JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

ANTIOXIDANT CHARACTERISTICS OF METHANOL AND ETHANOL EXTRACTS OF SELECTED VARIETIES OF *HUMULUS LUPULUS*

Nora Haring, Dana Urminská, Blažena Drábová*

Address(es): Doc. RNDr. Dana Urminská, CSc.,
Institute of Biotechnology, Faculty of Biotechnology and Food Science, Slovak Agricultural University in Nitra, Trieda Andreja Hlinku 2, 949 76 Nitra.

*Corresponding author: dana.urminska@uniag.sk

https://doi.org/10.55251/jmbfs.11108

ARTICLE INFO

Received 7. 3. 2024
Revised 5. 4. 2024
Accepted 17. 4. 2024
Published xx.xx.201x

Regular article

OPEN ACCESS

ABSTRACT

Hops are an important ingredient in beer composition. In recent times, attention has been focused on the positive effects of hops as a plant with a high content of substances with antioxidant properties. Knowing the total concentration of polyphenols or the total antioxidant capacity of individual hop varieties could contribute to the creation of the so-called functional beer with a high content of biologically active substances. The results showed a connection between the content of α-bitter acids and the total content of polyphenols in hops. The highest concentration of polyphenols, corresponding to 2.52 g/dm^3 of gallic acid, was the ethanol extract of the Polaris variety, prepared at a temperature of 100 °C from cryo-pulverized hops, whose content of α-bitter acids is up to 20 %. With the use of the ABTS cationic radical higher antioxidant activity, on average, was determined in the methanolic extracts, on the other hand, with the use of the DPPH radical, the ethanolic extracts showed a higher antioxidant activity. Using the ABTS method, the highest total antioxidant capacity was determined in the methanol extract of the Polaris variety, prepared from pulverized hops at a temperature of 100 °C, namely 25.50 mmol/dm^3 Trolox equivalents. The DPPH method had the highest antioxidant efficiency, 94.9 %, determined in the methanol extract of the Polaris variety prepared from cryo-pulverized hops by extraction at a temperature of 100 °C. By comparing the results of analyzes of several hop varieties, the Polaris variety appears to be suitable for the preparation of functional beer.

Keywords: hops, antioxidant capacity, polyphenols

INTRODUCTION

Common hop (*Humulus lupulus*) is a perennial herbaceous liana from the Cannabeaceae family cultivated by humans since time immemorial **(Alonso-Esteban *et al.*, 2019).** In brewing, the flowers of the female plants are used, which are 2.5-5 cm long, cone-shaped cones that develop shortly after pollination. Cones contain a diverse amount of phytochemicals in the glandular trichomes (so-called lupulin glands) on the lower part of the bracts, which are responsible for the effects of hops on human health **(Almaguer *et al.*, 2014; Liberatore *et al.*, 2018).** Lupulin is secreted from the lupulin glands **(Esslinger 2009),** in which the primary metabolites are bitter resins and aromatic substances, responsible for the characteristic taste and aroma of hops **(Stevens *et al.*, 1998).** Secondary metabolites are hop oils, resins, and polyphenols. The content of this metabolites in the dry matter of the hop cone ranges from 4-14 % **(Yan *et al.*, 2019).** Hop resins are divided into soft and hard resin fraction. Bitter acids, known as α-bitter acids mainly represented by humulone and β-bitter acids, mainly represented by lupulone, are included in the soft resin fraction. These substances have the most significant effect on the bitterness of beer **(Čermák *et al.*, 2015; Ban *et al.*, 2018).** In addition to hop resins, lupulin contains polyphenols, which can be divided into flavan-3-ols (catechin, epicatechin, proanthocyanidin), phenolic acids (ferulic acid), flavonols (quercetin and kaempferol) and in smaller quantities prenylated flavonoids (xanthohumol, desmethylxanthohumol, isoxanthohumol, 6-prenylnaringenin and 8-prenylnaringenin) **(Gorinstein *et al.*, 2007).** The content of prenylated flavonoids, which are currently of the greatest scientific interest, depends on the variety, agroecological conditions of growing and storing hops. Their amount is generally reported to be around 5 g in 100 g of dry hop cone mass **(Ciriminna *et al.*, 2018).** More specifically, **Karabin *et al.* (2012)** report that the content of xanthohumol is 0.2 - 1.1 g in 100 g dry matter of hop cone and according to **Miligan (2009)** 8-prenylnaringenin occurs only in very low concentrations. Xanthohumol and 8-prenylnaringenin, which is known as the most potent phytoestrogen of hops **(Sun *et al.*, 2022; Lecomte *et al.*, 2023)** are investigated as potential anticancer agents, with significant antiproliferative activity against cancer cells **(Busch *et al.*, 2015).** Xanthohumol has the potential of a drug that could slow the malignant progression of breast cancer **(Kim *et al.*, 2013).**

Hops have long been known in the pharmacopoeia as a plant that alleviates the symptoms of many health problems. Its use was essential in the treatment of toothache, leprosy, fever, during treatment of stomach problems, anxiety and it was even considered a medicament for insomnia **(Korpelainen *et al.*, 2012).** Significant use of hops is the action of its flavonoids in alleviating postmenopausal discomfort **(Keiler *et al.*, 2015).** Nowadays, it is confirmed that the bioactive substances of hops have a wide range of therapeutic and anti-inflammatory properties, the antimicrobial properties of hops against several microorganisms have also been proven **(Oliveira Neto *et al.*, 2017).** Biologically active substances of hops can act as scavengers of free radicals, metal chelating agents, modulators of enzymatic activity and inhibitors of cell proliferation **(Karabin *et al.*, 2015).** Thanks to these positive properties, these hop components are constantly being tested in primary or secondary prevention against some chronic degenerative diseases. The main role in these pathological processes is played by oxidation mechanisms in the organism **(Zugravu *et al.*, 2022).** Hops show a very high antioxidant activity, slowing down or preventing the oxidation of substrates, thereby reducing oxidative stress in cells and the organism **(Lang *et al.*, 2024).** However, the disadvantage of several natural antioxidants is that, in their free form are sensitive to modifications caused by various physicochemical factors, thereby losing their antioxidant properties **(Sharma *et al.*, 2023).** Based on these findings, there is an effort to prepare hop extracts with a high content of active polyphenols, which could find application in functional foods, nutraceuticals, pharmaceuticals, or cosmetics **(Astray *et al.*, 2020; Tronina *et al.*, 2020; Sun *et al.*, 2022).** However, it follows from the results of the research so far that for the assessment of the antioxidant potential of hops, the sources of variability in the results are the variety and growing conditions of hops, the method of solubilization and extraction of biologically active substances, as well as the analytical methods used. In general, it is necessary to realize that many substances in hops act synergistically, thus combining antioxidant effects with anti-inflammatory or antiproliferative effects **(Zugravu *et al.*, 2022).**

