targeted to increase SFE-CO_2 yield and recovery of valuable constituents from *Ella* hops under a significantly shorter extraction time. CCD-RSM was employed to optimize the SFE-CO_2 process by testing different experimental conditions at the higher P and T levels: P (25-45 MPa), T (40-60 °C), and τ (30-90 min). Within this region of operability, total SFE-CO_2 hop extract yield (RFI) ranged from 13.9 to 27.6 g/100 g HP, while the $TEAC_{ORAC}$ (RFII) were increasing from 252 to 375 mg TE/g HP or 1280.4 to 1847.7 mg/g E (Table 4), both well-fitting the predicted values of the designed models (Appendix 3).

Table 4. Central composite design matrix (levels of independent variables and variation levels) for SFE-CO2 optimization for extraction of non-polar constituents from *Ella* variety hop pellets

Levels and runs	**SFE-CO_2 parameters**			**CO_2 density*, kg/m^3**	**RF I: SFE-CO_2 extract yield**	**RF II: $TEAC_{ORAC}$**	
	P, MPa	T, °C	τ, min		g/ 100 g HP	mg TE/g HP	mg TE/g E
Center	35	50	60	899	23.24 ± 0.56	334.22 ± 14.15	1438.04 ± 60.89
Axial	25	50	60	834	20.15 ± 0.31	297.98 ± 19.92	1478.41 ± 98.86
Axial	35	40	60	935	25.12 ± 0.89	370.86 ± 12.90	1476.11 ± 51.35
Factorial	25	40	90	880	23.84 ± 1.32	351.05 ± 5.92	1472.32 ± 24.83
Factorial	25	60	30	787	13.85 ± 0.18	255.93 ± 7.42	1847.65 ± 53.57
Axial	35	50	90	899	25.74 ± 1.16	374.68 ± 5.43	1455.32 ± 21.1
Center	35	50	60	899	23.54 ± 0.56	338.53 ± 14.33	1437.98 ± 60.88
Center	35	50	60	899	24.22 ± 0.50	348.31 ± 14.74	1438.07 ± 60.86
Factorial	45	60	30	913	19.04 ± 0.62	304.38 ± 14.09	1598.21 ± 74.00
Factorial	45	40	90	975	27.57 ± 0.29	353.07 ± 19.91	1280.38 ± 72.22
Factorial	45	40	30	975	21.94 ± 0.47	304.98 ± 5.86	1389.70 ± 26.71
Axial	35	60	60	863	23.21 ± 0.54	355.29 ± 31.40	1530.37 ± 135.29
Factorial	25	60	90	787	22.72 ± 0.30	333.37 ± 9.10	1466.99 ± 40.05
Factorial	45	60	90	913	25.74 ± 0.66	347.98 ± 3.67	1351.59 ± 14.26
Center	35	50	60	899	23.14 ± 0.56	332.78 ± 14.09	1437.77 ± 60.89
Center	35	50	60	899	24.00 ± 0.55	345.15 ± 14.61	1437.92 ± 60.88
Axial	45	50	60	944	24.51 ± 0.23	335.77 ± 18.35	1369.65 ± 74.87
Center	35	50	60	899	24.52 ± 0.49	345.22 ± 2.21	1407.83 ± 9.01
Factorial	25	40	30	880	15.50 ± 0.27	251.79 ± 17.80	1623.87 ± 114.84
Axial	35	50	30	899	20.88 ± 1.28	308.17 ± 9.36	1475.57 ± 44.83

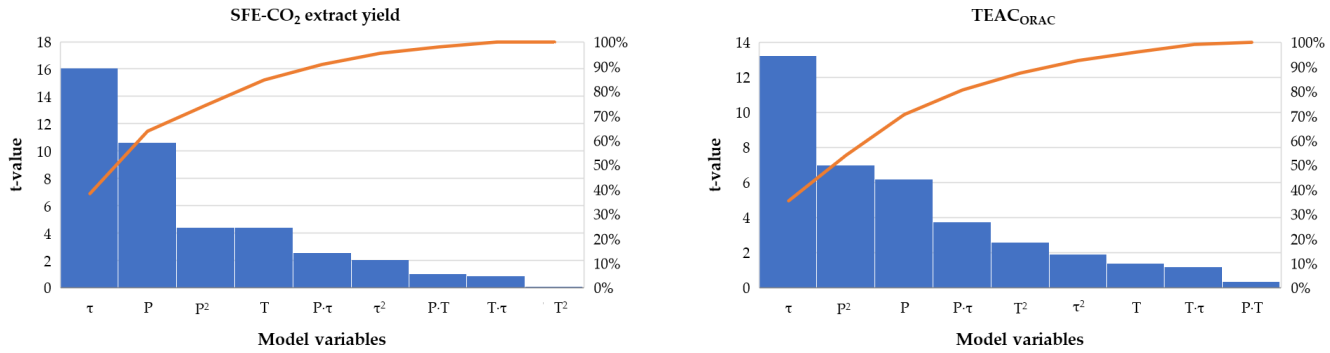
*: calculated using online Peace Software (http://www.peacesoftware.de/einigewerte/co2_e.html). SFE-CO_2: supercritical carbon dioxide extraction; ORAC: oxygen radical scavenging capacity; P: extraction pressure; RF: response factor; *τ:* extraction time; T: extraction temperature; TEAC: Trolox equivalent antioxidant capacity. Yields are expressed per 100 g of unextracted *Ella* hop pellets; TEAC values are expressed per g of unextracted *Ella* hop pellets and per g of extract, E: extract; HP: hop pellets.

Both models were reasonably reproducible with low variation coefficients (< 3%), high determination coefficients ($R^2 > 0.96$), and good agreement between the adjusted and predicted R^2 values (difference < 0.20), additionally confirming the good fit of the model to the experimental data (Appendix 2.) Based on the ANOVA (Appendix 4), developed models were statistically significant ($p < 0.05$) with *F*-values of 51.71 and 35.20 for RFI and RFII, respectively. Time (τ) was the primary extraction parameter responsible for the observed changes in both extract yield (*F*=258.72) and $TEAC_{ORAC}$ (*F*=175.15) under the different experimental conditions. The significance of other model terms for the extract yield decreased as follows: $P < P^2$ (showing the non-linear concave relationship between P and RFI) < T < Pτ interaction. The influence of other linear interactions (PT and Pτ) and second-order terms (T^2 and τ^2) were not significant. Besides τ, $TEAC_{ORAC}$ was mainly affected by the P^2 and P, to a lower extent by Pτ and T^2, while other factors and their interactions did not exert any significant input towards RFII. The Pareto charts (Figure 4) visualize these effects and indicate that three primary factors (τ, P, and P^2) together contributed to the >70% of the observed responses.

The following second-order polynomial regression equations describe the empirical relationship between the independent model variables and selected-response factors (coded factors):

$$Yield_{SFE-CO_2} = 23.92 + 2.27 \times P - 0.94 \times T + 3.44 \times r - 0.25 \times (PT) - 0.61 \times (Pr) + 0.20 \times (Tr) - 1.80 \times P^2 + 0.03 \times T^2 - 0.82 \times r^2 \quad (7)$$

$$Yield_{TEAC_{ORAC}} = 344.65 + 15.61 \times P - 3.48 \times T + 33.49 \times r + 0.98 \times (PT) - 10.63 \times (Pr) - 3.29 \times (Tr) - 33.70 \times P^2 + 12.50 \times T^2 - 9.15 \times r^2 \quad (8)$$



(A) **(B)**

Figure 4. Pareto charts ($p = 0.05$) for the main effects of SFE-CO_2 pressure (P), temperature (T) and time (τ) and their interactions on the *Ella* hop: (A) SFE-CO_2 extract yield (g/100 g HP); (B) oxygen radical scavenging capacity ($TEAC_{ORAC}$, mg TE/g HP).

3.1.2.2. Analysis of response surface plots

2D and 3D response surface plots visualize the effects of the independent variables on the extract yield (Appendix 5) and $TEAC_{ORAC}$ (Appendix 6). For example, the plots illustrating the effect of T and P, at fixed τ of 60 min indicated that the amount of the extract and $TEAC_{ORAC}$ did not exceed 20 g/100 g and 300 mg TE/g, respectively, at the minimal P (-1 level) of 25 MPa. Nevertheless, at 25 MPa and 40 °C already after 30 min was more efficient than 180 min extraction at 10 MPa, amounting to 166 and 216% of the final 10 MPa yield and $TEAC_{ORAC}$, respectively; the results for both responses after 90 min were by 5-43% higher than measured for 12.5 and 15 MPa after 240 min (Table 3, Table 4). It may be explained by the substantially higher CO_2 density and solvating power towards lipophilic

constituents at 25 MPa and 40 °C (880 kg/m^3) in comparison to 10, 12.5, and 15 MPa under the same extraction temperature (629, 705 and 780 kg/m^3, respectively).

The analysis of 2D and 3D response surface plots in Appendix 5(A) also outlined that combinations of 37-42 MPa and 40-45 °C augmented the yields to the maximum values (>26 g/100 g) within the selected region of operability. Maximum yield values were also reached due to the prolonged $\tau > 75$ min (Appendix 5B and C). Although the CO_2 diffusivity and solute vapour pressure are greater at higher temperatures [22], the yield reduction was observed > 45 °C at all tested P levels. It could be explained by the decreasing solvent density due to the T increase from 40 to 60 °C: by 11% at 25 MPa (from 880 to 787 kg/m^3), 8% at 35 MPa (from 935 to 863 kg/m^3), and 6% at 45 MPa (from 975 to 913 kg/m^3). Thus, the effect of density governs the retrograde behaviour of T in the *Ella* hop extraction model. Also, even high-end experimental P (45 MPa) remains lower than the so-called cross-over (inversion) P value, when the higher T would favor the extraction since increasing solute vapour pressure would outweigh the impact of decreasing CO_2 density [142].

For $TEAC_{ORAC}$, the plots with temperature and pressure effects at the fixed extraction time acquired a slight saddle shape (Appendix 6). The highest values were reached at the 35-40 MPa and 40-43 °C combinations, which overlapped favorably with the optimal T and low-to-middle range of the desired P for the maximum yield. Based on $TEAC_{ORAC}$, an even higher amount of radical scavengers were recovered by continuing extraction ($\tau > 80$ min), especially at T < 45 °C, which is in agreement with the observations for the yield.

3.1.2.3. SFE-CO_2 optimization for yield and in vitro antioxidant capacity

Based on the response surface plots and the predictive equations that describe the model, the SFE-CO_2 optimization was upgraded to obtain *Ella* hop extract combining a high yield (>26 g/100 g HP) and $TEAC_{ORAC}$ (> 360 mg TE/g HP) under the lowest possible P and shortest τ. For this task, the Design expert software suggested 37-38 MPa, up to 43 °C, 80-85 min. For example, 80 min extraction at 37 MPa and 43 °C yielded 26.3 g/100 g HP of the greenish-brown extract with the $TEAC_{ORAC}$ of 1481 mg TE/g E, equivalent to 390 mg TE/g HP (Table 3). Good agreement between the experimental and the predicted values under deduced optimal conditions additionally confirmed the suggested model's validity for both response factors (Appendix 7). Generally, maximum extract yields from *Ella* hops at 10-37 MPa were higher than the previously reported under the various experimental conditions; e.g., for *Hallertau Mittelfrüh* pellets it was 7 g/100 g at 20 MPa/55 °C/180 min [6]; for *Nugget* variety and five Chilean hop ecotype pellets 3-13 g/100 g at 20 MPa/40 °C/150 min [154]; for several unspecified *H. lupulus* samples 2-9 g/100 g at 30-35 MPa/250-300 min [7, 8]. Comparing the 10, 12.5, 15, and 37 MPa results (Table 3), an up to ~ 3-fold increase in SFE-CO_2 extract yield was obtained in ~4-fold shorter τ (80 versus 300 min) when optimized P of 37 MPa was applied. Similarly, the extract with high $TEAC_{ORAC}$ (1481 mg TE/g E) was produced at 37 MPa, remarkably reducing the τ of supercritical CO_2-soluble antioxidant constituents' recovery from hop pellets and up to 334% augmenting its content as compared to 10-15 MPa treatments (Table 3).

3.1.2.4. Bitter acid profile of hop extracts obtained under different SFE-CO_2 conditions

As reported in Table 5, α-and β-acids (soft resins) constituted the major portion of the hop SFE-CO_2 extracts, depending on process parameters from ~72 to 92%. Non-polar solvent-soluble uncharacterized soft resins, hop essential oil components, and waxy fraction could comprise the remaining 8-28% of the extract [1]. The percentage distribution of the individual constituents within

Table 5 Bitter acid, chlorophyll and carotenoid content of *Ella* hop SFE-CO_2 extracts obtained under the different experimental conditions.

Samples	SFE-CO_2 parameters			
	SFE-CO_2 I 10 MPa, 40 °C, 300 min	SFE-CO_2 II 12.5 MPa, 40 °C, 300 min	SFE-CO_2 III 15 MPa, 40 °C, 300 min	SFE-CO_2 IV 37 MPa, 43 °C, 80 min
Bitter acid content				
α-Bitter acids				
Cohumulone				
mg/g E	144.59 ± 2.89^{a}	182.60 ± 8.47^{b}	234.54 ± 0.24^{c}	186.95 ± 8.03^{b}
mg/g HP	13.49 ± 0.27^{a}	34.90 ± 1.62^{b}	51.81 ± 0.05^{c}	49.21 ± 2.11^{c}
Adhumulone + humulone				
mg/g E	246.37 ± 4.29^{a}	349.19 ± 17.43^{b}	360.15 ± 6.17^{b}	335.92 ± 2.27^{b}
mg/g HP	22.99 ± 0.40^{a}	66.73 ± 3.33^{b}	79.56 ± 1.36^{c}	88.41 ± 0.60^{d}
Total α-bitter acids				
mg/g E	390.96 ± 7.18^{a}	531.79 ± 25.90^{b}	594.69 ± 6.41^{c}	522.87 ± 10.30^{b}
mg/g HP	36.48 ± 0.67^{a}	101.63 ± 4.95^{b}	131.37 ± 1.42^{c}	137.62 ± 2.71^{c}
β-Bitter acids				
Colupulone				
mg/g E	217.38 ± 6.33^{b}	186.47 ± 7.83^{a}	212.82 ± 6.08^{b}	225.94 ± 13.05^{b}
mg/g HP	20.28 ± 0.59^{a}	35.63 ± 1.50^{b}	47.01 ± 1.34^{c}	59.47 ± 3.43^{d}
Adlupulone + lupulone				
mg/g E	109.69 ± 3.06^{a}	116.45 ± 6.38^{ab}	125.11 ± 1.68^{b}	119.09 ± 6.39^{ab}
mg/g HP	10.23 ± 0.29^{a}	22.25 ± 1.22^{b}	27.64 ± 0.37^{c}	31.34 ± 1.68^{d}
Total β-bitter acids				
mg/g E	327.07 ± 9.39^{ab}	302.92 ± 14.20^{a}	337.93 ± 4.40^{b}	345.03 ± 6.66^{b}
mg/g HP	30.51 ± 0.88^{a}	57.88 ± 2.71^{b}	74.65 ± 0.97^{c}	90.81 ± 1.75^{d}
Total hop bitter acids				
mg/g E	718.03 ± 16.57^{a}	834.71 ± 40.10^{b}	932.62 ± 2.01^{c}	867.90 ± 16.96^{b}
mg/g HP	66.99 ± 1.55^{a}	159.51 ± 7.66^{b}	206.02 ± 0.44^{c}	228.43 ± 4.46^{d}
α-acid/β-acid ratio	1.19	1.76	1.76	1.52
Pigment content				
Chlorophylls				
Chlorophyll A				
µg/g E	$-^{nd}$	10.60 ± 0.12^{a}	41.19 ± 0.14^{b}	146.13 ± 1.45^{c}
µg/g HP	$-^{nd}$	2.02 ± 0.02^{a}	9.10 ± 0.03^{b}	38.46 ± 0.38^{c}
Chlorophyll B				
µg/g E	$-^{nd}$	12.84 ± 1.16^{a}	20.48 ± 1.42^{b}	20.10 ± 0.83^{b}
µg/g HP	$-^{nd}$	2.45 ± 0.22^{a}	4.52 ± 0.31^{b}	5.29 ± 0.22^{c}
Total chlorophylls				
µg/g E	$-^{nd}$	23.43 ± 1.04^{a}	61.67 ± 1.28^{b}	166.23 ± 2.28^{c}
µg/g HP	$-^{nd}$	4.48 ± 0.20^{a}	13.62 ± 0.28^{b}	43.75 ± 0.60^{c}
Carotenoids				
Total carotenoids				
µg/g E	20.72 ± 1.18^{a}	76.80 ± 3.39^{b}	124.26 ± 0.59^{c}	235.12 ± 1.33^{d}
µg/g HP	1.93 ± 0.11^{a}	14.68 ± 0.65^{b}	27.45 ± 0.13^{c}	61.88 ± 0.35^{d}

$-^{nd}$: not detected; E: extract; HP: hop pellets; SFE-CO_2: supercritical carbon dioxide extraction; TEAC: Trolox equivalent antioxidant capacity; TE: Trolox equivalents; ORAC: oxygen radical scavenging capacity. Significant differences can be seen in the same row indicated by different superscript letters (one-way ANOVA and Tukey`s test $p < 0.05$).

the identified soft resins were as follows: adhumulone and humulone (25-36% of the total extract amount), colupulone (22-30%), cohumulone (20-25%), and finally, the sum of adlupulone and lupulone (13-15%). Considering extraction yields, 67.0-228.4 mg of these bitter acids were recovered from 1 gram of hop pellets. The recovery of soft resins from hops gradually increased by 71% increasing the P from 10 to 37 MPa (Table 5).

α-Bitter acids (humulones) comprised 54-64% of the total soft resins and were found in significantly varying amounts from ~391.0 to 594.9 mg/g E, corresponding to the recovery range of 36.5-137.6 mg/g HP (Table 5). Comparing these data with the manufacturer-provided α-bitter acid content in *Ella* hops (13.4 g/100 g HP), the recovery efficiency of SFE-CO_2 at 10, 12.5 and 15 MPa was 27, 76 and 98% of the declared content of humulones, respectively. However, higher P, up to 37 MPa enabled substantial shortening of the process, from 300 to 80 min. The percentage of β-bitter acid (lupulones) in the soft resin was lower, ~45% at 10 MPa, 36% at 12.5 and 15 MPa, and 40% at 37 MPa. Del Valle et al. [154] reported a similar ratio of α-/β- acids (1.2-1.7/1) in oleoresins from *Nugget*, *Osorno*, and *Elizalde Lake* variety hops. Humulone-rich (41%) antimicrobial extract was obtained from *Marynka* hop pellets by SFE-CO_2 at 30 MPa/50 °C [151]. The variations of SFE-CO_2 conditions had a lower effect on the concentration of β-acids than α-acids; the highest content of the former (345.0 mg/g E at 37 MPa) was only 14% larger than the lowest one (302.9 mg/g E at 12.5 MPa). However, the recovery of β-acids was highly dependent on the process pressure: thus, up to 3-fold, more lupulones (90.8 vs 30.5 mg/g HP) were recovered at 37 MPa than at lower pressures, which are most commonly used by the industry.

Numerous beneficial bioactivities both in vitro and in vivo were reported for bitter acids-rich preparations [2]. In general, high $TEAC_{ORAC}$ of *Ella* hop extracts (Table 3) were consistent with the previous reports showing prevalent links between strong in vitro oxygen radical scavenging capacity and high soft resin, mainly humulones, content in various hop extracts [70–72]. It is additionally supported by the calculated Pearson correlation coefficients (Appendix 8.), which indicate the significant positive correlation between the $TEAC_{ORAC}$ and cumulative α and β-bitter acid amount including the individual constituents within this group of bioactive components (> 0.97 and $p < 0.05$ for values expressed in mg/g HP).

3.1.2.5. Pigment profile of hop extracts obtained under different SFE-CO_2 conditions

The quantitative composition of the selected supercritical-CO_2 soluble pigments in hop extracts, namely chlorophylls and carotenoids, is reported in Table 5. Generally, the total amount of these pigments was very low compared to the bitter acid content and did not exceed 0.04% of the total extract mass. For example, the extract isolated at 10 MPa had only 20.7 μg/g of carotenoids, while chlorophylls were not detected (the colour of this extract was pale-yellow). The concentration of pigments in the extracts significantly increased by increasing P and at 37 MPa reached 166.2 and 235.1 μg/g E for chlorophylls and carotenoids, respectively. Consequently, the recovery of carotenoids at 37 MPa was 32, 4.2 and 2.3 times higher than at 10, 12.5 and 15 MPa, respectively. Although humulones are undoubtedly the major contributors to the overall $TEAC_{ORAC}$ of the extracts, chlorophylls and carotenoids, both of which have a well-documented antioxidant potential *in vitro* and biological systems [183], may also influence antioxidant capacity, which was higher for the extracts obtained at 15 and 37 MPa (Table 3). Chlorophyll A amounted to 45%, 67%, and 88% of the sum of all chlorophylls at 12.5, 15, and 37 MPa, respectively (Table 5.). The concentration of this compound significantly (~14-fold) increased from 10.6 μg/g E at 12.5 MPa to 146.1 μg/g E at 37 MPa, explaining the shift of SFE-CO_2 extract colour from yellow-orange to greenish-brown at

elevated P. The chlorophyll B content variations were less pronounced, ranging from 12.8 to 20.5 μg/g across different SFE-CO_2 extracts tested. Higher content of chlorophyll A compared to chlorophyll B (average ratio 7/3) was also characteristic for hydroethanolic extracts recently obtained from *Magnum*, *Marynka*, and *Lubelski* hop varieties [184]. The presence of chlorophylls in SFE-CO_2 extracts (without quantitative results) isolated from different hop varieties with pure CO_2 [154, 181] and with co-solvent ethanol [185] were previously reported in several articles. To the best of our knowledge, the effects of SFE-CO_2 parameters on the quantitative composition of chlorophylls in the extracts and their recovery rates have not been reported.

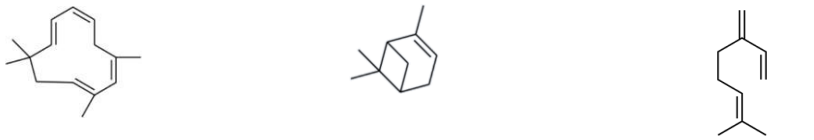
3.1.2.6. Volatile compound profile of hop extracts obtained under different SFE-CO_2 conditions

SPME-GC×GC-TOF-MS was employed to analyze the differences in the volatile compound composition of *Ella* hop lipophilic extracts obtained under different SFE-CO_2 conditions (Table 6). Quantitative assessment was based on the peak area (AU×10^7), which is dependent on the amount of eluting from the GC column compound and is relevant for comparison purposes (Appendix 9). The extract isolated at 10 MPa generated the highest total peak area, while pressure increase resulted in the lower peak area by 32 to 36%; however, it was not significantly different at 12.5, 15, and 37 MPa (Appendix 9). These findings may be explained by the dilution of volatile and GC-detectable fraction by the nonvolatile components, which were recovered at remarkably higher yields at the higher pressures (Table 6).

Comparing experimental mass spectra with various spectroscopic databases and retention indices with available literature data [169–175], 45 compounds of different chemical classes were identified in the tested SFE-CO_2 extracts: monoterpene hydrocarbons (8), oxygenated monoterpenes (1), sesquiterpene hydrocarbons (11), oxygenated sesquiterpenes (1), alcohols (2), aldehydes (1), ketones (2), fatty acids (4) and esters (15). Dietz et al. recently reported the importance of different fractions of hop essential oil constituents on the sensory flavor characteristics [186]. Consequently, the composition of volatiles may be important for developing various applications of hop extracts.

Sesquiterpenes represented the major fraction of volatiles, accounting for 33.8-38.7% of the total quantified by GC volatiles. Sesquiterpene hydrocarbons such as β-humulene (6.3-7.0%) seen in Figure 5, α-humulene (7.2-9.9%), and α-selinene (5.5-14.9%) with intense woody, spicy and pepper-like notes were found at the highest percentages in the headspace of extracts absorbed by SPME. These compounds were followed by herbal β-selinene (3.9-4.6%) and δ-cadinene (1.5-4.7%), and fruity α-ylangene (3.1-4.0%). Other identified sesquiterpene hydrocarbons individually contributed to less than 2% of the total GC peak area (Table 6). Yan and co-workers recently reported a high share of humulene, selinene, and cadinene in the overall sesquiterpene content (43%) for the *Ella* hop essential oil obtained by hydrodistillation [171]. Both the percentage content (Table 6) and peak areas (Appendix 9) indicate that sesquiterpene profile was dependent on extraction parameters, particularly P. For example, α-selinene significantly reduced from 14.9% (or 182×10^7 AU) at 10 MPa to 5.5% (or 49×10^7 AU) at 37 MPa. The extracts obtained at 10 and 12.5 MPa generated higher peak areas and percentage content of α-humulene (90-121×10^7 AU; on average, 9.8%) than at 15 and 37 MPa (67-72×10^7 AU; on average, 7.6%). The share of β-humulene in the headspace remained relatively stable, amounting to ~6.6% of the total GC peak area across the different extracts tested with no significant differences in AU at P >12.5 MPa. Only negligible amounts (< 0.5%) of oxygenated sesquiterpene caryophyllene oxide were found in extracts obtained up to 15 MPa (Table 5), while humulene epoxide II, previously reported in *Ella* hop essential oil at 0.4% [171], was not detected in these experiments.

The percentage of monoterpenes in the headspace increased from 10.5% (or 127×10^7 AU) to 17.6% (or 154×10^7 AU) when P was raised from 10 to 37 MPa (Table 6; Appendix 9). These changes were obtained due to the ~2-fold higher peak areas of herbal β-pinene (63×10^7 AU; 7.0%) and spicy β-myrcene (56×10^7 AU; 6.2%) at 37 MPa as compared to 10-15 MPa, both being the major identified monoterpene hydrocarbons. Linalool with the distinctively floral, citrus, woody, and green notes was the only identified oxygenated monoterpene in *Ella* hop SFE-CO_2 extracts. Its content did not significantly change at P >12.5 MPa, ~2.0% of the total GC peak area equivalent to ~19×10^7 AU across different extracts tested. As reported by Brendel et al., both myrcene and linalool are aroma-active constituents with the highest flavour dilution factor values among the other hop volatiles [174].



β-humulene β-pinene β-myrcene

Figure 5. Examples of the constituents from *Ella* hop essential oil

Recently, Duarte et al. suggested that the ratio of α-humulene/β-myrcene could be used as one of the parameters to differentiate between the aroma, bittering, and dual-purpose hops [187]. For *Ella* hop SFE-CO_2 extracts this ratio gradually decreased from 3.3/1 at 10 MPa to 2.5/1 at 12.5 MPa, 1.9/1 at 15 MPa, and 1.3 at 37 MPa (Table 6; Appendix 9), suggesting that P increase can shift the aroma profile of extracts from the dual-purpose hop typical characteristics towards the bitter hop-related ones [187]. The tunability of SFE-CO_2 parameters to produce hop extracts with the desired organoleptic properties was previously demonstrated by Van Opstaele et al. as well [11, 188].

Esters accounted for 12.2-22.3% of the identified headspace volatiles, with higher peak areas (Appendix 9) and percentages (Table 6) thus more pronounced fruity, green, and floral notes at P >10 MPa. Recent aroma profile analysis of *Ella* hop essential oil also indicated the presence of various esters at the total ~10% amount [171], which is comparable to the 10 MPa derived SFE-CO_2 sample. In agreement to the latter research [171], methyl-4-decenoate (5.5-11.1%) was the major the identified esters, followed by the pentyl 2-methylpropanoate (1.4-2.8%), methyl nonanoate (0.9-2.8%) and methyl octanoate (1.2-1.3%). Relatively high amounts (3-7%) of methyl-4-decenoate and pentyl 2-methylpropanoate were also characteristic of the essences obtained using hydrodistillation or SFE-CO_2 from *Galaxy*, *Topaz*, *Vic Secret*, *Super Pride*, *Hallertau Tradition*, *Saphir*, *Spalter Select*, and *Tettnanger* hops [171, 189]. The content of other volatiles in the headspace was rather low (Table 6; Appendix 9): alcohols (up to 2.3%), ketones (up to 1.3%), fatty acids (up to 1.0%), and aldehydes (up to 0.3%). 2-Undecanol (1.5-1.9%) with fresh, waxy, and cloth notes was the most abundant compound within this group of volatiles, previously identified in Portuguese hops' essential oils [169]. Fruity and waxy ketones 2-undecanone and 2-tridecanone comprised ~0.6% across different samples tested and were the predominant ketone fraction compounds for other hop varieties as well [171, 189].

Several identified major volatiles, particularly mono- and sesquiterpenes, are also known for their specific medicinal properties [4]. For example, direct radical scavenger myrcene shows antinociceptive and antimutagenic properties, acts protectively towards the inflammation and oxidation-induced brain, heart, and skin tissue damages. β-Pinene exhibits antidepressant, sedative, supraspinal antinociceptive, and antiproliferative activities, exert antiviral properties against the herpes simplex virus. Anticancer, anti-allergic, and anti-inflammatory activities have been reported for humulene and its derivatives [4].

Table 6. Volatile compound composition (% of the total GC peak area) of *Ella* hop SFE-CO_2 extracts obtained under different experimental conditions.

Compound	Exact Mass	RI_{exp}	RI_{lit}[A]	Odour type: description[B,C]	SFE-CO_2 conditions			
					SFE-CO_2 I 10 MPa, 40 °C, 300 min	**SFE-CO_2 II 12.5 MPa, 40 °C, 300 min**	**SFE-CO_2 III 15 MPa, 40 °C, 300 min**	**SFE-CO_2 IV 37 MPa, 43 °C, 80 min**
Monoterpenes, % of the total GC peak area								
α-Pinene	136.1252	950	946 [169]	Herbal: herbal, fresh, terpenic, fruity, sweet, green, pine, earthy, woody	0.09 ± 0.00^{a}	0.05 ± 0.00^{a}	0.08 ± 0.02^{a}	0.39 ± 0.03^{b}
Camphene	136.1252	971	972 [170]	Woody: camphoreous, cooling minty, citrus, green, spicy	0.02 ± 0.01^{a}	0.04 ± 0.00^{a}	0.06 ± 0.03^{a}	0.13 ± 0.05^{a}
β-Pinene	136.1252	1000	989 [190]	Herbal: cooling, dry, woody, piney, spicy, eucalyptus	3.21 ± 0.07^{a}	4.21 ± 0.05^{b}	3.74 ± 0.21^{b}	7.02 ± 0.08^{c}
β-Myrcene	136.1252	1000	995 [190]	Spicy: peppery, terpenic, balsamic, metallic, musty, fruity, ethereal, herbaceous, woody	3.02 ± 0.01^{a}	3.82 ± 0.04^{b}	3.81 ± 0.04^{b}	6.23 ± 0.17^{c}
p-Cymene	136.1252	1015	1015[172]	Terpenic: woody, fresh, terpenic, citrus, lemon, spicy	0.41 ± 0.01^{c}	0.20 ± 0.00^{b}	0.14 ± 0.00^{a}	0.39 ± 0.00^{c}
(*E*)-β-Ocimene	136.1252	1059	1052 [190]	Floral: herbal, mild, citrus, sweet, orange, lemon, tropical, green, woody	1.53 ± 0.02^{d}	1.34 ± 0.01^{c}	0.61 ± 0.00^{a}	1.18 ± 0.00^{b}
γ-Terpinene	136.1252	1074	1068 [170]	Terpenic: citrus, terpenic, herbal, oily, tropical, fruity, sweet	0.36 ± 0.04^{a}	0.36 ± 0.00^{a}	0.32 ± 0.00^{a}	-[ND]
Terpinolene	136.1252	1104	1105 [170]	Herbal: fresh, woody, sweet, piney, citrus, anise	0.08 ± 0.01^{a}	0.09 ± 0.00^{a}	0.11 ± 0.02^{a}	0.11 ± 0.00^{a}
β-Linalool	154.1358	1119	1109 [190]	Floral: citrus, orange, floral, sweet, rose, woody, green	1.78 ± 0.08^{a}	1.97 ± 0.04^{ab}	1.97 ± 0.02^{ab}	2.15 ± 0.14^{b}
Total monoterpenes					**10.50**	**12.08**	**10.84**	**17.60**
Sesquiterpenes, % of the total GC peak area								
α-Copaene	204.1878	1375	1374 [173]	Woody: woody, spicy, earthy	0.18 ± 0.01^{a}	0.24 ± 0.00^{c}	0.21 ± 0.00^{b}	-[ND]
α-Ylangene	204.1878	1401	1390[1169]	Fruity	3.14 ± 0.01^{a}	3.53 ± 0.04^{b}	3.47 ± 0.01^{b}	4.01 ± 0.00^{c}
β-Caryophyllene	204.1878	1438	1428 [169]	Spicy: musty, green, woody, clove, dry	0.64 ± 0.00^{b}	0.67 ± 0.00^{c}	0.61 ± 0.00^{a}	0.76 ± 0.00^{d}
Aromadendrene	204.1878	1439	1439 [173]	Sweet, dry	1.09 ± 0.01^{a}	1.00 ± 0.07^{a}	1.00 ± 0.00^{a}	1.08 ± 0.00^{a}
β-Humulene	204.1878	1457	1457[172]	-[NR]	6.30 ± 0.48^{a}	6.67 ± 0.30^{a}	6.98 ± 0.02^{b}	6.31 ± 0.22^{a}
α-Humulene	204.1878	1504	1505 [170]	Woody: woody, spicy, clove	9.88 ± 0.02^{b}	9.71 ± 0.08^{b}	7.23 ± 0.00^{a}	7.96 ± 0.78^{a}
β-Selinene	204.1878	1514	1524 [169]	Herbal	-[ND]	4.30 ± 0.00^{b}	4.61 ± 0.00^{c}	3.88 ± 0.00^{a}

α-Selinene	204.1878	1534	1533 [169]	Pepper, orange	14.86 ± 0.00^{c}	10.55 ± 0.00^{b}	5.90 ± 0.00^{a}	5.49 ± 0.01^{a}
δ-Cadinene	204.1878	1554	1556 [169]	Herbal: thyme, herbal, woody, dry	1.51 ± 0.00^{a}	1.58 ± 0.03^{a}	2.41 ± 0.01^{a}	4.65 ± 0.08^{b}
Calamenene	202.1722	1564	1562 [169]	Herbal, spicy	0.27 ± 0.00^{a}	0.31 ± 0.00^{b}	0.77 ± 0.00^{c}	0.84 ± 0.00^{d}
α-Calacorene	200.1565	1583	1590 [169]	Woody: dry, woody	0.21 ± 0.01^{c}	0.17 ± 0.00^{b}	0.20 ± 0.00^{c}	0.13 ± 0.00^{a}
Caryophyllene oxide	220.1827	1635	1617 [190]	Woody: sweet, fresh, dry, woody, spicy, fruity, sawdust, herbal	0.21 ± 0.00	-ND	0.41 ± 0.00	-ND
Total sesquiterpenes					**38.29**	**38.73**	**33.80**	**35.11**
Alcohols, % of the total GC peak area								
3-Methyl-2-buten-1-ol	86.0732	799	785[169]	Fruity: sweet, fruity, alcoholic, green	0.20 ± 0.00^{a}	0.44± 0.00^{b}	0.50 ± 0.00^{c}	-ND
2-Undecanol	170.1671	1314	1302 [169]	Waxy: fresh, waxy, cloth, sarsaparilla	1.74 ± 0.08 ^{b}	1.89 ± 0.13^{b}	1.80 ± 0.07^{b}	1.48 ± 0.13^{a}
Total alcohols					**1.94**	**2.33**	**2.3**	**1.48**
Aldehydes, % of the total GC peak area								
3-Methyl-2-butenal	84.0575	814	794 [169]	Fruity: sweet, fruity, pungent, nutty, almond, cherry	0.24 ± 0.01^{b}	0.24 ± 0.05^{b}	0.27 ± 0.06^{b}	0.04 ± 0.01^{a}
Total aldehydes					**0.24**	**0.24**	**0.27**	**0.04**
Ketones, % of the total GC peak area								
2-Undecanone	170.1671	1271	1294 [173]	Fruity: waxy, fruity, creamy, fatty, pineapple, orris, floral	0.63 ± 0.00^{a}	0.60 ± 0.02^{a}	0.64 ± 0.00^{a}	0.58 ± 0.00^{a}
2-Tridecanone	198.1984	1514	1504 [190]	Waxy: fatty, waxy, dairy, milky, coconut, nutty, herbal, earthy	0.67±0.00^{a}	0.64±0.00 ^{a}	-ND	-ND
Total ketones					**1.30**	**1.24**	**0.64**	**0.58**
Esters, % of the total GC peak area								
2-methylpropyl 2-methylpropanoate	144.1150	921	918 [190]	Fruity: ethereal, fruity, tropical, fruity, pineapple	0.08 ± 0.03^{a}	0.09 ± 0.04^{a}	0.16 ± 0.00^{ab}	0.27 ± 0.01^{b}
3-methylbutyl propanoate	144.1150	979	977 [190]	Fruity: sweet, fruity, apple, apple, raspberry, banana	0.56 ± 0.06 ^{a}	0.69 ± 0.07^{a}	0.59 ± 0.00^{a}	1.05 ± 0.10^{b}
Methyl hexanoate	130.0994	936	927 [169]	Fruity: fruity, pineapple, thinner, acetone	0.09 ± 0.00	0.10 ± 0.01	0.16 ± 0.00	0.11 ± 0.02
Pentyl 2-methylpropanoate	158.1307	1022	1020 [190]	Fruity: fruity, apple, banana, apricot, buttery	1.37 ± 0.02^{a}	1.52 ± 0.18^{a}	1.68 ± 0.09^{a}	2.76 ± 0.05^{b}
Methyl heptanoate	144.1150	1037	1030 [190]	Fruity: sweet, fruity, waxy, floral, berry, apple	0.56 ± 0.00^{a}	0.65 ± 0.05^{ab}	0.60 ± 0.04^{a}	0.77 ± 0.06^{b}
Methyl 6-methylheptanoate	158.1307	1096	1092 [169]	-^{NR}	0.72 ± 0.02^{a}	1.02 ± 0.00 ^{b}	1.14 ± 0.00^{c}	0.96 ± 0.05^{b}
2-Methylbutyl 3-methylbutanoate	172.1463	1111	1113 [169]	Fruity: herbal, earthy, apple, green	-ND	-ND	0.52 ± 0.06	0.63 ± 0.09

Methyl octanoate	158.1307	1135	1130 [190]	Waxy: waxy, green, sweet, orange, aldehydic, vegetable, herbal	1.16 ± 0.01^{a}	1.33 ± 0.08^{a}	1.28 ± 0.06^{a}	1.20 ± 0.05^{a}
Hexyl 2-methylpropanoate	172.1463	1158	1151 [190]	Green: sweet, green, fruity, apple, pear, grape, ripe, berry	0.15 ± 0.06^{a}	0.28 ± 0.00^{abc}	0.61 ± 0.04^{c}	0.36 ± 0.01^{b}
Heptyl propanoate	172.1463	1206	1207 [169]	Floral: rose, apricot	0.61 ± 0.03^{ab}	0.62 ± 0.01^{ab}	0.67 ± 0.00^{b}	0.56 ± 0.00^{a}
Methyl 8-nonenoate	170.1307	1222	1218 [190]	-^{NR}	-ND	0.55 ± 0.03^{a}	0.50 ± 0.00^{a}	0.49 ± 0.00^{a}
Methyl nonanoate	172.1463	1238	1229 [190]	Fruity: sweet, fruity, pear, waxy, tropical, winey	0.91 ± 0.01	2.18 ± 0.12^{bc}	2.83 ± 0.04^{c}	2.13 ± 0.00^{b}
Heptyl 2-methylpropanoate	186.1620	1255	1249 [190]	Fruity: fruity, sweet, green, warm, floral, tropical, chamomile, tea, green	0.39 ± 0.00	0.49 ± 0.00	0.53 ± 0.07^{a}	0.42 ± 0.02^{a}
2-Methylbutyl hexanoate	186.1620	1263	1246 [169]	Fruity: fruity, ethereal	0.06 ± 0.00	0.12 ± 0.00	-ND	-ND
Methyl 4-decenoate	184.1463	1322	1316 [190]	Fruity: fruity, pear, mango, fishy, peach, green	5.51 ± 0.04^{a}	10.89 ± 0.22^{c}	11.08 ± 0.27^{c}	8.38 ± 0.06^{b}
Total esters					**12.17**	**20.53**	**22.35**	**20.09**
Fatty acids, % of the total GC peak area								
2-Methylpropanoic acid	88.05240	778	762 [174]	Acidic: sour, cheesy, dairy, buttery, rancid, phenolic, fatty, sweaty	0.17 ± 0.00^{a}	0.17 ± 0.02^{a}	0.20 ± 0.02^{a}	0.26 ± 0.05^{a}
3-Methylbutanoic acid	102.0681	850	865 [174]	Cheesy: dairy, acidic, sour, pungent, fruity, fatty, sweaty, rancid	0.11 ± 0.01^{a}	0.13 ± 0.00^{a}	0.14 ± 0.00^{a}	0.11 ± 0.01^{a}
Heptanoic acid	130.0994	1089	1072 [169]	Cheesy: rancid, sour, cheesy, waxy, sweaty, fermented, pineapple, fruity	0.24 ± 0.00^{a}	0.41 ± 0.01^{c}	0.31 ± 0.02^{b}	0.28 ± 0.02^{ab}
Octanoic acid	144.1150	1189	1191 [175]	Fatty: fatty, waxy, rancid, oily, vegetable, cheesy	0.20 ± 0.00^{a}	0.25 ± 0.00^{c}	0.22 ± 0.00^{b}	0.28 ± 0.00^{d}
Total fatty acids					**0.72**	**0.96**	**0.87**	**0.93**

[A]: retention indexes (RI) reported for RTX-5 or equivalent column (± 20 units compared to the calculated RI_{exp}); [169] Martins et.al. J. Chemom., 2020, 34, e3285; [170] Rali et al. Molecules, 2007, 12, 3, 389-394; [190]Yan et al. Food Chem., 2019, 25, 15-23; [172]Frizzo et al. Flavour Fragr. J, 2001, 16, 286-288; [173] Adams, R.P. Identification of essential oil components by gas chromatography/mass spectrometry, ed. 4.1. 2017; [174]Brendel et.al. J.Agric.Food.Chem., 2019, 67, 12044-12053; [175]Alissandrakis et al. J.Agric.Food Chem., 2007, 55, 8152-8157.

