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BEER AS A SOURCE OF HOP PRENYLATED FLAVONOIDS, COMPOUNDS WITH ANTIOXIDANT, CHEMOPROTECTIVE AND PHYTOESTROGEN ACTIVITY

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ABSTRACT

Beer is an alcoholic beverage consumed worldwide, which is given its typical taste by the presence of hops. Hops contain an array technologically important substances, which are primarily represented by hop resins (humulones, lupulones, humulinones, hulupones, etc.), essential oils (humulene, myrcene, etc.) and tannins (phenolic compounds quercetin, catechin, etc.). In addition to their sensory properties, these molecules contribute to the biological and colloidal stability of beer with their antiseptic and antioxidant properties. Recently, an increased attention has been given to prenylated hop flavonoids, particularly xanthohumol, isoxanthohumol and 8-prenylnaringenin. Xanthohumol is a prenylated chalcone that exhibit antioxidant, anticancer and chemoprotective effects. Its only source in human nutrition is beer, nevertheless a large part of xanthohumol from hops is isomerized by heat to isoxanthohumol and desmethylxanthohumol, from which a racemic mixture of 6- and 8-prenylnaringenins is formed during the beer production. Xanthohumol is also converted to isoxanthohumol by digestion, leading to the formation of 8-prenylnaringenin that is being catalyzed by the enzymes of the intestinal microorganisms as well as liver enzymes. This substance is currently considered to be the most effective natural phytoestrogen.

Keywords: hops, xanthohumol, isoxanthohumol, prenylated flavonoids

INTRODUCTION

Beer is a global traditional fermented beverage containing carbohydrates, typical organic acids, vitamins, proteins and in particular hop substances.
The most popular type of beer in the world is the bottom-fermented beer, which is produced mainly by the brewer's yeast *Saccharomyces pastorianus* and the fermentation process takes place at temperatures ranging from 3.3 to 10 °C. The second type is the top-fermented beer, which is fermented with the help of the yeast *Saccharomyces cerevisiae* at temperatures from 20 to 24 °C. In comparison to other alcoholic beverages, classic beer has a higher nutritional value, mainly because of the presence of extracted substances from barley, flavor molecules from hops, as well as a relatively high content of minerals and nutrients such as calcium, sodium, potassium, and magnesium. In addition, by using malt, and especially hops, beer is enriched with naturally occurring antioxidants - phenolic compounds.
Antioxidants are substances that are present in foods and food products in lower concentrations when compared to oxidizable substrates, and act to cease or inhibit the oxidation process of such substrates. In beer, these compounds originate from malt (70%) and hops (30%). During the brewing and fermentation process, hops release three main metabolites into the beverage, namely bitter acids, prenylated chalcones and essential oils.
Common hops (*Humulus lupulus* L.) are botanically classified into the *Rosidae* subclass, the *Fabidae* group, the *Rosales* order and the *Cannabaceae* family. Several available varieties may be used for the brewing industry, which provide the beer with different flavors and aromas. Breweries use female cones exclusively, as these contain glandular trichomes located in the lupulin glands. These glands are considered to be the centre for the biosynthesis of secondary metabolites such as terpenoids, prenylated flavonoids, phenolic compounds, essential oils, resins and bitter acids. While all metabolites exhibit a certain biological activity, when it comes to the antioxidant potential and health benefits, the most interesting are prenylated chalcones, containing α- and β-unsaturated ketones.
Prenylated hop chalcones include xanthohumol, desmethylxanthohumol, xanthogalenol and isoxanthohumol, 6-prenylnaringenin, 8-prenylnaringenin and 8-geranylnaringenin. Out of these, xanthohumol stands out, as it is characterized by a high antioxidant activity, even stronger than resveratrol present in wine. The content of xanthohumol in dry hops may oscillate around 1%, while the amount of 8-prenylnaringenin is about ten times lower. An analytical complication lies in the fact that the exact concentration of each of these substances is difficult to be determined due to their isomerization. During the heat treatment, xanthohumol is isomerized to isoxanthohumol, while desmethylxanthohumol is isomerized to a racemic mixture of 6- and 8-prenylnaringenins.
Xanthohumol itself was not identified until 1957 and its favorable pharmacological properties, particularly its antioxidant, anti-inflammatory, antimicrobial, antiplasmodial and anticancer characteristics were not described until the 1990s.
The total content of biologically beneficial substances also depends on the actual processing of the common hops into their final form used in brewing. Hops can be used in the form of dried heads, hop pellets, extracts or isoextracts. Currently, the newest and most innovative method of processing hops lies in the use of liquid nitrogen, during which lupulin is extracted, containing most of the bitter and aromatic substances.
There are several methods to determine the content of prenylated flavonoids in hops or beer, based mainly on the principle of liquid chromatography. At present, liquid chromatography combined with tandem mass spectrometry are considered to be the most suitable.