MATERIAL A METHODS

Plant materials

Three varieties of common hops (*Humulus lupulus* L.) which differences in the declared content of bitter acids were analysed **(Html 1, Html 2, Html 3)** (Tab. 1).

Table 1 Overview of hop varieties

Variety	Country of origin	α-bitter acids	β-bitter acids	Aroma	Application	Time of hopping
Premiant	Czech Republic	7,3 %	3,5 %	hoppy, slightly spicy	all types of lagers, ALE, shout, porter and wheat beers	universal
Saaz late	Czech Republic	2,69 %	4 %	aromatical, hoppy	especially bottom-fermented beers, lagers	second and third hopping
Polaris	Germany	20 %	6,0 %	hint of pine and mint	special types of craft beers	universal

The homogenous preparation of hops was prepared by two procedures, namely mechanical homogenization, and cryo-pulverization after freezing with liquid nitrogen. During mechanical homogenization, 50 g of natural hop pellets were homogenized manually in a mortar (designation N). When preparing the cryo-pulverized sample, 200 ml of liquid nitrogen was added to 50 g of hop pellets. The frozen hops were then mechanically homogenized into a fine powder (designation C).

Extraction of prenylated flavonoids

The extraction system Dionex™ ASE™ 350 (Thermo Fisher Scientific, USA) was used for the extraction of hop flavonoids. The extraction was carried out with polar solvents 99.8 % (v) methanol and 99.9 % (v) ethanol in six repeated five-minute extraction cycles at a pressure of 10.5 MPa and a temperature of 50 °C, 100 °C, 150 °C or 200 °C.
Determination of the total polyphenols content and the total antioxidant capacity was carried out in 6 repetitions.

Determination of the total polyphenols content

Folin-Ciocalteu reagent was used for the determination of total polyphenols content. 25 µl of extract and 1 ml of Folin-Ciocalteu reagent (1:9, v/v) were added to 1 ml of demineralized water. The solution was vigorously vortexed. After 5 minutes, 1 ml of saturated Na_2CO_3 was added and let to incubation for 15 minutes at room temperature. The total polyphenols content was determined from the absorbance value at 515 nm using a calibration line (0.1–5.0 g/L gallic acid) in mg/L GAE.

Determination of the total antioxidant capacity by the ABTS method

The cationic radical $ABTS^{+}$• was generated by the reaction of 8 mmol/L 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) with 3 mmol/L $K_2S_2O_8$ in a ratio of 2:1 (v/v) in the dark at room temperature for 12 hours. Before determining the total antioxidant capacity, the $ABTS^{+}$• solution was diluted with 96% ethanol (v) so that the absorbance of the solution at a wavelength of 734 nm was equal to the maximum absorbance of the calibration line, prepared by determining the antioxidant capacity of 0 - 1 mmol/L Trolox. To determine the antioxidant capacity, 25 µl of extract was added to 2 ml of $ABTS^{+}$• solution. The reaction took place at room temperature for 30 minutes in the dark. The antioxidant capacity of the extract in mmol TE/L was determined from the absorbance value at 734 nm using the calibration line.

Antioxidant efficiency

The radical form of the compound 1,1-diphenyl-2-(2,4,6-trinitrophenyl-hydrazyl) (DPPH) was prepared by diluting 0.608 mmol/L DPPH with 99.8 % methanol (1:5, v/v). To determine the antioxidant activity of the extract, 200 µl of the sample was added to 3.8 ml of DPPH•. After 30 minutes, the absorbance was measured at 515 nm. The control contained 200 µl of extraction reagent instead of the sample. DPPH inhibition (%) = $[(A_0-A_1)/A_0]x100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the test.

RESULTS AND DISCUSSION

Three varieties of common hops (*Humulus lupulus* L.) were analysed, which differ significantly in the content of bitter acids. The Saaz Late variety has a low content of these substances (2.69 % α-bitter acids and 4 % β-bitter acids), the average content of bitter acids is typical for the Premiant variety (7.3 % α-bitter acids and 3.5 % β -bitter acids), on the other hand, the Polaris variety is characterized by a high content of bitter acids (20 % α-bitter acids and 6 % β-bitter acids).