[B]: Odour descriptions obtained from Pherobase database (https://www.pherobase.com/);[C]: Odour descriptions obtained from The Goodscent Company database (http://www.thegoodscentscompany.com/); -ND: not detected; -^{NR}: not reported. Significant differences can be seen in the same row indicated by different superscript letters (one-way ANOVA and Tukey`s test $p < 0.05$).

3.2. Optimization of hop SFE-CO_2 process for high bitter acid content

3.2.1. Central composite design and model analysis

Hop bitter acids have strong antioxidant, antimicrobial, anticarcinogenic, and other beneficial health effects [2]. As previously discussed in Chapter 1., the extraction of α and β-acids were affected differently by the increase of pressure from 10 to 37 MPa. Therefore the SFE-CO_2 optimization experiments were further continued to evaluate the impact of extraction parameters P, T and τ on the α-and β-bitter acid profile in order to maximize the recovery of these valuable constituents from hop pellets. Additionally, the α/β ratio can also be of use as it is a good indicator of how do the extraction parameters influence the extraction of the two main bitter acid groups. Since the variations in SFE-CO_2 conditions might alter the extraction of specific bitter acids and change overall α/β acid ratio, products with different functional properties and wide-range applications could be obtained. For example, bitter acids display selective inhibition of microorganisms [52, 58]. Generally, higher antimicrobial activity is reported for β-acids, attributed to the higher number and length of the acyl- or prenyl-side chains [2]. However, taking into consideration antioxidant potential and sedative effects, the α-acids exert higher activity [69, 109].

Based on the previous SFE-CO_2 optimization to maximize the extraction yield and recovery of antioxidants (Chapter 3.1), CCD-RSM with the same variable factors, namely P (25-45 MPa), T (40-60°C), and τ (30-90 min) were employed (Table 7). Within the region of operability, the content of total α acids (RFV) ranged from 79.7 to 176.6 mg/g HP, consisting of 27.9-63.7 mg/g HP cohumulone (RFI) and 51.8-112.9 mg/g HP humulone+adhumulone (RFII). Total β-acids ranged from 40.4-89.1 mg/g HP, from which colupulone (RFIII) and lupulone+adlupulone (RFIV) amounted to 24.3-57.8 and 16.1-31.3 mg/g HP, respectively. Consequently, total bitter acid content (RFVII) increased from 128.4 to 236.3 mg/g HP. Per gram of extract these values were equal to: 194.3 229.0 cohumulone 332.6 -411.8 humulone+adhumulone, 528.9 - 640.8 total α acids, 110.2-243.5 colupulone, 73.2 - 128.9 lupulone+adlupulone, 184.1 - 372.4 total beta acids and 714.4 -925.0 total bitter acids (Appendix 10). The data in Table 7 indicate that the α/β ratio (RFVIII) changed 2-fold from 1.5 to 3.1 under the different SFE-CO_2 conditions.

The DOE software was utilized to establish the models of the response factors from the provided data. However, based on the ANOVA analysis, the lack of fit for RFII, RFIII, RF IV and RF VI was significant, despite the significance of the models implied by their F-value (Appendix 12). Additionally, the predicted R^2 was not as close to the adjusted R^2 as one might normally expect. Nevertheless, the signal-to-noise ratio indicated by adequate precision was acceptable, therefore the models could be used to navigate the design space (Appendix 11).

Thus, the models (Appendix 12) were reduced to increase the precision [191] by eliminating some of the non-significant parameters (Appendix 14), as suggested by the DOE software. The experimental values fitted the predicted values of the reduced models (Appendix 15). Individual and total α-acid (RFI, RFII and RFV), also total bitter acid (RFVII) models were reproducible with low variation coefficients (< 5%); ~5-7% variation coefficients were obtained for β-acid models (RFIII, RFIV, and RFVI). All reduced models were statistically significant ($p<0.0001$), had high determination coefficients (>0.95), F values ranged from 22.8 to 33.7 and the difference between the adjusted and predicted R^2 values were < 0.29 (Appendix 13).

Table 7. Central composite design matrix (levels of independent variables and variation levels) for SFE-CO_2 optimization for α- and β-bitter acid extraction from *Ella* variety hop pellets

Levels and runs	**PLE-EtOH parameters**			**RFI Cohumulone**	**RFII Humulone +Adhumulone**	**RFIII: Colupulone**	**RFIV: Lupulone +Adlupulone**	**RFV: Total α-acids**	**RFVI: Total β-acids**	**RFVII: Total acids**	**RFVIII: α/β ratio**
	P, MPa	T, °C	τ, min	mg/g HP	mg/g HP	mg/g HP	mg/g HP	mg/g HP	mg/g HP	mg/g HP	
Center	35	50	60	46.73 ± 2.13	84.41 ± 1.58	50.10 ± 1.76	28.82 ± 0.55	131.14 ± 0.55	78.92 ± 1.21	210.06 ± 1.76	1.66 ± 0.02
Axial	25	50	60	39.14 ± 3.10	72.91 ± 7.21	40.16 ± 2.45	21.61 ± 1.27	112.05 ± 10.31	61.77 ± 3.72	173.82 ± 14.03	1.81 ± 0.06
Axial	35	40	60	51.63 ± 0.58	88.90 ± 3.20	51.88 ± 1.12	29.58 ± 1.19	140.53 ± 3.78	81.46 ± 2.32	221.99 ± 6.10	1.73 ± 0.00
Factorial	25	40	90	49.29 ± 3.13	88.85 ± 3.45	48.73 ± 6.17	27.07 ± 1.12	138.14 ± 6.58	75.80 ± 7.30	213.94 ± 13.88	1.83 ± 0.09
Factorial	25	60	30	27.95 ± 1.23	51.77 ± 1.51	30.86 ± 0.99	17.77 ± 0.32	79.72 ± 0.28	48.63 ± 1.31	128.35 ± 1.59	1.64 ± 0.04
Axial	35	50	90	51.87 ± 1.13	97.15 ± 6.95	57.80 ± 0.99	31.26 ± 1.01	149.02 ± 8.08	89.06 ± 2.00	238.08 ± 6.08	1.67 ± 0.13
Center	35	50	60	47.34 ± 2.16	85.49 ± 1.60	50.74 ± 1.78	29.07 ± 0.56	132.83 ± 0.56	79.81 ± 1.23	212.64 ± 1.78	1.66 ± 0.02
Center	35	50	60	48.71 ± 2.22	87.96 ± 1.65	52.21 ± 1.84	29.91 ± 0.57	136.67 ± 0.57	82.12 ± 1.26	218.79 ± 1.83	1.66 ± 0.02
Factorial	45	60	30	43.60 ± 0.64	78.40 ± 0.34	33.70 ± 0.29	18.58 ± 0.04	122.00 ± 0.98	52.28 ± 0.25	174.28 ± 1.23	2.33 ± 0.01
Factorial	45	40	90	63.69 ± 2.74	112.94 ± 0.00	36.61 ± 3.33	23.06 ± 1.29	176.63 ± 2.74	59.67 ± 4.62	236.30 ± 7.36I	2.97 ± 0.18
Factorial	45	40	30	44.68 ± 2.35	78.70 ± 8.34	24.32 ± 4.07	16.06 ± 2.12	123.38 ± 10.69	40.38 ± 6.19	163.76 ± 16.88	3.07 ± 0.21
Axial	35	60	60	49.56 ± 0.38	88.25 ± 1.39	47.44 ± 1.95	27.28 ± 0.05	137.81 ± 1.01	74.72 ± 2.00	212.53 ± 0.99	1.85 ± 0.06
Factorial	25	60	90	44.63 ± 0.37	80.18 ± 3.25	43.84 ± 3.07	24.95 ± 0.07	124.81 ± 3.62	68.79 ± 3.14	193.60 ± 6.76	1.81 ± 0.03
Factorial	45	60	90	51.48 ± 4.06	97.95 ± 1.19	35.90 ± 2.03	22.45 ± 0.76	149.43 ± 2.87	58.35 ± 2.79	207.78 ± 5.65	2.56 ± 0.07
Center	35	50	60	46.53 ± 2.12	84.04 ± 1.57	49.88 ± 1.75	28.57 ± 0.55	130.57 ± 0.55	78.45 ± 1.20	209.02 ± 1.75	1.66 ± 0.02
Center	35	50	60	48.26 ± 2.20	87.17 ± 1.63	51.73 ± 1.82	29.64 ± 0.57	135.43 ± 0.57	81.37 ± 1.25	216.80 ± 1.82	1.66 ± 0.02
Axial	45	50	60	48.09 ± 1.59	81.53 ± 3.58	27.01 ± 1.23	18.48 ± 0.83	129.62 ± 4.87	45.49 ± 2.06	175.11 ± 2.81	2.85 ± 0.24
Center	35	50	60	49.31 ± 2.25	89.05 ± 1.67	52.86 ± 1.86	30.28 ± 0.58	138.36 ± 0.58	83.14 ± 1.28	221.50 ± 1.86	1.66 ± 0.02
Factorial	25	40	30	30.37 ± 0.35	55.28 ± 1.27	37.74 ± 2.21	19.98 ± 0.54	85.65 ± 0.92	57.72 ± 2.74	143.37 ± 3.67	1.49 ± 0.05
Axial	35	50	30	41.15 ± 4.56	72.67 ± 4.25	41.04 ± 1.93	22.32 ± 0.51	113.82 ± 8.81	63.36 ± 2.45	177.18 ± 11.26	1.79 ±0.07

SFE-CO_2: supercritical carbon dioxide extraction; RFI: Cohumulone, mg/g HP; RFII: Humulone + adhumulone, mg/g HP; RFIII: Colupulone, mg/g HP; RFIV: Lupulone+adlupulone, mg/g HP; RFV: Total α-acids, mg/g HP; RFVI: Total β-acids, mg/g HP; RFVII: Total bitter acids, mg/g HP; RF VIII: α / β ratio; HP: hop pellets; P: extraction pressure; RF: response factor; τ: extraction time; T: extraction temperature.

The main parameters, P, T and τ were significant ($p<0.05$) in all RFs except T in β-acid models (RFIII, RFIV and RFVI) and τ for α/β ratio (RFVIII). Extraction time (τ) was the main parameter responsible for the observed changes in the α-acid models RFI, RFII and RFV, similarly affecting the total bitter acid content (RFVII). Other significant terms in these models were P and the quadratic effect of P, followed by T. Additional significant term for RFI and RFV is the linear Tτ interaction. Pareto charts (Figure 6, Figure 7) visualize the dominant effects of τ, P and P^2, contributing 33-, 31-, 7% and 35-, 31-, 14% and 36-, 32-, 11% of observed RFI, RFII and RFV responses, respectively, and to 40-, 24-, 15% of RFVII responses.

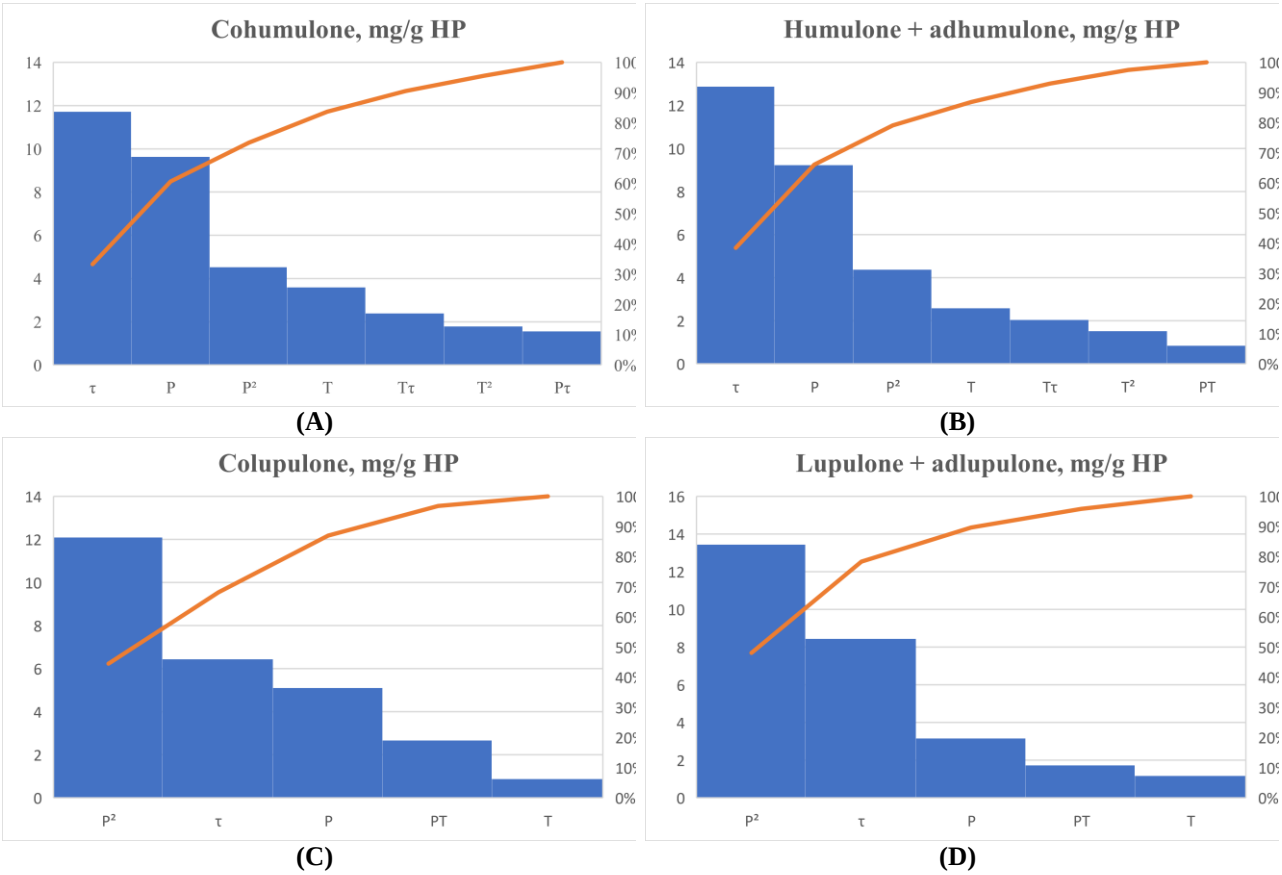


Figure 6. Pareto charts ($p = 0.05$) for the studied effects of SFE-CO_2 pressure (P), temperature (T), time (τ) and their interactions on the *Ella* hop: (A) RFI: cohumulone mg/g HP; (B) RFII: humulone+adhumulone mg/g HP; (C) RFIII: colupulone mg/g HP; (D) RFIV: lupulone+adlupulone mg/g HP; HP: hop pellets

In all three effects were responsible for 72%, 80% 79% and 79% of observed RFI, RFII, RFV and RFVII responses, respectively. In contrast, β-acid models were influenced primarily by the quadratic effect of P, and less so, but significantly by the linear PT interaction, similarly to the α/β-acid ratio.

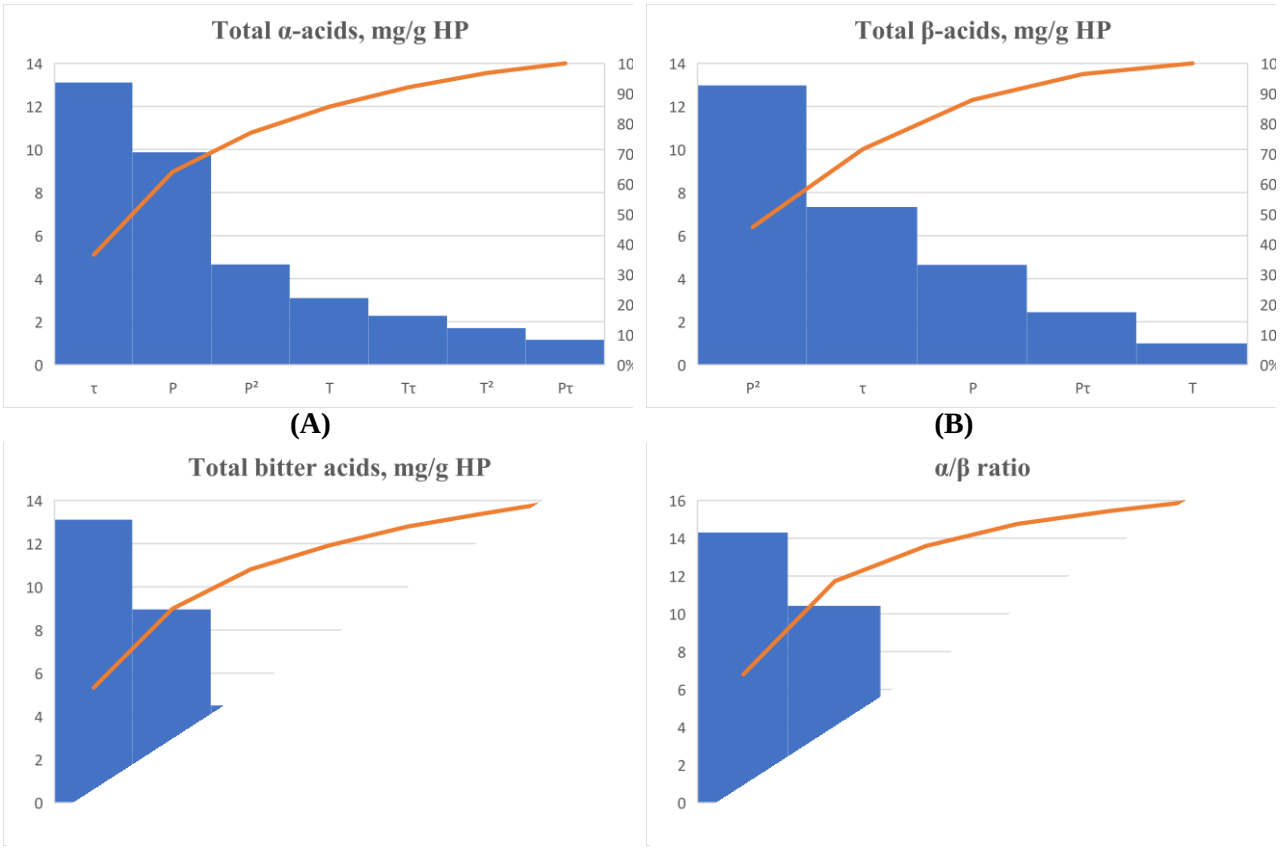


Figure 7. Pareto charts (p = 0.05) for the studied effects of SFE-CO_2 pressure (P), temperature (T), time (τ) and their interactions on the *Ella* hop: (A) RFV total α-acids mg/g HP; (B) RFVI: total β-acids mg/g HP; (C) RFVII: total bitter acids mg/g HP; (D) RFVIII: α/ β ratio; HP: hop pellets

The relationship between the independent model variables and selected-response factors is described by the second-order polynomial regression equations for the reduced models in coded factores:

$$Cohumulone = 47.71 + 6.02 \times P - 2.24 \times T + 7.32 \times r - 1.09 \times Pr - 1.67\,Tr - 5.00 \times (P^2) + 1.98\,(T^2) \quad (9)$$

$$Humulone + adhumulone = 85.92 + 10.05 \times P - 2.81 \times T + 14.03 \times r - 2.48 \times Tr - 8.42 \times (P^2) + 2.94 \times (T^2) \quad (10)$$

$$Colupulone = 50.57 - 4.38 \times P - 0.75 \times T + 5.52 \times r + 2.55 \times PT - 14.68 \times (P^2) \quad (11)$$

$$Lupulone + adlupulone = 28.67 - 1.28 \times P - 0.47 \times T + 3.41 \times r + 0.78 \times PT - -7.67 \times (P^2) \quad (12)$$

$$Total\ \alpha\ acids = 133.64 + 16.07 \times P - 5.06 \times T + 21.35 \times r - 2.11 \times Pr - 4.15 \times Tr - 13.42 \times (P^2) + 4.91 \times (T^2) \quad (13)$$

$$Total\ \beta\ acids = 79.24 - 5.65 \times P - 1.23 \times T + 8.93 \times r + 3.34 \times PT - 22.35 \times (P^2) \quad (14)$$

$$Total\ acids = 212.61 + 10.42 \times P - 6.28 \times T + 30.28 \times r - 3.72 \times Pr - 5.55 \times Tr - 36.57 \times (P^2) + 6.22 \times (T^2) \quad (15)$$

$$\alpha/\beta\ ratio = 1.7 + 0.52 \times P - 0.09 \times T + 0.052 \times r - 0.16 \times PT - 0.05 \times Pr + 0.54 \times (P^2) \quad (16)$$

3.2.2. Analysis of the response surface plots

Essentially, the response surface plots of α acid-related models RF I, RF II and RF V follow a similar pattern (Appendix 16). High-yield extraction of cohumulone (>60 mg/g HP), humulone+adhumulone (>100 mg/g HP), and total α-acids (>160 mg/g HP) can be observed after prolonged SFE-CO_2 (> 85 min) at the lowest experimental T (40-41°C). Pressure exerts great influence on the presence of α-acids in the extracts as well, as it can be seen in Appendix 16: the RFI, -II and V reach the beforementioned levels at >35 MPa and >85 minutes. Alternatively, levels of the colupulone (RFIII) peak between 28 to 37 MPa (>55 mg/g HP) while lupulone+adlupulone (RFIV) narrows this intervallum to 31-36 MPa (>32 mg/g HP) and total β-acids (RFVI) to 32-35 MPa (>90 mg/g HP). In

contrast to the plots of RFV, the parameter of extraction time only slightly altered the extracted amount of total β-acids (RFVI) compared to the change induced by pressure (Appendix 17).

The 2D and 3D surface plots of RFVII are suitable for the restriction of the SFE-CO_2 parameters (P, T and τ) to identify the ranges that extract α- and β-acids together at an optimum. Extraction temperature should not exceed 40-41 °C, as all RFs, except for RFVIII, tended to decrease at higher T. On account of the α-acids, more effective extraction was above 35 MPa (Appendix 16), while the α- acid to β-acids ratio increased above 37 MPa (Appendix 18B), therefore the optimal recovery of total bitter acid content could be identified at 35-37 MPa (Appendix 18A). Extraction of bitter acid is facilitated by increasing extraction time to >85 min.

3.2.3. Optimization of bitter acid content

Based on the above-discussed impact of various SFE-CO_2 parameters on the extraction of hop bitter acids seen in Figure 8, optimal SFE-CO_2 conditions previously determined for the highest SFE-CO_2 extract yield and recovery of antioxidant compounds from hop pellets (37 MPa, 43 °C, 80 min) could be further upgraded to fulfill the goal of bitter acid recovery as well. For this task, parameters P and τ were set as „minimize“, T as „in range”. RFI to RFVII were set as „maximize“ indicating the lower limit as follows: cohumulone >55 mg/g HP, humulone + adhumulone >100 mg/g HP; total α-acids >155 mg/g HP, colupulone >50 mg/g HP, lupulone+adlupulone > 25 mg/g HP, total β-bitter acids >75 mg/g HP, and total bitter acids > 230 mg/g HP. The α/β ratio was set as „in range“. The suggested conditions by the Design expert software were 36 MPa, 40 °C and 90 min, yielding 25.3 g/100 g HP of SFE-CO_2 extract. Under these optimal conditions, optimized SFE-CO_2 extract contained 247.0 mg/g E cohumulone (62.4 mg/g HP), 403.7 mg/g E of humulone and adhumulone (102.0 mg/g HP), 202.1 of colupulone (51.1 mg/h HP), 122.2 mg/g E lupulone and adlupulone (30.9 mg/g HP), shown in Table 8. Thus total α- and β-acids amounted to 650.7 and 324.4mg/g E, equivalent to 164.4 and 82.0 mg/g HP, respectively. As follows, the total bitter acids reached 975.1 mg/g E or 246.4 mg/g HP, with a 2.01 α/β ratio. These experimental values under the determined optimal conditions were in good agreement with the predicted ones, additionally pointing out the model validity to optimize bitter acid recovery from hops in the tested experimental P, T and τ range (Appendix 19).

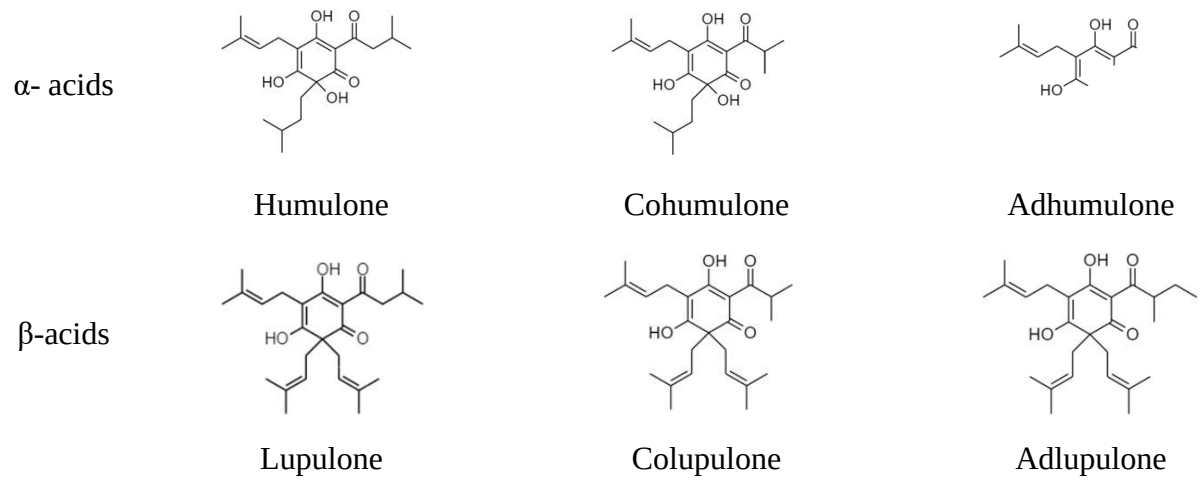


Figure 8. Hop bitter acids that were quantified through HPLC-DAD in this work

Comparing to the *Ella* hop SFE-CO_2 extract obtained at 37 MPa and 43 °C, the extraction of α-acids was more favored, increasing the recovery per gram HP by 19.5%. Efficient recovery is further proven

by exceeding the manufacturer-provided average minimum α-bitter acid content of *Ella* hop pellets (13.4 g/100 g HP) by 22%. In general, commercial *Ella* hops contain 13-19 % α-acids and 5-9 % β-acids [192], these ranges are given considering that reported values might differ slightly based on the homogeneity of the harvest and the extraction and analysis method applied [193]. In Europe, the Analytica EBC (European Brewery Convention), is the authority on bitter acid analysis of hops, the EBC - Method 7.7. describes the HPLC procedure. Important to note that the antioxidant capacity, 1472.3 mg TE/Gg E, or 372.0 mg TE/g HP was not significantly different from the SFE-CO_2 extract, obtained at 37 MPa and 43 °C. Furthermore, the yield of 25.3 g/100 g HP (Table 8) is comparable to the of 26.3 g/100 g HP achieved through SFE-CO_2 at 37 MPa and 43 °C (Table 3).

Enrichment of α-acids (mainly humulone) through the SFE-CO_2 process has been reported in earlier works. For example, Zekovic et al. [14] concentrated 41% of humulone in the extract from the bittering variety *Magnum* hop cones after 150 min of two-stage SFE-CO_2 at 30 MPa and 40 °C. Under the similar SFE-CO_2 conditions (30 MPa, 50 °C) extracts with 41% of α-acid and 19.5 % β-acid were produced from dual-purpose hops *Marynka* [151]. A longer extraction time of 240 min at 25 MPa and 48 °C yielded 53% of α-acid from *Citra* hop pellets [145]. The *Citra* hop extraction was also included in the US patent by John I. Haas, Inc., describing the manufacture of oil-rich hop extract with α-acid content from 15 to 70 % from various hops, however, requiring significantly longer extraction time (4.5 hours). Compared to the previously discussed works substantially shorter extraction time (90 min) led to 65.1 and 32,4 % of α- and β-acid content, respectively.

Table 8. Yield (g/100 g hop pellets), bitter acid composition and $TEAC_{ORAC}$ of *Ella* variety hop SFE-CO_2 extracts obtained at 36 MPa, 40 °C, 90 min

Sample		**SFE-CO_2V 36 MPa, 40 °C, 90 min**
Yield	g/100 g HP	25.27 ±0.28
Bitter acid content		
α-Bitter acids		
Cohumulone	mg/g E	246.97 ± 7.40
	mg/g HP	62.41 ± 1.87
Humolone + adhumulone	mg/g E	403.72 ± 14.43
	mg/g HP	102.02 ± 3.65
Total α-bitter acids	mg/g E	650.69 ± 21.83
	mg/g HP	164.43 ± 5.52
β-Bitter acids		
Colupulone	mg/g E	202.13 ± 19.98
	mg/g HP	51.08 ± 5.05
Lupulone+adlupulone	mg/g E	122.24 ± 4.32
	mg/g HP	30.89 ± 1.09
Total β-bitter acids	mg/g E	324.37 ± 24.29
	mg/g HP	81.97 ± 6.14
Total bitter acids	mg/g E	975.06 ± 2.46
	mg/g HP	246.40 ± 0.62
α/β-acid ratio		2.01 ± 0.22
In vitro oxygen radical absorbance capacity		
$TEAC_{ORAC}$	mg TE/g E	1472.27 ± 34.19
	mg TE/g HP	372.04 ± 8.64

Results are expressed as mean ± SD, n=. SFE-CO_2: supercritical carbon dioxide extraction; TEAC: Trolox equivalent antioxidant capacity; TE: Trolox equivalents; ORAC: oxygen radical absorbance capacity; E: hop extract; HP: hop pellets

3.2.4. Antimicrobial activity of hop extract obtained under optimized SFE-CO_2 conditions

The antimicrobial activity of bitter acid-rich SFE-CO_2 V (36 MPa, 40 °C, 90 min) extract was tested by agar well diffusion assay against three bacteria and one yeast strain that are human pathogens and cause problems in the food and medical fields. The gram-positive bacteria, *S. aureus* was chosen as a tested microorganism, for the many virulence factors it produces and the proposed danger of emerging and strengthening antibiotic resistance in some strains. The gram-negative bacteria, *E. coli* (ATTC 8739) and the toxin-producing *E. coli* (NTC 12900) were included for similar reasons. *C. albicans*, responsible for 75% of all candidal infections, were also tested. Extracts were applied up to 50 mg/mL, which is a practical limit for antimicrobial assays.

The gram-positive *S. aureus* **(**ATCC 25923) was strongly inhibited by the extract at 50 mg/mL concentration; the inhibition zone reached 36.9 mm in diameter (Table 9). Lower tested concentrations of 25 and 10 mg/mL produced significantly lower inhibition zones, 34.0-32.7 mm respectively, which were not significantly different from each other. The gram-negative bacteria, *E. coli* (ATTC 8739) was less affected by SFE-CO_2 V; inhibition zones were ranging from 15.1 to 17.1 mm, as the concentration of the extract was increased from 10 to 25 mg/mL. In both cases, the difference between the inhibition zones of 50- and 10 mg/mL concentrations is relatively small. The toxin-producing *E. coli* (NTC 129000) and the yeast *C. albicans* (ATCC 90028) were not inhibited by SFE-CO_2 V up to 50 mg/mL concentration in this experiment.

Table 9. Antibacterial activity of hop SFE-CO_2 extracts obtained at 36 MPa, 40 °C, 90 min using agar well diffusion (AWD) method

Antimicrobial activity	**SFE-CO_2 V (36 MPa, 40°C, 90 min)**	
	Inhibition zone (mm)	
Extract concentration	*S. aureus* (ATCC 25923)	*E.coli* (ATTC 8739)
10 mg/mL	32.67 ± 0.67^{a}	15.11 ± 0.51^{a}
25 mg/mL	34.00 ± 1.33^{a}	16.67 ± 0.67^{ab}
50 mg/mL	36.89 ± 0.19^{b}	17.11 ± 0.84^{b}

Results are expressed as mean ± SD, n=3; SFE-CO_2: supercritical carbon dioxide extraction.; Inhibition zone expressed as the diameter of inhibition zone + diameter of well (mm). Different letters indicate significantly different values in columns ($p < 0.05$) based on one-way ANOVA and Tukey's test.