BEER

Beer as a fermented beverage has a very long history (8000 years), while the basic brewing methods have not changed significantly. A more sophisticated and efficient method of beer production was introduced only in the period of the industrial revolution (**Guido, 2019**). Following water and tea, beer is the third most frequently consumed drink in the world. Currently, the popularity of small craft breweries or home brewing is increasing, which is gradually outpacing the demand for commercially brewed beer (**Matanjević *et al.*, 2019**).
Beer is an alcoholic beverage produced using yeast and three raw materials, which are water, malt and hops. The main steps of the beer production process include the preparation of malt and its milling, wiping, mashing, lautering, wort boiling, wort clarification, fermentation, storage and filtration. The basic

qualitative characterization of beer is divided by **Gonzales-Viejo *et al.* (2016)** into a visual and a sensory one. The primary attribute that consumers rate is visual, which includes the color of the beer, the overall appearance, the volume and stability of the foam, or the clarity of the beer. The sensory attributes comprise the perception and drinkability of beer, including the content of alcohol and carbon dioxide, aroma, mouthfeel, bitterness, or the presence of unpleasant tastes in beer.
The color, turbidity and fermentation process are all taken into account when classifying beer into individual categories. The color of beer is generally determined by the color of the malt, which depends on the color of the grain. Eventually, barley grain acquires color during the malt production and roasting due to the Maillard reaction or caramelization (**Lukinac *et al.*, 2019**). Thus, beer can take on a vast array of shades of yellow, red, brown to black. Depending on the brewer's yeast used and the fermentation temperature, three main categories may be distinguished:

- bottom-fermented beers (lagers),
- top-fermented beers (ALEs),
- spontaneously fermented beers (**Ebienfa *et al.*, 2015**).