The total polyphenols content

The method of determining the total polyphenols content in plant material by reducing the Folin-Ciocalteu reagent was used by several authors **(Sánchez-Rangel *et al.*, 2013; Blainski *et al.*, 2013; Kamboj *et al.*, 2015; Lamuela-Raventós, 2017)**. Methanol and ethanol extracts, prepared from pellets of natural hops homogenized mechanically (N) and from hops cryo-pulverized using liquid nitrogen (C), were analyzed. This modification of the sample was carried out because there are currently several commercial hop preparations available in the form of Cryohops®, modified by this method. It is a procedure in which, before pelleting, fresh hop heads are first frozen with liquid nitrogen under a low oxygen content at a temperature of -28 ºC and only after freezing are they pelleted **(Tembo, 2020).** Publications by several authors indicate that such processing of hops should preserve a higher content of biologically active components of hops. Their content should be up to twice that of hops processed by classic pelleting **(Stokholm & Shellhammer, 2020).**
The highest total polyphenols content was determined in the ethanol extracts (Figure 1) of the Polaris variety, namely in the extract obtained at a temperature of 100 °C (2.52 g/L GAE in extract C, respectively 2.51 g/L in extract N). Cryo-pulverization had no significant effect on the extractability of total polyphenols from hops. The biggest differences were determined in the Saaz Late extract at an extraction temperature of 150 °C, when compared to the N homogenate due to cryo-pulverization, total polyphenols content in the extract increased by 16 %, and in the Premiant C extract, total polyphenols content increased by 10% at a temperature of 100 °C. The most suitable extraction temperature when using ethanol as an extraction agent was 100 °C.

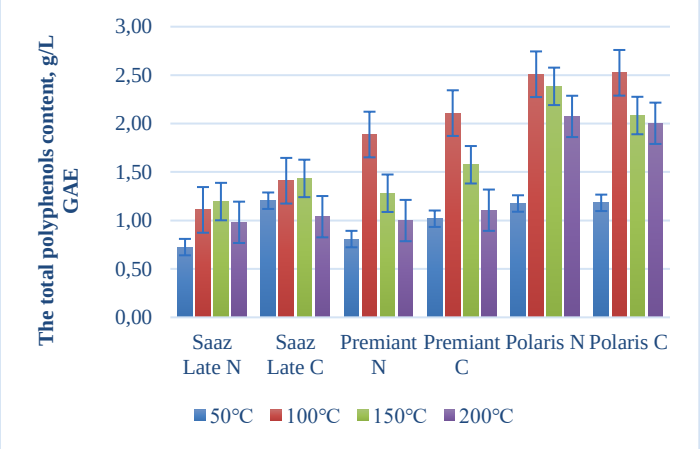

Figure 1 The total polyphenols content in ethanol extracts of hops

In extracts prepared using methanol, the mass yield of polyphenols was lower than in ethanol extracts (Figure 2). Also in this case, the highest total polyphenols content was determined in the Polaris C extract (2.43 g/L GAE). The mass yield of polyphenols was lower by 3.6 % compared to the ethanol extract. The highest total polyphenols content was obtained at a temperature of 100 °C. When comparing N and C methanol extracts, the mass yield of polyphenols due to the cryo-pulverization of Saaz Late was higher by 20 %, Premiant by 3 % and Polaris by 6 %. Processing hop pellets into extracts can be an effective way to reduce the amount of hops used in the production of beers enriched with polyphenols **(Bramforth, 2006)**. In the past, hexane, methanol and dichloromethane were used as extraction agents, currently subcritical CO_2 and ethanol are mainly used in terms of safety and environmental impact **(Kowalczyk *et al.*, 2013).** Due to its non-polar nature, subcritical CO_2 extraction is selective for aromatic components and hop resins, not for polyphenols **(Mander *et al.*, 2010).** Substances with a polar character, including polyphenols, are dissolved by ethanol **(Stevens *et al.*, 2004)**, which is also confirmed by the results of this work.
In total polyphenols content, there are no significant differences in the recovery of these substances using the extraction agents ethanol and methanol. From the point of view of toxicity, this is a significant finding, preferring ethanol. Methanol, ethanol, propanol, ethyl acetate, water or acetone are recommended for the extraction of phenolics **(Antolovich *et al.*, 2000).** However, their use in the food industry is limited by European legislation, with regard to the possible content of residues in extracted foods **(Varlet *et al.*, 2014).**

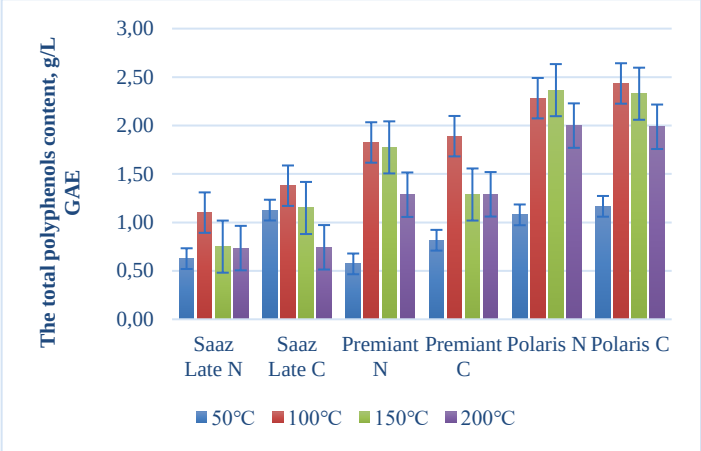

Figure 2 The total polyphenols content in methanol extracts of hops

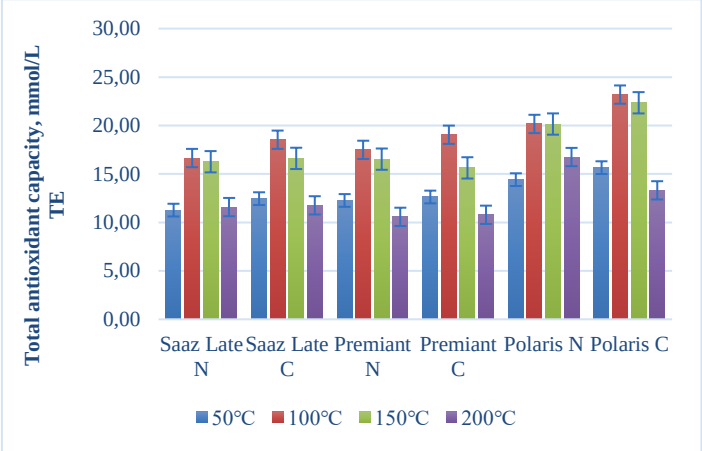

Figure 3 The total antioxidant capacity of ethanol extracts of hops

There is a connection between the amount of α-bitter acids and the total content of polyphenols in hops. In the analyzed varieties, the total content of polyphenols in ethanol and methanol extracts obtained at 100 °C decreases in the order Polaris > Premiant > Saaz Late.