Bocquet et al. [52] in a review on the antimicrobial effect of hop reported a generally stronger antibacterial effect of various hop extracts against gram-positive bacteria, and low or none against gram-negative bacteria. Strong inhibition of different and even methillin resistant *S. aureus* strains by either extract of hops or purified hop components was previously published by other researchers [194–196]. Practical applications of hop SFE-CO_2 extracts include antibacterial all-purpose cleaners formulated by Wasilewski et al. from *Marynka* variety hop pellets at 30 MPa and 50°C. The cleaner liquid at 5 mg/mL hop extract was effective against *S. aureus* (ATCC 259239) up to 29 mm IZ [197], which is only 11.2 % lower compared to bitter-acid enriched *Ella* hop SFE-CO_2 extract at 10 mg/mL (Table 9). In antimicrobial edible chitosan-gelatin films, incorporation of hop extract (concentration 1.5 mg/mL in the film), was more effective against *Bacillus subtilis* (19.0 mm) and *Listeria monocytogenes* (18.8 mm), than *ShigElla sonnei* (16.8mm) or *E.coli* (14.8 mm) [198]. In comparison bitter acid-enriched SFE-CO_2 extract from *Ella* hops, at 10 mg/mL inhibited *E.coli* similarly (15.1 mm, Table 9) to the chitosan-gelatin hop extract (0.15%) film, the differences in the applied matrixes presumably influence the results as chitosan also provides some antibacterial effect. The same

S.aureus strain was inhibited by purified lupulones 10-times more effectively (minimal inhibitory concentration MIC 0.1 μg/mL) than by purified humulones, other acne-causing bacteria *P. acnes*, *S.pyogenes* and *K. rhizophila* was also affected to different inhibitory levels [70]. Furthermore, lupulone extract (40% lupulone in gylcol) as an antibacterial ingredient in meat preparations was significantly more effective against gram-positive *S. aureus* and *L. monocytogenes* (MIC 1-6 ppm) than gram-negative *E. coli* or *SalmonElla enterica* (MIC 625-5000 ppm). Higher activities were observed when pH was decreased from 7.0 to 5.0, in agreement with the weak acid nature of β-acids, and compared to humulone extract (20% in glycol). In conclusion, optimization based on bitter acid content may be relevant in modulating the desired antimicrobial properties of hop extracts, and the properties of the applied matrix should be taken into consideration.

3.2.5. The effect of bitter acid optimized SFE-CO_2 extract on skin cell viability[2]

In line with in vitro antioxidant potential and antimicrobial properties, the effect of bitter acid-enriched *Ella* hop SFE-CO_2 (36 MPa, 40 °C, 90 min) extract on the viability of cancerous and healthy skin cells was evaluated. Keratinocytes, melanocytes, Langerhans cells, and Merkel cells are the four main cell types that make up the most outer layer of human skin, the epidermis. The middle layer - called dermis - is comprised mainly of fibroblasts that produce the components of intercellular material and ensure physiological functions of the skin [199]. Therefore, a stable transformed adherent human keratinocyte cell line (HaCaT) was chosen for the epidermis- and a fibroblast cell line (HDFa) for the dermis model. For the effect on skin cancer, the cell line (A375) was used, which originates from human malignant melanomas (which develop from melanocytes) [200]. The results were expressed by relative cell viability (%) in comparison with control cells not treated with the extract.

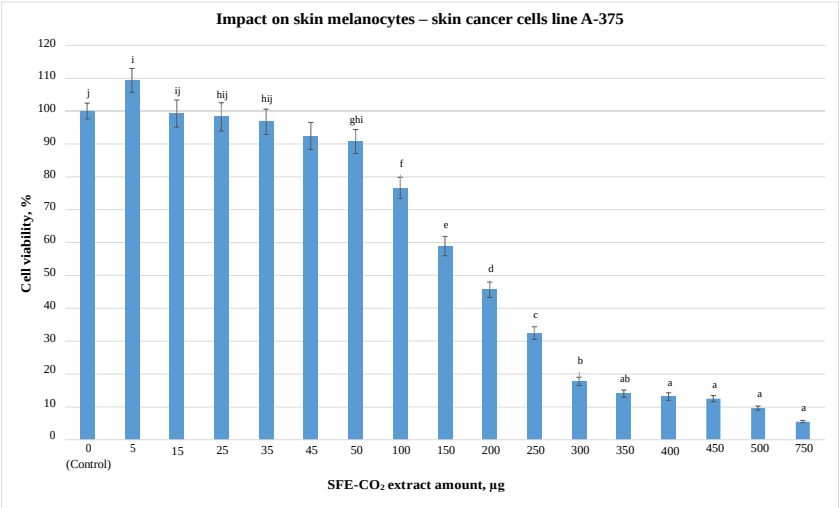
Firstly, the anticarcinogenic effect of bitter acid-enriched SFE-CO_2 V extract was tested on the skin cancer cell line (A375) (Figure 9.A). Up to 45 μg extract did not cause a significant reduction in cell viability as compared to the control. When the cells were affected by >100 μg amounts, the viability of cells decreased to 76.6%, which was statistically significantly different from the controls ($p<0,05$). From that point every 50 μg increase up to 250 μg of SFE-CO_2 V exerted significantly enhanced toxic effects on the cancer cells, viability decreased to 32.5 %. The final tested extract amount (750 μg reduced cell viability to 5.5 %.

Next, the healthy cell lines were tested; the impact of SFE-CO_2 V on the keratinocytes is shown in Figure 9 (B). The obtained results indicate that the cell viability was not affected significantly only with the lowest extract amount of 5 μg, while higher tested amounts caused significant reductions as compared to the control. A steady drop in cell viability from 88 to 24% was observed at a range of 15-100 μg, from 17 to 4% at 200-500 μg, finally reaching ~1% at the highest amount of 750 μg in these experiments. The decline in cell viability from 34.5 to 0.6 % was caused by the amounts > 50 μg.

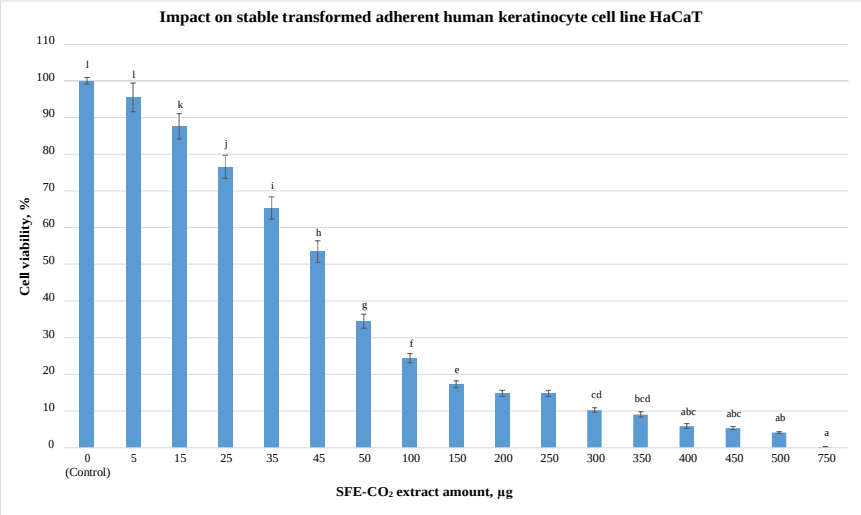
In contrast, with up to 45 μg of extract, the viability of healthy fibroblast cells compared to control did not change significantly after treatment (Figure 9.C). However, the effect of 50 μg caused a significant ($p<0.005$) decrease from 87.4 to 68.0 %.

[2] Reported results were provided by the research group of Prof. Dr. Kristina Ramanauskienė from the Lithuanian University of Health Sciences, Kaunas

(A)



(B)



(C)

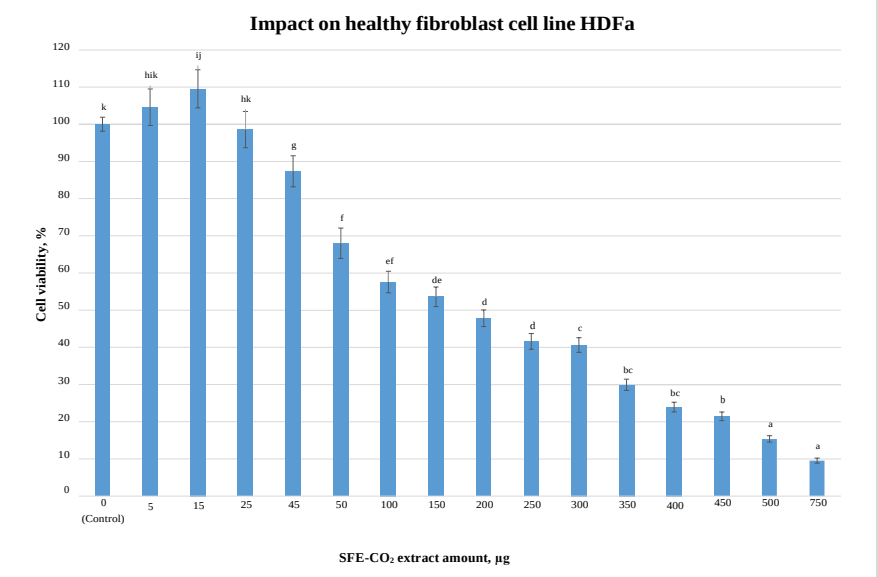


Figure 9. Relative cell viability (%) of stable transformed adherent human keratinocyte cell line HaCaT (A), fibroblast cell line HDFa (B), and skin cancer cell line A375 (C) after treatment of different SFE-CO_2 V amount (5 mg/mL concentration)

From 100 to 200 μg there was no significant difference between the effect of extracts on cell viability (from 57.6% to 47.8%). By 750 μg the viability decreased by an app. 90.5% compared to control.

The reduction of skin cancer cell viability by the bitter acid-enriched SFE-CO_2 V extract is promising. Nevertheless, the obtained results indicate that the extract can reduce the viability of healthy skin cells as well. Thus, *Ella* hop SFE-CO_2 extract could serve as a bioactive ingredient to develop dermatological phytopharmaceutical formulations, however, the effective concentrations and mode of application such as localized should be taken into consideration.

Carcinogenesis has been linked to increased cell proliferation and angiogenesis (the formation of new blood vessels), governed by vascular endothelial growth factor (VEGF) [200]. Philips et al. [201] studied the effect of a hop extract (containing bitter acids and phenolics) in vitro, and observed significantly reduced cell viability to 55-17% of melanomas (CRL-1619) at 0.01, 0.04, 0.2% concentrations and 71% of fibroblasts cells at 0.2% concentration, respectively. A stronger effect was reported for the purified constituents of the extract, namely humulone, lupulone, xantho-flavonoid, XN and IXN, the cell viability of fibroblast decreased to 41, 50, 49, 29 and 23%, respectively. The authors concluded that the melanoma cells were reduced without antioxidant cellular effect and no increase in VEGF was found. Although the cell viability of fibroblasts was reduced, the antioxidant activity within the cell and expression of VEGF was elevated, which means a possible angiogenic effect. The exact composition of the hop extract is not disclosed, therefore the direct comparison to the results of *Ella* hop SFE-CO_2 V research extract is difficult. However, *Ella* SFE-CO_2 and the hop extract of Philips et al. [201] indicated cell reduction of both melanoma and fibroblast type cells, governed by applied concentration and in the case of Philips et al., whether they are present as sole or in a mixture.

3.3. Optimization of PLE-EtOH of hop SFE-CO_2 residue

3.3.1. Central composite design and model analysis

Recovery of polar compounds with high antioxidant potential was achieved through PLE-EtOH from *Ella* hop residue remaining after bitter acid content optimized pilot-scale SFE-CO_2 (36 MPa, 40°C, 90 min). Supercritical CO_2 favors the extraction of non-polar constituents, the residue still contains more polar hop phytochemicals, such as polyphenols, which can be extracted and capitalized on further [151]. PLE-EtOH utilizes high-pressure conditions, which facilitates extraction, thus reduces extraction time and improves the yield of valuable bioactive compounds [158]. Selectivity for phenolics is possible through process optimization, an important aspect as extraction temperature and time can play part in the undesirable degradation or isomerization of target compounds [12]. The polarity of polyphenols influences their solubility, the use of ethanol, a green and GRAS solvent, less polar than water may aid their recovery [202]. PLE-EtOH extracts were expected to be rich in flavonoids, e.g. mainly xanthohumol and other known hop flavonoids in 10-100 less-fold amount [87]. Xanthohumol is unique to *Humulus lupulus* and provides anticarcinogenic, antimicrobial and antioxidant effects, furthermore, XN is the precursor of other bioactive prenylated chalcones such IXN and 8-PN [40].

The major PLE parameters, namely T (40-100°C) and τ (15-45 min) were optimized via CCD-RSM to maximize the recovery of polar antioxidants and the selected prenylflavonoids from *Ella* hop residue after SFE-CO_2 at high yields. The efficacy of PLE to extract antioxidant compounds was

assessed by TPC and $TEAC_{ORAC}$ of samples, which are common *in vitro* assays to measure the antioxidant potential of various plant extracts [74]. Total phenolic content (TPC) measured by Folin-Ciocalteu's assay is based on an oxidation/reduction reaction, therefore it has been utilized as an antioxidant capacity measurement method for plant materials [75]. TPC measured the general reducing power of samples, while ORAC allowed to evaluate more specific antioxidant properties such as measuring the radical chain-breaking of antioxidants, based on the hop polar antioxidant compound ability to scavenge biologically relevant oxygen radicals via hydrogen donation [166]. Biologically active major hop prenylflavonoids, namely XN, IXN and 8-PN [203], were selected as phytochemical composition markers for further PLE-EtOH optimization.

However, the HPLC-DAD analysis indicated that IXN and 8-PN were present only in trace amounts in PLE-EtOH extracts, thus these compounds were excluded from further optimization experiments. Data in Table 10 indicate that within the selected region of operability, PLE-EtOH hop extract yield (RFI) ranged from 11.1 to 21.8 g/100g HR, TPC (RFII and RFIII) from 174.9 to 217.55 mg GAE/g E and from 19.7 to 39.7 mg GAE/g HR, $TEAC_{ORAC}$ (RFIV and RFV) increased from 1824.8 to 2588.2 mg TE/g E and 273.50 to 417.79 mg TE/g HR, XN content (RFVI and RFVII) rose from 50.6 to 83.3 mg/g E and 9.14 to 10.3 mg/g HR. It could be observed that The PLE conditions had a negligible effect on the recovery of XN from hop residue after SFE-CO_2 (RFVII) with only ~12% variation between the highest and lowest value and > 90% of the XN obtained already at the lowest T (40°C) and τ (15 min).

Low variation coefficients (<3.78%), high determination coefficients ($R^2 > 0.97$), similar adjusted and predicted R^2 values (difference < 0.089), and good fit of the experimental data to the predicted values were obtained for RFI (Yield, g/100 g HR), RFIII (TPC, Mg GAE/g HR), RFV ($TEAC_{ORAC}$, mg TE/g HR) and RFVI (XN content, mg/g E) models (Appendix 20. Fit statistics parameters for the selected models of *Ella* hop PLE-EtOH extraction optimization. In the case of RFII (TPC, mg GAE/g E) and RFVII (XN content, mg/g HR) negative predicted R^2 values implied that the overall mean may be a better predictor of response than the current models, while RFIV ($TEAC_{ORAC}$, mg TE/g E) had lower determination coefficient and substantial difference between the predicted and adjusted coefficients (Appendix 20). The four well-fitting models, namely RFI, -RFIII, RFV and RFVI, were statistically significant, with the F-values of 57.85, 79.39, 83.25 and 137.42, respectively (Appendix 22). Temperature (T) was the primary extraction parameter responsible for the observed changes in RFI (*F*=267.4, RFIII (*F*=286.7), RFV (*F*=346.5) and RFVI (*F*=541.7) values under the different experimental conditions. For RF I model only the second-order term of temperature (T^2) was significant, additionally to that of temperature (T), while other terms had no significant input. This was not the case in the rest of the working models, where other linear interactions and quadratic effects of terms were also significant in the following order: $T > T^2 > \tau > \tau^2$for RFIII; $T > T^2 > T\tau > \tau^2$ for RFV; $T > T^2 > \tau$ for RFVI. Pareto charts visually depict the massive effect of T and T^2, which contributed to 75-85% of observed RFI, RFIII RFV and RFVI responses (Figure 10). The below reported the second-order polynomial regression equations in coded factors to describe the empirical relationship between the independent model variables (T and τ) and selected-response factors (RFI, RFII, RFV and RFVI) within the selected region of PLE operability:

$$Yield_{PLE-EtOH} = 18.07 + 4.38 \times T + 0.60 \times r - 0.42 \times Tr - 1.34 \times (T^2) - 0.18 \times (r^2) \quad (17)$$

$$TPC = 37.62 + 8.14 \times T + 1.49 \times r - 1.18 \times Tr - 5.61 \times (T^2) - 1.68 \times (r^2) \quad (18)$$

$$TEAC_{ORAC} = 369.10 + 55.99 \times T - 6.54 \times r - 12.24 \times Tr - 32.57 \times (T^2) - 12.10 \times (r^2) \quad (19)$$

$$Xanthohumol\ content = 52.48 + 15.20 \times T - 1.74 \times r + 1.66 \times Tr + 9.85 \times (T^2) + 1.08 \times (r^2) \quad (20)$$

Table 10. Central composite design matrix (levels of independent variables and variation levels) for PLE-EtOH optimization for extraction of polar constituents from *Ella* hop residue after optimized SFE-CO_2 (36 MPa, 40°C) and values of observed responses

Levels and runs	**PLE-EtOH parameters**		**RFI: PLE-EtOH yield**	**RFII: TPC**	**RFIII: TPC**	**RFIV: $TEAC_{ORAC}$**	**RFV: $TEAC_{ORAC}$**	**RF VI: XN content**	**RF VII: XN content**
	T, °C	τ, min	g/100 g HR	mg GAE/g E	mg GAE/g HR	mg TE/g E	mg TE/g of HR	mg/g E	mg/g HR
Factorial	100	15	20.44 ± 0.81	188.30 ± 2.87	38.77 ± 0.23	2043.98 ± 46.45	417.79 ± 9.49	48.17 ± 1.34	9.85 ± 0.27
Axial	100	30	21.75 ± 0.99	174.92 ± 3.40	38.64 ± 0.35	1824.81 ± 65.88	396.90 ± 14.33	47.34 ± 1.39	10.30 ± 0.30
Factorial	40	15	11.09 ± 0.25	177.88 ± 3.64	19.73 ± 0.40	2588.28 ± 149.20	287.04 ± 16.55	83.30 ± 3.01	9.29 ± 0.33
Center	70	30	18.85 ± 0.81	206.73 ± 3.57	38.97 ± 0.67	1991.54 ± 27.74	375.41 ± 5.23	52.05 ± 1.00	9.94 ± 0.19
Axial	70	45	18.90 ± 1.43	190.79 ± 1.86	36.06 ± 0.35	1939.11 ± 289.39	366.49 ± 54.79	50.59 ± 0.95	9.56 ± 0.18
Factorial	40	45	13.11 ± 0.74	193.19 ± 3.58	25.33 ± 0.47	2328.65 ± 118.42	305.27 ± 15.52	76.52 ± 1.87	10.03 ± 0.24
Axial	70	15	17.65 ± 1.41	191.51 ± 2.80	33.63 ± 0.49	2227.97 ± 142.69	393.24 ± 25.19	54.11 ± 1.69	9.55 ± 0.30
Factorial	100	45	20.77 ± 0.10	190.91 ± 0.26	39.66 ± 0.05	1865.35 ± 21.45	387.06 ± 4.45	48.01 ± 0.87	9.97 ± 0.18
Center	70	30	17.05 ± 0.81	217.55 ± 1.58	37.09 ± 0.27	2199.26 ± 33.28	374.96 ± 5.82	53.71 ± 1.35	9.14 ± 0.23
Center	70	30	17.48 ± 0.81	216.16 ± 1.21	37.78 ± 0.21	2113.52 ± 121.26	369.44 ± 21.20	53.70 ± 1.32	9.37 ± 0.23
Center	70	30	17.92 ± 0.81	214.88 ± 1.03	38.51 ± 0.18	2019.46 ± 11.75	361.89 ± 2.11	51.84 ± 1.95	9.29 ± 0.35
Center	70	30	18.29 ± 0.81	207.29 ± 0.00	37.91 ± 0.00	2003.62 ± 28.68	366.46 ± 5.25	53.49 ± 0,82	9.74 ± 0.15
Axial	40	30	12.48 ± 0.70	185.90 ± 4.20	23.20 ± 0.52	2191.51 ± 130.55	273.50 ± 16.29	74.90 ± 0.77	9.35 ± 0.10

PLE-EtOH: pressurized liquid extraction with ethanol; SFE-CO_2: supercritical carbon dioxide extraction; TPC: total phenolic content; GAE: gallic acid equivalents; TEAC: Trolox equivalent antioxidant capacity; ORAC: oxygen radical absorance capacity; TE: Trolox equivalents; RF: response factor E: extract; HR: hop residue after SFE-CO_2 (36 MPa, 40°C, 90 min) ; XN: xanthohumol

Pearson correlation coefficients > 0.90 (Appendix 26) indicate a strong positive correlation between PLE-EtOH yield (RFI) and recovery of antioxidants, measured as TPC (RFIII) and $TEAC_{ORAC}$ (RFV) per gram of HR. A positive correlation (0.85, $p<0.05$) also existed between ORAC values and XN content (RFIV and RFVI, respectively), expressed per gram of E (Appendix 27).

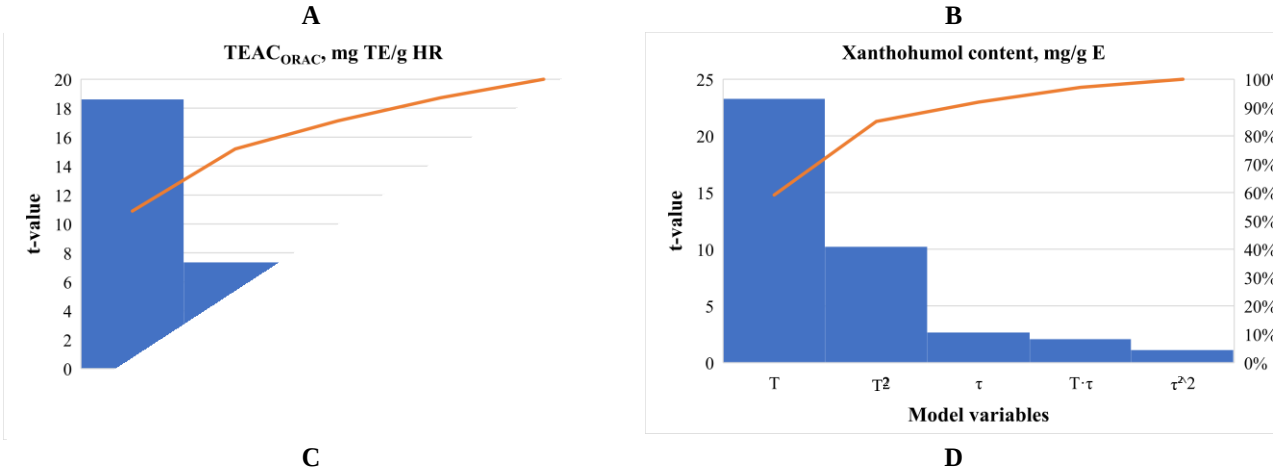


Figure 10. Pareto charts ($p = 0.05$) for the main effects of PLE-EtOH temperature (T), time (τ) and their interactions on the *Ella* hop: (A) RFI PLE-EtOH yield (g/100 g HR); (B) RFIII TPC (mg GAE/g HR), (C) RFV $TEAC_{ORAC}$ (mg TE/ g HR); (D) RFVI, xanthohumol content (mg/g E)

3.3.2. Analysis of response surface plots

2D and 3D response surface plots (Appendix 23) were created to visualize the effects of the T and τ on the RFs in the significant models (RFI, RFIII, RFV and RFVI). Yield and the antioxidant capacity indices followed an increasing trend by the elevation of T towards their maximum, exceeding 20 g/100 g HR (Appendix 23A), 40 mg GAE/g HR (Appendix 23B) and 400 mg TE/g HR (Appendix 23C), respectively. The response surface for RFI was close to linear, the increase of T had a positive effect, while time as a function of T had little influence on the extraction yield.

For RFIII, the response surface plots were more complex, maximum values (>40 mg GAE/g HR) were reached at T> 81 °C and τ of 20-45 min, however, τ had less of a significant effect (Appendix 23B). Based on the second-order polynomial regression equation and the significance of the linear Tτ interaction for RF V, the 3D response surface shows that $TEAC_{ORAC}$ reached 400 mg TE/g HR at less than 25 min and T > 75 °C (Appendix 23C). This reduction of the possible T and τ combinations to recover maximum amounts of antioxidants when RFV is involved may be attributed to the difference between the antioxidant effects of extracted components, as proposed from the specificity of the

$TEAC_{ORAC}$ assay, which is based on the hydrogen atom-, whereas TPC on electron-transfer reactions (Prior, 2015). The concave-shaped 3D plot of RFVI (Appendix 23D) showed that XN content per gram of E significantly reduced due to the higher T: from >80 mg/g E at 40-42 °C to 50-70 mg/g E at 51-80 °C and < 50 mg/g E at >80 °C. These findings may be explained by the so-called dilution of XN by other polar components, extracted at the higher T from hop residue after SFE-CO_2. Generally, the increase of τ from 15 to 45 min had a negligible effect on XN content in PLE extracts (Appendix 23D): a small 7-8% reduction was obtained only at 40-70 °C range with no differences at the high-end experimental T (100 °C).

3.3.3. PLE-EtOH optimization by the desirability function

Xanthohumol is one of the most researched compounds of *Humulus lupulus* plants for the reason of its varied beneficial bioactivities. Therefore, standardized extracts rich in xanthohumol produced under economically optimized conditions with safe and non-toxic solvent can be the effective ingredients of functional food and pharmaceutical including cosmetical products. The predicted equations and the response surface plots of the significant models were utilized to optimize PLE-EtOH and in under the lowest possible T and shortest τ: (1) to obtain xanthohumol-enriched (>80 mg/g E) PLE-EtOH extract (2) to extract polar hop antioxidant constituents (>35 mg GAE/g HR and >350 mg TE/g HR) at high yields (>20 g/100 g HR). As suggested by the Design expert software, the first task can be implemented extracting hop residue after SFE-CO_2 at the economically desirable low-end T (40 °C) and τ (15 min), while T >85°C and τ >18 min favor high-yield extraction of polar antioxidants. Table 11 summarizes the experimental yields, the antioxidant potential and xanthohumol content under the following optimal PLE-EtOH conditions.

Table 11. Yields, antioxidant activity indices (TPC, $TEAC_{ORAC}$, $TEAC_{ABTS}$), xanthohumol content of *Ella* hop PLE-EtOH extracts obtained under the optimized experimental conditions

Samples		**PLE-EtOH parameters**	
		PLE-EtOH I 40°C, 15 min	PLE-EtOH II 85°C, 18 min
Yield	g/100 g HR	11.27 ± 0.68^{a}	20.52 ± 0.55^{b}
	g/100 g HP	8.42 ± 0.51^{a}	15.33 ± 0.41^{b}
***In vitro* antioxidant activity**			
TPC	mg GAE/g E	187.98 ± 1.76^{a}	191.47 ± 2.19^{b}
	mg GAE/g HR	21.19 ± 0.20^{a}	39.29 ± 0.45^{b}
	mg GAE/g HP	15.82 ± 0.15^{a}	29.35 ± 0.18^{b}
$TEAC_{ORAC}$	mg TE/g E	2623.65 ± 108.31^{a}	1936.49 ± 34.22^{b}
	mg TE/g HR	295.68 ± 12.21^{a}	397.37 ± 7.02^{b}
	mg TE/g HP	220.91 ± 9.12^{a}	296.86 ± 0.34^{b}
$TEAC_{ABTS}$	mg TE/g E	513.14 ± 27.66^{a}	550.45 ± 28.12^{b}
	mg TE/g HR	57.83 ± 3.12^{a}	112.95 ± 5.77^{b}
	mg TE/g HP	43.21 ± 0.77^{a}	84.38 ± 4.31^{b}
Prenylflavonoid content			
Xanthohumol	mg/g E	83.45 ± 7.16^{a}	45.74 ± 1.98^{b}
	mg/g HR	9.41 ± 0.81^{a}	9.39 ± 0.41^{a}
	mg/g HP	7.03 ± 0.60^{a}	7.01 ± 0.30^{a}

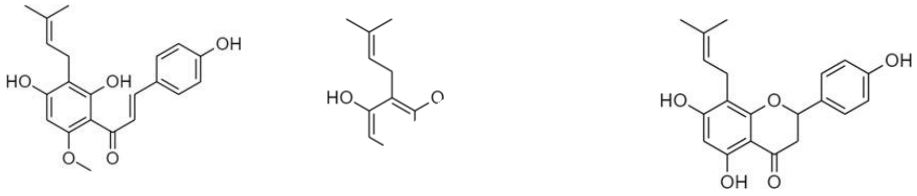
PLE-EtOH: pressurized liquid extraction with ethanol; TPC: total phenolic content, GAE: gallic acid equivalents; ORAC: oxygen radical absorbance capacity; TEAC: Trolox equivalent antioxidant capacity; E.: extract; HR. hop residue after SFE-CO_2 (36 MPa, 40°C, 90 min); HP: hop pellets. Results are expressed as mean ± SD. Different superscript letters in the same row indicate significantly different values ($p < 0.05$) based on an unpaired t-test.

As given in Table 11, 11.3 g/100 g HR deep green colored extract with 83.5 mg/g E of xanthohumol was obtained at 40 °C and 15 min (PLE-EtOH-I), while 20.5 g/100 g HR of a darker green-colored extract with 39.3 mg GAE/g HR and 397.4 mg TE/g HR were produced at 85 °C and 18 min (PLE-EtOH-II). Good agreement between the experimental and the predicted values under deduced optimal conditions additionally confirmed the validity of RFI, RFIII, RFV and RFVI models (Appendix 24, Appendix 25.). A PLE process with water, pressurized hot water extraction (PHWE) conditions were optimized for high isoxanthohumol content (150°C, 10.3 Mpa, 30 min) and found to be compared to be more selective for isoxanthohumol (ISX) than PLE with subcritical ethanol or a sequence of three solvents (hexane, ethanol and water). XN enrichment of PLE extract produced with ethanol as solvent from the residue obtained after SFE-CO_2 hasn't been reported in the literature available at the writing of this work. This residue is a substantial waste material left after SFE-CO_2 extraction takes place, based on our results it can be used to produce high-value-added hop products such as xanthohumol enriched PLE-EtOH extract that may be incorporated into food, nutraceuticals, pharmaceuticals and cosmetics as an active ingredient.

3.3.4. Xanthohumol content of hop extracts obtained under different PLE-EtOH conditions

The XN content of optimized PLE-EtOH extracts was analyzed. The results in Table 11 indicate that xanthohumol level was 82% higher in PLE-EtOH I extract (83.5 mg/g E) as compared to PLE-EtOH II sample (45.7 mg/g E). However, the values per gram of HR and HP, calculated taking into consideration extraction yields, did not significantly change, amounting to 9.4 mg/g HR equivalent to 7.0 mg/g HP. Thus, PLE-EtOH was efficient to recover this prenylflavonoid from *Ella* hop residue after SFE-CO_2 already at the lowest tested T and τ.

Rój et al. (2015) extracted spent *Marynka* hops SFE-CO_2 residue with supercritical CO_2 without ethanol at 100 MPa and 50°C and obtained extract containing 6.5% of xanthohumol among other constituents [151]. In MWE a higher, 19.1 mg/g dry hop cones, while in UAE 8 mg of XN similar to the levels in PLE-EtOHI and -II, was achieved from Italian wild hops [204]. Previously, Gil-Ramírez et al. (2012) performed pressurized hot water extraction (PHWE) at 50 - 200 °C and fixed 30 min to obtain isoxanthohumol-rich hop extracts from Spanish hops. The authors reported that IXN was only detected at higher temperatures (150-200°C), while XN was present throughout this temperature range decreasing as T was elevated. The highest IXN (see Figure 11) content (2.3 mg/g HP) was reached by PHWE at 150°C, 30 min (6 cycles), which produced low XN content (0.11 mg/g HP). For comparison, PLE-EtOH at the same conditions contained 17-fold more XN (1.9 mg/g HP) and app. third of IXN (0.7 mg/g HP), the IXN/XN ratio decreased substantially (from 21 to 0.35). XN content in the PLE-EtOH extracts of this research was more than three times greater in comparison (Table 11). The higher temperature and the presence of water are effective for IXN enrichment by PLE, this may be confirmed by the beer brewing example, where XN thermally isomerizes into its derivative IXN during wort boiling [205]. Consequently, the choice of solvent and extraction temperature greatly influences composition of the final extract. In PLE, lower temperatures favor extraction of XN and presence of water may be beneficial to increase their levels, but more research is needed to identify the ethanol:water ratio ideal for XN extraction.



Xanthohumol Isoxanthohumol 8-prenylnaringenin

Figure 11. Selected phenolic compounds of *Humulus lupulus*

An extract rich in XN, besides the much-studied properties of antioxidant, anticarcinogenic, antimicrobial and other, can be the precursor for further transformation products of XN e.g. isoxanthohumol, desmethylxanthohumol, 8-prenylnaringenin, etc. These derivatives in some cases have stronger or other new effects such as the estrogenic activity of 8-prenylnaringenin [206].

3.3.5. Pigment content of hop extracts obtained under different PLE-EtOH conditions

The qualitative and quantitative composition of pigments in hop PLE-EtOH extracts is reported in Table 12. Total chlorophyll content and carotenoid contents were ~0.4 % and ~0.1% of the total PLE-EtOH-I and PLE-EtOH-II extract mass, respectively. Recalculating these values per gram of HR shows an increase of 111 and 85% in total chlorophyll and total carotenoid content, respectively, in PLE-EtOH-II as compared to PLE-EtOH-I.

Table 12. Pigment content (chlorophyll and carotenoids) of *Ella* hop PLE-EtOH extracts obtained under the optimized experimental conditions

Samples		**PLE-EtOH parameters**	
		PLE-EtOH I 40°C, 15 min	PLE-EtOH II 85°C, 18 min
Pigment content			
	µg/g E	3081.17 ± 2.40[a]	3534.92 ± 3.07 [b]
Chlorophyll A	µg/g HR	347.25 ± 0.27[a]	725.37 ± 0.63[b]
	µg/g HP	259.43 ± 0.20[a]	541.90 ± 0.47 [b]
	µg/g E	574.01 ± 4.25[a]	707.03 ± 5.62 [b]
Chlorophyll B	µg/g HR	64.69 ± 0.48[a]	145.08 ± 1.15 [b]
	µg/g HP	48.33 ± 0.36[a]	108.39 ± 0.47 [b]
	µg/g E	3655.18 ± 5.19[a]	4241.95± 2.55[b]
Total chlorophylls	µg/g HR	411.94 ± 0.58[a]	870.45 ± 0.52[b]
	µg/g HP	307.77 ± 0.44[a]	650.29 ± 0.39[b]
	µg/g E	687.40 ± 1.26[a]	697.40 ± 1.44[b]
Total carotenoid	µg/g HR	77.47 ± 0.14[a]	143.17 ± 0.29[b]
	µg/g HP	57.87 ± 1.06[a]	106.91 ± 0.22[b]

PLE-EtOH: pressurized liquid extraction with ethanol; E.: extract; HR. hop residue after SFE-CO_2 (36 MPa, 40°C, 90 min); HP: hop pellets. Results are expressed as mean ± SD. Different superscript letters in the same row indicate significantly different values ($p < 0.05$) based on an unpaired t-test.

The extracts were dominated by chlorophyll A (83% of total chlorophyll content: 347 µg/g of HR for PLE-EtOH I) and 725 µg/g of HR for PLE-EtOH II. Similarly, ~2-fold higher chlorophyll B (145 µg/g HP) and carotenoid (106.9 µg/g HP) content was extracted after PLE-ETOH at 85 °C and 15 min in comparison to 40 °C and 15 min partially explaining higher antioxidant recovery after PLE-EtOH II. In comparison to *Ella* hop SFE-CO_2 extracts, ~22 and a 25-fold increase in total chlorophylls

content, which translates to 7 and 15-fold elevation in terms of HP in PLE-EtOH-I and -II, respectively. The total carotenoid content was measured ~3-fold higher in both extracts. While the content in hop pellets for PLE-EtOH-I was similar, and PLE-EtOH-II was 2-fold higher compared to bitter acid-enriched SFE-CO_2 extract (Table 5). Both chlorophylls and carotenoids were extensively studied for their antioxidant effect [183], thus may have bearing on the antioxidant potential of the extracts even at lower levels.

3.3.6. Antioxidant activity of hop extracts obtained under different PLE-EtOH conditions

The antioxidant properties of *Ella* hop ethanolic extracts under the two determined optimal extraction conditions, namely PLE-EtOH-I (40 °C, 15 min) and PLE-EtOH-II (85 °C, 18 min), are reported in Table 11. In addition to TPC and ORAC measurements, the $ABTS^{\bullet+}$ scavenging capacity of *Ella* hop PLE-EtOH extracts was evaluated too. The ABTS assay is based on hydrogen atom transfer, and also on single-electron transfer. Compounds with lower redox potential than ABTS•+ (0.68 V) like phenolic compounds reduce it. To its advantage is that hydrophilic, as well as lipophilic antioxidant potential, can be measured over a wide pH range [74]. It is beneficial to apply different antioxidant activity assessments to better understand the mechanisms behind the hop extract antioxidant potential and to compare the experimental values with the previously published data.