According to **Nešpor *et al.* (2019)** specific features of bottom-fermented beers involve yeasts of the genus *Saccharomyces pastorianus*, which are effective within the temperature range of 0 - 13 °C. During fermentation, they fall to the bottom of the fermentation vessel. The fermentation time is longer and following this step the beer is left to stand in the lager cellar, thanks to which bottom-fermented beers are characterized by a balanced flavor. These types of beers include Pilsner lager, Vienna-style lager, light draft types, Bock and Märzen (**Dredge, 2019**).
The fermentation process of top-fermented beers takes place at higher temperatures of 13 - 24 °C using the brewer's yeast *Saccharomyces cerevisiae*. During fermentation, yeasts stay and aggregate on the surface, where they form the so-called beer blanket. The fermentation and storage time are shorter when compared to bottom-fermented beers (**Pajic, 2019**). Interestingly, higher fermentation temperature is accompanied by the formation of more fermentation by-products, particularly esters and phenolic compounds (**Lasanta *et al.*, 2020**). **Liguori *et al.* (2020)** state that top-fermented beers have a higher maltiness, mild yeasty taste and fruit aromas. These are distinctive beers, often hopped with aromatic hops. Examples include India Pale Ale (IPA), American Pale Ale (APA), English Pale Ale (EPA) (**Bamforth, 2020**), Belgian Ale (**Poelmans *et al.*, 2019**), wheat beer (Weizen) (**Meyerding *et al.*, 2019**), American Black Ale (**Malin, 2019**), porter and shout (**Cornell, 2020**).
Spontaneously fermented beers are traditionally produced in Belgium. This is a small group of beers that are not fermented by standard yeast strains, but on the contrary, they are allowed to be freely colonized by wild yeasts of the genus *Brettanomyces*. These yeasts are found in old wooden barrels that were previously used to store wine or sherry. The maturation of such beers is very long, sometimes up to 3 years (**Tyakht *et al.*, 2021**). Currently, starter cultures of *Brettanomyces bruxellensis*, *Pediococcus damnosus*, *Pediococcus parvulus* and *Acetobacter pasteurianus* are used to produce such fermented beers (**De Roos *et al.*, 2018**). The beer taste is unconventional, sour, cider-like, with a dry finish. These include lambic, shrub, geuze or faro beers (**Dysvik *et al.*, 2020**).
Beer is, among other substances, forming its essence, also a source of substances with antioxidant properties. Antioxidants in beer come from raw materials, malt and hops, but their final concentration depends on the brewing method. These compounds are important in the brewing process as they support the viability of yeasts and protect wort as well as beer from oxidation, thus extending its shelf life. Both endogenous and exogenous, added antioxidants may be present in beer (**Rahman *et al.*, 2020**). Endogenous beer antioxidants include particularly polyphenols (catechins), phenolic acids (ferulic acid), chemical products of the Maillard reaction and the enzymes superoxide dismutase, catalase and glutathione peroxidase. An exogenous antioxidant is, for example, L-ascorbic acid and sulfur dioxide (**Neto *et al.*, 2017**). According to **Mudura *et al.* (2018)** catechins, epicatechins, ferulic and caffeic acids extracted from malt, represent the main antioxidant activity in beer. **Zhao (2014)** states that the total content of polyphenols is 250 - 500 mg/L light beer and 489 mg/L dark beer. The content of these compounds increases in the order of non-alcoholic beer <lager <pilsner beer <wheat beer <ALE type beer. Furthermore, hop-containing flavonoid derivatives are found in beer, with higher levels of flavonoids in dark beers, as they contain the Maillard reaction products that inhibit the isomerization of prenylflavonoid compounds to substances with a lower biological activity (**Martinez-Gomez *et al.*, 2020**).