In methanol extracts from seven varieties of hops (Styrian Golding, Hersbrucker, Hallertau Blanc, Cascade Cryo Hops®, Pacifica Jade, Pacifica, Topaz) total polyphenols ranged from 27.4 to 49.8 mg GAE/g dried hops **(Iannone *et al.*, 2022).** Different authors report very different contents of total polyphenols in the methanol extract, e.g. is 2.39 mg GAE/g **(Fărcaş *et al.*, 2017),** 7.4 µg GAE/g (**Maliar *et al.*, 2017).**

Polyphenols are generally considered to be thermally labile compounds **(Rajbhar *et al.*, 2015).** In traditional extraction procedures, the highest yields of these substances are obtained at temperatures of 60 - 80 °C **(Casazza *et al.*, 2012).** On the other hand, high pressure extraction studies prefer temperatures above 100 °C. For example, increasing the extraction temperature to 180 - 200 °C meant an increase in both total polyphenols content and antioxidant capacity in the canola flour extract **(Nandasiri *et al.*, 2019). Ibañez *et al.* (2003)** carried out a sequential extraction of medicinal rosemary at temperatures of 100, 150 and 200 °C, in which they used water as a solvent. They analyzed the extracts by HPLC and found that polar phenolic compounds were extracted at low temperatures, while less polar phenolic compounds were extracted at higher temperatures. Water's polarity was reduced by higher temperatures, allowing it to solvate non-polar compounds and extract them. The study of total polyphenols content showed that an extraction temperature higher than 100 °C reduces total polyphenols content in hop extracts. Thermal degradation of these substances takes place, while this process also involves treatment, or material homogenization and type of solvent. Several authors (**Lapornik *et al.*, 2005; Turkmen *et al.*, 2006; Turkmen *et al.*, 2007; Xi *et al.*, 2009; Inglett *et al.*, 2010)** state that the extraction of polyphenolic substances will increase by using dilute solvents, e.g. ethanol : water 1:1 (v/v). **Kowalczyk *et al.* (2013)** found that a 50 % aqueous ethanol solution was a better solvent for these substances from plant materials than pure ethanol.

The total antioxidant capacity

Several methods are described for determining the antioxidant capacity of substances **(Wang *et al.*, 1996; Ghiselli *et al.*, 2000; Bartosz, 2003; Sies, 2007).** The reactions of antioxidants with transition metals or direct reactions of quenching or scavenging of free radicals are most often used in them. They are methods of evaluating the ability of substances to eliminate radicals and methods that assess the redox properties of substances **(Harasym *et al.*, 2014).** ABTS cation radical scavenging assay was used for the analysis of hop extracts. **(Ozgen *et al.*, 2006)**. The designation TEAC method (Trolox Equivalent Antioxidant Capacity, **Proestos *et al.*, 2009)**, is based on the fact that the antioxidant activity of the sample is compared with the antiradical activity of the standard substance 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox).

Of the ethanol extracts, Polaris C extracted at 100 °C had the highest total antioxidant capacity (23.19 mmol/L TE, Figure 3). For comparison, the difference between the same prepared Polaris C and Polaris N extract was 13 % against N. The lowest antioxidant capacity was determined in the Saaz Late N extract (11.27 mmol/L TE in the extract obtained at 50 °C), which corresponds to the declared lowest content of α-bitter acids from the analyzed hops (2.69 %). Cryo-pulverization of hops increased the ethanol extractability of substances with antioxidant action at the most suitable temperature of 100 °C in the analyzed hops by 11.8 %. **Arsene *et al.* (2015)** found a direct correlation of antioxidant activities and the total polyphenols content in the hop's ethanolic extract. A good antiradical activity, corresponding to 0.78 - 0.99 mg/ml was determined in methanol extracts of hops, which had a higher polyphenolic content of 7.12 mg GAE/g (**Keskin *et al.*, 2019)**.

Polaris C methanol extract obtained at 100 °C (25.50 mmol/L TE, Figure 4) had the highest antioxidant capacity, while Saaz Late N extracted at 150 °C (10.30 mmol/L TE) had the lowest antioxidant capacity. Cryo-pulverization of hops increased the methanol extractability of substances with antioxidant action at the most suitable temperature of 100 °C in the analyzed hops by 15.2 %.

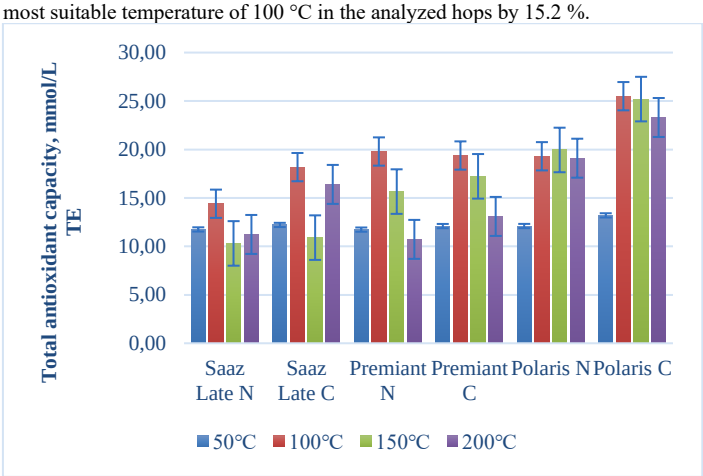

Figure 4 total antioxidant capacity of methanol extracts of hops

The ABTS method was used to determine the antioxidant activity in methanol extracts of Styrian Golding, Hersbrucker, Hallertau Blanc, Cascade Cryo Hops®, Pacifica Jade, Pacifica, Topaz hops. The Hallertau Blanc variety had the highest activity, 1.036 µmol TE/mg of dry hop, on the other hand, the extract from the Cascade variety showed the lowest antioxidant capacity, only 0.114 µmol TE/mg of dry hop (**Iannone *et al.*, 2022**).