The obtained TPC results indicate that 167.9 and 191.5 mg GAE/g E was equal to 21.2 and 39.3 mg GAE/g HR (or 15.8 and 29.5 mg GAE/g HP) for PLE-EtOH I and -II, respectively. These values are similar to TPC of ~34 mg GAE/g of hops, obtained after optimized hydroethanolic ultrasound-assisted extraction of Brazilian *Cascade* hops at 52 °C [207]. Higher total phenolic content was obtained by microwave-assisted extraction and ultrasound extraction when ethanol was used (20.3 and 93.4 mg GAE/g hop, respectively) compared to 50 % ethanol-water or only water from wild hops [204]. Optimized extraction with natural deep eutectic solvent from Greek hops (119 mg GAE/g hops) [135], SFE-CO_2 with EtOH as modifier from spent hops (87-91 mg GAE/g of hops, 925.5-866.6 mg/g E) [143].

Between RF III (TPC, mg GAE/g R) and RF V ($TEAC_{ORAC}$, mg TE/g R) the Pearson correlation coefficient was significant (Appendix 26). PLE-EtOH II optimized for high yield and antioxidant activity is higher by an app. 85 % in TPC (39.29 mg GAE/g R), 34 % in $TEAC_{ORAC}$ value (397.37 mg TE/g R) and 95% in $TEAC_{ABTS+}$ (112.95 mg TE/g R) compared to PLE-EtOH I. $TEAC_{ABTS+}$ presents ~4-fold less TE mg/g R compared to $TEAC_{ORAC}$ for both PLE hop extracts. This may be explained by the $TEAC_{ORAC}$ reacting with more and especially with phenolic compounds compared to $TEAC_{ABTS+}$ [208, 209].

Higher antioxidant potential observed after 18 min of PLE-EtOH at 85 °C leads to the assumption that higher PLE temperature favors the extraction of a complex mixture of various phenolics that undergo redox-type reactions and are responsible for the detected antioxidant effect. Xanthohumol already has notable AA, the total ORAC value (modified method to measure fat- and water-soluble antioxidant capacity) was found to be proportionate to green tea catechin, Polyphenon 60, and even higher than the effect of vitamin C or E [70]. Gerhauser (2009a) identified, naringenin and isoxanthohumol Notably, quercetin and epicatechin gallate have been identified by Gerhauser (2009a) as the most active hop phenolic compounds in $TEAC_{ORAC}$ assay, at least twice as active as XN [76]. Additionally, the flavanone and were twice and similarly active, respectively, compared to XN. Caffeic and chlorogenic acids and isoxanthohumol were similarly active as XN, while ferulic, gallic acids and naringenin showed higher activity in $TEAC_{ORAC}$ assay. Besides flavonoids, the

carotenoids were reported to scavenge singlet oxygen and peroxyl radicals [210, 211]. Carotenoids and chlorophylls are elemental for photosynthesis, safeguard against damage caused by photo-oxidation. The lipophilic carotenoids can behave as antioxidants in electron-, hydrogen atom transfer reactions, and form radical adducts [183]. Their presence presumably augments the antioxidant activity of PLE-EtOH-II compared to PLE-EtOH-I.

3.3.7. Antimicrobial activity of PLE-EtOH hop extracts

Due to the presence of various phenolic compounds, PLE-EtOH extracts obtained from hop residues after bitter acid extraction can still provide a measurable antimicrobial inhibition. The broad spectrum of biological effects, including the selective antibacterial, antiviral and antifungal properties, has been ascribed mainly to xanthohumol, catechins, flavonols, multifidol glucosides, prenylflavonoids, phenolic acids and stilbenes [51, 70]. The effect of hop phenolic compounds against bacteria, fungi, and protozoan parasites is connected to their ability to penetrate the cell membrane, accumulate in the cell, and further influence bacterial cell surface hydrophobicity [59]. The assembly of adhesins in the cell wall can be inhibited mainly in gram-positive bacteria, this way the adhesion step for biofilm development could be disrupted. *S. aureus* was chosen as a tested microorganism, for the many virulence factors it produces and the proposed danger of emerging and strengthening antibiotic resistance in some strains. From the gram-positive, *E. coli* (ATTC 8739) and the toxin-producing (NTC 12900) were included for similar reasons. The pathogenic yeast responsible for 75% of all candidal infections, *C. albicans* were also tested. Extracts were applied up to 50 mg/mL, which is a practical limit for antimicrobial assays.

The obtained data indicated that tested hop PLE-EtOH extracts did not affect the growth of *E. coli* (ATTC 8739), *E coli* (NTC 12900) and *C. albicans* (ATCC 90028), however, showed inhibitory effect against the *S. aureus* (ATCC 25923) in the agar well diffusion assay (see Table 13). The highest primary inhibition zone of 22.7 mm was achieved by subjecting *S. aureus* to 25 mg/mL of PLE-EtOH II, obtained after 18 min of PLE at 85 °C. Hop extract prepared at lower PLE temperature and shorter time (40 °C, 15 min) generated slightly smaller primary inhibition zones without significant differences at the tested 10-50 mg/mL concentration range (~21.1 mm). Interestingly, *E. coli* strains are usually resistant to hop extract preparations[195], but low to medium antibacterial activity was also observed in some studies [212], also mentioned in an extensive review by Bocquet et al.[52].

Table 13. Antibacterial activity of PLE-EtOH extracts on *S. aureus* (ATCC 25923) using agar well diffusion (AWD) assay

Extract concentration	Inhibition zone	PLE-EtOH parameters	
		PLE-EtOH I 40°C, 15 min	PLE-EtOH II 85°C, 18 min
10 mg/mL	Primary, mm	21.22 ± 1.26 ^{abc}	18.89 ± 0.19 ^{b}
25 mg/mL	Primary, mm	21.00 ± 0.67 ^{abc}	22.67 ± 1.33 ^{c}
50 mg/mL	Primary, mm	21.11 ± 0.51 ^{abc}	19.89 ± 0.84 ^{ab}

Inhibition zone expressed as the diameter of inhibition zone + diameter of well (mm). Results are expressed as mean ± SD, n=3; PLE-EtOH: pressurized liquid extraction with ethanol; -nd: not detected; Different superscript letters in the table indicate significantly different values ($p < 0.05$) for the primary inhibition zone based on one-way ANOVA and Tukey's test.

Previously, Wendakoon et al. [63] reported 32-36 mm inhibition zones on the identical *S. aureus* strain for various hydroethanolic extracts (50-90%, v/v) after cold percolation of hops at room temperature. Kobus-Cisowska et al. [213] presented smaller, 4-8 mm *S.aureus* (ATCC 25923)

inhibition zones for *Magnum* and *Marynka* hop hydroethanolic extracts (40% ethanol, v/v %) obtained in a three-step liquid-solid extraction method. The antibacterial effect is not only influenced by the type of solvent [126] but also by the different extraction methods used. However, without IC_{50} values the direct comparison of inhibition zones is rather complicated, since variations in the analytical procedures, for example, not the identical well diameter or amount of sample deposited, can account for some of the observed differences.

Yamaguchi et al. [70] showed that the most common bacteria causing primary or secondary skin or soft tissue infections *P. acnes, S.aureus, S. epidermis, S. pyogenes* and *K. rhizophila* were inhibited by purified XN at MIC 3, 1, 3, 1, 1 mg/mL, respectively. Also, hydroalcoholic hop extract containing significant gallic acid, resveratrol and rutin amounts reduced biofilm formation of multidrug-resistant *S. epidermis* and *Cutibacterium acnes* [214]. While Rozalski et al. [62] demonstrated detrimental effects on *S. aureus* (ATCC 29213) biofilms, pure XN decreased viability by 86.5%, hop extract (51% XN) by 75%, and same hop extract deprived of XN by 42.8%. When hop cone extract and pure XN concentrations were increased, 97-99% eradication was achieved. Purified XN displayed inhibition on mainly gram-positive bacteria *Staphylococcus,* but also on the *Streptococci* e.g. *Sc. mutans* and others which typically cause dental caries were more affected than by typical mouthwash ingredients menthol or eucalyptus [215]. Other possible PLE-EtOH compounds, gallic, caffeic and ferulic acids are hop phenolic acids that were effective against some gram-positive (e.g., *S. aureus, Lysteria monocytogenes*) and gram-negative bacteria (e.g., *Pseudomonas aeruginosa*) [2].

Hop polyphenol-rich extracts can be used as natural bioactive ingredients in the cosmetic industry to develop novel phytodermatological preparations for prophylaxis and treatment of various skin infections, for example, acne, which initial stage pathogenesis is primarily conditioned by *S. aureus* [70].

Conclusions

1. The following results from SFE-CO_2 optimization were obtained:

1.1. The models for yield (F=51.17) ORAC values (F=35.20), cohumulone (F=39.00), humulone and adhumulone (F=40.50), colupulone (F=44.34), lupulone and adlupulone (F=53.26), total α-acids (F=44.21), total β-acids (F=50.19), total bitter acids (F=44.27) and α/β ratio (F=56.41) were significant and reproducible. τ, P and P^2 together contributed to the >70 % of the observed changes except for colupulone, lupulone and adlupulone and total β-acids influenced primarily by P^2, and less so, but significantly by PT, similarly to the α/β-acid ratio.

1.2. Optimal conditions (37 MPa, 43 °C, 80 min) yielded 26.3 g/100 g of an extract with 1481 mg TE/g E ORAC. SFE-CO_2 extract (37 MPa, 43 °C, 80 min) contained 867.9 mg/g E total bitter acid content, 1.5/1 α- to β-bitter acid ratio. Under the corrected SFE-CO_2 conditions (36 MPa, 40 °C, 90 min), recovery of bitter acids yielded 25.27 g/100 g extract with 650.7 mg/g E total α-acids and 324.4 mg/g E β-acids, equivalent to 975.1 mg/g E or 246.4 mg/g HP total bitter acid content, with a 2.01 α/β ratio. Compared to SFE-CO_2 extract obtained at 37 MPa, 43 °C and 80 min, the extraction of α-acids was more favored, increasing the recovery per gram HP by 19.5%.

2. With optimized SFE-CO_2 conditions, ~3-fold higher extraction yield and antioxidant recovery were achieved under significantly shorter extraction time compared to common commercially applied one-stage SFE-CO_2 at 10-15 MPa and 40°C.

3. Substantially shortened exhaustive extraction of bitter acids from hop pellets was achieved compared to the classical commercial one-stage SFE-CO_2 at 10-15 MPa and 40°C. Lipophilic pigments content (carotenoids and chlorophylls) was negligible (< 0.04% of the total extract mass). The major identified volatile compounds were monoterpene hydrocarbons β-pinene (up to 7.0%) and β-myrcene (up to 6.2%), sesquiterpene hydrocarbons β-humulene (up to 7.0%), α-humulene (up to 9.9%), and α-selinene (up to 14.9%), also unsaturated ester methyl-4-decenoate (up to 11.1%), providing fruity, herbal, spicy and woody odour to the hop SFE-CO_2 extracts.

4. SFE-CO_2 extract (36 MPa, 40 °C, 90 min) showed antimicrobial activity against *S. aureus* (ATCC 25923) up to 36.9 mm and *E.coli* (ATTC 8739) up to 17.11 mm inhibition zone diameter at 50 mg/ml extract concentration. Did not inhibit the growth of *E. coli* (NTC 12900) and *C. albicans* (ATCC 90028). Results also indicate that it significantly decreased cell viability of skin cancer cell line (375) at 100 µg by 23.4%, and further at 750 µg to 94.5%. Viability of healthy keratinocyte was reduced significantly at 15 µg by 12%, and at 100 µg by 76%, while fibroblasts were affected significantly at 50 µg by 12.6%, and at 750 µg by 90.5%.

5. The following results from PLE-EtOH process optimization were obtained

5.1. Statistically significant and reproducible models were obtained for yield (F=57.85) and the following antioxidant indices: TPC (mg GAE/g HR; F=79.39), $TEAC_{ORAC}$ (mg TE/g HR; F=83.25) and the XN content (mg/g E; F=137.42). The effect of T and T^2 contributed to 75-85% of observed responses.

5.2. Optimal conditions were separated, maximized yield coupled with strong antioxidant potential (85 °C,18 min) 20.5 g/100 g HR extract with 39.3 mg GAE/g HR and 397.4 mg TE/g HR was obtained, while maximized XN content (40 °C, 15 min) yielded 11.3 g/100 g HR extract with 83.5 mg/g E of XN.

6. PLE-EtOH-I (40 °C, 15 min) contained 82% higher XN content, as compared to PLE-EtOH-II (85 °C,18 min), ~ 9.4 mg/g HR and 7.0 mg/g HP. Recovery of XN from *Ella* hop residue after SFE-CO_2 was achieved already at the lowest tested temperature and time. Total chlorophyll content and carotenoid content were ~0.4 % (40 °C, 15 min) and ~0.1% (85 °C,18 min). Higher activity by an app. 85 % in TPC (39.29 mg GAE/g R), 34 % in $TEAC_{ORAC}$ value (397.37 mg

TE/g R) and 95% in $TEAC_{ABTS+}$ (112.95 mg TE/g R) was observed in PLE-EtOH-II (85 °C,18 min) compared to PLE-EtOH-I (40 °C, 15 min). The hop PLE-EtOH extracts inhibited *S. aureus* (ATCC 25923) at 10 to 50 mg/ml concentrations to 21.00-22.67 mm inhibition zone diameter. The extracts did not inhibit the growth of *E.coli* (ATTC 8739), *E. coli* (NTC 12900) and *C. albicans* (ATCC 90028).

List of references

1. ALMAGUER, Cynthia, Christina SCHÖNBERGER, Martina GASTL, Elke K. ARENDT a Thomas BECKER. Humulus lupulus - a story that begs to be told. A review. *Journal of the Institute of Brewing* [online]. 2014, **120**(4), 289–314. ISSN 20500416. Dostupné z: doi:10.1002/jib.160
2. KARABÍN, Marcel, Tereza HUDCOVÁ, Lukáš JELÍNEK a Pavel DOSTÁLEK. Biologically Active Compounds from Hops and Prospects for Their Use. *Comprehensive Reviews in Food Science and Food Safety* [online]. 2016, **15**(3), 542–567. ISSN 15414337. Dostupné z: doi:10.1111/1541-4337.12201
3. HRNČIČ, Maša Knez, Eva ŠPANINGER, Iztok Jože KOŠIR, Željko KNEZ a Urban BREN. Hop compounds: Extraction techniques, chemical analyses, antioxidative, antimicrobial, and anticarcinogenic effects. *Nutrients* [online]. 2019, **11**(2), 1–37. ISSN 20726643. Dostupné z: doi:10.3390/nu11020257
4. NUUTINEN, Tarmo. Medicinal properties of terpenes found in Cannabis sativa and Humulus lupulus. *European Journal of Medicinal Chemistry* [online]. 2018, **157**, 198–228. ISSN 17683254. Dostupné z: doi:10.1016/j.ejmech.2018.07.076
5. LIN, Mengfei, Diying XIANG, Xiaoyang CHEN a Heqiang HUO. Role of Characteristic Components of Humulus lupulus in Promoting Human Health. *Journal of Agricultural and Food Chemistry* [online]. 2019, **67**(30), 8291–8302. ISSN 15205118. Dostupné z: doi:10.1021/acs.jafc.9b03780
6. KUPSKI, Sérgio Carlos, Elissandro Jair KLEIN, Edson Antônio DA SILVA, Fernando PALÚ, Reginaldo GUIRARDELLO a Melissa Gurgel Adeodato VIEIRA. Mathematical modeling of supercritical CO2 extraction of hops (Humulus lupulus L.). *Journal of Supercritical Fluids* [online]. 2017, **130**(June), 347–356. ISSN 08968446. Dostupné z: doi:10.1016/j.supflu.2017.06.011
7. CAMPALANI, Carlotta, Francesco CHIOGGIA, Emanuele AMADIO, Michele GALLO, Flavio RIZZOLIO, Maurizio SELVA a Alvise PEROSA. Supercritical CO2extraction of natural antibacterials from low value weeds and agro-waste. *Journal of CO2 Utilization* [online]. 2020, **40**(February), 101198. ISSN 22129820. Dostupné z: doi:10.1016/j.jcou.2020.101198
8. FORMATO, Andrea, Monica GALLO, Domenico IANNIELLO, Domenico MONTESANO a Daniele NAVIGLIO. Supercritical fluid extraction of α- And β-acids from hops compared to cyclically pressurized solid-liquid extraction. *Journal of Supercritical Fluids* [online]. 2013, **84**, 113–120. ISSN 08968446. Dostupné z: doi:10.1016/j.supflu.2013.09.021
9. SANZ, Vanesa, María Dolores TORRES, José M. LÓPEZ VILARIÑO a Herminia DOMÍNGUEZ. What is new on the hop extraction? *Trends in Food Science and Technology* [online]. 2019, **93**(June), 12–22. ISSN 09242244. Dostupné z: doi:10.1016/j.tifs.2019.08.018
10. VAN OPSTAELE, Filip, Koen GOIRIS, Gert DE ROUCK, Guido AERTS a Luc DE COOMAN. Production of novel varietal hop aromas by supercritical fluid extraction of hop pellets - Part 2: Preparation of single variety floral, citrus, and spicy hop oil essences by density programmed supercritical fluid extraction. *Journal of Supercritical Fluids* [online]. 2012, **71**, 147–161. ISSN 08968446. Dostupné z: doi:10.1016/j.supflu.2012.06.004
11. VAN OPSTAELE, Filip, Koen GOIRIS, Gert DE ROUCK, Guido AERTS a Luc DE COOMAN. Production of novel varietal hop aromas by supercritical fluid extraction of hop pellets - Part 1: Preparation of single variety total hop essential oils and polar hop essences. *Journal of Supercritical Fluids* [online]. 2012, **69**, 45–56. ISSN 08968446. Dostupné z: doi:10.1016/j.supflu.2012.05.009
12. HERRERO, Miguel, María CASTRO-PUYANA, José A. MENDIOLA a Elena IBAÑEZ. Compressed fluids for the extraction of bioactive compounds. *TrAC - Trends in Analytical Chemistry* [online]. 2013, **43**(2), 67–83. ISSN 18793142. Dostupné z: doi:10.1016/j.trac.2012.12.008
13. *Beermaverick.com* [online]. Dostupné z: https://beermaverick.com/highest-alpha-acid-aa-hops/
14. ZEKOVIĆ, Zoran, Ivana PFAF-ŠOVLJANSKI a Olgica GRUJIĆ. Supercritical fluid extraction of hops. *Journal of the Serbian Chemical Society* [online]. 2007, **72**(1), 81–87. ISSN 03525139. Dostupné z: doi:10.2298/JSC0701081Z
15. *European Commission - Hops Overview* [online]. Dostupné z: https://ec.europa.eu/info/food-farming-fisheries/plants-and-plant-products/plant-products/hops_en
16. JÓŹWIAK, Grzegorz, Barbara MAJER-DZIEDZIC, Justyna KWIECIŃSKA a Monika WAKSMUNDZKA-HAJNOS. Comparison of the microbiological activities of different varieties of hop (humulus lupulus) extracts by thin-layer chromatography-direct bioautography. *Journal of Planar Chromatography - Modern TLC* [online]. 2017, **30**(2), 126–130. ISSN 17890993. Dostupné z: doi:10.1556/1006.2017.30.2.6

17. SREČEC, Siniša, Tonci REZIC, Božidar SANTEK a Veljko MARIĆ. Hop pellets type 90: Influence of manufacture and storage on losses of α-acids. *Acta Alimentaria - ACTA ALIMENT* [online]. 2009, **38**, 141–147. Dostupné z: doi:10.1556/AAlim.2008.0014
18. HATFIELD, Gabrielle. Encyclopedia of Folk Medicine: Old World and New World Traditions. In: *Journal of American Folklore* [online]. 2003, s. 392. ISBN 1576078744. Dostupné z: doi:10.2307/20487662
19. SANBORN C. BROWN. *Wines and Beers of Old New England: A How to-Do-It History*. B.m.: University Press of New England, 1998. ISBN 0874511488.
20. ROBERTS, T. R. *Hops* [online]. B.m.: Elsevier Inc., 2016. ISBN 9780128004685. Dostupné z: doi:10.1016/B978-0-12-799954-8.00003-4
21. SALANȚĂ, Liana, Maria TOFANĂ, Sonia SOCACI, Elena MUDURA, Carmen POP, Anamaria POP a Anca FĂRCAŞ. Evaluation and comparison of aroma volatile compounds in hop varieties grown in Romania. *Romanian Biotechnological Letters*. 2015, **20**(6), 11049–11056. ISSN 12245984.
22. CRELLIN, John. Gabrielle Hatfield, Encyclopedia of folk medicine: old world and new world traditions, Santa Barbara, CA, ABC–CLIO, 2004, pp. xx, 392, illus., £56.95 (hardback 1-57607-874-4). *Medical History* [online]. 2006, **50**, 141–142. Dostupné z: doi:10.1017/S0025727300009704
23. VLIETINCK, A.J. Assessment Report on Humulus Lupulus L., Flos. *European Medicines Agency*. 2014, **44**(May), 1–38.
24. BIENDL, Martin, Bernard ENGELHARD, Adrian FORSTER, Andreas GAHR, Andon LUTZ, Willi MITTER, Roland SCHMIDT a Christina SCHÖNBERGER. *Hops Their Cultivation, Composition and Usage*. Nuremberg, Germany: Fachverlag Hans Carl GmbH, 2014. ISBN 978-3-418-00904-2.
25. MILLIGAN, S R, J C KALITA, A HEYERICK, H RONG, L DE COOMAN a D DE KEUKELEIRE. Identification of a potent phytoestrogen in hops (Humulus lupulus L.) and beer. *The Journal of clinical endocrinology and metabolism* [online]. 1999, **84**(6), 2249–2252. ISSN 0021-972X (Print). Dostupné z: doi:10.1210/jcem.84.6.5887
26. KARABIN, Marcel, Tereza HUDCOVA, Lukas JELINEK a Pavel DOSTALEK. Biotransformations and biological activities of hop flavonoids. *Biotechnology Advances* [online]. 2014, **33**(6), 1063–1090. ISSN 07349750. Dostupné z: doi:10.1016/j.biotechadv.2015.02.009
27. PIELECH-PRZYBYLSKA, Katarzyna, Maria BALCEREK, Agnieszka NOWAK, Maciej WOJTCZAK, Agata CZYZOWSKA, Urszula DZIEKONSKA-KUBCZAK a Piotr PATELSKI. The effect of different starch liberation and saccharification methods on the microbial contaminations of distillery mashes, fermentation efficiency, and spirits quality. *Molecules* [online]. 2017, **22**(10), 1–20. ISSN 14203049. Dostupné z: doi:10.3390/molecules22101647
28. BALCEREK, Maria, Katarzyna PIELECH-PRZYBYLSKA, Urszula DZIEKOŃSKA-KUBCZAK, Piotr PATELSKI a Ewelina STRAK. Fermentation results and chemical composition of agricultural distillates obtained from rye and barley grains and the corresponding malts as a source of amylolytic enzymes and starch. *Molecules* [online]. 2016, **21**(10). ISSN 14203049. Dostupné z: doi:10.3390/molecules21101320
29. POLLACH, Günter, Walter HEIN a David BEDDIE. Application of hop β-acids and rosin acids in the sugar industry. *Zuckerindustrie*. 2002, **127**(12), 921–930. ISSN 03448657.
30. RÜCKLE, L. Hop acids as natural antibacterials in ethanol fermentation. *International Sugar Journal* [online]. 2005, **107**(1275), 162–165. ISSN 0020-8841. Dostupné z: https://www.cabdirect.org/cabdirect/abstract/20053047285
31. LEITE, Inácio R., Jonas R. FARIA, Líbia D.S. MARQUEZ, Miria H.M. REIS, Miriam M. DE RESENDE, Eloízio J. RIBEIRO a Vicelma L. CARDOSO. Evaluation of hop extract as a natural antibacterial agent in contaminated fuel ethanol fermentations. *Fuel Processing Technology* [online]. 2013, **106**, 611–618. ISSN 03783820. Dostupné z: doi:10.1016/j.fuproc.2012.09.050
32. ESKIN, Michael a Snait TAMIR. *Dictionary of nutraceuticals and functional foods*. B.m.: Crc Press, 2005. ISBN 0203486854.
33. COOPER, Andrew J., Anthony W. DALLMIER a Howard Raymond BARNES. United States Patent - COMPOSITIONS CONTAINING HOPS EXTRACT AND THEIR USE IN WATER SYSTEMS AND PROCESS STREAMS TO CONTROL BIOLOGICAL FOULING [online]. 2019. Dostupné z: https://patents.google.com/patent/US6379720B1/en
34. DEGRANDI-HOFFMAN, Gloria, Fabiana AHUMADA, Gene PROBASCO a Lloyd SCHANTZ. The effects of beta acids from hops (Humulus lupulus) on mortality of Varroa destructor (Acari: Varroidae). *Experimental and Applied Acarology* [online]. 2012, **58**(4), 407–421. ISSN 01688162. Dostupné z: doi:10.1007/s10493-012-9593-2

35. MOŠKRIČ, Ajda, Jernej BUBNIČ, Maja Ivana Smodiš ŠKERL a Janez PREŠERN. Potential positive effects of hop beta acids on parasitic mite varroa destructor control in honey bee colonies. *Journal of Hygienic Engineering and Design*. 2018, **24**, 21–28. ISSN 18578489.
36. GARDEA-TORRESDEY, J, M HEJAZI, K TIEMANN, J PARSONS, M DUARTE-GARDEA a J HENNING. ulus lupulus) agricultural by-products for the reduction of aqueous lead(II) environmental health hazards. *Journal of Hazardous Materials*. 2002, **91**(1–3), 95–112.
37. *SpecialChem - Humulus Lupulus Extract* [online]. Dostupné z: https://cosmetics.specialchem.com/inci/humulus-lupulus-extract
38. VOGT, Otmar a Jan OGONOWSKI. The effect of selected supercritical CO 2 plant extract addition on user properties of shower gels. 2014, **16**(4), 51–54.
39. HUGHES, P. S. a W. J. SIMPSON. Production and Composition of Hop Products. *MBAA Technical Quarterly*. 1993, **30**(August), 146–154.
40. ZANOLI, Paola a Manuela ZAVATTI. Pharmacognostic and pharmacological profile of Humulus lupulus L. *Journal of Ethnopharmacology* [online]. 2008, **116**(3), 383–396. ISSN 03788741. Dostupné z: doi:10.1016/j.jep.2008.01.011
41. VAN CLEEMPUT, Marjan, Ko CATTOOR, Karolien DE BOSSCHER, Guy HAEGEMAN, Denis DE KEUKELEIRE a Arne HEYERICK. Hop (Humulus lupulus)-derived bitter acids as multipotent bioactive compounds. *Journal of Natural Products* [online]. 2009, **72**(6), 1220–1230. ISSN 01633864. Dostupné z: doi:10.1021/np800740m
42. HASELEU, G., INTELMANN, D., AND HOFMANN, T. Identification and RP-HPLC-ESI-MS/MS quantitation of bitter-tasting β-acid trans- formation products in beer. *J. Agric. Food Chem.* 2009, (57), 7480–7489.
43. BRIGGS, D. E., BOULTON, C. A., BROOKES, P. A., AND STEVENS, R. The chemistry of hop constituents, in Brewing – Science and Practice. In: . Boca Raton, FL.: CRC Press, 2009, s. 255–305.
44. STEVENS, Jan F. a Jonathan E. PAGE. Xanthohumol and related prenylflavonoids from hops and beer: To your good health! *Phytochemistry* [online]. 2004, **65**(10), 1317–1330. ISSN 00319422. Dostupné z: doi:10.1016/j.phytochem.2004.04.025
45. DILLER, Reinhard A., Herbert M. RIEPL, Oliver ROSE, Corazon FRIAS, Günter HENZE a Aram PROKOP. Desmethylxanthohumol from hops, chemistry and biological effects. *Beer in Health and Disease Prevention* [online]. 2008, 703–709 [vid. 2019-11-18]. Dostupné z: doi:10.1016/b978-0-12-373891-2.00071-7
46. STEVENS, Jan F., Alan W. TAYLOR a Max L. DEINZER. Quantitative analysis of xanthohumol and related prenylflavonoids in hops and beer by liquid chromatography-tandem mass spectrometry. *Journal of Chromatography A* [online]. 1999, **832**(1–2), 97–107. ISSN 00219673. Dostupné z: doi:10.1016/S0021-9673(98)01001-2
47. BARTMAŃSKA, Agnieszka, Ewa WAŁECKA-ZACHARSKA, Tomasz TRONINA, Jaroslaw POPŁOŃSKI, Sandra SORDON, Ewa BRZEZOWSKA, Jacek BANIA a Ewa HUSZCZA. Antimicrobial properties of spent hops extracts, flavonoids isolated therefrom, and their derivatives. *Molecules* [online]. 2018, **23**(8), 1–12. ISSN 14203049. Dostupné z: doi:10.3390/molecules23082059
48. ALEC, Ž a S LOVENIA. *International Hop Growers' Convention* [online]. 2019. Dostupné z: http://www.hmelj-giz.si/ihgc/doc/2019 AUG IHGC EC Report_final.pdf
49. HEINLEIN, Anja a Andrea BUETTNER. Monitoring of biotransformation of hop aroma compounds in an in vitro digestion model. *Food and Function* [online]. 2012, **3**(10), 1059–1067. ISSN 2042650X. Dostupné z: doi:10.1039/c2fo30061c
50. BOCQUET, Laetitia, Sevser SAHPAZ, Natacha BONNEAU, Claire BEAUFAY, Séverine MAHIEUX, Jennifer SAMAILLIE, Vincent ROUMY, Justine JACQUIN, Simon BORDAGE, Thierry HENNEBELLE, Feng CHAI, Joëlle QUETIN-LECLERCQ, Christel NEUT a Céline RIVIÈRE. Phenolic compounds from humulus lupulus as natural antimicrobial products: New weapons in the fight against methicillin resistant staphylococcus aureus, leishmania mexicana and trypanosoma brucei strains. *Molecules* [online]. 2019, **24**(6). ISSN 14203049. Dostupné z: doi:10.3390/molecules24061024
51. GERHÄUSER, Clarissa. Broad spectrum antiinfective potential of xanthohumol from hop (Humulus lupulus L.) in comparison with activities of other hop constituents and xanthohumol metabolites. *Molecular Nutrition and Food Research* [online]. 2005, **49**(9), 827–831. ISSN 16134125. Dostupné z: doi:10.1002/mnfr.200500091
52. BOCQUET, Laetitia, Sevser SAHPAZ a Céline RIVIÈRE. An Overview of the Antimicrobial Properties of Hop [online]. 2018, 31–54. Dostupné z: doi:10.1007/978-3-319-67045-4_2

53. NATARAJAN, P., S. KATTA, I. ANDREI, V. BABU RAO AMBATI, M. LEONIDA a G. J. HAAS. Positive antibacterial co-action between hop (Humulus lupulus) constituents and selected antibiotics. *Phytomedicine* [online]. 2008, **15**(3), 194–201. ISSN 09447113. Dostupné z: doi:10.1016/j.phymed.2007.10.008
54. DUMAS, Elizabeth R., Amy E. MICHAUD, Chantal BERGERON, Jennifer L. LAFRANCE, Susan MORTILLO a Stefan GAFNER. Deodorant effects of a supercritical hops extract: Antibacterial activity against Corynebacterium xerosis and Staphylococcus epidermidis and efficacy testing of a hops/zinc ricinoleate stick in humans through the sensory evaluation of axillary deodorancy. *Journal of Cosmetic Dermatology* [online]. 2009, **8**(3), 197–204. ISSN 14732130. Dostupné z: doi:10.1111/j.1473-2165.2009.00449.x
55. BONILLA, J., L. ATARÉS, M. VARGAS a A. CHIRALT. Edible films and coatings to prevent the detrimental effect of oxygen on food quality: Possibilities and limitations. *Journal of Food Engineering* [online]. 2012, **110**(2), 208–213. ISSN 02608774. Dostupné z: doi:10.1016/j.jfoodeng.2011.05.034
56. BAJIĆ, Marijan, Helena JALŠOVEC, Andrea TRAVAN, Uroš NOVAK a Blaž LIKOZAR. Chitosan-based films with incorporated supercritical CO2 hop extract: Structural, physicochemical, and antibacterial properties. *Carbohydrate Polymers* [online]. 2019, **219**(May), 261–268. ISSN 01448617. Dostupné z: doi:10.1016/j.carbpol.2019.05.003
57. KOWALCZYK, Dariusz a Martin BIENDL. Physicochemical and antioxidant properties of biopolymer/candelilla wax emulsion films containing hop extract - A comparative study. *Food Hydrocolloids* [online]. 2016, **60**, 384–392. ISSN 0268005X. Dostupné z: doi:10.1016/j.foodhyd.2016.04.010
58. HAMM, Alison, Daniel MANTER, Jay KIRKWOOD, Lisa WOLFE, Kimberly COX-YORK a Tiffany WEIR. The Effect of Hops (Humulus lupulus L.) Extract Supplementation on Weight Gain, Adiposity and Intestinal Function in Ovariectomized Mice [online]. 2019 [vid. 2019-11-18]. Dostupné z: doi:10.20944/PREPRINTS201910.0359.V1
59. ALVESALO, J, H VUORELA, P TAMMELA, M LEINONEN, P SAIKKU a P VUORELA. Inhibitory effect of dietary phenolic compounds on Chlamydia pneumoniae in cell cultures [online]. 2006, **71**, 735–741. Dostupné z: doi:10.1016/j.bcp.2005.12.006
60. SAAVEDRA, Maria J, Anabela BORGES, Carla DIAS, Alfredo AIRES, Richard N BENNETT, Eduardo S ROSA a Manuel SIMÕES. Antimicrobial Activity of Phenolics and Glucosinolate Hydrolysis Products and their Synergy with Streptomycin against Pathogenic Bacteria Antimicrobial Activity of Phenolics and Glucosinolate Hydrolysis Products and their Synergy with Streptomycin against [online]. 2010, (September 2015). Dostupné z: doi:10.2174/1573406411006030174
61. KANG, Jiamu, Qianqian LI, Liu LIU, Wenyuan JIN, Jingfan WANG a Yuyang SUN. The specific effect of gallic acid on Escherichia coli biofilm formation by regulating pgaABCD genes expression. 2018, 1837–1846.
62. ROZALSKI, Marcin, Bartlomiej MICOTA, Beata SADOWSKA, Anna STOCHMAL, Dariusz JEDREJEK, Marzena WIECKOWSKA-SZAKIEL a Barbara ROZALSKA. Antiadherent and antibiofilm activity of humulus lupulus L. derived products: New pharmacological properties. *BioMed Research International* [online]. 2013, **2013**. ISSN 23146133. Dostupné z: doi:10.1155/2013/101089
63. WENDAKOON, Chitra a Daniel GAGNON. Evaluation of Selected Medicinal Plants Extracted in Different Ethanol Concentrations for Antibacterial Activity against Human Pathogens. 2012, **1**(2), 60–68.
64. WENDAKOON, Chitra, Daniel GAGNON a Matt KOENIG. Hops (Humulus lupulus) Strobile Extract and Its Major Components Show Strong Antibacterial Activity against Methicillin-Resistant Staphylococcus aureus. 2018, **7**(1), 12–18.
65. CERMAK, Pavel, Jana OLSOVSKA, Alexandr MIKYSKA, Martin DUSEK, Zuzana KADLECKOVA, Jiri VANICEK, Otakar NYC, Karel SIGLER, Vanda BOSTIKOVA a Pavel BOSTIK. Strong antimicrobial activity of xanthohumol and other derivatives from hops (Humulus lupulus L.) on gut anaerobic bacteria. *Apmis* [online]. 2017, **125**(11), 1033–1038. ISSN 16000463. Dostupné z: doi:10.1111/apm.12747
66. DI SOTTO, Antonella, Paola CHECCONI, Ignacio CELESTINO, Marcello LOCATELLI, Stefania CARISSIMI, Marta DE ANGELIS, Valeria ROSSI, Dolores LIMONGI, Chiara TONIOLO, Lucia MARTINOLI, Silvia DI GIACOMO, Anna Teresa PALAMARA a Lucia NENCIONI. Antiviral and antioxidant activity of a hydroalcoholic extract from humulus lupulus L. *Oxidative Medicine and Cellular Longevity* [online]. 2018, **2018**. ISSN 19420994. Dostupné z: doi:10.1155/2018/5919237
67. JIROVETZ, Leopold, Stefanie BAIL a Gerhard BUCHBAUER. Antimicrobial testings, gas

chromatographic analysis and olfactory evaluation of an essential oil of hop cones (Humulus lupulus L.) from Bavaria and some of its. *Scientia ...* [online]. 2006, **201**, 189–201. Dostupné z: http://www.scipharm.at/download.asp?id=192%5Cnhttp://www.scipharm.at/download.asp?id=192&lid=1