Basic raw materials for beer production

The basic raw materials for beer production include water, malt and hops. The fermentation process is provided by the brewer's yeasts.
Water can significantly affect or change the overall profile of beer. Therefore, in addition to hygienic standards, water hardness, acidity and salt content are important parameters to be considered. Water for the brewing industry should not contain chlorine, alkali carbonates and large amounts of nitrates, manganese or iron (**Punčochářová *et al.*, 2019**). **Novotný (2019)** explains that calcium and magnesium ions are involved in accelerating the isomerization of bitter substances, besides being essential for the yeast metabolism and interacting with malt phosphates, thereby lowering the pH of the mash. Chlorides accentuate the malty character and fullness of beer, while in turn, sulphates promote the taste of hops. According to **Kunatha (2012)**, iron provides beer with an undesirable flavor and is responsible for slowing down the fermentation processes. Micro- and macroelements that are irreplaceable for yeasts should come from malt and hops, and not from the water.
Barley is most often used for malting because of its high ratio of starch to protein, while the husks of the grain are responsible for typical malt flavors. Other cereals may be used as well, such as wheat, oats, rye, sorghum or millet, which, following malting, can serve not only for brewing purposes, but also for the production of distillates, or in other sectors of the food industry (**MacLeod *et al.*, 2016**).
Within non-traditional malts, an increased attention has been paid to sorghum and millet malt, as they have a significantly higher lysine content and a better protein digestibility than conventional cereal malts. However, the malting of sorghum leads to the release of hydrogen cyanide, which must be removed in the form of gaseous cyanide by drying the malt at a temperature above 30 °C. These raw materials are also more susceptible to the growth of microscopic fibrous fungi, which may produce mycotoxins (**Taylor *et al.*, 2017**).
The role of malting is to alter the physical structure of the barley grain in order to allow the activation of constitutive as well as the synthesis of inductive enzymes (**De Schepper *et al.*, 2019**). Good malting varieties are characterized by a uniform germination, during which hydrolytic enzymes are activated, which degrade large molecules of starch, proteins and nucleic acids into sugars, amino acids and nucleotides. These ingredients are important as they support the fermentation process during the beer production (**Henry, 2016**). **Koren *et al.* (2020)** consider the color to be one of the most important properties of malt. In the brewing industry, EBC (European Brewery Convention) units are used to evaluate the color, based on measuring the absorbance of beer at a wavelength of 430 nm. After shredding, the malt is placed into the wiping boiler and subsequently mixed with hot water to form wort. This is then pumped to the mashing boiler, where the decomposition of biopolymers continues, and nutrients are released for the yeasts to growth and multiplicate. The outcome of the chemical and physical changes is wort (**Saluri *et al.*, 2019**).
The main task of the hopping plant is to add hops, which provide the beer with its typical bitter taste. Gradually, excess water evaporates, whereby the wort acquires the required density, the enzymatic activity terminates, the wort is then sterilized and enriched with aromas and hop essential oils. The pH decreases, which positively affects the coagulation of proteins, the color intensity increases by 1 - 1.5 EBC units per hour. There are Maillard reaction products from malt which with released reducing substances increased the stability of beer (**Silva *et al.*, 2019**). The quantity of hops added depends on the desired bitterness of the beer (**Boronat *et al.*, 2020**). IBU (International Bitterness Units) are units by which the content of bitter substances in mg/L of beer is expressed by a spectrophotometric determination of the isohumulone concentration. The process should not exceed 120 minutes because beer could acquire an old taste and the color of the wort could increase too much (**Brendel *et al.*, 2020**). **Novotný (2019)** states that there are also so-called unconventional hop methods, e. g. mashing hops and cold hops. The aim of such processes is to increase the aromatic profile of beer and the content of healthy prenylated flavonoids and α-bitter acids. However, these undergo isomerizations at high temperatures (**Machado *et al.*, 2019**).
Based on DNA analyzes, commercially used brewer's yeasts have been divided by **Gallone *et al.* (2016)** into five groups:

- Asian phyla used to produce sake rice wine,
- Phyla derived from wine production (wine yeast),
- Phyla originating from the bakery industry,
- Brewer's yeast used in continental Europe (Germany, Belgium),
- Brewer's yeast used in the United Kingdom and the United States of America.

The yeasts *Saccharomyces cerevisiae* and *Saccharomyces pastorianus*, which evolved by interspecific fusion crossings between *Saccharomyces cerevisiae* and *Saccharomyces bayanus*, are most often used in the beer production (**Aquilani *et al.*, 2015**). Yeasts are used in the brewing process either in a fresh (liquid) or a dehydrated (dried) state. Another attribute when choosing the right yeast is the type of fermentation where a distinction is made between bottom fermentation yeasts (bottom fermented beers) or top fermentation yeasts (top fermented beers). Bottom brewer's yeasts are used for the production of lagers, with a fermentation temperature of 5 - 15 °C. With this type of fermentation, the process is slower and the fermentation is less turbulent. To this day, it is the most commonly used method of beer production. Top fermenting yeasts are used for the production of ALE, Shout or wheat beers. **Goncalves *et al.* (2016)** state that such beers ferment at a temperature range of 15 - 25 °C, the primary fermentation is stormy, the yeasts form a beer blanket on the surface, but over time they will settle as well. A

significant feature of these yeasts is the formation of aromatic substances, e. g. a typical banana aroma of wheat beers.