Antioxidant efficiency

The ability to scavenge the DPPH radical was widely used to determine the antioxidant activity of crude extracts or purified compounds from plants. The results are expressed as percentage inhibition of radical activity. The highest antioxidant efficiency of all analyzed extracts, up to 94.9 %, was shown by the Polaris C methanol extract prepared from cryo-pulverized hops by extraction at a temperature of 100 °C (Figure 5 and 6). Highly effective substances with antioxidant activity were extracted with methanol from the hops Polaris N (by extraction at a temperature of 100 °C 91.6 % inhibition) and Polaris C (by extraction at a temperature of 100 °C 78.6 % inhibition), ethanol from hops Premiant C (by extraction at at a temperature of 100 °C 88.76 % inhibition). **Krofta *et al.* (2008)** were determined differences in the values of antioxidant activity in four Czech and ten world hop varieties by the DPPH radical. The highest antioxidant activities (from 70 to 80 %) were determined in hops Saaz and Spalter Select. V ostatných chmeľoch bola antioxidant activity in the scope of 40 to 60 %. The comparison of the antioxidant efficiency of the Premiant variety is interesting. Fresh green hop Premiant were pressed, wrapped in paper and than stored. Hot water was used for extract preparation. The antioxidant efficiency of such an extract was 53.7 % (**Krofta *et al.*, 2008).** While in the extracts prepared in this work from pelleted Premiant hops, the highest antioxidant activity of Premiant C, 88.71 %, was determined in the ethanol extract. By analyzing water and ethanol extracts (at 90 °C) of commercially used hops (No. 9910403006 Taiwan Tobacco and Liquor Corporation), it was found that the highest antioxidant activity expressed by 50 % inhibitory concentration, which indicates required concentration to scavenge 50 % of DPPH• aqueous extracts of hops, namely

456.08 µg/ml. In contrast, the extract prepared using 55 % ethanol had an activity of only 184.9 µl/ml **(Wu *et al.*, 2020).**

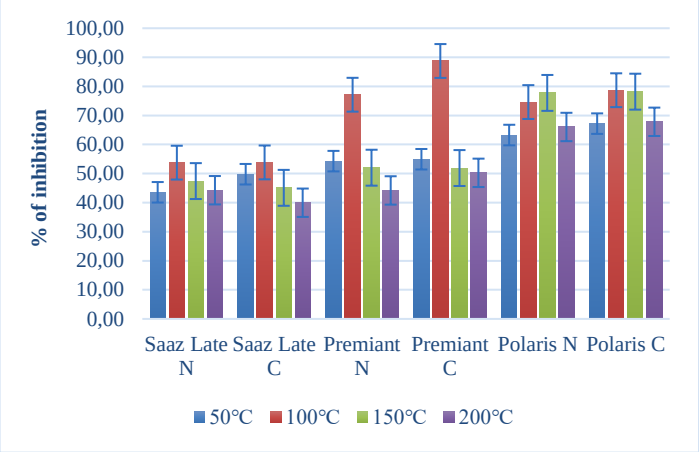

Figure 5 Antioxidant efficiency of ethanol extracts of hops

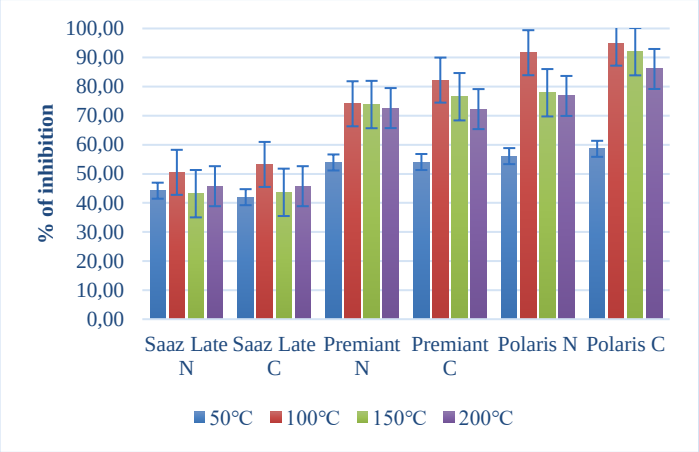

Figure 6 Antioxidant efficiency of methanol extracts of hops

CONCLUSION

Currently, in brewing practice, in addition to classic cold hopping, beer is enriched with biologically active substances with an antioxidant effect, as well as the addition of pure hop extracts. The results of the work showed a connection between the content of α-bitter acids and the total content of polyphenols in hops. Ethanol extraction under high pressure at 100 °C is an effective preparation in which an extract with an application-interesting content of antioxidants is obtained. Cryo-pulverization of hops increases the yield of these substances, which supports the use of Cryohops® in brewing practice. Of the hop varieties that were analyzed, the Polaris variety appears to be the most suitable for the preparation of beer with functional properties.

Acknowledgements: This work was supported by the Slovak Research and Development Agency under the Contract no. APVV-21-0206.