68. TOBE, H, M KUBOTA, M YAMAGUCHI, T KOCHA a T AOYAGI. Apoptosis to HL-60 by humulone. *Bioscience, biotechnology, and biochemistry* [online]. 1997, **61**(6), 1027–1029. ISSN 0916-8451 (Print). Dostupné z: doi:10.1271/bbb.61.1027
69. VAN CLEEMPUT, Marjan, Ko CATTOOR, Karolien DE BOSSCHER, Guy HAEGEMAN, Denis DE KEUKELEIRE a Arne HEYERICK. Hop (Humulus lupulus)-derived bitter acids as multipotent bioactive compounds. *Journal of Natural Products* [online]. 2009, **72**(6), 1220–1230. ISSN 01633864. Dostupné z: doi:10.1021/np800740m
70. YAMAGUCHI, Naoto, Keiko SATOH-YAMAGUCHI a Mitsunori ONO. In vitro evaluation of antibacterial, anticollagenase, and antioxidant activities of hop components (Humulus lupulus) addressing acne vulgaris. *Phytomedicine* [online]. 2009, **16**(4), 369–376. ISSN 09447113. Dostupné z: doi:10.1016/j.phymed.2008.12.021
71. GORJANOVIĆ, Stanislava, Ferenc T. PASTOR, Radica VASIĆ, Miroslav NOVAKOVIĆ, Mladen SIMONOVIĆ, Sonja MILIĆ a Desanka SUŽNJEVIĆ. Electrochemical versus spectrophotometric assessment of antioxidant activity of hop (Humulus lupulus L.) products and individual compounds. *Journal of Agricultural and Food Chemistry* [online]. 2013, **61**(38), 9089–9096. ISSN 00218561. Dostupné z: doi:10.1021/jf401718z
72. TING, Patrick L., Lance LUSK, Jay REFLING, Susan KAY a David RYDER. Identification of antiradical hop compounds. In: *Journal of the American Society of Brewing Chemists* [online]. 2008, s. 116–126. ISSN 03610470. Dostupné z: doi:10.1094/ASBCJ-2008-0310-01
73. OLECH, Marta a Natalia NOWACKA-JECHALKE. Plant Polyphenols as Chemopreventive Agents. In: *Polyphenols in Human Health and Disease* [online]. 2013, s. 1289–1307. ISBN ISBN: 978-0-12-398472-2. Dostupné z: doi:10.1016/B978-0-12-398456-2.00086-4
74. PRIOR, Ronald L., Xianli WU a Karen SCHAICH. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry* [online]. 2005, **53**(10), 4290–4302. ISSN 00218561. Dostupné z: doi:10.1021/jf0502698
75. KOWALCZYK, Dariusz, Michał ŚWIECA, Joanna CICHOCKA a Urszula GAWLIK-DZIKI. The phenolic content and antioxidant activity of the aqueous and hydroalcoholic extracts of hops and their pellets. *Journal of the Institute of Brewing* [online]. 2013, **119**(3), 103–110. ISSN 20500416. Dostupné z: doi:10.1002/jib.73
76. GERHÄUSER, Clarissa. Phenolic Beer Compounds to Prevent Cancer Provided for non-commercial research and educational use only . Not for reproduction , distribution or commercial use [online]. 2008, (September 2008). Dostupné z: doi:10.1016/B978-0-12-373891-2.00068-7
77. CALLEJA, Miguel Angel, Jose María VIEITES, Trinidad MONTERO-MELÉNDEZ, María Isabel TORRES, María José FAUS, Angel GIL a Antonio SUÁREZ. The antioxidant effect of β-caryophyllene protects rat liver from carbon tetrachloride-induced fibrosis by inhibiting hepatic stellate cell activation. *The British journal of nutrition* [online]. 2013, **109**(3), 394–401. ISSN 1475-2662 (Electronic). Dostupné z: doi:10.1017/S0007114512001298
78. DAHHAM, Saad S, Yasser M TABANA, Muhammad A IQBAL, Mohamed B K AHAMED, Mohammed O EZZAT, Aman S A MAJID a Amin M S A MAJID. The Anticancer, Antioxidant and Antimicrobial Properties of the Sesquiterpene β-Caryophyllene from the Essential Oil of Aquilaria crassna. *Molecules (Basel, Switzerland)* [online]. 2015, **20**(7), 11808–11829. ISSN 1420-3049 (Electronic). Dostupné z: doi:10.3390/molecules200711808
79. MANNERING, G J, J A SHOEMAN a L B DELORIA. Identification of the antibiotic hops component, colupulone, as an inducer of hepatic cytochrome P-4503A in the mouse. *Drug metabolism and disposition: the biological fate of chemicals*. 1992, **20**(2), 142–147. ISSN 0090-9556 (Print).
80. LAMY, Virginie, Stamatiki ROUSSI, Mehdi CHAABI, Francine GOSSÉ, Annelise LOBSTEIN a Francis RAUL. Lupulone, a hop bitter acid, activates different death pathways involving apoptotic TRAIL-receptors, in human colon tumor cells and in their derived metastatic cells. *Apoptosis : an international journal on programmed cell death* [online]. 2008, **13**(10), 1232–1242. ISSN 1573-675X (Electronic). Dostupné z: doi:10.1007/s10495-008-0250-5
81. STEPHAN, T E, E O NGO a L M NUTTER. Hexahydrocolupulone and its antitumor cell proliferation activity in vitro. *Biochemical pharmacology* [online]. 1998, **55**(4), 505–514. ISSN 0006-2952 (Print). Dostupné z: doi:10.1016/s0006-2952(97)00469-3

82. MOON, Young Jin, Xiaodong WANG a Marilyn E. MORRIS. Dietary flavonoids: Effects on xenobiotic and carcinogen metabolism. *Toxicology in Vitro* [online]. 2006, **20**(2), 187–210. ISSN 08872333. Dostupné z: doi:10.1016/j.tiv.2005.06.048
83. HENDERSON, M C, C L MIRANDA, J F STEVENS, M L DEINZER a D R BUHLER. In vitro inhibition of human P450 enzymes by prenylated flavonoids from hops, Humulus lupulus. *Xenobiotica* [online]. 2000, **30**(3), 235–251. ISSN 0049-8254. Dostupné z: doi:10.1080/004982500237631
84. DASHWOOD, Roderick H., Melinda C. MYZAK a Emily HO. Dietary HDAC inhibitors: Time to rethink weak ligands in cancer chemoprevention? *Carcinogenesis* [online]. 2006, **27**(2), 344–349. ISSN 01433334. Dostupné z: doi:10.1093/carcin/bgi253
85. MONTEIRO, Rosário, Ana FARIA, Isabel AZEVEDO a Conceição CALHAU. Modulation of breast cancer cell survival by aromatase inhibiting hop (Humulus lupulus L.) flavonoids. *The Journal of Steroid Biochemistry and Molecular Biology* [online]. 2007, **105**(1–5), 124–130 [vid. 2019-11-18]. ISSN 0960-0760. Dostupné z: doi:10.1016/J.JSBMB.2006.11.026
86. BALUNAS, M. J., SU, B., BRUEGGEMEIER, R. W., & KINGHORN, A. D. Natural products as aromatase inhibitors. *Anti-Cancer Agents in Medicinal Chemistry*. 2008, **8(6)**, 646–682.
87. CHADWICK, L.R. R., G.F. F. PAULI a N.R. R. FARNSWORTH. The pharmacognosy of Humulus lupulus L. (hops) with an emphasis on estrogenic properties. *Phytomedicine* [online]. 2006, **13**(1–2), 119–131 [vid. 2019-11-19]. ISSN 09447113. Dostupné z: doi:10.1016/j.phymed.2004.07.006
88. ALLSOPP, Philip, Sam POSSEMIERS, David CAMPBELL, Chris GILL a Ian ROWLAND. A comparison of the anticancer properties of isoxanthohumol and 8-prenylnaringenin using in vitro models of colon cancer. *BioFactors* [online]. 2013, **39**(4), 441–447. ISSN 09516433. Dostupné z: doi:10.1002/biof.1084
89. SELIGER, Jan Moritz, Hans-jörg Jörg MARTIN, Edmund MASER a Jan HINTZPETER. Potent inhibition of human carbonyl reductase 1 (CBR1) by the prenylated chalconoid xanthohumol and its related prenylflavonoids isoxanthohumol and 8-prenylnaringenin. *Chemico-Biological Interactions* [online]. 2019, **305**, 156–162. ISSN 18727786. Dostupné z: doi:10.1016/j.cbi.2019.02.031
90. COLGATE, Emily C., Cristobal L. MIRANDA, Jan F. STEVENS, Tammy M. BRAY a Emily HO. Xanthohumol, a prenylflavonoid derived from hops induces apoptosis and inhibits NF-kappaB activation in prostate epithelial cells. *Cancer Letters* [online]. 2007, **246**(1–2), 201–209 [vid. 2019-11-18]. ISSN 0304-3835. Dostupné z: doi:10.1016/J.CANLET.2006.02.015
91. KOOSHA, Sanaz, Zahurin MOHAMED, Ajantha SINNIAH, Zaridatul Aini IBRAHIM, Atefehalsadat SEYEDAN a Mohammed A. ALSHAWSH. Antiproliferative and apoptotic activities of 8-prenylnaringenin against human colon cancer cells. *Life Sciences* [online]. 2019, **232**(April), 116633. ISSN 18790631. Dostupné z: doi:10.1016/j.lfs.2019.116633
92. HWANG, Eunson, Hien T.T. NGO, Bom PARK, Seul A. SEO, Jung Eun YANG a Tae Hoo YI. Myrcene, an Aromatic Volatile Compound, Ameliorates Human Skin Extrinsic Aging via Regulation of MMPs Production. *American Journal of Chinese Medicine* [online]. 2017, **45**(5), 1113–1124. ISSN 0192415X. Dostupné z: doi:10.1142/S0192415X17500604
93. FIDYT, Klaudyna, Anna FIEDOROWICZ, Leon STRZĄDAŁA a Antoni SZUMNY. B-Caryophyllene and B-Caryophyllene Oxide—Natural Compounds of Anticancer and Analgesic Properties. *Cancer Medicine* [online]. 2016, **5**(10), 3007–3017. ISSN 20457634. Dostupné z: doi:10.1002/cam4.816
94. CHANG, Mei Yin a Yi Ling SHEN. Linalool exhibits cytotoxic effects by activating antitumor immunity. *Molecules* [online]. 2014, **19**(5), 6694–6706. ISSN 14203049. Dostupné z: doi:10.3390/molecules19056694
95. CHERNG, Jaw Ming, Den En SHIEH, Wen CHIANG, Mei Yin CHANG a Lien Chai CHIANG. Chemopreventive effects of minor dietary constituents in common foods on human cancer cells. *Bioscience, Biotechnology and Biochemistry* [online]. 2007, **71**(6), 1500–1504. ISSN 09168451. Dostupné z: doi:10.1271/bbb.70008
96. RAVIZZA, R., M.B. GARIBOLDI, R. MOLTENI a E. MONTI. nalool, a plant-derived monoterpene alcohol, reverses doxorubicin resistance in human breast adenocarcinoma cells. *Oncology Reports* [online]. 2008, **20**, 625–630. Dostupné z: doi:10.3892/or_00000051
97. HAN, Hee Dong, Young Jae CHO, Sung Keun CHO, Yeongseon BYEON, Hat Nim JEON, Hye Sun KIM, Byoung Gie KIM, Duk Soo BAE, Gabriel LOPEZ-BERESTEIN, Anil K. SOOD, Byung Cheol SHIN, Yeong Min PARK a Jeong Won LEE. Linalool-incorporated nanoparticles as a novel anticancer agent for epithelial ovarian carcinoma. *Molecular Cancer Therapeutics* [online]. 2016, **15**(4), 618–627. ISSN 15388514. Dostupné z: doi:10.1158/1535-7163.MCT-15-0733-T

98. ERKKOLA, R., S. VERVARCKE, S. VANSTEELANDT, P. ROMPOTTI, D. DE KEUKELEIRE a A. HEYERICK. A randomized, double-blind, placebo-controlled, cross-over pilot study on the use of a standardized hop extract to alleviate menopausal discomforts. *Phytomedicine* [online]. 2010, **17**(6), 389–396. ISSN 0944-7113. Dostupné z: doi:10.1016/j.phymed.2010.01.007
99. MINECKA, Aldona, Maria ZYCH a Ilona KACZMARCZYK-SEDLAK. 8-Prenylnaringenin from hop (Humulus lupulus L.) – a panacea for menopause? *Herba Polonica* [online]. 2018, **63**(4), 34–44. ISSN 2449-8343. Dostupné z: doi:10.1515/hepo-2017-0023
100. SCHAEFER, Olaf, Michael HÜMPEL, Karl Heinrich FRITZEMEIER, Rolf BOHLMANN a Wolf Dieter SCHLEUNING. 8-Prenyl naringenin is a potent ERα selective phytoestrogen present in hops and beer. *Journal of Steroid Biochemistry and Molecular Biology* [online]. 2003, **84**(2–3), 359–360. ISSN 09600760. Dostupné z: doi:10.1016/S0960-0760(03)00050-5
101. MILLIGAN, S.R. Downloaded from https://academic.oup.com/jcem/article-abstract/85/12/4908/2856299 by Kyoto University Medical Library user on 20 February 2019 RAPID COMMUNICATIONS. 2000, **85**(12), 4908–4911.
102. OVERK, Cassia R., Jian GUO, Lucas R. CHADWICK, Daniel D. LANTVIT, Alberto MINASSI, Giovanni APPENDINO, Shao Nong CHEN, David C. LANKIN, Norman R. FARNSWORTH, Guido F. PAULI, Richard B. VAN BREEMEN a Judy L. BOLTON. In vivo estrogenic comparisons of Trifolium pratense (red clover) Humulus lupulus (hops), and the pure compounds isoxanthohumol and 8-prenylnaringenin. *Chemico-Biological Interactions* [online]. 2008, **176**(1), 30–39. ISSN 00092797. Dostupné z: doi:10.1016/j.cbi.2008.06.005
103. SOBSTYL, Małgorzata, Wiesława BEDNAREK, Joanna TKACZUK-WŁACH, Jacek SOBSTYL a Grzegorz JAKIEL. Objawy naczynioruchowe w menopauzie - Diagnostyka i leczenie. *Przeglad Menopauzalny*. 2011, **15**(3), 254–259. ISSN 16438876.
104. FREEDMAN, Robert R. Imaging and menopausal hot flashes. *Temperature* [online]. 2016, **3**(3), 361–361. ISSN 2332-8940. Dostupné z: doi:10.1080/23328940.2016.1208318
105. MOORE, Thea R, Rachel B FRANKS a Carol FOX. Review of Efficacy of Complementary and Alternative Medicine Treatments for Menopausal Symptoms [online]. 2017. Dostupné z: doi:10.1111/jmwh.12628
106. TONOB, Dunia a Melissa K MELBY. Broadening our perspectives on complementary and alternative medicine for menopause: A narrative review. *Maturitas* [online]. 2017, **99**, 79–85. ISSN 0378-5122. Dostupné z: doi:10.1016/j.maturitas.2017.01.013
107. HÜMPEL, Michael, Päivi ISAKSSON, Olaf SCHAEFER, Ulrike KAUFMANN, Paolo CIANA, Adriana MAGGI a Wolf Dieter SCHLEUNING. Tissue specificity of 8-prenylnaringenin: Protection from ovariectomy induced bone loss with minimal trophic effects on the uterus. *Journal of Steroid Biochemistry and Molecular Biology* [online]. 2005, **97**(3), 299–305. ISSN 09600760. Dostupné z: doi:10.1016/j.jsbmb.2005.05.009
108. KEILER, Annekathrin M., Oliver ZIERAU a Georg KRETZSCHMAR. Hop extracts and hop substances in treatment of menopausal complaints. *Planta Medica* [online]. 2013, **79**(7), 576–579. ISSN 00320943. Dostupné z: doi:10.1055/s-0032-1328330
109. SCHILLER, H., A. FORSTER, C. VONHOFF, M. HEGGER, A. BILLER a H. WINTERHOFF. Sedating effects of Humulus lupulus L. extracts. *Phytomedicine* [online]. 2006, **13**(8), 535–541. ISSN 09447113. Dostupné z: doi:10.1016/j.phymed.2006.05.010
110. SAHIN, Sümeyye, Volker EULENBURG, Wolfgang KREIS, Carmen VILLMANN a Monika PISCHETSRIEDER. Three-Step Test System for the Identification of Novel GABAA Receptor Modulating Food Plants. *Plant Foods for Human Nutrition* [online]. 2016, **71**(4), 355–360. ISSN 15739104. Dostupné z: doi:10.1007/s11130-016-0566-1
111. MEISSNER, Oliver a Hanns HÄBERLEIN. Influence of xanthohumol on the binding behavior of GABAA receptors and their lateral mobility at hippocampal neurons. *Planta Medica* [online]. 2006, **72**(7), 656–658. ISSN 00320943. Dostupné z: doi:10.1055/s-2006-931609
112. BENKHEROUF, Ali Y., Nora LOGRÉN, Tamara SOMBORAC, Maaria KORTESNIEMI, Sanna L. SOINI, Baoru YANG, Outi M.H. SALO-AHEN, Oskar LAAKSONEN a Mikko UUSI-OUKARI. Hops compounds modulatory effects and 6-prenylnaringenin dual mode of action on GABAA receptors. *European Journal of Pharmacology* [online]. 2020, **873**(January), 172962. ISSN 18790712. Dostupné z: doi:10.1016/j.ejphar.2020.172962
113. BENKHEROUF, Ali Y., Sanna L. SOINI, Monika STOMPOR a Mikko UUSI-OUKARI. Positive allosteric modulation of native and recombinant GABAA receptors by hops prenylflavonoids. *European Journal of Pharmacology* [online]. 2019, **852**(September 2018), 34–41. ISSN 18790712.

Dostupné z: doi:10.1016/j.ejphar.2019.02.034
114. ZANOLI, P., M. RIVASI, M. ZAVATTI, F. BRUSIANI a M. BARALDI. New insight in the neuropharmacological activity of Humulus lupulus L. *Journal of Ethnopharmacology* [online]. 2005, **102**(1), 102–106. ISSN 03788741. Dostupné z: doi:10.1016/j.jep.2005.05.040
115. GUZMÁN-GUTIÉRREZ, S. L., R. GÓMEZ-CANSINO, J. C. GARCÍA-ZEBADÚA, N. C. JIMÉNEZ-PÉREZ a R. REYES-CHILPA. Antidepressant activity of Litsea glaucescens essential oil: Identification of β-pinene and linalool as active principles. *Journal of Ethnopharmacology* [online]. 2012, **143**(2), 673–679. ISSN 03788741. Dostupné z: doi:10.1016/j.jep.2012.07.026
116. BLAND, Jeffrey S., Deanna MINICH, Robert LERMAN, Gary DARLAND, Joseph LAMB, Matthew TRIPP a Neile GRAYSON. Isohumulones from hops (Humulus lupulus) and their potential role in medical nutrition therapy. *PharmaNutrition* [online]. 2015, **3**(2), 46–52. ISSN 22134344. Dostupné z: doi:10.1016/j.phanu.2015.03.001
117. ANO, Yasuhisa, Ayaka HOSHI, Tatsuhiro AYABE, Rena OHYA, Shinichi UCHIDA, Koji YAMADA, Keiji KONDO, Shiho KITAOKA a Tomoyuki FURUYASHIKI. Iso-a-acids, the bitter components of beer, improve hippocampus-dependent memory through vagus nerve activation. *FASEB Journal* [online]. 2019, **33**(4), 4987–4995. ISSN 15306860. Dostupné z: doi:10.1096/fj.201801868RR
118. MORIMOTO-KOBAYASHI, Yumie, Kazuaki OHARA, Hiroshi ASHIGAI, Tomoka KANAYA, Kumiko KOIZUMI, Fumitoshi MANABE, Yuji KANEKO, Yoshimasa TANIGUCHI, Mikio KATAYAMA, Yasuyuki KOWATARI a Sumio KONDO. Matured hop extract reduces body fat in healthy overweight humans: A randomized, double-blind, placebo-controlled parallel group study. *Nutrition Journal* [online]. 2016, **15**(1), 1–13. ISSN 14752891. Dostupné z: doi:10.1186/s12937-016-0144-2
119. WALKER, Jessica, Johannes HELL, Kathrin I. LISZT, Michael DRESEL, Marc PIGNITTER, Thomas HOFMANN a Veronika SOMOZA. Identification of beer bitter acids regulating mechanisms of gastric acid secretion. *Journal of Agricultural and Food Chemistry* [online]. 2012, **60**(6), 1405–1412. ISSN 00218561. Dostupné z: doi:10.1021/jf204306z
120. MAHAPATRA, Debarshi Kar a Sanjay Kumar BHARTI. Therapeutic potential of chalcones as cardiovascular agents. *Life Sciences* [online]. 2016, **148**, 154–172. ISSN 18790631. Dostupné z: doi:10.1016/j.lfs.2016.02.048
121. CASASCHI, Adele, Geoffrey K. MAIYOH, Brent K. RUBIO, Rachel W. LI, Khosrow ADELI a Andre G. THERIAULT. The Chalcone Xanthohumol Inhibits Triglyceride and Apolipoprotein B Secretion in HepG2 Cells. *The Journal of Nutrition* [online]. 2004, **134**(6), 1340–1346. ISSN 0022-3166. Dostupné z: doi:10.1093/jn/134.6.1340
122. LIU, Ming, Hua YIN, Ge LIU, Jianjun DONG, Zhonghua QIAN a Jinlai MIAO. Xanthohumol, a prenylated chalcone from beer hops, acts as an α-glucosidase inhibitor in vitro. *Journal of Agricultural and Food Chemistry* [online]. 2014, **62**(24), 5548–5554. ISSN 15205118. Dostupné z: doi:10.1021/jf500426z
123. LUÍS, Carla, Raquel COSTA, Ilda RODRIGUES, Ângela CASTELA, Pedro COELHO, Susana GUERREIRO, Joana GOMES, Celso REIS a Raquel SOARES. Xanthohumol and 8-prenylnaringenin reduce type 2 diabetes–associated oxidative stress by downregulating galectin-3. *Porto Biomedical Journal* [online]. 2019, **4**(1). ISSN 2444-8664. Dostupné z: https://journals.lww.com/pbj/Fulltext/2019/02000/Xanthohumol_and_8_prenylnaringenin_reduce_type_2.1.aspx
124. DI VITO, Clara, Alessandra BERTONI, Michela NALIN, Sara SAMPIETRO, Manuela ZANFA a Fabiola SINIGAGLIA. The phytoestrogen 8-prenylnaringenin inhibits agonist-dependent activation of human platelets. *Biochimica et Biophysica Acta - General Subjects* [online]. 2012, **1820**(11), 1724–1733. ISSN 03044165. Dostupné z: doi:10.1016/j.bbagen.2012.06.018
125. GRANT, Herbert L. United States Patent Office. 1974, 8–10.
126. DŪDĖNAS, Mantas, Žydrūnas STANIUS, Vilma KAŠKONIENĖ, Audrius MARUŠKA, Ona RAGAŽINSKIENĖ a Kęstutis OBELEVIČIUS. The influence of various organic solvents and their concentrations for the extraction efficiency of phenolic compounds from Hop (Humulus lupulus L.) leaves and cones. In: [online]. 2019, s. 90. Dostupné z: https://hdl.handle.net/20.500.12259/99473
127. KARABÍN, Marcel, Vojtěch HANKO, Jakub NEŠPOR, Lukáš JELÍNEK a Pavel DOSTÁLEK. Hop tannin extract: a promising tool for acceleration of lautering. *Journal of the Institute of Brewing* [online]. 2018, **124**(4), 374–380. ISSN 20500416. Dostupné z: doi:10.1002/jib.502
128. JELIAZKOVA, Ekaterina, Valtcho D. ZHELJAZKOV, Miroslava KAČÁNIOVA, Tess ASTATKIE a Babu L. TEKWANI. Sequential elution of essential oil constituents during steam distillation of hops

(Humulus lupulus L.) and influence on oil yield and antimicrobial activity. *Journal of Oleo Science* [online]. 2018, **67**(7), 871–883. ISSN 13473352. Dostupné z: doi:10.5650/jos.ess17216

129. VÁZQUEZ-ARAÚJO, Laura, Raquel RODRÍGUEZ-SOLANA, Sandra M. CORTÉS-DIÉGUEZ a José M. DOMÍNGUEZ. Use of hydrodistillation and headspace solid-phase microextraction to characterize the volatile composition of different hop cultivars. *Journal of the Science of Food and Agriculture* [online]. 2013, **93**(10), 2568–2574. ISSN 00225142. Dostupné z: doi:10.1002/jsfa.6078
130. EYRES, Graham a Jean Pierre DUFOUR. Hop essential oil: Analysis, chemical composition and odor characteristics. *Beer in Health and Disease Prevention* [online]. 2008, 239–254. Dostupné z: doi:10.1016/b978-0-12-373891-2.00022-5
131. RAPINEL, Vincent, Aziadé CHEMAT, Cyrille SANTERRE, Justine BELAY, Farnaz HANAEI, Nadine VALLET, Laurence JACQUES a Anne Sylvie FABIANO-TIXIER. 2-Methyloxolane As a Bio-Based Solvent for Green Extraction of Aromas From Hops (Humulus Lupulus L.). *Molecules* [online]. 2020, **25**(7). ISSN 14203049. Dostupné z: doi:10.3390/molecules25071727
132. RETTBERG, Nils a Leif GARBE. Starting hop lipidomics – Isolation and characterization of non-polar , neutral and. 2013, (March 2019).
133. TYŚKIEWICZ, Katarzyna, Roman GIEYSZTOR, Marcin KONKOL, Jan SZAŁAS a Edward RÓJ. Essential oils from Humulus lupulus scCO2 extract by hydrodistillation and microwave-assisted hydrodistillation. *Molecules* [online]. 2018, **23**(11). ISSN 14203049. Dostupné z: doi:10.3390/molecules23112866
134. BARTOSZ, Grzegorz a Miguel HERRERO. *Case Studies on Selected Natural Food Antioxidants* [online]. 2018. ISBN 9781439882412. Dostupné z: doi:10.1201/b15062-20
135. LAKKA, Achillia, Ioanna KARAGEORGOU, Olga KALTSA, Georgia BATRA, Eleni BOZINOU, Stavros LALAS a Dimitris MAKRIS. Polyphenol Extraction from Humulus lupulus (Hop) Using a Neoteric Glycerol/L-Alanine Deep Eutectic Solvent: Optimisation, Kinetics and the Effect of Ultrasound-Assisted Pretreatment. *AgriEngineering* [online]. 2019, **1**(3), 403–417. Dostupné z: doi:10.3390/agriengineering1030030
136. FDA. *FDA - GRAS - Carbon dioxide* [online]. Dostupné z: https://ecfr.io/Title-21/Section-184.1240
137. SKALA, Dejan a Irena ZIZOVIC. Supercritical fluid extraction: Application in the food industry [online]. 2010, (January 2002), 2008–2010. Dostupné z: doi:10.2298/HEMIND0205179S
138. DANLAMI, Jibrin Mohammed, Agus ARSAD, Muhammad Abbas Ahmad ZAINI a Hanizam SULAIMAN. A comparative study of various oil extraction techniques from plants. *Reviews in Chemical Engineering* [online]. 2014, **30**(6), 605–626. ISSN 01678299. Dostupné z: doi:10.1515/revce-2013-0038
139. ROVETTO, Laura J a Niccolo V AIETA. Supercritical carbon dioxide extraction of cannabinoids from Cannabis sativa L . plant material The Journal of Supercritical Fluids Supercritical carbon dioxide extraction of cannabinoids from Cannabis. *The Journal of Supercritical Fluids* [online]. 2017, **129**(March), 16–27. ISSN 0896-8446. Dostupné z: doi:10.1016/j.supflu.2017.03.014
140. LACK, E a H SEIDLITZ. 5 Commercial scale decaffeination of coffee and tea using supercritical C02. 1993.
141. DZINGELEVIČIUS, Nerijus, Audrius MARUŠKA, Ona RAGAŽINSKIENĖ a Kęstutis OBELEVIČIUS. Optimization of hop essential oil extraction by means of supercritical CO2. *Biologija* [online]. 2011, **57**(2), 63–69. ISSN 1392-0146. Dostupné z: doi:10.6001/biologija.v57i2.1830
142. ESSIEN, Sinemobong O., Brent YOUNG a Saeid BAROUTIAN. Recent advances in subcritical water and supercritical carbon dioxide extraction of bioactive compounds from plant materials. *Trends in Food Science and Technology* [online]. 2020, **97**(January), 156–169. ISSN 09242244. Dostupné z: doi:10.1016/j.tifs.2020.01.014
143. VEIGA, Bruno Aguilar, Fabiane HAMERSKI, Mathias Porsmose CLAUSEN, Massimiliano ERRICO, Agnes DE PAULA SCHEER a Marcos Lúcio CORAZZA. Compressed fluids extraction methods, yields, antioxidant activities, total phenolics and flavonoids content for Brazilian Mantiqueira hops. *Journal of Supercritical Fluids* [online]. 2021, **170**(May 2020), 105155. ISSN 08968446. Dostupné z: doi:10.1016/j.supflu.2020.105155
144. KUPSKI, Sérgio Carlos, Elissandro Jair KLEIN, Edson Antônio DA SILVA, Fernando PALÚ, Reginaldo GUIRARDELLO a Melissa Gurgel Adeodato VIEIRA. Mathematical modeling of supercritical CO2 extraction of hops (Humulus lupulus L.). *Journal of Supercritical Fluids* [online]. 2017, **130**(June), 347–356. ISSN 08968446. Dostupné z: doi:10.1016/j.supflu.2017.06.011
145. BYELASHOV, Alex. *United States (12) Patent Application Publication (10) Pub*. 2018.
146. LAWS, D. R.J., N. A. BATH, J. A. PICKETT, C. S. ENNIS a A. G. WHELDON. Preparation of Hop

Extracts Without Using Organic Solvents. *Journal of the Institute of Brewing* [online]. 1977, **83**(1), 39–40. ISSN 20500416. Dostupné z: doi:10.1002/j.2050-0416.1975.tb03791.x

147. GOIRIS, Koen, Marjan DE RIDDER, Gert DE ROUCK, Annick BOEYKENS, Filip VAN OPSTAELE, Guido AERTS, Luc DE COOMAN a Denis DE KEUKELEIRE. The Oxygenated Sesquiterpenoid Fraction of Hops in Relation to the Spicy Hop Character of Beer. *Journal of the Institute of Brewing* [online]. 2002, **108**(1), 86–93. ISSN 00469750. Dostupné z: doi:10.1002/j.2050-0416.2002.tb00129.x
148. JACKOWSKI, J., M. HUREJ, E. RÓJ, J. POPŁOŃSKI, L. KOŠ NY a E. HUSZCZA. Antifeedant activity of xanthohumol and supercritical carbon dioxide extract of spent hops against stored product pests. *Bulletin of Entomological Research* [online]. 2015, **105**(4), 456–461. ISSN 14752670. Dostupné z: doi:10.1017/S0007485315000255
149. FORSTER, D. A, J. SCHULMEYR, R. SCHMIDT, K. SIMON, M. KETTERER a B. FORCHHAMMER. Method for preparing a xanthohumol enriched hop extract and use thereof. EP1424385. 2003.
150. GUO-QING, H E, Xiong HAO-PING, Chen QI-HE a Ruan HUI. Optimization of conditions for supercritical fluid extraction of flavonoids from hops (Humulus lupulus L .) * [online]. 2005, (10), 999–1004. Dostupné z: doi:10.1631/jzus.2005.B0999
151. RÓJ, Edward, Vanja M. TADIĆ, Dušan MIŠIĆ, Irena ŽIŽOVIĆ, Ivana ARSIĆ, Agnieszka DOBRZYŃSKA-INGER a Dorota KOSTRZEWA. Supercritical carbon dioxide hops extracts with antimicrobial properties. *Open Chemistry* [online]. 2015, **13**(1), 1157–1171. ISSN 23915420. Dostupné z: doi:10.1515/chem-2015-0131
152. DZINGELEVIČIUS, Nerijus, Audrius MARUŠKA, Ona RAGAŽINSKIENĖ a Kęstutis OBELEVIČIUS. Optimization of hop essential oil extraction by means of supercritical CO2. *Biologija* [online]. 2011, **57**(2), 63–69. ISSN 1392-0146. Dostupné z: doi:10.6001/biologija.v57i2.1830
153. FUMINORI, Katono, Yonezawa DAISAKU a Inui TAKAKO. *HOP EXTRACT AND METHOD FOR PRODUCING SAME*. 2018.
154. DEL VALLE, José M., Oscar RIVERA, Osvaldo TEUBER a M. Teresa PALMA. Supercritical CO2 extraction of Chilean hop (Humulus lupulus) ecotypes. *Journal of the Science of Food and Agriculture* [online]. 2003, **83**(13), 1349–1356. ISSN 00225142. Dostupné z: doi:10.1002/jsfa.1547
155. LANGEZAAL, Carlos R., Amitabh CHANDRA, Stavros T. KATSIOTIS, Johannes J.C. SCHEFFER a Andre B. DE HAAN. Analysis of supercritical carbon dioxide extracts from cones and leaves of a Humulus lupulus L cultivar. *Journal of the Science of Food and Agriculture* [online]. 1990, **53**(4), 455–463. ISSN 10970010. Dostupné z: doi:10.1002/jsfa.2740530404
156. TYŚKIEWICZ, Katarzyna, Marcin KONKOL a R EDWARD. The Application of Supercritical Fluid Extraction in Phenolic Compounds Isolation from Natural Plant Materials [online]. 2018. Dostupné z: doi:10.3390/molecules23102625
157. PLAZA, Merichel a Charlotta TURNER. Pressurized hot water extraction of bioactives. *TrAC - Trends in Analytical Chemistry* [online]. 2015, **71**, 39–54. ISSN 18793142. Dostupné z: doi:10.1016/j.trac.2015.02.022
158. MUSTAFA, Arwa a Charlotta TURNER. Pressurized liquid extraction as a green approach in food and herbal plants extraction: A review. *Analytica Chimica Acta* [online]. 2011, **703**(1), 8–18. ISSN 00032670. Dostupné z: doi:10.1016/j.aca.2011.07.018
159. ČULÍK, Jiří, Marie JURKOVÁ, Tomáš HORÁK, Pavel ČEJKA, Vladimír KELLNER, Josef DVOŘÁK, Pavel KARÁSEK a Michal ROTH. Extraction of bitter acids from hops and hop products using Pressurized Solvent Extraction (PSE). *Journal of the Institute of Brewing* [online]. 2009, **115**(3), 220–225. ISSN 00469750. Dostupné z: doi:10.1002/j.2050-0416.2009.tb00372.x
160. LIGOR, Magdalena, Mantas STANKEVIČIUS, Anna WENDA-PIESIK, Kestutis OBELEVIČIUS, Ona RAGAŽINSKIENE, Žydrunas STANIUS, Audrius MARUŠKA a Bogusław BUSZEWSKI. Comparative Gas Chromatographic-Mass Spectrometric Evaluation of Hop (Humulus lupulus L.) Essential Oils and Extracts Obtained Using Different Sample Preparation Methods. *Food Analytical Methods* [online]. 2014, **7**(7), 1433–1442. ISSN 1936976X. Dostupné z: doi:10.1007/s12161-013-9767-5
161. GIL-RAMÍREZ, Alicia, José Antonio MENDIOLA, Elena ARRANZ, Alejandro RUÍZ-RODRÍGUEZ, Guillermo REGLERO, Elena IBÁÑEZ a Francisco R. MARÍN. Highly isoxanthohumol enriched hop extract obtained by pressurized hot water extraction (PHWE). Chemical and functional characterization. *Innovative Food Science and Emerging Technologies* [online]. 2012, **16**, 54–60. ISSN 14668564. Dostupné z: doi:10.1016/j.ifset.2012.04.006

162. KRAUSE, Elizabeth, Yang YUAN, Atieh HAJIRAHIMKHAN, Huali DONG, Birgit M. DIETZ, Dejan NIKOLIC, Guido F. PAULI, Judy L. BOLTON a Richard B. VAN BREEMEN. Biological and chemical standardization of a hop (Humulus lupulus) botanical dietary supplement. *Biomedical Chromatography* [online]. 2014, **28**(6), 729–734. ISSN 10990801. Dostupné z: doi:10.1002/bmc.3177
163. SYRPAS, Michail, Jolita BUKAUSKAITĖ, Ričardas PAŠKAUSKAS, Loreta BAŠINSKIENĖ a Petras Rimantas VENSKUTONIS. Recovery of lipophilic products from wild cyanobacteria (Aphanizomenon flos-aquae) isolated from the Curonian Lagoon by means of supercritical carbon dioxide extraction. *Algal Research* [online]. 2018, **35**, 10–21. ISSN 22119264. Dostupné z: doi:10.1016/j.algal.2018.08.006
164. KITRYTE, V., A. LAURINAVIČIENE, M. SYRPAS, A. PUKALSKAS a P.R. VENSKUTONIS. Modeling and optimization of supercritical carbon dioxide extraction for isolation of valuable lipophilic constituents from elderberry (Sambucus nigra L.) pomace. *Journal of CO2 Utilization* [online]. 2020, **35**. ISSN 22129820. Dostupné z: doi:10.1016/j.jcou.2019.09.020
165. KITRYTĖ, Vaida, Aistė NARKEVIČIŪTĖ, Laura TAMKUTĖ, Michail SYRPAS, Milda PUKALSKIENĖ a Petras Rimantas VENSKUTONIS. Consecutive high-pressure and enzyme assisted fractionation of blackberry (Rubus fruticosus L.) pomace into functional ingredients: Process optimization and product characterization. *Food Chemistry* [online]. 2020, **312**. ISSN 18737072. Dostupné z: doi:10.1016/j.foodchem.2019.126072
166. L. PRIOR, Ronald, Ha HOANG, Liwei GU, Xianli WU, Mara BACCHIOCCA, Luke HOWARD, Maureen HAMPSCH-WOODILL, Dejian HUANG, Boxin OU a Robert JACOB. Assays for Hydrophilic and Lipophilic Antioxidant Capacity (oxygen radical absorbance capacity (ORACFL)) of Plasma and Other Biological and Food Samples. *Journal of Agricultural and Food Chemistry* [online]. 2003, **51**(11), 3273–3279. Dostupné z: doi:10.1021/jf0262256
167. RE, Roberta, Nicoletta PELLEGRINI, Anna PROTEGGENTE, Ananth PANNALA, Min YANG a Catherine RICE-EVANS. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine* [online]. 1999, **26**(9), 1231–1237. ISSN 0891-5849. Dostupné z: doi:https://doi.org/10.1016/S0891-5849(98)00315-3
168. LICHTENTHALER, Hartmut K. a Claus BUSCHMANN. Chlorophylls and Carotenoids: Measurement and Characterization by UV-VIS Spectroscopy. *Current Protocols in Food Analytical Chemistry* [online]. 2001, **1**(1), F4.3.1-F4.3.8. ISSN 25725599. Dostupné z: doi:10.1002/0471142913.faf0403s01
169. MARTINS, Zita E., Júlio C. MACHADO, Sara C. CUNHA, Ana Maria BARATA a Isabel M.P.L.V.O. FERREIRA. A chemometric approach to compare Portuguese native hops with worldwide commercial varieties. *Journal of Chemometrics* [online]. 2020, (September 2019), 1–16. ISSN 1099128X. Dostupné z: doi:10.1002/cem.3285
170. RALI, Topul, Stewart WOSSA, David LEACH a Peter WATERMAN. Volatile Chemical Constituents of Piper aduncum L and Piper gibbilimbum C. DC (Piperaceae) from Papua New Guinea. *Molecules* [online]. 2007, **12**(3), 389–394. ISSN 1420-3049. Dostupné z: doi:10.3390/12030389
171. YAN, Dan Dan, Yong Foo WONG, Robert A. SHELLIE, Philip J. MARRIOTT, Simon P. WHITTOCK a Anthony KOUTOULIS. Assessment of the phytochemical profiles of novel hop (Humulus lupulus L.) cultivars: A potential route to beer crafting. *Food Chemistry* [online]. 2019, **275**(September 2018), 15–23. ISSN 18737072. Dostupné z: doi:10.1016/j.foodchem.2018.09.082
172. FRIZZO, C. D., L. A. SERAFINI, E. DELLACASSA, D. LORENZO a P. MOYNA. Essential oil of Baccharis uncinella DC. From Southern Brazil. *Flavour and Fragrance Journal* [online]. 2001, **16**(4), 286–288. ISSN 08825734. Dostupné z: doi:10.1002/ffj.998
173. ADAMS, Robert.P. Identification of Essential Oil Components by Gas Chromatograpy/Mass Spectrometry, 4th Edition. *Illinois USA: Allured Publishing Corporation, Carol Stream*. 2007, 804–806.
174. BRENDEL, Sabrina, Thomas HOFMANN a Michael GRANVOGL. Characterization of Key Aroma Compounds in Pellets of Different Hop Varieties (Humulus lupulus L.) by Means of the Sensomics Approach. *Journal of Agricultural and Food Chemistry* [online]. 2019. ISSN 15205118. Dostupné z: doi:10.1021/acs.jafc.9b05174
175. ALISSANDRAKIS, Eleftherios, Petros A. TARANTILIS, Paschalis C. HARIZANIS a Moschos POLISSIOU. Comparison of the volatile composition in thyme honeys from several origins in Greece. *Journal of Agricultural and Food Chemistry* [online]. 2007, **55**(20), 8152–8157. ISSN 00218561. Dostupné z: doi:10.1021/jf071442y
176. DHOOGHE, Liene, Tania NAESSENS, Arne HEYERICK, Denis DE KEUKELEIRE, Arnold J.

VLIETINCK, Luc PIETERS a Sandra APERS. Quantification of xanthohumol, isoxanthohumol, 8-prenylnaringenin, and 6-prenylnaringenin in hop extracts and derived capsules using secondary standards. *Talanta* [online]. 2010, **83**(2), 448–456 [vid. 2019-11-19]. ISSN 00399140. Dostupné z: doi:10.1016/j.talanta.2010.09.041

177. LOŽIENE, Kristina, Petras R. VENSKUTONIS, Aušra ŠIPAILIENE a Juozas LABOKAS. Radical scavenging and antibacterial properties of the extracts from different Thymus pulegioides L. chemotypes. *Food Chemistry* [online]. 2007, **103**(2), 546–559. ISSN 03088146. Dostupné z: doi:10.1016/j.foodchem.2006.08.027
178. VAN MEERLOO, Johan, Gertjan J L KASPERS a Jacqueline CLOOS. Cell sensitivity assays: the MTT assay. *Methods in molecular biology (Clifton, N.J.)* [online]. 2011, **731**, 237–245. ISSN 1940-6029 (Electronic). Dostupné z: doi:10.1007/978-1-61779-080-5_20
179. SYRPAS, Michail, Jolita BUKAUSKAITĖ, Kristina RAMANAUSKIENĖ, Jū Ratė KAROSIENĖ, Daiva MAJIENĖ, Loreta BAŠINSKIENĖ a Petras Rimantas VENSKUTONIS. Ultrasound-Assisted Extraction and Assessment of Biological Activity of Phycobiliprotein-Rich Aqueous Extracts from Wild Cyanobacteria (Aphanizomenon flos-aquae). *Journal of Agricultural and Food Chemistry* [online]. 2019. ISSN 15205118. Dostupné z: doi:10.1021/acs.jafc.9b05483
180. SYRPAS, Michail, Kiran SUBBARAYADU, Vaida KITRYTĖ a Petras Rimantas VENSKUTONIS. High-pressure extraction of antioxidant-rich fractions from Shrubby cinquefoil (Dasiphora fruticosa L. Rydb.) leaves: Process optimization and extract characterization. *Antioxidants* [online]. 2020, **9**(6). ISSN 20763921. Dostupné z: doi:10.3390/antiox9060457
181. SCHUSTER, Julian Jonathan, Leo Alexander BAHR, Leonhard FEHR, Marlene TIPPELT, Josef SCHULMEYR, Andreas WUZIK a Andreas Siegfried BRAEUER. Online monitoring of the supercritical CO2 extraction of hop. *Journal of Supercritical Fluids* [online]. 2018, **133**(August 2017), 139–145. ISSN 08968446. Dostupné z: doi:10.1016/j.supflu.2017.10.006
182. PRIOR, Ronald L. Oxygen radical absorbance capacity (ORAC): New horizons in relating dietary antioxidants/bioactives and health benefits. *Journal of Functional Foods* [online]. 2015, **18**, 797–810. ISSN 17564646. Dostupné z: doi:10.1016/j.jff.2014.12.018
183. PÉREZ-GÁLVEZ, Antonio, Isabel VIERA a María ROCA. Carotenoids and chlorophylls as antioxidants. *Antioxidants* [online]. 2020, **9**(6), 1–39. ISSN 20763921. Dostupné z: doi:10.3390/antiox9060505
184. KOBUS-CISOWSKA, Joanna, Daria SZYMANOWSKA-POWAŁOWSKA, Oskar SZCZEPANIAK, Dominik KMIECIK, Monika PRZEOR, Anna GRAMZA-MICHAŁOWSKA, Judyta CIELECKA-PIONTEK, Małgorzata SMUGA-KOGUT a Piotr SZULC. Composition and in vitro effects of cultivars of humulus lupulus L. Hops on cholinesterase activity and microbial growth. *Nutrients* [online]. 2019, **11**(6). ISSN 20726643. Dostupné z: doi:10.3390/nu11061377
185. MASEK, Anna, Ewa CHRZESCIJANSKA, Anna KOSMALSKA a Marian ZABORSKI. Characteristics of compounds in hops using cyclic voltammetry, UV-VIS, FTIR and GC-MS analysis. *Food Chemistry* [online]. 2014, **156**, 353–361. ISSN 03088146. Dostupné z: doi:10.1016/j.foodchem.2014.02.005
186. DIETZ, Christina, David COOK, Colin WILSON, Ray MARRIOTT a Rebecca FORD. Sensory properties of supercritical CO2 fractions extracted from Magnum hop essential oil. *Journal of the Institute of Brewing* [online]. 2020, **126**(3), 263–279. ISSN 20500416. Dostupné z: doi:10.1002/jib.612
187. DUARTE, Lucas Mattos, Tatiane Lima AMORIM, Richard Michael GRAZUL a Marcone Augusto Leal DE OLIVEIRA. Differentiation of aromatic, bittering and dual-purpose commercial hops from their terpenic profiles: An approach involving batch extraction, GC–MS and multivariate analysis. *Food Research International* [online]. 2020, **138**. ISSN 18737145. Dostupné z: doi:10.1016/j.foodres.2020.109768
188. VAN OPSTAELE, Filip, Koen GOIRIS, Gert DE ROUCK, Guido AERTS a Luc DE COOMAN. Production of novel varietal hop aromas by supercritical fluid extraction of hop pellets - Part 2: Preparation of single variety floral, citrus, and spicy hop oil essences by density programmed supercritical fluid extraction. *Journal of Supercritical Fluids* [online]. 2012, **71**, 147–161. ISSN 08968446. Dostupné z: doi:10.1016/j.supflu.2012.06.004
189. VAN OPSTAELE, Filip, Brecht DE CAUSMAECKER, Guido AERTS a Luc DE COOMAN. Characterization of novel varietal floral hop aromas by headspace solid phase microextraction and gas chromatography-mass spectrometry/olfactometry. *Journal of Agricultural and Food Chemistry* [online]. 2012, **60**(50), 12270–12281. ISSN 00218561. Dostupné z: doi:10.1021/jf304421d
190. YAN, Dan Dan, Yong Foo WONG, Robert A. SHELLIE, Philip J. MARRIOTT, Simon P.

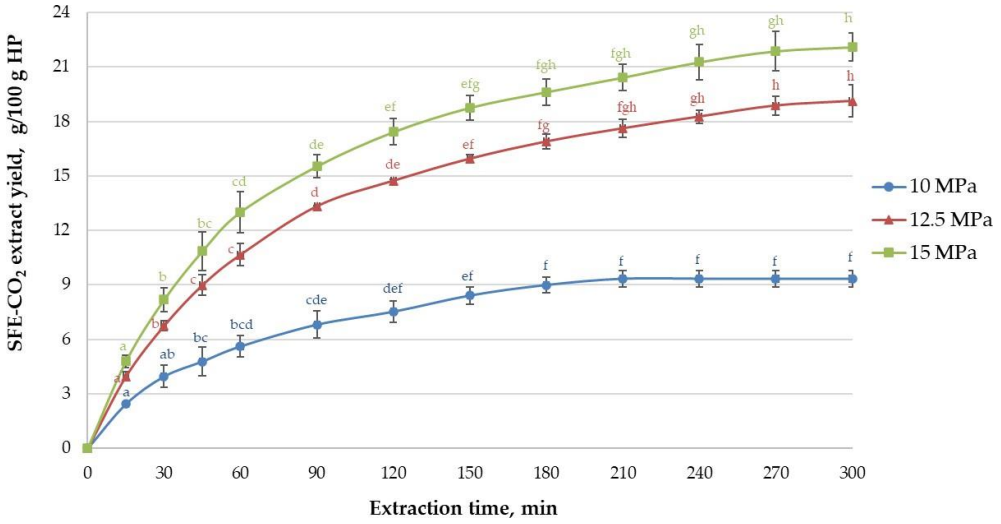
WHITTOCK a Anthony KOUTOULIS. Assessment of the phytochemical profiles of novel hop (Humulus lupulus L.) cultivars: A potential route to beer crafting. *Food Chemistry* [online]. 2019, **275**(September 2018), 15–23. ISSN 18737072. Dostupné z: doi:10.1016/j.foodchem.2018.09.082
191. AYDAR, Alev. Statistical Methods in Optimization of Food Materials. In: . 2018.
192. *Ella variety - Beermaverick* [online]. Dostupné z: https://beermaverick.com/hop/ella/
193. SALANTA, Liana-claudia, Maria TOFANA a Sonia SOCACI. Hop Pellets Bitter Acids -Comparison of Different Determination Methods Hop Pellets Bitter Acids - Comparison of Different Determination Methods. 2011, (April 2015).
194. VIDMAR, Mateja, Mario HERCEZI, Neda LAZI, Veronika ABRAM, Barbara CEH, Valentina BUCIK, Sonja Smole MOˇ, Iztok J KOˇ, Milica KAˇ a Lea DEMˇ. A comparison of antioxidant and antimicrobial activity between hop leaves and hop cones [online]. 2015, **64**, 124–134. Dostupné z: doi:10.1016/j.indcrop.2014.11.008
195. KRAMER, B., J. THIELMANN, A. HICKISCH, P. MURANYI, J. WUNDERLICH a C. HAUSER. Antimicrobial activity of hop extracts against foodborne pathogens for meat applications. *Journal of Applied Microbiology* [online]. 2015, **118**(3), 648–657. ISSN 13652672. Dostupné z: doi:10.1111/jam.12717
196. STAVRI, Michael, Ruth SCHNEIDER, Gemma O'DONNELL, Doris LECHNER, Franz BUCAR a Simon GIBBONS. The antimycobacterial components of hops (Humulus lupulus) and their dereplication. *Phytotherapy Research* [online]. 2004, **18**(9), 774–776. ISSN 0951418X. Dostupné z: doi:10.1002/ptr.1527
197. WASILEWSKI, Tomasz, Dominik CZERWONKA, Urszula PIOTROWSKA, Artur SEWERYN, Zofia NIZIOŁ-ŁUKASZEWSKA a Marcin SOBCZAK. Use of hop cone extract obtained under supercritical co2 conditions for producing antibacterial all-purpose cleaners. *Green Chemistry Letters and Reviews* [online]. 2018, **11**(4), 419–428. ISSN 17517192. Dostupné z: doi:10.1080/17518253.2018.1526975
198. XU, Dan, Ting CHEN a Yumei LIU. The physical properties, antioxidant and antimicrobial activity of chitosan–gelatin edible films incorporated with the extract from hop plant. *Polymer Bulletin* [online]. 2020, (0123456789). ISSN 14362449. Dostupné z: doi:10.1007/s00289-020-03294-1
199. VENUS, Matt, Jacqueline WATERMAN a Ian MCNAB. Basic physiology of the skin. *Surgery (Oxford)* [online]. 2010, **28**(10), 469–472. ISSN 0263-9319. Dostupné z: doi:https://doi.org/10.1016/j.mpsur.2010.07.011
200. PENTA, Dhanamjai, Bagganahalli S SOMASHEKAR a Syed MUSTHAPA. Epigenetics of skin cancer : Interventions by selected bioactive phytochemicals [online]. 2018, (September 2017), 42–49. Dostupné z: doi:10.1111/phpp.12353
201. PHILIPS, Neena, Philips SAMUEL, Tiana LOZANO, Ana GVALADZE, Benito GUZMAN, Halyna SIOMYK a Gerhard HAAS. Madridge Effects of Humulus lupulus extract or its Components on Viability , Lipid Peroxidation , and expression of Vascular Endothelial Growth Factor in Melanoma Cells and Fibroblasts [online]. nedatováno, **1**(1), 15–19. Dostupné z: doi:10.18689/mjcr-1000103
202. CLARKE, Coby J., Wei Chien TU, Oliver LEVERS, Andreas BRÖHL a Jason P. HALLETT. Green and Sustainable Solvents in Chemical Processes. *Chemical Reviews* [online]. 2018, **118**(2), 747–800. ISSN 15206890. Dostupné z: doi:10.1021/acs.chemrev.7b00571
203. CHADWICK, L R, G F PAULI a N R FARNSWORTH. The pharmacognosy of Humulus lupulus L. (hops) with an emphasis on estrogenic properties. *Phytomedicine : international journal of phytotherapy and phytopharmacology* [online]. 2006, **13**(1–2), 119–131. ISSN 0944-7113 (Print). Dostupné z: doi:10.1016/j.phymed.2004.07.006
204. CARBONE, Katya, Valentina MACCHIONI, Greta PETRELLA a Daniel Oscar CICERO. Exploring the potential of microwaves and ultrasounds in the green extraction of bioactive compounds from Humulus lupulus for the food and pharmaceutical industry. *Industrial Crops and Products* [online]. 2020, **156**(April), 112888. ISSN 09266690. Dostupné z: doi:10.1016/j.indcrop.2020.112888
205. CARVALHO, Daniel a Luis F GUIDO. Fundamentals and Health Benefits of Xanthohumol , a Natural Product Derived from Hops and Beer [online]. 2009, (June). Dostupné z: doi:10.1177/1934578X0900400501
206. ŠTULÍKOVÁ, Kateřina, Marcel KARABÍN, Jakub NEŠPOR a Pavel DOSTÁLEK. Therapeutic perspectives of 8-prenylnaringenin, a potent phytoestrogen from hops. *Molecules* [online]. 2018, **23**(3). ISSN 14203049. Dostupné z: doi:10.3390/molecules23030660
207. ALMEIDA, Aline da Rosa, Matheus Vinícius de Oliveira Brisola MACIEL, Michelle Heck MACHADO, Giovana Carolina BAZZO, Rafael Dutra DE ARMAS, Vinicius Bittencourt VITORINO,

Luciano VITALI, Jane Mara BLOCK a Pedro Luiz Manique BARRETO. Bioactive compounds and antioxidant activities of Brazilian hop (Humulus lupulus L.) extracts. *International Journal of Food Science and Technology* [online]. 2020, **55**(1), 340–347. ISSN 13652621. Dostupné z: doi:10.1111/ijfs.14311

208. PEREZ, D D, F LEIGHTON, A ASPEE, C ALIAGA a E LISSI. A comparison of methods employed to evaluate antioxidant capabilities. *Biological research* [online]. 2000, **33**(2), 71–77. ISSN 0716-9760 (Print). Dostupné z: doi:10.4067/s0716-97602000000200006
209. ZULUETA, Ana, Maria J. ESTEVE a Ana FRÍGOLA. ORAC and TEAC assays comparison to measure the antioxidant capacity of food products. *Food Chemistry* [online]. 2009, **114**(1), 310–316. ISSN 03088146. Dostupné z: doi:10.1016/j.foodchem.2008.09.033
210. BÖHM, Fritz, Ruth EDGE a T George TRUSCOTT. Interactions of dietary carotenoids with singlet oxygen (1O2) and free radicals: potential effects for human health. *Acta biochimica Polonica*. 2012, **59**(1), 27–30. ISSN 1734-154X (Electronic).
211. STAHL, Wilhelm a Helmut SIES. Antioxidant activity of carotenoids. *Molecular aspects of medicine* [online]. 2004, **24**, 345–351. Dostupné z: doi:10.1016/S0098-2997(03)00030-X
212. ABRAM, Veronika, Barbara ČEH, Mateja VIDMAR, Mario HERCEZI, Neda LAZIĆ, Valentina BUCIK, Sonja Smole MOŽINA, Iztok J. KOŠIR, Milica KAČ, Lea DEMŠAR a Nataša POKLAR ULRIH. A comparison of antioxidant and antimicrobial activity between hop leaves and hop cones. *Industrial Crops and Products* [online]. 2015, **64**, 124–134. ISSN 09266690. Dostupné z: doi:10.1016/j.indcrop.2014.11.008
213. KOBUS-CISOWSKA, Joanna, Daria SZYMANOWSKA-POWAŁOWSKA, Oskar SZCZEPANIAK, Judyta CIELECKA-PIONTEK, Małgorzata SMUGA-KOGUT a Piotr SZULC. Composition and In Vitro Effects of Cultivars of Humulus lupulus L. Hops on Cholinesterase Activity and Microbial Growth. 2019, 1–14.
214. DI LODOVICO, Silvia, Luigi MENGHINI, Claudio FERRANTE, Erica RECCHIA, Juliana CASTRO-AMORIM, Paula GAMEIRO, Luigina CELLINI a Lucinda J. BESSA. Hop Extract: An Efficacious Antimicrobial and Anti-biofilm Agent Against Multidrug-Resistant Staphylococci Strains and Cutibacterium acnes. *Frontiers in Microbiology* [online]. 2020, **11**(August), 1–12. ISSN 1664302X. Dostupné z: doi:10.3389/fmicb.2020.01852
215. BHATTACHARYA, Sanchita, Salima VIRANI, Mashenka ZAVRO a Gerhard J. HAAS. Inhibition of Streptococcus mutans and other oral Streptococci by hop (Humulus lupulus L.) constituents. *Economic Botany* [online]. 2003, **57**(1), 118–125. ISSN 00130001. Dostupné z: doi:10.1663/0013-0001(2003)057[0118:IOSMAO]2.0.CO;2

Appendices

Appendix 1. The influence of the extraction time on the *Ella* hop SFE-CO_2 extract yield (g/100 g pellets) at 10-15 MPa pressure at 40 °C. SFE-CO_2: supercritical carbon dioxide extraction. Different superscript letters indicate significant differences for each graph individually (one-way ANOVA and Tukey`s test $p < 0.05$).

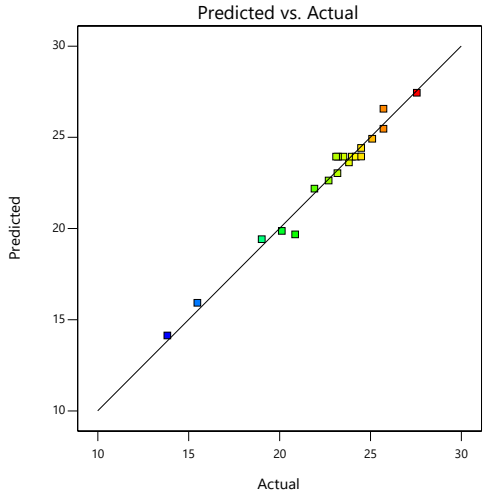


Appendix 2. Fit statistics parameters for the quadratic models of *Ella* hop SFE-CO_2 extract yield (RFI) and $TEAC_{ORAC}$ (RFII).

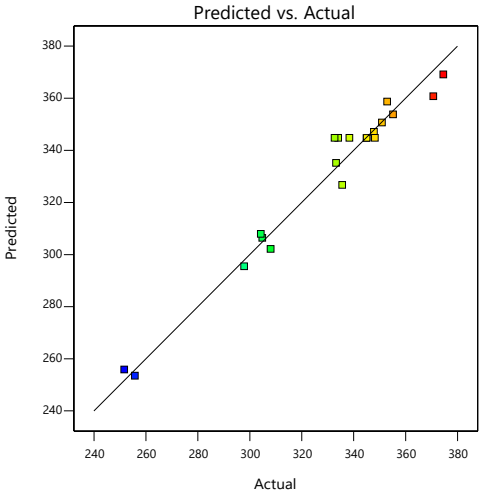
Fit statistics parameters	RFI: Extract yield, g/100 g HP	RFII: $TEAC_{ORAC}$, mg TE/g HP
Standard deviation	0.6763	8.00
Mean	22.62	329.48
C.V. %	2.99	2.43
R^2	0.9787	0.9694
Adjusted R^2	0.9596	0.9419
Predicted R^2	0.8859	0.8538
Adequate precision	27.8325	20.4273

HP: hop pellets; SFE-CO_2: supercritical carbon dioxide extraction; ORAC: oxygen radical scavenging capacity; RF: response factor; TEAC: Trolox equivalent antioxidant capacity.

Appendix 3. Predicted vs. Actual values of reduced quadratic models of (A): extraction yield (RFI, g/100 g HP) and (B): TEAC$_{ORAC}$ (RFII, mg TE/g HP)



(A)



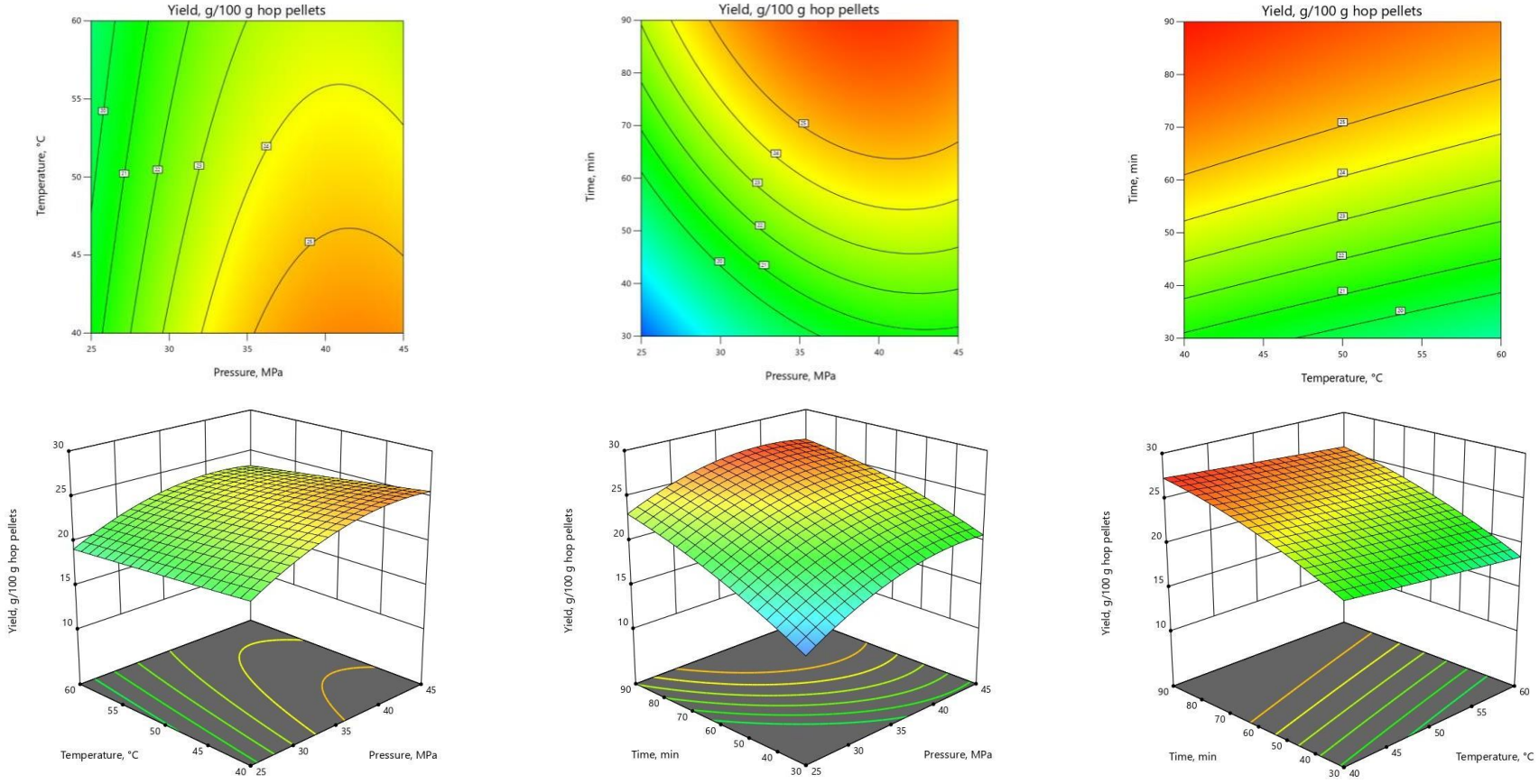
(B)

Appendix 4. Analysis of variance of the regression parameters for the response surface quadratic models of *Ella* hop SFE-CO2 extract yield (RFI) and TEACORAC (RFII).

Source	SS	df	MS	F-value	p-value
RFI: Extract yield, g/100 g HP					
Model	210.66	9	23.41	51.17	< 0.0001*
P (pressure, MPa)	51.71	1	51.71	113.06	< 0.0001*
T (temperature, °C)	8.85	1	8.85	19.36	0.0013*
τ (time, min)	118.34	1	118.34	258.72	< 0.0001*
PT	0.4802	1	0.4802	1.05	0.3297**
Pτ	2.98	1	2.98	6.51	0.0288*
Tτ	0.3200	1	0.3200	0.6996	0.4224**
P^2	8.93	1	8.93	19.52	0.0013*
T^2	0.0030	1	0.0030	0.0066	0.9368**
τ^2	1.86	1	1.86	4.06	0.0716**
Residual	4.57	10	0.4574		
Lack of Fit	3.03	5	0.6051	1.95	0.2399**
Pure Error	1.55	5	0.3097		
Cor Total	215.23	19			
RFII $TEAC_{ORAC,}$ mg TE/g HP					
Model	20284.94	9	2253.88	35.20	< 0.0001*
P (pressure, MPa)	2435.47	1	2435.47	38.03	0.0001*
T (temperature, °C)	121.10	1	121.10	1.89	0.1991**
τ (time, min)	11215.80	1	11215.80	175.15	< 0.0001*
PT	7.70	1	7.70	0.1203	0.7359**
Pτ	903.34	1	903.34	14.11	0.0037*
Tτ	86.53	1	86.53	1.35	0.2721**
P^2	3123.23	1	3123.23	48.77	< 0.0001*
T^2	429.66	1	429.66	6.71	0.0269*
τ^2	230.26	1	230.26	3.60	0.0872**
Residual	640.35	10	64.03		
Lack of Fit	432.78	5	86.56	2.08	0.2196**
Pure Error	207.57	5	41.51		
Cor Total	20925.29	19			

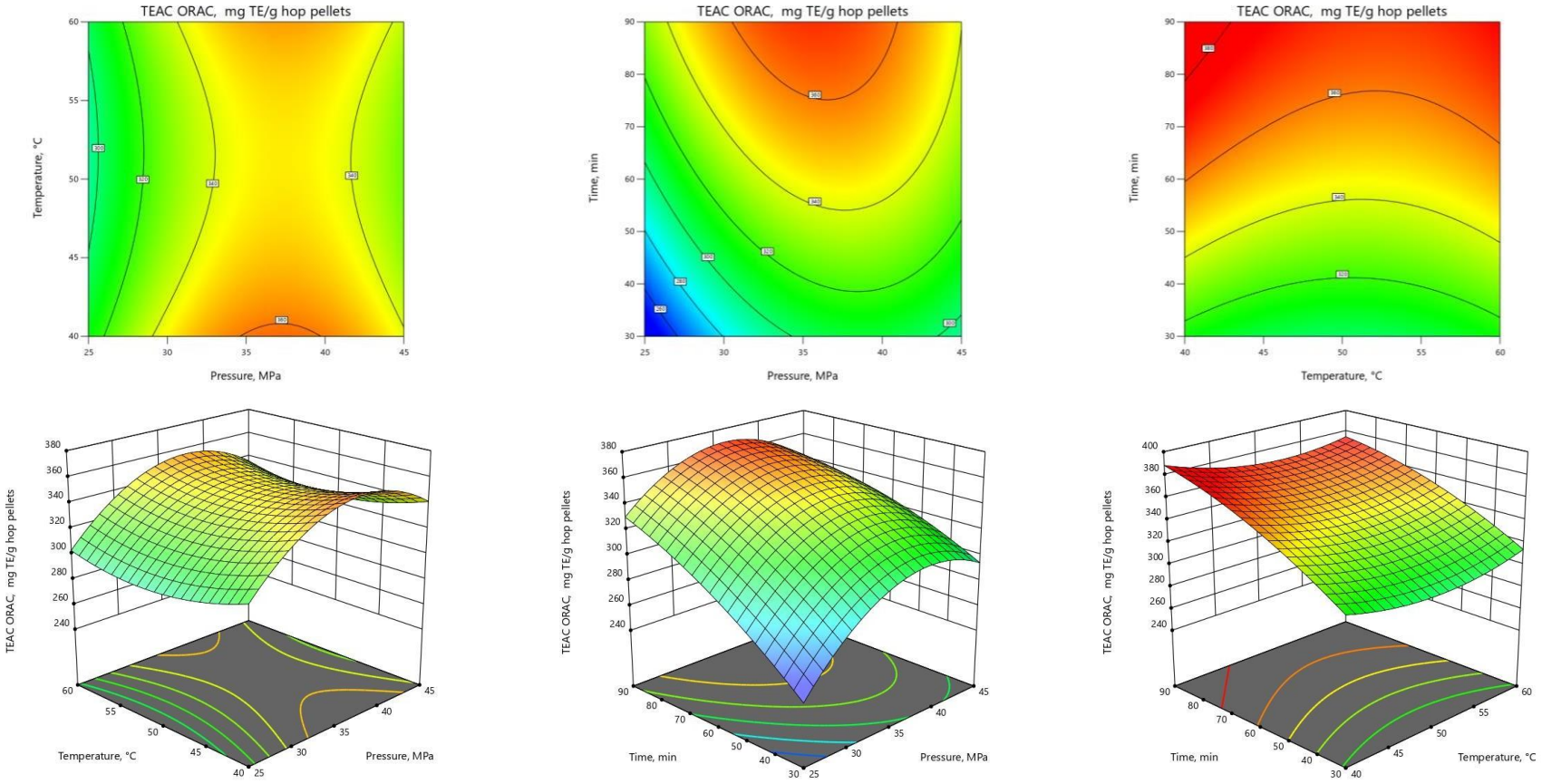
*: significant; **: not significant; HP: hop pellets; SS: sum of square; df: degree of freedom; MS: mean square; F: Fisher value. SFE-CO_2: supercritical carbon dioxide extraction; ORAC: oxygen radical scavenging capacity; P: extraction pressure; RF: response factor; *τ:* extraction time; T: extraction temperature; TEAC: Trolox equivalent antioxidant capacity.

Appendix 5. Response surface 3D and 2D plots showing the effects of independent variables pressure (P), temperature (T) and time (τ) on the *Ella* hop SFE-CO_2 extract yield (g/100 g HP)



(A) effect of T and P (τ_{const}= 60 min) **(B) effect of τ and P (T_{const}= 50°C)** **(C) effect of τ and T (P_{const}= 35 MPa)**

Appendix 6. Response surface 3D and 2D plots showing the effects of independent variables pressure (P), temperature (T) and time (τ) on the *Ella* hop SFE-CO_2 extract $TEAC_{ORAC}$ (mg TE/g HP).



(A) effect of T and P (τ_{const}= 60 min) **(B) effect of τ and P (T_{const}= 50°C)** **(C) effect of τ and T (P_{const}= 35 MPa)**

Appendix 7. Confirmation parameters for the quadratic models of *Ella* hop SFE-CO_2 extract yield (RFI) and $TEAC_{ORAC}$ (RFII).

Confirmation parameters	RFI: Extract yield, g/100 g HP	RFII: $TEAC_{ORAC}$, mg TE/g HP
Predicted mean	26.7641	373.2250
Predicted median	26.7641	373.2250
Standard deviation	0.6763	8.0022
SE Pred.	0.7558	8.9434
95% PI (low)	25.0800	353.2980
Experimental data mean	26.3200	389.8400
95% PI (high)	28.4483	393.1530

HP: hop pellets; SE Pred.: standard deviation associated with the prediction of an individual observation; SFE-CO_2: supercritical carbon dioxide extraction; ORAC: oxygen radical scavenging capacity; PI: prediction interval; RF: response factor; TEAC: Trolox equivalent antioxidant capacity.

Appendix 8. Analysis of correlation between *in vitro* oxygen radical scavenging capacity ($TEAC_{ORAC}$) values and different phytochemical composition indices of *Ella* variety hop SFE-CO_2 extracts obtained under the different experimental conditions

Phytochemical composition indices	Pearson correlation coefficients	
	$TEAC_{ORAC}$, mg TE/g extract	$TEAC_{ORAC}$, mg TE/g pellets
Total bitter acid content, mg/g of extract or pellets	0.8664	0.9934**
α-Acid content, mg/g of extract or pellets:	0.7473	0.9799*
Cohumulone	0.8240	0.9671*
Adhumulone + humulone	0.6462	0.9774*
β-acid content, mg/g of extract or pellets:	0.7570	0.9976**
Colupulone	0.4866	0.9912**
Adlupulone + lupulone	0.8871	0.9941**
Total carotenoid content, μg/g of extract or pellets	0.7943	0.9064
Total chlorophyll content, μg/g of extract or pellets:	0.7479	0.8398
Chlorophyll A	0.6968	0.7958
Chlorophyll B	0.8803	0.9981**

SFE-CO_2: supercritical carbon dioxide extraction; *: correlation is significant at the $P<0.05$ level (two-tailed). Pearson correlation coefficients were calculated using GraphPad Prism 7.04 software (2017).

Appendix 9. Volatile compound composition (GC peak area arbitrary units × 10^7) of *Ella* hop SFE-CO2 extracts obtained under different experimental conditions.