REFERENCES

Almaguer, C., Schönberger, C., Gastl, M., Arendt, E. K., & Becker, T. (2014). Humulus lupulus–a story that begs to be told. A review. *Journal of the Institute of Brewing*, *120*(4), 289-314. **https://dx.doi.org/10.1002/jib.160**

Alonso-Esteban, J. I., Pinela, J., Barros, L., Ćirić, A., Soković, M., Calhelha, R. C., & Ferreira, I. C. (2019). Phenolic composition and antioxidant, antimicrobial and cytotoxic properties of hop (Humulus lupulus L.) Seeds. *Industrial Crops and Products*, *134*, 154-159. **https://dx.doi.org/10.1016/j.indcrop.2019.04.001**

Antolovich, M., Prenzler, P., Robards, K., & Ryan, D. (2000). Sample preparation in the determination of phenolic compounds in fruits. *Analyst*, *125*(5), 989-1009. **https://dx.doi.org/10.1039/b000080i**

Astray, G., Gullón, P., Gullón, B., Munekata, P. E., & Lorenzo, J. M. (2020). Humulus lupulus L. as a natural source of functional biomolecules. *Applied Sciences*, *10*(15), 5074. **https://dx.doi.org/10.3390/app10155074**

Ban, Z., Qin, H., Mitchell, A. J., Liu, B., Zhang, F., Weng, J. K., & Wang, G. (2018). Noncatalytic chalcone isomerase-fold proteins in Humulus lupulus are auxiliary components in prenylated flavonoid biosynthesis. *Proceedings of the national Academy of Sciences*, *115*(22), E5223-E5232. **https://dx.doi.org/10.1073/pnas.1802223115**

Bartosz, G. (2003). Total antioxidant capacity. *Advances in clinical chemistry*, *37*, 219-292. **https://dx.doi.org/10.1016/s0065-2423(03)37010-6**

Blainski, A., Lopes, G. C., & De Mello, J. C. P. (2013). Application and analysis of the Folin Ciocalteu method for the determination of the total phenolic content from Limonium brasiliense L. *Molecules*, *18*(6), 6852-6865. **https://dx.doi.org/10.3390/molecules18066852**

Bamforth, C. (Ed.). (2006). *Brewing: new technologies*. Woodhead Publishing. **https://dx.doi.org/10.1201/9781439824603**

Busch, C., Noor, S., Leischner, C., Burkard, M., Lauer, U. M., & Venturelli, S. (2015). Anti-proliferative activity of hop-derived prenylflavonoids against human cancer cell lines. *Wiener Medizinische Wochenschrift (1946)*, *165*(11-12), 258-261. **https://dx.doi.org/10.1007/s10354-015-0355-8**

Casazza, A. A., Aliakbarian, B., Sannita, E., Perego, P. (2012). High-pressure high-temperature extraction of phenolic compounds from grape skins. *International journal of food science & technology*, *47*(2), 399-405. **https://dx.doi.org/10.1111/j.1365-2621.2011.02853.x**

Cirimina, R., Albanese, L., Stefano, V., Delisi, R., Avellone, G., Meneguzzo, F., Pagliaro, M. (2018). Beer produced via hydrodynamic cavitation retains higher amounds of xanthohumol and other hops prenylflavonoids. *LWT*, 91, 160-167. **https://dx.doi.org/10.1016/j.lwt.2018.01.037**

Čermák, P., Palečková, V., Houška, M., Strohalm, J., Novotna, P., Mikyška, A., & Sikorova, M. (2015). Inhibitory effects of fresh hops on Helicobacter pylori strains. *Czech Journal of Food Sciences*, *33*(4), 302-307. **https://dx.doi.org/10.17221/261/2014-cjfs**

Esslinger, H. M. (Ed.). (2009). *Handbook of brewing: processes, technology, markets*. John Wiley & Sons. **https://dx.doi.org/10.1002/9783527623488**

Ghiselli, A., Serafini, M., Natella, F., & Scaccini, C. (2000). Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. *Free Radical Biology and Medicine*, *29*(11), 1106-1114. **https://dx.doi.org/10.1016/s0891-5849(00)00394-4**

Gorinstein, S., Caspi, A., Libman, I., Leontowicz, H., Leontowicz, M., Tashma, Z., & Trakhtenberg, S. (2007). Bioactivity of beer and its influence on human metabolism. *International journal of food sciences and nutrition*, *58*(2), 94-107. **https://dx.doi.org/10.1080/09637480601108661**

Harasym, J., & Oledzki, R. (2014). Effect of fruit and vegetable antioxidants on total antioxidant capacity of blood plasma. *Nutrition*, *30*(5), 511-517. **https://dx.doi.org/10.1016/j.nut.2013.08.019**

Iannone, M., Ovidi, E., Vitalini, S., Laghezza Masci, V., Marianelli, A., Iriti, M., & Garzoli, S. (2022). From hops to craft beers: Production process, vocs profile characterization, total polyphenol and flavonoid content determination and antioxidant activity evaluation. *Processes*, *10*(3), 517. **https://dx.doi.org/10.3390/pr10030517**

Ibañez, E., Kubátová, A., Señoráns, F. J., Cavero, S., Reglero, G., Hawthorne, S. B. (2003). Subcritical water extraction of antioxidant compounds from rosemary plants. *Journal of agricultural and food chemistry*, *51*(2), 375-382. **https://dx.doi.org/10.1021/jf025878j**

Inglett, G. E., Rose, D. J., Chen, D., Stevenson, D. G., & Biswas, A. (2010). Phenolic content and antioxidant activity of extracts from whole buckwheat (Fagopyrum esculentum Möench) with or without microwave irradiation. *Food Chemistry*, *119*(3), 1216-1219. **https://dx.doi.org/10.1016/j.foodchem.2009.07.041**

Karabín, M., Hudcova, T., Jelinek, L., & Dostalek, P. (2015). Biotransformations and biological activities of hop flavonoids. *Biotechnology advances*, *33*(6), 1063-1090. **https://dx.doi.org/10.1016/j.biotechadv.2015.02.009**

Keiler, A. M., Dörfelt, P., Chatterjee, N., Helle, J., Bader, M. I., Vollmer, G., & Zierau, O. (2015). Assessment of the effects of naringenin-type flavanones in uterus and vagina. *The Journal of Steroid Biochemistry and Molecular Biology*, *145*, 49-57. **https://dx.doi.org/10.1016/j.jsbmb.2014.10.001**