Compound	Exact Mass	RI_{exp}	RI_{lit}[A]	Odour type: description[B,C]	SFE-CO_2 conditions			
					SFE-CO_2 I 10 MPa, 40 °C, 300 min	SFE-CO_2 II 12.5 MPa, 40 °C, 300 min	SFE-CO_2 III 15 MPa, 40 °C, 300 min	SFE-CO_2 IV 37 MPa, 43 °C, 80 min
Monoterpenes GC peak area AU, × 10^7								
α-Pinene	136.1252	950	946 [169]	Herbal: herbal, fresh, terpenic, fruity, sweet, green, pine, earthy, woody	1.1±0.0^a	0.5±0.1^a	0.7±0.2^a	3.5±0.3^b
Camphene	136.1252	971	972 [170]	Woody: camphoreous, cooling minty, citrus green spicy	0.2±0.1^a	0.4±0.0^a	0.6±0.3^a	1.2±0.5^b
β-Pinene	136.1252	1000	989 [190]	Herbal: cooling, dry, woody, piney, eucalyptus, spicy	39.4±0.9^a	39.1±0.5^a	34.6±1.9^a	63.2±0.7^b
β-Myrcene	136.1252	1000	995 [190]	Spicy: peppery, terpenic, balsamic, metallic, musty, fruity, ethereal, herbaceous, woody	37.1±0.1^a	35.4±0.4^a	35.3±0.4^a	56.1±1.5^b
p-Cymene	136.1252	1015	1015[172]	Terpenic: woody, fresh, terpenic, citrus, lemon, spicy	5.0±0.1^d	1.9±0.0^b	1.3±0.0^a	3.5±0.0^a
(*E*)-β-Ocimene	136.1252	1059	1052 [190]	Floral: herbal, mild, citrus, sweet, orange, lemon, tropical, green, woody	18.8±0.3^d	12.4±0.1^c	5.7±0.0^a	10.6±0.0^b
γ-Terpinene	136.1252	1074	1068 [170]	Terpenic: citrus, terpenic, herbal, oily, tropical, fruity, sweet	4.4±0.5^b	3.3±0.0^b	3.0±0.0^a	–ND
Terpinolene	136.1252	1104	1105 [170]	Herbal: fresh, woody, sweet, piney, citrus, anise	1.0±0.1^a	0.8±0.0^a	1.0±0.2^a	1.0±0.0^a
β-Linalool	154.1358	1119	1109 [190]	Floral: citrus, orange, floral, sweet, rose, woody, green	21.8±1.0^b	18.3±0.4^a	18.2±0.2^a	19.4±1.3^{ab}
Total monoterpenes					**127.5**	**111.2**	**Total monoterpenes**	**153.8**
Sesquiterpenes GC peak area AU, × 10^7								
α-Copaene	204.1878	1375	1374 [173]	Woody: woody, spicy, earthy	2.2±0.1^a	2.2±0.0^a	1.9±0.0^a	–ND
α-Ylangene	204.1878	1401	1390[169]	Fruity	38.5±0.1^c	32.7±0.4^a	32.1±0.1^a	36.1±0.0^b

Compound	Exact Mass	RI_{exp}	RI_{lit}[A]	Odour type: description[B,C]	SFE-CO_2 conditions			
					SFE-CO_2 I 10 MPa, 40 °C, 300 min	SFE-CO_2 II 12.5 MPa, 40 °C, 300 min	SFE-CO_2 III 15 MPa, 40 °C, 300 min	SFE-CO_2 IV 37 MPa, 43 °C, 80 min
β-Caryophyllene	204.1878	1438	1428 [169]	Spicy: musty, green, woody, clove, dry	7.9±0.0^{d}	6.2±0.0^{b}	5.7±0.0^{a}	6.8±0.0^{c}
Aromadendrene	204.1878	1439	1439 [173]	Sweet, dry	13.4±0.1^{b}	9.3±0.7^{a}	9.3±0.0^{a}	9.7±0.0^{a}
β-Humulene	204.1878	1457	1457[172]	-NR	77.3±5.9^{b}	61.9±2.8^{ab}	64.6±0.2^{ab}	56.8±2.0^{a}
α-Humulene	204.1878	1504	1505 [170]	Woody: woody, spicy, clove	121.2±0.3^{c}	90.1±0.7^{b}	66.9±0.0^{a}	71.7±7.0^{a}
β-Selinene	204.1878	1514	1524 [169]	Herbal	-ND	39.9±0.0^{b}	42.7±0.0^{c}	34.9±0.0^{a}
α-Selinene	204.1878	1534	1533 [169]	Pepper, orange	181.6±0.0^{d}	97.4±0.0^{c}	54.6±0.0^{b}	49.4±0.1^{a}
δ-Cadinene	204.1878	1554	1556 [169]	Herbal: thyme, herbal, woody, dry	18.5±0.0^{b}	14.7±0.3^{a}	22.3±0.1^{c}	41.9±0.7^{d}
Calamenene	202.1722	1564	1562 [169]	Herbal, spicy	3.3±0.0^{b}	2.9±0.0^{a}	7.1±0.0^{c}	7.6±0.0^{d}
α-Calacorene	200.1565	1583	1590 [169]	Woody: dry, woody	2.6±0.1^{c}	1.6±0.0^{b}	1.9±0.0^{b}	1.2±0.0^{a}
Caryophyllene oxide	220.1827	1635	1617 [190]	Woody: sweet, fresh, dry, woody, spicy, fruity, sawdust, herbal	2.6±0.0^{a}	-ND	3.8±0.0^{b}	-ND
Total sesquiterpenes					**469.0**	**358.8**	**Total sesquiterpenes**	**316.0**
Alcohols GC peak area AU, × 10^{7}								
3-Methyl-2-buten-1-ol	86.0732	799	785[169]	Fruity: sweet, fruity, alcoholic, green	2.5±0.0^{a}	4.1±0.0^{b}	4.6±0.0^{c}	-ND
2-Undecanol	170.1671	1314	1302 [169]	Waxy: fresh, waxy, cloth, sarsaparilla	21.3±1.0^{c}	17.5±1.2^{b}	16.7±0.7^{b}	13.3±1.2^{a}
Total alcohols					**23.8**	**21.6**	**Total alcohols**	**13.3**
Aldehydes GC peak area AU, × 10^{7}								
3-Methyl-2-butenal	84.0575	814	794 [169]	Fruity: sweet, fruity, pungent, nutty, almond, cherry	2.9±0.1^{b}	2.2±0.5^{b}	2.5±0.6^{b}	0.4±0.1^{a}
Total aldehydes					**2.9**	**2.2**	**Total aldehydes**	**0.4**
Ketones GC peak area AU, × 10^{7}								
2-Undecanone	170.1671	1271	1294 [173]	Fruity: waxy, fruity, creamy, fatty, pineapple, orris, floral	7.7±0.0^{c}	5.6±0.2^{ab}	5.9±0.0^{b}	5.2±0.0^{a}

Compound	Exact Mass	RI_{exp}	RI_{lit}[A]	Odour type: description[B,C]	SFE-CO_2 conditions			
					SFE-CO_2 I 10 MPa, 40 °C, 300 min	SFE-CO_2 II 12.5 MPa, 40 °C, 300 min	SFE-CO_2 III 15 MPa, 40 °C, 300 min	SFE-CO_2 IV 37 MPa, 43 °C, 80 min
2-Tridecanone	198.1984	1514	1504 [190]	Waxy: fatty, waxy, dairy, milky, coconut, nutty, herbal, earthy	8.2±0.0^{b}	5.9±0.0^{a}	_ND	_ND
Total ketones					**15.9**	**11.5**	**Total ketones**	**5.2**
Esters GC peak area AU, × 10^7								
2-methylpropyl 2-methylpropanoate	144.1150	921	918 [190]	Fruity: ethereal, fruity, tropical fruit, pineapple	1.0±0.4^{a}	0.83±0.37^{a}	1.48±0.00^{ab}	2.43±0.09^{b}
3-methylbutyl propanoate	144.1150	979	977 [190]	Fruity: sweet, fruity, apple, apple, raspberry, banana	6.9±0.7^{ab}	6.40±0.65^{a}	5.46±0.00^{a}	9.45±0.9^{b}
Methyl hexanoate	130.0994	936	927 [169]	Fruity: fruity, pineapple, thinner, acetone	1.1±0.0^{abc}	0.93±0.09^{ab}	1.48±0.00^{c}	0.99±0.18^{b}
Pentyl 2-methylpropanoate	158.1307	1022	1020 [190]	Fruity: fruity, apple, banana, apricot, buttery	16.8±0.3^{a}	14.1±1.67^{a}	15.55±0.83^{a}	24.84±0.45^{b}
Methyl heptanoate	144.1150	1037	1030 [190]	Fruity: sweet, fruity, waxy, floral, berry, apple	6.87±0.00^{a}	6.03±0.46^{a}	5.55±0.37^{a}	6.93±0.54^{a}
Methyl 6-methylheptanoate	158.1307	1096	1092 [169]	-NR	8.83±0.25^{a}	9.46±0.00^{a}	10.55±0.00^{b}	8.64±0.45^{a}
2-Methylbutyl 3-methylbutanoate	172.1463	1111	1113 [169]	Fruity: herbal, earthy, apple, green	_ND	_ND	4.81±0.56^{a}	5.67±0.81^{a}
Methyl octanoate	158.1307	1135	1130 [190]	Waxy: waxy, green, sweet, orange, aldehydic, vegetable, herbal	14.23±0.12^{b}	12.34±0.74^{ab}	11.85±0.56^{a}	10.80±0.45^{a}
Hexyl 2-methylpropanoate	172.1463	1158	1151 [190]	Green: sweet, green, fruity, apple, pear, grape, ripe, berry	1.84±0.74^{a}	2.60±0.00^{a}	5.65±0.37^{b}	3.24±0.09^{a}
Heptyl propanoate	172.1463	1206	1207 [169]	Floral: rose, apricot	7.48±0.37^{c}	5.75±0.09^{ab}	6.20±0.00^{b}	5.04±0.00^{a}
Methyl 8-nonenoate	170.1307	1222	1218 [190]	-NR	_ND	5.1±0.28^{b}	4.63±0.00^{ab}	4.41±0.00^{a}
Methyl nonanoate	172.1463	1238	1229 [190]	Fruity: sweet, fruity, pear, waxy, tropical, winey	11.16±0.12^{a}	20.22±1.11^{b}	26.19±0.37^{c}	19.17±0.00^{b}
Heptyl 2-methylpropanoate	186.1620	1255	1249 [190]	Fruity: fruity, sweet, green, warm, floral, tropical, chamomile, tea, green	4.78±0.00^{a}	4.55±0.00^{a}	4.9±0.65^{a}	3.78±0.18^{a}
2-Methylbutyl hexanoate	186.1620	1263	1246 [169]	Fruity: fruity, ethereal	0.74±0.00^{a}	1.11±0.00^{b}	_ND	_ND
Methyl 4-decenoate	184.1463	1322	1316 [190]	Fruity: fruity, pear, mango, fishy, peach, green	67.59±0.49^{a}	100.18±2.04^{c}	102.54±2.5^{c}	75.43±0.54^{b}

Compound	Exact Mass	RI_{exp}	RI_{lit}[A]	Odour type: description[B,C]	SFE-CO_2 conditions			
					SFE-CO_2 I 10 MPa, 40 °C, 300 min	SFE-CO_2 II 12.5 MPa, 40 °C, 300 min	SFE-CO_2 III 15 MPa, 40 °C, 300 min	SFE-CO_2 IV 37 MPa, 43 °C, 80 min
Total esters					**149.29**	**189.59**	**Total esters**	**180.84**
Fatty acids GC peak area AU, × 10^7								
2-Methylpropanoic acid	88.05240	778	762 [174]	Acidic: sour, cheesy, dairy, buttery, rancid, phenolic, fatty, sweaty	2.09±0.00[a]	1.58±0.19[a]	1.85±0.19[a]	2.34±0.45[a]
3-Methylbutanoic acid	102.0681	850	865 [174]	Cheesy: dai y, acidic, sour, pungent, fruity, fatty, sweaty, rancid	1.35±0.12[a]	1.21±0.00[a]	1.30±0.00[a]	0.99±0.09[a]
Heptanoic acid	130.0994	1089	1072 [169]	Cheesy: rancid, sour, cheesy, waxy, sweaty, fermented, pineapple, fruity	2.94±0.00[a]	3.80±0.09[b]	2.87±0.19[a]	2.52±0.18[a]
Octanoic acid	144.1150	1189	1191 [175]	Fatty: fatty, waxy, rancid, oily, vegetable, c eesy	2.45±0.00[d]	2.32±0.00[b]	2.04±0.00[a]	2.52±0.00[c]
Total fatty acids					**8.83**	**8.90**	**8.05**	**8.37**
Total GC peak area AU, × 10^7					**1226.67 ±21.92[b]**	**927.56±7.08[a]**	**925.47±6.20[a]**	**900.15±5.63[a]**

AU: arbitrary units; [A]: retention indexes (RI) reported for RTX-5 or equivalent column (± 20 units compared to the calculated RI_{exp}); [169] Martins et.al. J. Chemom., 2020, 34, e3285; [170] Rali et al. Molecules, 2007, 12, 3, 389-394; [190]Yan et al. Food Chem., 2019, 25, 15-23; [172]Frizzo et al. Flavour Fragr. J, 2001, 16, 286-288; [173] Adams, R.P. Identification of essential oil components by gas chromatography/mass spectrometry, ed. 4.1. 2017; [174]Brendel et.al. J.Agric.Food.Chem., 2019, 67, 12044-12053; [175]Alissandrakis et al. J.Agric.Food Chem., 2007, 55, 8152-8157. [B]: Odour descriptions obtained from Pherobase database (https://www.pherobase.com/); [C]: Odour descriptions obtained from The Goodscent Company database (http://www.thegoodscentscompany.com/); -[NI]: not identified; -[ND]: not detected; -[NR]: not reported. Different superscript letters in the same row indicate significant differences (one-way ANOVA and Tukey`s test $p < 0.05$).

Appendix 10. Central composite design matrix (levels of independent variables and variation levels) for SFE-CO_2 optimization for α- and β-bitter acid extraction from *Ella* variety hop pellets

Levels and runs	PLE-EtOH parameters			RFI Cohumulone	RFII Humulone +Adhumulone	RFIII: Colupulone	RFIV: Lupulone +Adlupulone	RFV: Total α-acids	RFVI: Total β-acids	RFVII: Total acids	RFVIII: α/β ratio
	P, MPa	T, °C	τ, mi	mg/g E	mg/g E	mg/g E	mg/g E	mg/g E	mg/g E	mg/g E	
Center	35	50	60	201.08 ± 9.17	363.21 ± 6.8	215.58 ± 7.57	124.01 ± 2.37	564.29 ± 2.37	339.59 ± 5.21	903.87 ± 7.57	1.66 ± 0.02
Axial	25	50	60	194.24 ± 15.38	361.84 ± 35.78	199.31 ± 12.16	107.25 ± 6.30	556.08 ± 1.54	306.55 ± 18.46	862.63 ± 20.00	1.81 ± 0.06
Axial	35	40	60	205.53 ± 2.31	353.9 ± 12.74	206.53 ± 4.46	117.75 ± 4.74	559.43 ± 15.05	324.28 ± 9.24	883.72 ± 24.28	1.73 ± 0.00
Factorial	25	40	90	206.75 ± 13.13	372.69 ± 14.47	204.4 ± 25.88	113.55 ± 4.70	579.45 ± 27.6	317.95 ± 30.62	897.4 ± 16.28	1.83 ± 0.09
Factorial	25	60	30	201.81 ± 8.88	373.79 ± 10.9	222.82 ± 7.15	128.3 ± 2.31	575.6 ± 2.02	351.12 ± 9.46	926.71 ± 11.48	1.64 ± 0.04
Axial	35	50	90	201.52 ± 4.39	377.43 ± 27.00	224.55 ± 3.85	121.45 ± 3.92	578.94 ± 31.39	346 ± 7.77	924.94 ± 23.62	1.67 ± 0.13
Center	35	50	60	201.1 ± 9.18	363.17 ± 6.80	215.55 ± 7.56	123.49 ± 2.38	564.27 ± 2.38	339.04 ± 5.23	903.31 ± 7.56	1.66 ± 0.02
Center	35	50	60	201.11 ± 9.17	363.17 ± 6.81	215.57 ± 7.60	123.49 ± 2.35	564.29 ± 2.35	339.06 ± 5.20	903.34 ± 7.56	1.66 ± 0.02
Factorial	45	60	30	228.99 ± 3.36	411.76 ± 1.79	177 ± 1.52	97.58 ± 0.21	640.76 ± 5.15	274.58 ± 1.31	915.34 ± 6.46	2.33 ± 0.01
Factorial	45	40	90	231.01 ± 9.94	409.5 ± 0.00	132.79 ± 12.08	83.64 ± 4.68	640.66 ± 9.94	216.43 ± 16.76	857,09 ± 26,69	2.97 ± 0.18
Factorial	45	40	30	203.65 ± 10.71	358.71 ± 38.01	110.85 ± 18.55	73.20 ± 9.66	562.35 ± 3.14	184.05 ± 28.21	746.4 ± 31.36	3.07 ± 0.21
Axial	35	60	60	213.53 ± 1.64	380.22 ± 5.99	204.39 ± 8.40	117.54 ± 0.22	593.75 ± 4.35	321.93 ± 8.62	915.68 ± 4.27	1.85 ± 0.06
Factorial	25	60	90	196.43 ± 1.63	352.9 ± 14.30	192.96 ± 13.51	109.82 ± 0.31	549.34 ± 15.93	302.77 ± 13.82	852.11 ± 29.75	1.81 ± 0.03
Factorial	45	60	90	200 ± 15.77	380.54 ± 4.62	139.47 ± 7.89	87.22 ± 2.95	580.54 ± 11.15	226.69 ± 10.84	807.23 ± 21.95	2.56 ± 0.07
Center	35	50	60	201.08 ± 9.16	363.18 ± 6.78	215.56 ± 7.56	123.47 ± 2.38	564.26 ± 2.38	339.02 ± 5.19	903.28 ± 7.56	1.66 ± 0.02
Center	35	50	60	201.08 ± 9.17	363.21 ± 6.79	215.54 ± 7.58	123.5 ± 2.38	564.29 ± 2.38	339.04 ± 5.21	903.33 ± 7.58	1.66 ± 0.02
Axial	45	50	60	196.21 ± 6.49	332.64 ± 14.61	110.20 ± 5.02	75.4 ± 3.39	528.85 ± 19.87	185.6 ± 8.40	714.44 ± 11.46	2.85 ± 0.24
Center	35	50	60	201.1 ± 9.18	363.17 ± 6.81	215.58 ± 7.59	123.49 ± 2.37	564.27 ± 2.37	339.07 ± 5.22	903.34 ± 7.59	1.66 ± 0.02
Factorial	25	40	30	195.94 ± 2.26	356.65 ± 8.19	243.48 ± 14.26	128.90 ± 3.48	552.58 ± 5.94	372.39 ± 17.68	924.97 ± 23.68	1.49 ± 0.05
Axial	35	50	30	197.08 ± 21.84	348.04 ± 20.35	196.55 ± 9.24	106.90± 2.44	545.11 ± 42.19	303.45 ± 11.73	848.56 ± 6.03	1.79 ±0.07

SFE-CO_2: supercritical carbon dioxide extraction; RFI: Cohumulone, mg/g E; RFII: Humulone + adhumulone, mg/g E; RFIII: Colupulone, mg/g E; RFIV: Lupulone+adlupulone, mg/g E; RFV: Total α-acids, mg/g E; RFVI: Total β-acids, mg/g E; RFVII: Total bitter acids, mg/g E; RF VIII: α / β ratio; E: extract; P: extraction pressure; RF: response factor; τ: extraction time; T: extraction temperature.

Appendix 11. Fit statistics parameters for the not reduced models of SFE-CO_2 optimization for extraction of bitter acid content from *Ella* variety hop, content of cohumulone (RF I), humulone + adhumulone (RF II), colupulone (RF III), lupulone+adlupulone (RF IV), total α acids (RF V), total β acids (RF VI) and total bitter acids (RF VII), and α/β ratio (RF VIII)

Fit statistics parameters	RF I	RF II	RF III	RF IV	RF V	RF VI	RF VII	RF VIII
Suggested model	Quadratic	Quadratic	Quadratic	Quadratic	Quadratic	Quadratic	Quadratic	Quadratic
Std. Dev.	1.89	3.75	2.84	1.32	5.43	4.07	7.65	0.1276
Mean	46.20	83.18	43.23	24.84	129.38	68.06	197.44	1.97
C.V. %	4.08	4.50	6.57	5.33	4.20	5.97	3.87	6.48
R^2	0.9680	0.9600	0.9535	0.9617	0.9653	0.9579	0.9659	0.9649
Adjusted R^2	0.9393	0.9239	0.9117	0.9272	0.9341	0.9200	0.9352	0.9334
Predicted R^2	0.6504	0.6791	0.5379	0.7668	0.6912	0.6343	0.6684	0.6566
Adeq. recision	23.6799	20.3432	15.8015	16.2471	22.2474	16.3249	19.6128	16.1245

Std. Dev.: standard deviation; C.V.: coefficient of variation SFE-CO_2: supercritical carbon dioxide extraction; RF: response factor; HP: hop pellets

Appendix 12. Analysis of variance of the regression parameters for the response surface quadratic models before reduction, content of cohumulone (RF I), humulone + adhumulone (RF II), colupulone (RF III), lupulone+adlupulone (RF IV), total α acids (RF V), total β acids (RF VI) and total bitter acids (RF VII) and α/β ratio (RF VIII) of *Ella* variety hop SFE-CO_2 extract

Source	SS	df	MS	F-value	p-value
RF I: Cohumulone, mg/g HP					
Model	1076.71	9	119.63	33.66	< 0.0001*
P-Pressure	361.92	1	361.92	101.82	< 0.0001*
T-Temperature	50.36	1	50.36	14.17	0.0037*
τ-Time	535.97	1	535.97	150.78	< 0.0001*
PT	4.82	1	4.82	1.36	0.2712**
Pτ	9.48	1	9.48	2.67	0.1334**
Tτ	22.34	1	22.34	6.29	0.0311*
P^2	53.92	1	53.92	15.17	0.0030*
T^2	17.91	1	17.91	5.04	0.0486*
τ^2	6.46	1	6.46	1.82	0.2072**
Residual	35.55	10	3.55		
Lack of Fit	29.26	5	5.85	4.65	0.0584**
Pure Error	6.29	5	1.26		
Cor Total	1112.26	19			
RF II: Humulone+ adhumulone, mg/g HP					
Model	3365.55	9	373.95	26.64	< 0.0001*
P-Pressure	1010.63	1	1010.63	71.99	< 0.0001*
T-Temperature	79.07	1	79.07	5.63	0.0391*
τ-Time	1967.01	1	1967.01	140.12	< 0.0001*
PT	1.21	1	1.21	0.0861	0.7752**
Pτ	8.38	1	8.38	0.5973	0.4575**
Tτ	49.25	1	49.25	3.51	0.0905**
P^2	185.83	1	185.83	13.24	0.0045*
T^2	27.02	1	27.02	1.92	0.1955**
τ^2	0.7738	1	0.7738	0.0551	0.8191**
Residual	140.38	10	14.04		
Lack of Fit	119.99	5	24.00	5.88	0.0371*
Pure Error	20.39	5	4.08		
Cor Total	3505.93	19			
RF III: Colupulone, mg/g HP					
Model	1654.69	9	183.85	22.80	< 0.0001*
P-Pressure	191.76	1	191.76	23.78	0.0006*
T-Temperature	5.69	1	5.69	0.7050	0.4207**
τ-Time	304.92	1	304.92	37.82	0.0001*
PT	52.22	1	52.22	6.48	0.0291*
Pτ	11.23	1	11.23	1.39	0.2652**
Tτ	8.20	1	8.20	1.02	0.3370**
P^2	650.15	1	650.15	80.63	< 0.0001*
T^2	1.34	1	1.34	0.1667	0.6917**
τ^2	0.5796	1	0.5796	0.0719	0.7941**
Residual	80.64	10	8.06		

Source	SS	df	MS	F-value	p-value
Lack of Fit	73.43	5	14.69	10.19	0.0117*
Pure Error	7.20	5	1.44		
Cor Total	1735.33	19			
RF IV Lupulone + adlupulone, mg/g HP					
Model	439.10	9	48.79	27.89	< 0.0001*
P-Pressure	16.26	1	16.26	9.29	0.0123*
T-Temperature	2.23	1	2.23	1.27	0.2855**
τ-Time	116.14	1	116.14	66.38	< 0.0001*
PT	4.87	1	4.87	2.78	0.1263**
Pτ	1.44	1	1.44	0.8259	0.3848**
Tτ	1.16	1	1.16	0.6602	0.4354**
P^2	159.41	1	159.41	91.11	< 0.0001*
T^2	1.64	1	1.64	0.9352	0.3563**
τ^2	2.07	1	2.07	1.19	0.3017**
Residual	17.50	10	1.75		
Lack of Fit	15.27	5	3.05	6.87	0.0271*
Pure Error	2.22	5	0.4449		
Cor Total	456.60	19			
RF V Total α-acids, mg/g HP					
Model	8216.35	9	912.93	30.93	< 0.0001*
P-Pressure	2582.13	1	2582.13	87.48	< 0.0001*
T-Temperature	255.64	1	255.64	8.66	0.0147*
τ-Time	4556.52	1	4556.52	154.38	< 0.0001*
PT	10.86	1	10.86	0.3679	0.5577**
Pτ	35.70	1	35.70	1.21	0.2972**
Tτ	137.94	1	137.94	4.67	0.0559**
P^2	439.97	1	439.97	14.91	0.0032*
T^2	88.92	1	88.92	3.01	0.1133**
τ^2	11.71	1	11.71	0.3968	0.5429**
Residual	295.15	10	29.52		
Lack of Fit	245.82	5	49.16	4.98	0.0513**
Pure Error	49.33	5	9.87		
Cor Total	8511.50	19			
RF VI Total β-acids, mg/g HP					
Model	3761.68	9	417.96	25.29	< 0.0001*
P-Pressure	319.68	1	319.68	19.35	0.0013*
T-Temperature	15.03	1	15.03	0.9096	0.3627**
τ-Time	797.45	1	797.45	48.26	< 0.0001*
PT	88.98	1	88.98	5.38	0.0427*
Pτ	20.74	1	20.74	1.25	0.2888**
Tτ	15.51	1	15.51	0.9388	0.3554**
P^2	1453.43	1	1453.43	87.96	< 0.0001*
T^2	5.95	1	5.95	0.3598	0.5619**
τ^2	0.4613	1	0.4613	0.0279	0.8706**
Residual	165.24	10	16.52		
Lack of Fit	147.83	5	29.57	8.49	0.0174*
Pure Error	17.42	5	3.48		
Cor Total	3926.92	19			
RF VII Total bitter acids, mg/g HP					
Model	16569.61	9	1841.07	31.47	< 0.0001*

Source	SS	df	MS	F-value	p-value
P-Pressure	1084.72	1	1084.72	18.54	0.0015*
T-Temperature	394.64	1	394.64	6.75	0.0266*
τ-Time	9166.36	1	9166.36	156.67	< 0.0001*
PT	37.67	1	37.67	0.6439	0.4410**
Pτ	110.86	1	110.86	1.89	0.1987**
Tτ	245.98	1	245.98	4.20	0.0675**
P^2	3492.72	1	3492.72	59.70	< 0.0001*
T^2	140.86	1	140.86	2.41	0.1518**
τ^2	16.82	1	16.82	0.2875	0.6035**
Residual	585.06	10	58.51		
Lack of Fit	459.71	5	91.94	3.67	0.0901**
Pure Error	125.35	5	25.07		
Cor Total	17154.67	19			
RF VIII α/β-acid ratio					
Model	4.48	9	0.4978	30.57	< 0.0001*
P-Pressure	2.70	1	2.70	166.04	< 0.0001*
T-Temperature	0.0810	1	0.0810	4.97	0.0498*
τ-Time	0.0270	1	0.0270	1.66	0.2266**
PT	0.2048	1	0.2048	12.58	0.0053*
Pτ	0.0180	1	0.0180	1.11	0.3172**
Tτ	0.0032	1	0.0032	0.1965	0.6670**
P^2	0.8457	1	0.8457	51.93	< 0.0001*
T^2	0.0006	1	0.0006	0.0357	0.8539**
τ^2	0.0057	1	0.0057	0.3489	0.5678**
Residual	0.1628	10	0.0163		
Lack of Fit	0.1628	5	0.0326		
Pure Error	0.0000	5	0.0000		
Cor Total	4.64	19			

*: significant; **: not significant; SS: sum of square; df: degree of freedom; MS: mean square; F: Fisher value. SFE-CO_2: supercritical carbon dioxide extraction; ORAC: oxygen radical scavenging capacity; P: extraction pressure; RF: response factor; *τ:* extraction time; T: extraction temperature; HP: hop pellets

Appendix 13. Fit statistics parameters for the reduced models of SFE-CO_2 optimization for extraction of bitter acid content from *Ella* variety hop, content of cohumulone (RF I), humulone + adhumulone (RF II), colupulone (RF III), lupulone+adlupulone (RF IV), total α acids (RF V), total β acids (RF VI) and total bitter acids (RF VII), and α/β ratio (RF VIII)

Fit statistics parameters	RF I	RF II	RF III	RF IV	RF V	RF VI	RF VII	RF VIII
Suggested model	Quadratic	Quadratic	Quadratic	Quadratic	Quadratic	Quadratic	Quadratic	Quadratic
Std. Dev.	1.98	3.44	2.71	1.28	5.15	3.85	7.30	0.1149
Mean	46.20	83.18	43.23	24.84	129.38	68.06	197.44	1.97
C.V. %	4.28	4.14	6.28	5.14	3.98	5.66	3.70	5.84
R^2	0.9579	0.9594	0.9406	0.9501	0.9627	0.9472	0.9627	0.9630
Adjusted R^2	0.9333	0.9357	0.9194	0.9322	0.9409	0.9283	0.9410	0.9459
Predicted R^2	0.8005	0.8414	0.8299	0.8931	0.8444	0.8586	0.8329	0.8627
Adeq. recision	24.9413	24.6878	21.4672	22.9885	26.1011	22.7517	22.9063	21.5293

Std. Dev.: standard deviation; C.V.: coefficient of variation; SFE-CO_2: supercritical carbon dioxide extraction; RF: response factor; HP: hop pellets

Appendix 14. Analysis of variance of the regression parameters for the response surface reduced quadratic models of cohumulone, humulone+adhuulone, colupulone, lupulone and adlupulone, total α acid, total β acid and total bitter acid content (mg/g HP) and α/β acid ratio of *Ella* variety hop SFE-CO_2 extract

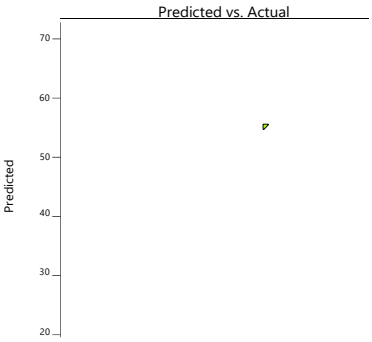
Source	SS	df	MS	F-value	p-value
RF I: Cohumulone, mg/g HP					
Model	1065.43	7	152.20	39.00	< 0.0001*
P-Pressure	361.92	1	361.92	92.74	< 0.0001*
T-Temperature	50.36	1	50.36	12.90	0.0037*
τ-Time	535.97	1	535.97	137.34	< 0.0001*
Pτ	9.48	1	9.48	2.43	0.1450**
T	22.34	1	22.34	5.73	0.0340*
P^2	80.10	1	80.10	20.52	0.0007*
T^2	12.51	1	12.51	3.20	0.0987**
Residual	46.83	12	3.90		
Lack of Fit	40.54	7	5.79	4.61	0.0559**
Pure Error	6.29	5	1.26		
Cor Total	1112.26	19			
RF II: Cohumulone+ adhumulone, mg/g HP					
Model	3363.57	7	480.51	40.50	< 0.0001*
P-Pressure	1010.63	1	1010.63	85.19	< 0.0001*
T-Temperature	79.07	1	79.07	6.67	0.0240*
τ-Time	1967.01	1	1967.01	165.80	< 0.0001*
Pτ	8.38	1	8.38	0.7067	0.4170**
Tτ	49.25	1	49.25	4.15	0.0643**
P^2	226.83	1	226.83	19.12	0.0009*
T^2	27.58	1	27.58	2.32	0.1533**
Residual	142.36	12	11.86		
Lack of Fit	121.97	7	17.42	4.27	0.0646**
Pure Error	20.39	5	4.08		
Cor Total	3505.93	19			
RF III: Colupulone, mg/g HP					
Model	1632.25	5	326.45	44.34	< 0.0001*
P-Pressure	191.76	1	191.76	26.04	0.0002*
T-Temperature	5.69	1	5.69	0.7721	0.3944**
τ-Time	304.92	1	304.92	41.41	< 0.0001*
PT	52.22	1	52.22	7.09	0.0185*
P^2	1077.66	1	1077.66	146.36	< 0.0001*
Residual	103.08	14	7.36		
Lack of Fit	95.88	9	10.65	7.39	0.0201*
Pure Error	7.20	5	1.44		
Cor Total	1735.33	19			
RF IV Lupulone + adlupulone, mg/g HP					
Model	433.79	5	86.76	53.26	< 0.0001*
P-Pressure	16.26	1	16.26	9.98	0.0070*
T-Temperature	2.23	1	2.23	1.37	0.2618**
τ-Time	116.14	1	116.14	71.29	< 0.0001*
PT	4.87	1	4.87	2.99	0.1059**

Source	SS	df	MS	F-value	p-value
P^2	294.30	1	294.30	180.65	< 0.0001*
Residual	22.81	14	1.63		
Lack of Fit	20.58	9	2.29	5.14	0.0431*
Pure Error	2.22	5	0.4449		
Cor Total	456.60	19			
RF V Total α-acids, mg/g HP					
Model	8193.78	7	1170.54	44.21	< 0.0001*
P-Pressure	2582.13	1	2582.13	97.52	< 0.0001*
T-Temperature	255.64	1	255.64	9.66	0.0091*
τ-Time	4556.52	1	4556.52	172.09	< 0.0001*
Pτ	35.70	1	35.70	1.35	0.2681**
Tτ	137.94	1	137.94	5.21	0.0415*
P^2	576.52	1	576.52	21.77	0.0005*
T^2	77.22	1	77.22	2.92	0.1134**
Residual	317.72	12	26.48		
Lack of Fit	268.39	7	38.34	3.89	0.0771**
Pure Error	49.33	5	9.87		
Cor Total	8511.50	19			
RF VI Total β-acids, mg/g HP					
Model	3719.42	5	743.88	50.19	< 0.0001*
P-Pressure	319.68	1	319.68	21.57	0.0004*
T-Temperature	15.03	1	15.03	1.01	0.3310**
τ -Time	797.45	1	797.45	53.80	< 0.0001*
PT	88.98	1	88.98	6.00	0.0280*
P^2	2498.28	1	2498.28	168.55	< 0.0001*
Residual	207.50	14	14.82		
Lack of Fit	190.09	9	21.12	6.06	0.0307*
Pure Error	17.42	5	3.48		
Cor Total	3926.92	19			
RF VII Total bitter acids, mg/g HP					
Model	16515.12	7	2359.30	44.27	< 0.0001*
P-Pressure	1084.72	1	1084.72	20.35	0.0007*
T-Temperature	394.64	1	394.64	7.40	0.0186*
τ -Time	9166.36	1	9166.36	171.99	< 0.0001*
Pτ	110.86	1	110.86	2.08	0.1748**
Tτ	245.98	1	245.98	4.62	0.0528**
P^2	4278.54	1	4278.54	80.28	< 0.0001*
T^2	124.18	1	124.18	2.33	0.1528**
Residual	639.55	12	53.30		
Lack of Fit	514.20	7	73.46	2.93	0.1273**
Pure Error	125.35	5	25.07		
Cor Total	17154.67	19			
RF VIII α/β-acid ratio					
Model	4.47	6	0.7452	56.41	< 0.0001*
P-Pressure	2.70	1	2.70	204.67	< 0.0001*
T-Temperature	0.0810	1	0.0810	6.13	0.0278*
τ-Time	0.0270	1	0.0270	2.05	0.1761**
PT	0.2048	1	0.2048	15.50	0.0017*
Pτ	0.0180	1	0.0180	1.37	0.2634**
P^2	1.44	1	1.44	108.73	< 0.0001*

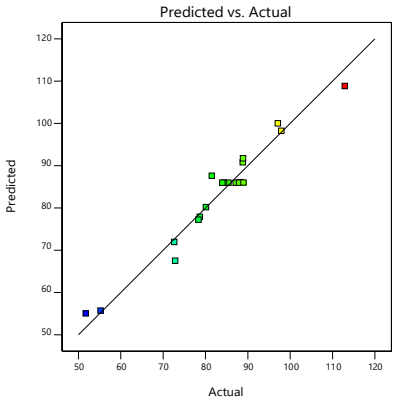
Source	SS	df	MS	F-value	p-value
Residual	0.1718	13	0.0132		
Lack of Fit	0.1718	8	0.0215		
Pure Error	0.0000	5	0.0000		
Cor Total	4.64	19			

*: significant; **: not significant; SS: sum of square; df: degree of freedom; MS: mean square; F: Fisher value. SFE-CO_2: supercritical carbon dioxide extraction; ORAC: oxygen radical scavenging capacity; P: extraction pressure; RF: response factor; τ: extraction time; T: extraction temperature; HP: hop pellets

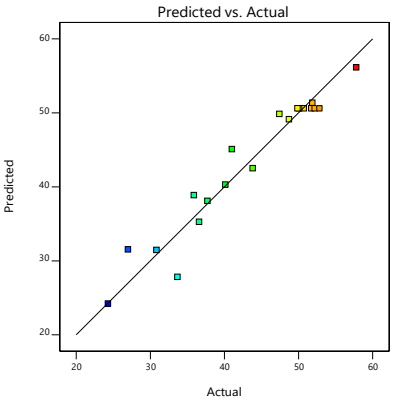
Appendix 15. Predicted vs. Actual values of reduced quadratic models of cohumulone (RFI, mg/g HP), humulone+adhuulone (RFII, mg/g HP), colupulone (RFIII, mg/g HP), lupulone + adlupulone (RFIV, mg/g HP), total α acid (RFV, mg/g HP), total β acid (RFVI, mg/g HP), and total bitter acid content (RFVII, mg/g HP), and α/β acid ratio (RFVIII) of *Ella* variety hop SFE-CO2 extract