Keskin, Ş., Şirin, Y., Çakir, H. E., & Keskin, M. (2019). An investigation of Humulus lupulus L.: Phenolic composition, antioxidant capacity and inhibition properties of clinically important enzymes. *South African journal of botany*, *120*, 170-174. **https://dx.doi.org/10.1016/j.sajb.2018.04.017**

Kim, S. Y., Lee, I. S., & Moon, A. (2013). 2-Hydroxychalcone and xanthohumol inhibit invasion of triple negative breast cancer cells. *Chemico-biological interactions*, *203*(3), 565-572. **https://dx.doi.org/10.1016/j.cbi.2013.03.012**

Korpelainen, H., & Pietiläinen, M. (2021). Hop (*Humulus lupulus* L.): Traditional and present use, and future potential. *Economic botany*, *75*(3-4), 302-322. **https://dx.doi.org/10.1016/j.cbi.2013.03.012**

Kowalczyk, D., Świeca, M., Cichocka, J., & Gawlik-Dziki, U. (2013). The phenolic content and antioxidant activity of the aqueous and hydroalcoholic extracts of hops and their pellets. *Journal of the Institute of Brewing*, *119*(3), 103-110. **https://dx.doi.org/10.1002/jib.73**

Krofta, K., Mikyška, A., & Hašková, D. (2008). Antioxidant characteristics of hops and hop products. *Journal of the Institute of Brewing*, *114*(2), 160-166. **https://dx.doi.org/10.1002/j.2050-0416.2008.tb00321.x**

Lamuela-Raventós, R. M. (2017). Folin-Ciocalteu method for the measurement of total phenolic content and antioxidant capacity. *Meas. Antioxid. Act. Capacit. Recent Trends Appl*, 107-115. **https://dx.doi.org/10.1002/9781119135388.ch6**

Lang, Y., Gao, N., Zang, Z., Meng, X., Lin, Y., Yang, S., & Li, B. (2024). Classification and antioxidant assays of polyphenols: A review. *Journal of Future Foods*, *4*(3), 193-204. **https://dx.doi.org/10.1016/j.jfutfo.2023.07.002**

Lapornik, B., Prošek, M., & Wondra, A. G. (2005). Comparison of extracts prepared from plant by-products using different solvents and extraction time. *Journal of food engineering*, *71*(2), 214-222. **https://dx.doi.org/10.1016/j.jfoodeng.2004.10.036**

Lecomte, M., Tomassi, D., Rizzoli, R., Tenon, M., Berton, T., Harney, S., & Fança-Berthon, P. (2023). Effect of a Hop Extract Standardized in 8-Prenylnaringenin on Bone Health and Gut Microbiome in Postmenopausal Women with Osteopenia: A One-Year Randomized, Double-Blind, Placebo-Controlled Trial. *Nutrients*, *15*(12), 2688. **https://dx.doi.org/10.3390/nu15122688**

Liberatore, C. M., Mattion, G., Rodolfi, M., Ganino, T., Fabbri, A., & Chiancone, B. (2018). Chemical and physical pre-treatments to improve in vitro seed germination of Humulus lupulus L., cv. Columbus. *Scientia horticulturae*, *235*, 86-94. **https://dx.doi.org/10.1016/j.scienta.2018.02.077**

Maliar, T., Nemeček, P., Ürgeová, E., Maliarová, M., Nesvadba, V., Krofta, K., & Kraic, J. (2017). Secondary metabolites, antioxidant and anti-proteinase activities of methanolic extracts from cones of hop (Humulus lupulus L.) cultivars. *Chemical Papers*, *71*, 41-48. **https://dx.doi.org/10.1007/s11696-016-0034-2**

Mander, L., Liu, H. W. (Ben). (2010). Introduction. *Comprehensive natural products II: chemistry and biology* (Vol. 1). **https://dx.doi.org/10.1016/b978-008045382-8.09004-3**

Milligan, S. R. (2009). Reproductive and Estrogenic Effects of 8-Prenylnaringenin in Hops. *Beer in Health and Disease Prevention*, 711–723. **https://dx.doi.org/10.1016/b978-0-12-373891-2.00072-9**

Nandasiri, R., Eskin, N. M., Thiyam-Höllander, U. (2019). Antioxidative polyphenols of canola meal extracted by high pressure: impact of temperature and solvents. *Journal of food science*, *84*(11), 3117-3128. **https://dx.doi.org/10.1111/1750-3841.14799**

Oliveira Neto, J. R., Lopes de Macedo, I. Y., Lopes de Oliveira, N. R., de Queiroz Ferreira, R., & de Souza Gil, E. (2017). Antioxidant capacity and total phenol content in hop and malt commercial samples. *Electroanalysis*, *29*(12), 2788-2792. **https://dx.doi.org/10.1002/elan.201700492**

Ozgen, M., Reese, R. N., Tulio, A. Z., Scheerens, J. C., & Miller, A. R. (2006). Modified 2, 2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method to measure antioxidant capacity of selected small fruits and comparison to ferric reducing antioxidant power (FRAP) and 2, 2 '-diphenyl-1-picrylhydrazyl (DPPH) methods. *Journal of Agricultural and Food Chemistry*, *54*(4), 1151-1157. **https://dx.doi.org/10.1021/jf051960d**

Proestos, C., & Komaitis, M. (2009). Antioxidant capacity of hops. In *Beer in Health and Disease Prevention* (pp. 467-474). Academic Press. **https://dx.doi.org/10.1016/b978-0-12-373891-2.00045-6**

Sánchez-Rangel, J. C., Benavides, J., Heredia, J. B., Cisneros-Zevallos, L., & Jacobo-Velázquez, D. A. (2013). The Folin–Ciocalteu assay revisited: improvement of its specificity for total phenolic content determination. *Analytical Methods*, *5*(21), 5990-5999. **https://dx.doi.org/10.1039/c3ay41125g**

Sharma, S., Strickland, D. A., Hay, F., & Pethybridge, S. J. (2023). First Report of Halo Blight on Hop Caused by *Diaporthe humulicola* in New York. *Plant Disease*, *107*(1), 216. **https://dx.doi.org/10.1094/pdis-01-22-0202-pdn**

Sies, H. (2007). Total antioxidant capacity: appraisal of a concept. *The Journal of nutrition*, *137*(6), 1493-1495. **https://dx.doi.org/10.1093/jn/137.6.1493**

Stevens, J. F., Miranda, C. L., Buhler, D. R., Deinzer, M. L. (1998). Chemistry and biology of hop flavonoids. *Journal of the American Society of Brewing Chemists*, *56*(4), 136-145. **https://dx.doi.org/10.1094/asbcj-56-0136**

Stevens, J. F., & Page, J. E. (2004). Xanthohumol and related prenylflavonoids from hops and beer: to your good health!. *Phytochemistry*, *65*(10), 1317-1330. **https://dx.doi.org/10.1016/j.phytochem.2004.04.025**

Sun, S., Wang, X., Yuan, A., Liu, J., Li, Z., Xie, D., Zhang, H. (2022). Chemical constituents and bioactivities of hops (Humulus lupulus L.) and their effects on beer-related microorganisms. *Food and Energy Security*, *11*(2), e367. **https://dx.doi.org/10.1002/fes3.367**

Tronina, T., Popłoński, J., & Bartmańska, A. (2020). Flavonoids as phytoestrogenic components of hops and beer. *Molecules*, *25*(18), 4201. **https://dx.doi.org/10.3390/molecules25184201**

Turkmen, N., Sari, F., & Velioglu, Y. S. (2006). Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin–Ciocalteu methods. *Food chemistry*, *99*(4), 835-841. **https://dx.doi.org/10.1016/j.foodchem.2005.08.034**

Turkmen, N., Velioglu, Y. S., Sari, F., & Polat, G. (2007). Effect of extraction conditions on measured total polyphenol contents and antioxidant and antibacterial activities of black tea. *Molecules*, *12*(3), 484-496. **https://dx.doi.org/10.3390/12030484**

Varlet, V., Smith, F., & Augsburger, M. (2014). New trends in the kitchen: Propellants assessment of edible food aerosol sprays used on food. *Food chemistry*, *142*, 311-317. **https://dx.doi.org/10.1016/j.foodchem.2013.07.036**

Wang, H., Cao, G., & Prior, R. L. (1996). Total antioxidant capacity of fruits. *Journal of agricultural and food chemistry*, *44*(3), 701-705. **https://dx.doi.org/10.1021/jf950579y**

Wu, C. N., Sun, L. C., Chu, Y. L., Yu, R. C., Hsieh, C. W., Hsu, H. Y., Cheng, K. C. (2020). Bioactive compounds with anti-oxidative and anti-inflammatory activities of hop extract. *Food chemistry*, 330, 127244. **https://dx.doi.org/10.1016/j.foodchem.2020.127244**

Xi, J., Shen, D., Zhao, S., Lu, B., Li, Y., & Zhang, R. (2009). Characterization of polyphenols from green tea leaves using a high hydrostatic pressure extraction. *International Journal of Pharmaceutics*, *382*(1-2), 139-143. **https://dx.doi.org/10.1016/j.ijpharm.2009.08.023**

Yan, D., Wong, Y. F., Shellie, R. A., Marriott, P. J., Whittock, S. P., & Koutoulis, A. (2019). Assessment of the phytochemical profiles of novel hop (Humulus lupulus L.) cultivars: A potential route to beer crafting. *Food chemistry*, *275*, 15-23. **https://dx.doi.org/10.1016/j.foodchem.2018.09.082**

Zugravu, C. A., Bohiltea, R. E., Salmen, T., Pogurschi, E., & Otelea, M. R. (2022). Antioxidants in Hops: Bioavailability, Health Effects and Perspectives for New Products. *Antioxidants*, *11*(2), 241. **https://dx.doi.org/10.3390/antiox11020241**

Article: Arsene, A. L., Rodino, S., Butu, A., Petrache, P., Iordache, O., & Butu, M. (2015). Study on antimicrobial and antioxidant activity and phenolic content of ethanolic extract of Humulus lupulus. *Farmacia*, *63*(6), 851-857.

Article: Fărcaş, A., Tofană, M., Socaci, S., Scrob, S., Salanţă, L., & Borşa, A. (2013). Preliminary study on antioxidant activity and polyphenols content in discharged waste from beer production. *J. Agroaliment. Process. Technol*, *19*, 319-324.

Article: Kamboj, A., Gupta, R., Rana, A., & Kaur, R. (2015). Application and analysis of the Folin Ciocalteu method for the determination of the total phenolic content from extracts of Terminalia bellerica. *European Journal of Biomedical and Pharmaceutical Sciences*, *2*(3), 201-215.

Article: Karabin, M., Hudcova, T., Jelinek L., Dostalek, P. (2012). The importance of hop prenylflavonoids for human health. *Chemical Lists*, 106(12), 1095-1103.

Article: Rajbhar, K. A. R. I. S. H. M. A., Dawda, H., Mukundan, U. S. H. A. (2015). Polyphenols: Methods of extraction. *Sci. Revs. Chem. Commun*, *5*(1), 1-6.

Technical Brief: Stokholm, A., & Shellhammer, T. H. (2020). Hop Creep–Technical Brief. *Brewers Association: Boulder, CO, USA*.

Doctoral dissertation: Tembo, G. M. (2020). Effects of storage conditions on alpha acid degradation of Indiana Grown Hops (*Humulus lupulus*) (Doctoral dissertation, Purdue University Graduate School).

Html 1: https://www.maroma.sk/premiant-pelety

Html 2: https://obchod.svet-piva.cz/zbozi/chmel-saaz-late-100g

Html 3: https://www.uvartesipivo.sk/https/www-uvartesipivo-sk/Chmel-Polaris-100g-d77-htm