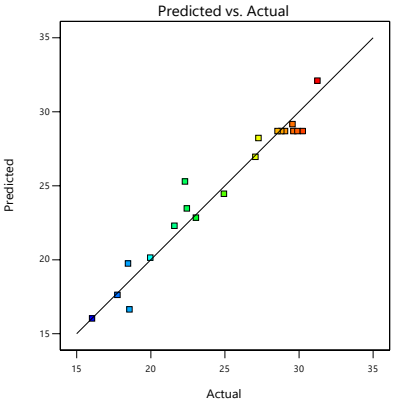
RFI



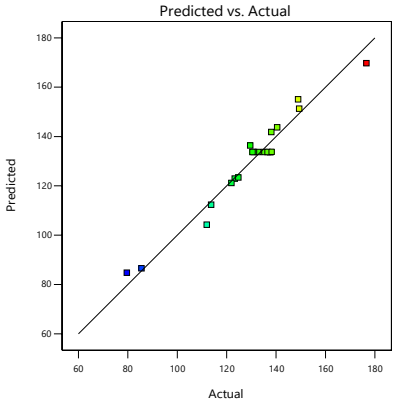
RFII



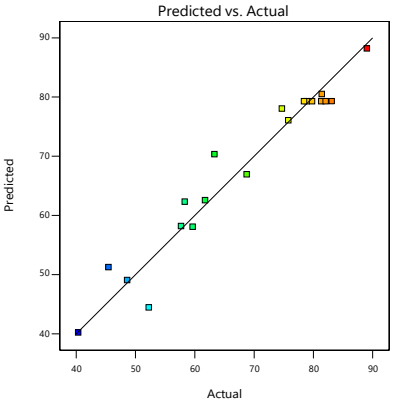
RFIII



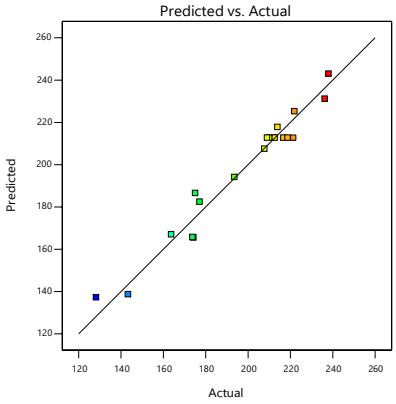
RFIV



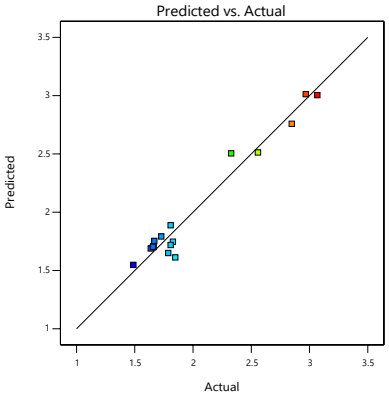
RFV



RFVI

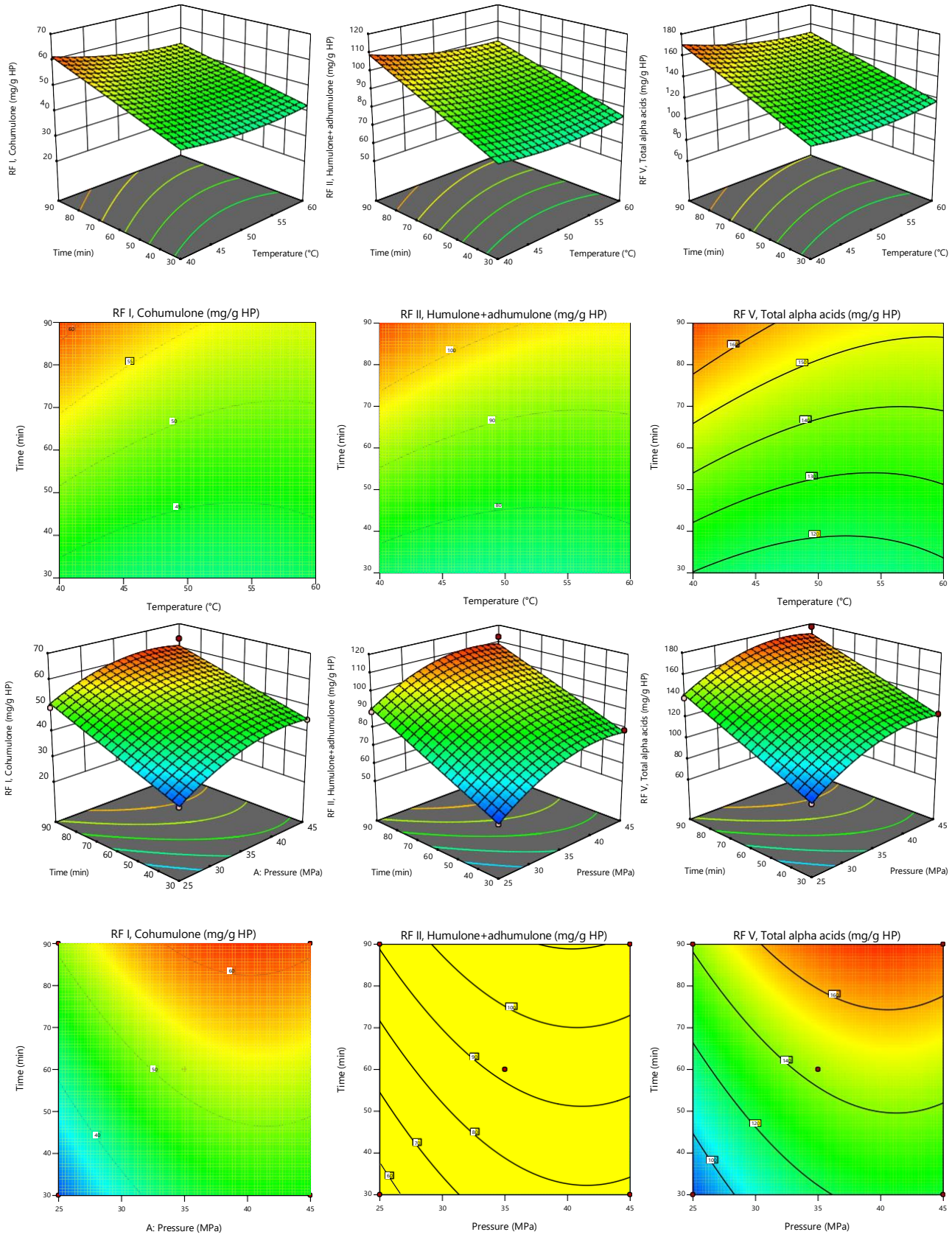


RFVII



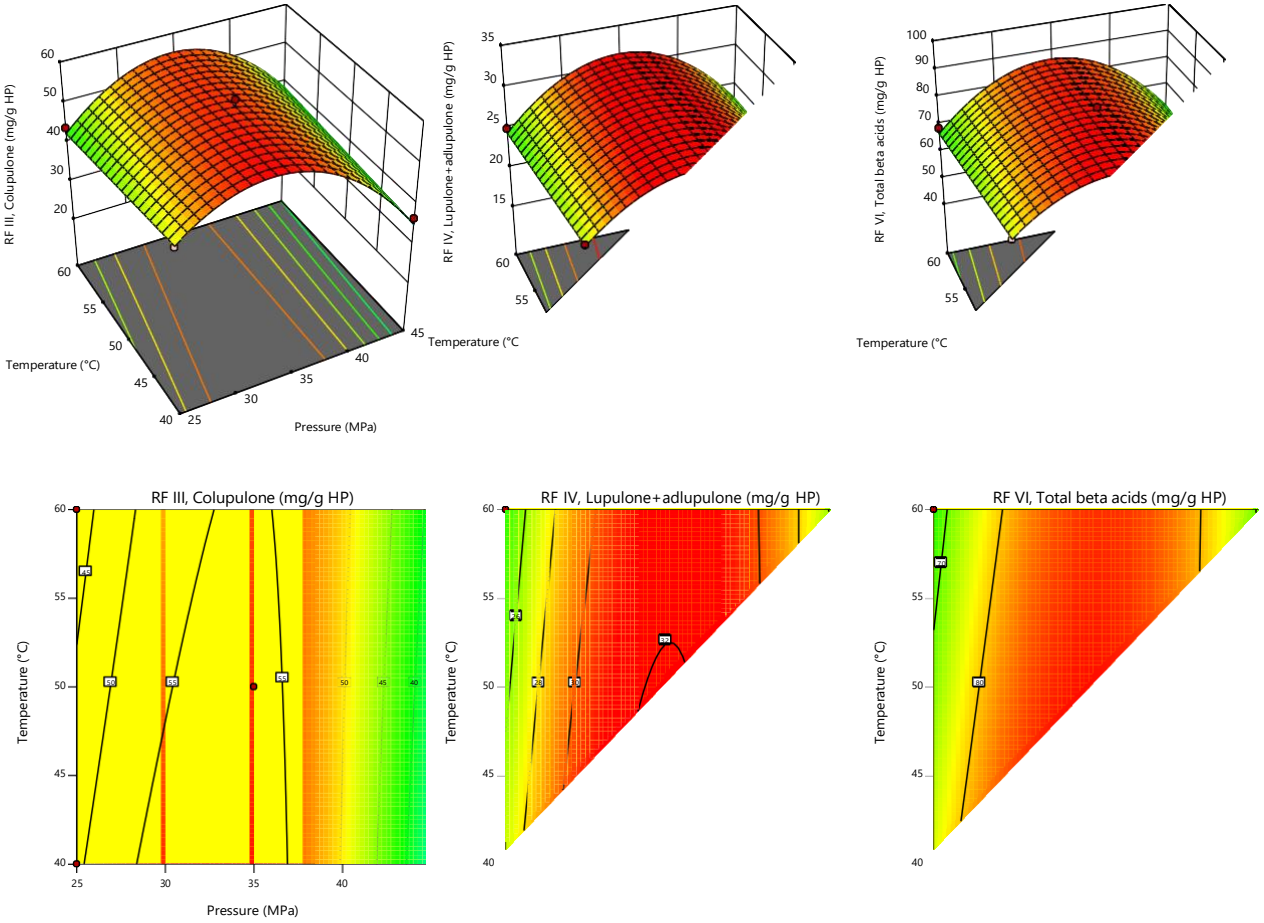
RFVIII

Appendix 16. Response surface 3D and 2D plots showing the effects of SFE-CO_2 pressure (P), temperature (T) and time (τ) on the *Ella* hop: (A) RFI cohumulone mg/g HP; (B) RFII humulone and adhumulone mg/g HP; (C) RFV: total α-acids mg/g HP



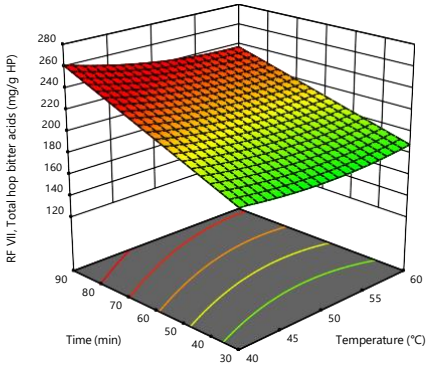
(A) **(B)** **(C)**

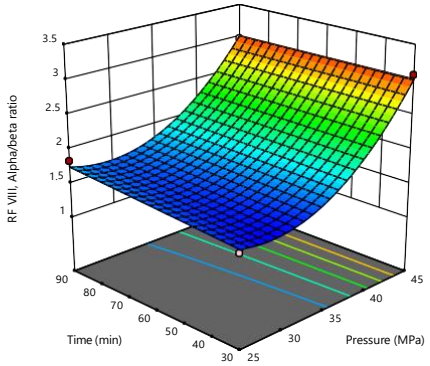
Appendix 17. Response surface 3D and 2D plots showing the effects of SFE-CO_2 pressure (P) and temperature (T) on the *Ella* hop: (A) RFIII: colupulone mg/g HP; (B) RFIV: lupulone and adlupulone mg/g HP; (C) RFVI: total β-acids mg/g HP

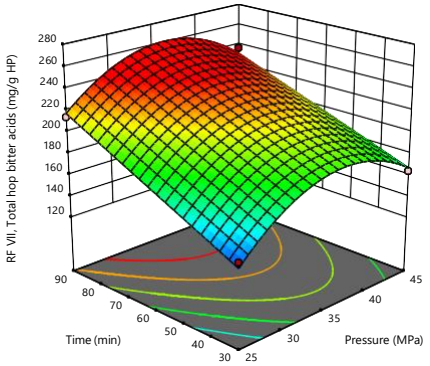


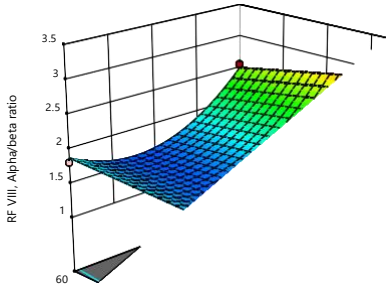
(A) **(B)** **(C)**

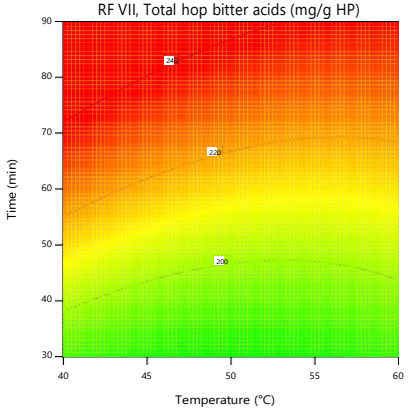
Appendix 18. Response surface 3D and 2D plots showing the effects of SFE-CO_2 pressure (P), temperature (T) and time (τ) on the *Ella* hop: (A) RFVII: total bitter acids mg/g HP; (B) RFVIII: α/β ratio; HP: hop pellets

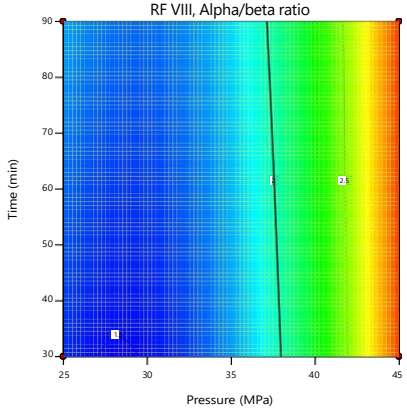


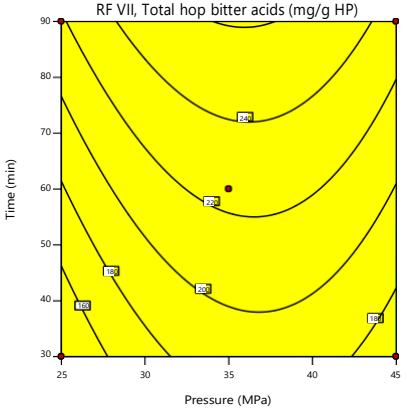


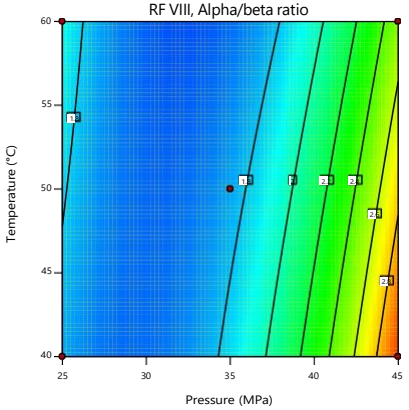












(A) **(B)** **(A)** **(B)**

Appendix 19. Confirmation parameters for the quadratic models of *Ella* variety hop SFE-CO_2 extract, content of cohumulone (RF I), humulone + adhumulone (RF II), colupulone (RF III), lupulone+adlupulone (RF IV), total α acids (RF V), total β acids (RF VI) and total bitter acids (RF VII), and α/β ratio (RF VIII)

Confirmation parameters	RF I	RF II	RF III	RF IV	RF V	RF VI	RF VII	RF VIII
Predicted Mean	58.5769	103.597	55.4237	31.7651	162.174	87.1888	249.363	1.88224
Predicted Median	58.5769	103.597	55.4237	31.7651	162.174	87.1888	249.363	1.88224
Std.Dev.	1.88537	3.74673	2.83965	1.32275	5.43281	4.06503	7.64893	0.127612
SE Pred.	2.30842	4.58744	3.47682	1.61955	6.65184	4.97716	9.36523	0.156246
95% PI low	53.4334	93.3754	47.6768	28.1565	147.353	76.099	228.495	1.5341
Data Mean	62.41	102.02	51.08	30.89	164.43	81.97	246.4	2.02
95% PI high	63.7203	113.818	63.1705	35.3737	176.995	98.2786	270.23	2.23038

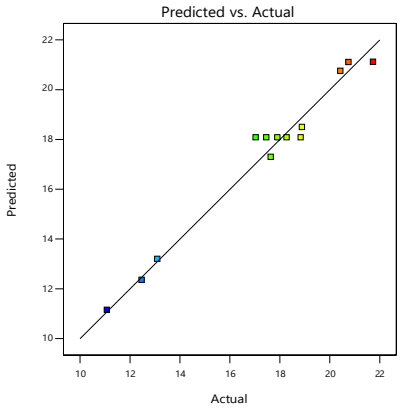
Std. Dev.: standard deviation; SE Pred.: standard deviation associated with the prediction of an individual observation; PI: prediction interval; SFE-CO_2: supercritical carbon dioxide extraction; RF I: response factor; HP: hop pellets

Appendix 20. Fit statistics parameters for the selected models of *Ella* hop PLE-EtOH extraction optimization

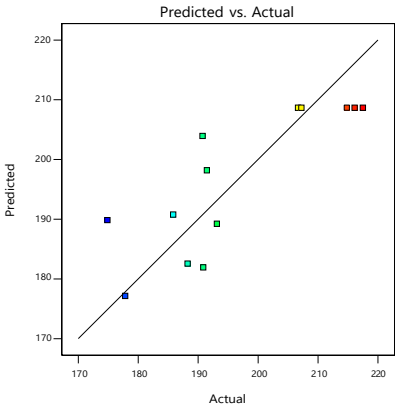
Fit statistics parameters	RFI	RFII	RFIII	RFIV	RFV	RFVI	RFVII
Suggested model	Quadratic	Quadratic	Quadratic	Linear	Quadratic	Quadratic	Linear
Std. Dev.	0.6561	10.51	1.18	104.80	7.37	1.60	0.3210
Mean	17.37	196.62	34.25	2102.85	359.65	57.52	9.64
C.V. %	3.78	5.35	3.44	4.98	2.05	2.78	3.33
R^2	0.9764	0.6882	0.9827	0.7858	0.9835	0.9899	0.3163
Adjusted R^2	0.9595	0.4656	0.9703	0.7430	0.9716	0.9827	0.1795
Predicted R^2	0.9157	-1.0430	0.8973	0.5648	0.8832	0.9303	-0.1709
Adeq. Precision	22.3575	4.4124	25.7859	13.9136	28.5413	32.0888	5.0152

Std. Dev.: standard deviation; C.V.: coefficient of variation; RF: response factor;RF I: PLE-EtOH extract yield, g/100 g HR; RFII: TPC, mg GAE/g E; RFIII: TPC, mg GAE/g HR; RFIV: $TEAC_{ORAC}$, mg TE/g E; RFV: $TEAC_{ORAC}$, mg TE/g HR; RFVI: Xanthohumol content, mg/g E; RFVII: Xanthohumol content, mg/g HR.

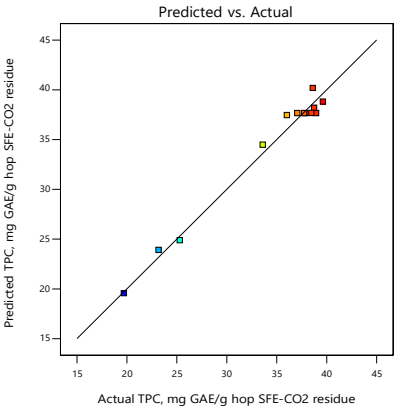
Appendix 21. Predicted vs. Actual values of models of RF I: PLE-EtOH extract yield (g/100 g HR), RFII: TPC (mg GAE/g E), RFIII: TPC (mg GAE/g HR), RFIV: $TEAC_{ORAC}$ (mg TE/g E), RFV: $TEAC_{ORAC}$ (mg TE/g HR), RFVI: XN content (mg/g E), RFVII: XN content (mg/g HR) of *Ella* variety hop PLE-EtOH extract



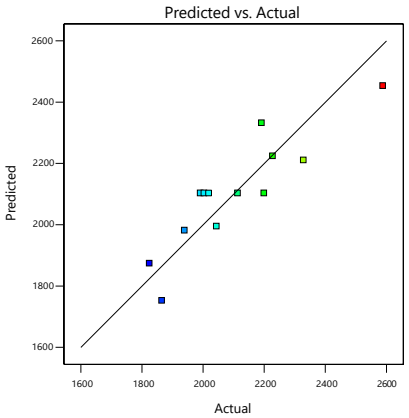
RFI



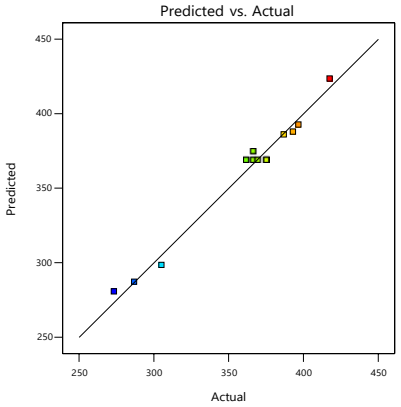
RFII



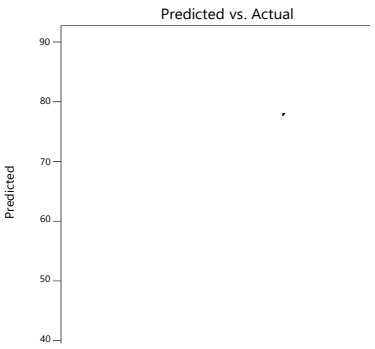
RFIII



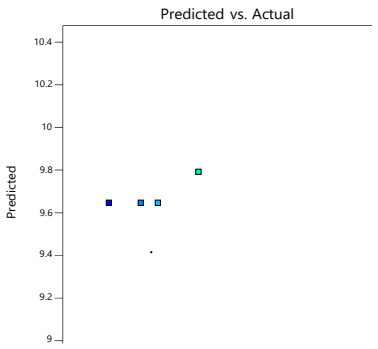
RFIV



RFV



RFVI



RFVII

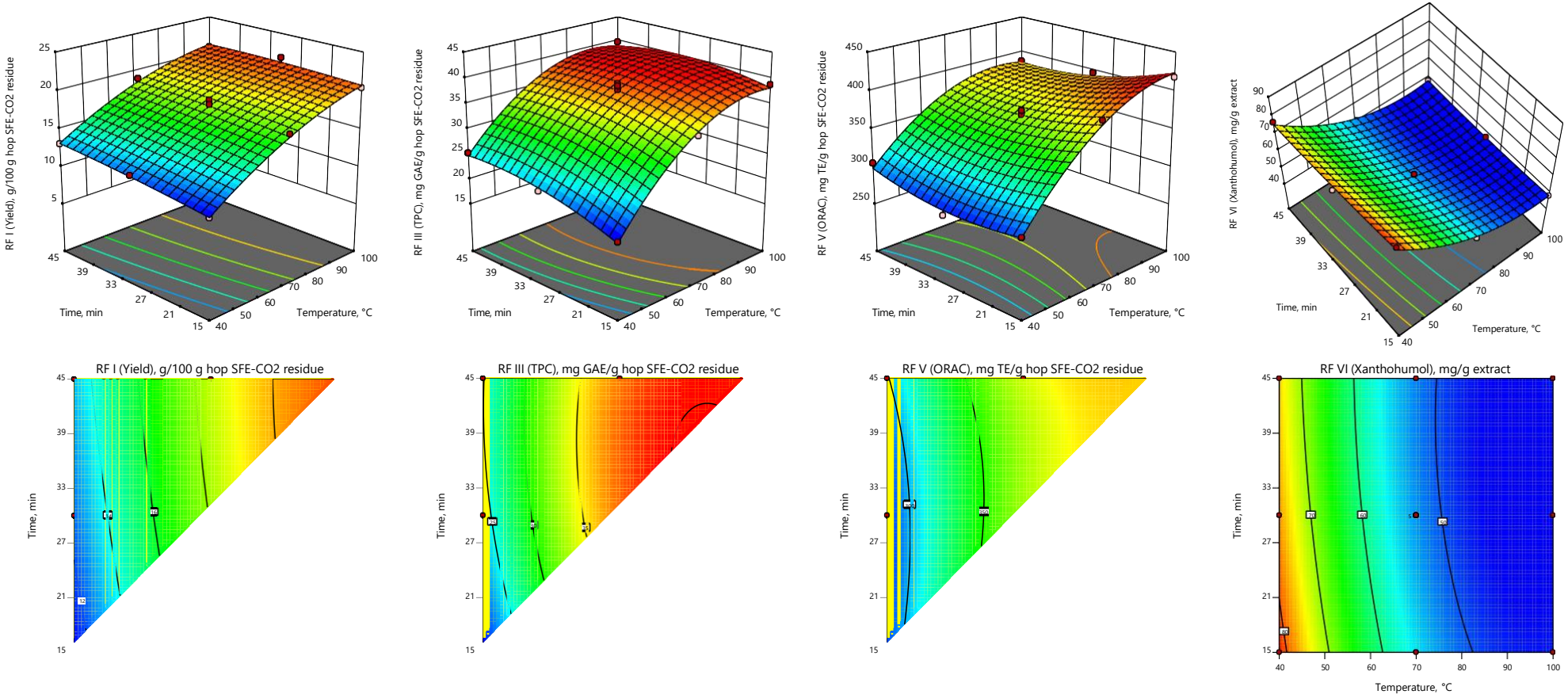
Appendix 22. Analysis of variance of the regression parameters for the response surface models of *Ella* hop PLE- EtOH extract yield (g/100 g HR), TPC (mg GAE/g E and HR), $TEAC_{ORAC}$ (mg TE/g E and HR) and xanthohumol content (mg/g E and HR)

Source	SS	df	MS	F-value	p-value
RFI:Yield, g/100g HR					
Model	124.53	5	24.91	57.85	< 0.0001*
T-Temperature	115.11	1	115.11	267.37	< 0.0001*
τ -Time	2.16	1	2.16	5.02	0.0601**
Tτ	0.7140	1	0.7140	1.66	0.2387**
T^2	4.98	1	4.98	11.58	0.0114*
τ^2	0.0929	1	0.0929	0.2159	0.6563**
Residual	3.01	7	0.4305		
Lack of Fit	1.06	3	0.3538	0.7248	0.5879**
Pure Error	1.95	4	0.4881		
Cor Total	127.54	12			
RFII: TPC, mg GAE/g E					
Model	1706.89	5	341.38	3.09	0.0867**
T-Temperature	1.34	1	1.34	0.0122	0.9153**
τ -Time	49.31	1	49.31	0.4464	0.5255**
Tτ	40.32	1	40.32	0.3651	0.5648**
T^2	928.77	1	928.77	8.41	0.0230*
τ^2	159.44	1	159.44	1.44	0.2686**
Residual	773.16	7	110.45		
Lack of Fit	668.17	3	222.72	8.48	0.0330*
Pure Error	105.00	4	26.25		
Cor Total	2480.05	12			
RFIII: TPC, mg GAE/g R					
Model	549.78	5	109.96	79.39	< 0.0001*
T-Temperature	397.07	1	397.07	286.70	< 0.0001*
τ -Time	13.26	1	13.26	9.58	0.0175*
Tτ	5.55	1	5.55	4.00	0.0855**
T^2	86.82	1	86.82	62.69	< 0.0001*
τ^2	7.81	1	7.81	5.64	0.0493*
Residual	9.69	7	1.38		
Lack of Fit	7.62	3	2.54	4.90	0.0793**
Pure Error	2.07	4	0.5180		
Cor Total	559.48	12			
RFIV: $TEAC_{ORAC}$, mg TE/g E					
Model	4.029E+05	2	2.015E+05	18.34	0.0005*
T-Temperature	3.148E+05	1	3.148E+05	28.66	0.0003*
τ -Time	88117.25	1	88117.25	8.02	0.0178*
Residual	1.098E+05	10	10983.10		
Lack of Fit	78214.41	6	13035.74	1.65	0.3270**
Pure Error	31616.55	4	7904.14		
Cor Total	5.127E+05	12			
RFV: $TEAC_{ORAC}$, mg TE/ g HR					
Model	22595.47	5	4519.09	83.25	< 0.0001*
T-Temperature	18809.28	1	18809.28	346.52	< 0.0001*
τ -Time	256.76	1	256.76	4.73	0.0661**

Source	SS	df	MS	F-value	p-value
Tτ	599.27	1	599.27	11.04	0.0127*
T^2	2929.84	1	2929.84	53.98	0.0002*
τ^2	404.04	1	404.04	7.44	0.0294*
Residual	379.97	7	54.28		
Lack of Fit	248.16	3	82.72	2.51	0.1976**
Pure Error	131.81	4	32.95		
Cor Total	22975.43	12			
RFVI: Xanthohumol content, mg/g E					
Model	1758.44	5	351.69	137.42	< 0.0001*
T-Temperature	1386.24	1	1386.24	541.68	< 0.0001*
τ -Time	18.24	1	18.24	7.13	0.0320*
Tτ	10.96	1	10.96	4.28	0.0773**
T^2	267.74	1	267.74	104.62	< 0.0001*
τ^2	3.20	1	3.20	1.25	0.3006**
Residual	17.91	7	2.56		
Lack of Fit	14.44	3	4.81	5.54	0.0658**
Pure Error	3.47	4	0.8684		
Cor Total	1776.35	12			
RFVII: Xanthohumol content, mg/g HR					
Model	0.4766	2	0.2383	2.31	0.1494**
T-Temperature	0.3504	1	0.3504	3.40	0.0949**
τ -Time	0.1261	1	0.1261	1.22	0.2944**
Residual	1.03	10	0.1030		
Lack of Fit	0.5886	6	0.0981	0.8884	0.5737**
Pure Error	0.4417	4	0.1104		
Cor Total	1.51	12			

PLE-EtOH: pressurized liquid extraction with ethanol; TPC: total phenolic content; GAE: gallic acid equivalents; TEAC: Trolox equivalent antioxidant capacity; ORAC: oxygen radical absorance capacity; TE: Trolox equivalents; RF: response factor E: extract; HR: hop residue after SFE-CO_2(36 MPa, 40°C, 90 min);

Appendix 23. Response surface 3D and 2D plots showing the effects of PLE-EtOH temperature (T) and time (τ) on the *Ella* hop: (A) RFI, PLE-EtOH yield (g/100 g HR); (B) RFIII, TPC (mg GAE/g HR), (C) RFV, TEAC$_{ORAC}$ (mg TE/ g HR); (D) RFVI, xanthohumol content (mg/g E)



A **B** **C** **D**

Appendix 24. Confirmation parameters for the selected models of *Ella* hop PLE-EtOH extraction under the optimal conditions of 40 °C and 15 min

Fit statistics parameters	RFI	RFII	RFIII	RFIV	RFV	RFVI	RFVIII
Predicted mean	11.1430	177.0820	19.5286	2453.0900	286.9370	81.9970	9.2580
Predicted median	11.1430	177.0820	19.5286	2453.0900	286.9370	81.9970	9.2580
Std. Dev.	0.6561	10.5096	1.1768	104.8000	7.3670	1.5997	0.3209
SE Pred.	0.8779	14.0618	1.5746	124.4550	9.8577	2.1404	0.3811
95% PI (low)	9.0671	143.8310	15.8053	2175.7800	263.6270	76.9356	8.4086
Exp. data mean	11.2700	187.9800	21.1900	2700.2300	304.3200	83.4500	9.4100
95% PI (high)	13.2189	210.3330	23.2519	2730.3900	310.2470	87.0583	10.1073

Std. Dev.: standard deviation; Exp. Data mean: experimental data mean; SE Pred.: standard deviation associated with the prediction of an individual observation; PI: prediction interval; RF: response factor;RF I: PLE-EtOH extract yield, g/100 g HR; RFII: TPC, mg GAE/g E; RFIII: TPC, mg GAE/g HR; RFIV: $TEAC_{ORAC}$, mg TE/g E; RFV: $TEAC_{ORAC}$, mg TE/g HR; RFVI: xanthohumol content, mg/g E; RFVII: Xanthohumol content, mg/g HR;; E: extract; HR: hop residue after SFE-CO_2 (36 MPa, 40°C, 90 min)

Appendix 25. Confirmation parameters for the selected models of *Ella* hop PLE-EtOH extraction under the optimal conditions of 85 °C and 18 min

Fit statistics parameters	RF I	RF II	RF III	RF IV	RF V	RF VI	RF VIII
Predicted mean	19.4981	197.8790	38.4874	2085.2800	406.8230	48.7596	9.6495
Predicted median	19.4981	197.8790	38.4874	2085.2800	406.8230	48.7596	9.6495
Std. Dev.	0.6561	10.5096	1.1768	104.8000	7.3676	1.5997	0.3210
SE Pred.	0.7491	11.9985	1.3436	116.0050	8.4113	1.8264	0.3553
95% PI (low)	17.7268	169.5080	35.3104	1826.8000	386.9330	44.4409	8.8578
Exp. data mean	20.5200	191.4700	39.2900	1936.4900	397.3700	45.7400	9.3900
95% PI (high)	21.2694	226.2510	41.6644	2343.7500	426.7120	53.0782	10.4411

Std. Dev.: standard deviation; Exp. Data mean: experimental data mean; SE Pred.: standard deviation associated with the prediction of an individual observation; PI: prediction interval; RF: response factor; RFI: PLE-EtOH extract yield, g/100 g HR; RFII: TPC, mg GAE/g E; RFIII: TPC, mg GAE/g HR; RFIV: $TEAC_{ORAC}$, mg TE/g E; RFV: $TEAC_{ORAC}$, mg TE/g HR; RFVI: xanthohumol content, mg/g E; RFVII: Xanthohumol content, mg/g HR; E: extract; HR: hop residue after SFE-CO_2 (36 MPa, 40°C, 90 min)

Appendix 26. Analysis of correlation between *Ella* hop PLE-EtOH extract yield (g/100 g HR), TPC (mg GAE/g HR), $TEAC_{ORAC}$ (mg TE/g HR) and xanthohumol content (mg/g HR)

Response factors	RFI	RFIII	RFV	RFVII
RFI	1	0.9340****	0.9292****	0.5005 ^{ns}
RFIII		1	0.9049****	0.2848 ^{ns}
RFV			1	0.3535 ^{ns}
RFVII				1

RF: response factor; RFI: PLE-EtOH extract yield, g/100 g HR; RFIII: TPC, mg GAE/g HR; RFV: $TEAC_{ORAC}$, mg TE/g HR; RFVII: Xanthohumol content, mg/g HR; HR: hop residue after SFE-CO_2 (36 MPa, 40°C, 90 min); GAE: gallic acid equivalents; TEAC: Trolox equivalent antioxidant capacity; TE: Trolox equivalents; TPC: total phenolic content; ORAC: oxygen radical absorbance capacity; *: correlation is significant at the $P<0.05$ level (two-tailed). Pearson correlation coefficients were calculated using GraphPad Prism X software (X).

Appendix 27. Analysis of correlation between *Ella* hop PLE-EtOH extract TPC (mg GAE/g E), $TEAC_{ORAC}$ (mg TE/g E) andxanthohumol content (mg/g E)

Response factors	RFII	RFIV	RFVI
RF II	1	-0.1152 ^{ns}	-0.3241 ^{ns}
RFIV		1	0.8538***
RFVI			1

RF: response factor; RFII: total phenolic content, mg GAE/g E; RFIV: $TEAC_{ORAC}$, mg TE/g E; RFVI: Xanthohumol content, mg/g E; E: extract; GAE: gallic acid equivalents; TEAC: Trolox equivalent antioxidant capacity; TE: Trolox equivalents; TPC: total phenolic content; ORAC: oxygen radical absorbance capacity; ^{ns}: correlation is not significant; *: correlation is significant at the P<0.05 level (two-tailed). Pearson correlation coefficients were calculated GraphPad Prism 7.04 software (2017).

List of publications

List of scientific publications on the topic of the master thesis published in the Clarivate Analytics WOS database:

1. **Nóra Emilia Nagybákay**, Michail Syrpas, Vaiva Vilimaitė, Laura Tamkutė, Audrius Pukalskas, Petras Rimantas Venskutonis and Vaida Kitrytė. Optimized supercritical CO_2 extraction enhances the recovery of valuable lipophilic antioxidants and other constituents from dual-purpose hop (*Humulus lupulus* L.) variety *Ella* // *Antioxidants*, 2021, status: under revision (I.F. 5.014 (2019)).

List of presentations on the master thesis topic

1. **Nóra Emilia Nagybákay**, Michail Syrpas, Vaiva Vilimaitė, Laura Tamkutė, Audrius Pukalskas, Petras Rimantas Venskutonis and Vaida Kitrytė. Optimization of supercritical carbon dioxide extraction to recover valuable lipophilic fraction from *Ella* hops (*Humulus lupulus* L.) // Student conference of Chemistry and chemical technology, May 16, 2021, Kaunas, Lithuania. // Awarded first prize of the section on food, agro- and biochemistry - II

2. **Nóra Emilia Nagybákay**, Vaida Kitrytė, Michail Syrpas, Audrius Pukalskas, Aušra Šipailienė, Petras Rimantas Venskutonis, Kristina Perminaitė, Daiva Majienė, Kristina Ramanauskienė. Bitter acid and antioxidant-rich *Ella* hop extract // "Technorama 2021": from vision to innovation!, May 28, 2021. Kaunas, Lithuania. Innovation catalogue/ Santaka Valley. Kaunas: Kaunas University of Technology, 2021

List of presentations on other topic

1. Nóra Emilia Nagybákay, Shreya Pravin Kumar, Simona Sedláčková. Line of 4 seasons – instant buckwheat porridge // "Technorama 2020": from vision to innovation. Kaunas: Kaunas University of Technology. 2020. p. 117-119