Hops provide beer with a unique bitterness and aroma (**Rettberg *et al.*, 2018**). The common hop (*Humulus lupulus* L.) is a dioecious liana whose cones collected from female plants are used in the beer production (**Nuutinen, 2018**). The lupulin glands host the biosynthesis of secondary metabolites, which are considered to be biologically active substances. According to **Zhuang *et al.* (2017)** the most interesting of these seem to be prenylated chalcones. These compounds ultimately improve the properties of beer such as its preservation and taste profile, particularly its bitterness (**Karabín *et al.*, 2015**).

One of the most important quality characteristics of hops is the content of bitter acids. Therefore, hop varieties are primarily divided into aromatic (fine) and bitter (with a high content of bitter acids). Dual purpose hops represent an interesting alternative. These are pleasantly aromatic with a high bitterness (**Brendel *et al.*, 2020**). According to the growing season, hops are divided into early, semi-early and late varieties (**Marceddu *et al.*, 2020**). The final general characteristic of hops, according to which the individual varieties differ, is the color of the shoot - the hops may be green or red. European hops from Poland, the Czech Republic, Germany or Slovenia are among the redheads, including the most famous Žatec redhead from the Czech Republic, which has a characteristic delicate aroma. Greens include hops grown in Australia, the United Kingdom and the United States. The best known in this category is the American variety Cascade, which is characterized by strong citrus, floral and grapefruit aromas (**Novotný, 2019**).

It is estimated that currently only about 10% of breweries use actual hop cones to hop their beer. **Tronina *et al.* (2020)** explain this activity by the fact that dried heads have lower yields and chemical stability, are less homogeneous and present with higher demands for their storage and packaging.

Due to a high concentration of hop compounds, various hop products are more popular. These are easy to store, pack and transport. Hop pellets are among the most popular, their production consists in drying and subsequent crushing of hop cones at a low temperature and without access to oxygen. The powder is then pelleted under pressure in an inert gas environment. Nevertheless, because of compression friction, the temperature can rise up to 65 °C, which may affect the individual components of the hops (**Sharp *et al.*, 2017**). Pellets are generally referred to as 90, 45 and 30, which determines the percentage yield of granules from hop cones. Type 90 means that 90 kg of granules with the same composition as the original hops were obtained from 100 kg of cones (**Bober *et al.*, 2020**). Hop extracts are made from ground hops by extraction, most often with ethanol or supercritical CO_2. This is the most concentrated form, however not all substances are preserved in the same amount and the beer taste may differ from a beverage in which conventional hops or pellets were used. Beer made with the addition of hop extracts is often depleted of polyphenols (**Okafor *et al.*, 2016**). Hop isolates are widely used for a fast brewing since these are modified to contain soluble iso-bitter substances. As such, they do not have to undergo boiling to release the bitter substances (**Roberts, 2016**). One of the most innovative methods of processing hops is the so-called cryohops. Lupulin (most bitter and aromatic substance) is extracted from hops using liquid nitrogen, and the residual bitter-depleted hops can be used instead of fine hops **(Novotný, 2019).**

BIOLOGICALLY ACTIVE SUBSTANCES OF HOPS

The most technologically important components of hops are the hop resins, tannins and essential oils. Hop resins are divided into soft (soluble in n-hexane) and hard resins (insoluble in n-hexane) (**Steenackers *et al.*, 2015**). The group of soft resins includes prenylated derivatives of phloroglucinol, α-bitter acids (humulones), β-bitter acids (lupulones), γ-bitter acids (humulinones), δ-bitter acids (hulupones) and non-specific soft resins. Hard resins comprise prenylated chalcones and flavanones, γ-resins and δ-resins.

Bitter hop acids

Bitter acids, which are divided into α-acids and β-acids, are essential for brewing. Chemically, these are derivatives of prenylated phloroglucinols (**Alfonso-Esteban *et al.*, 2019**). The most common are α-acids, which represent 2 - 15% (wt.) of the dry mass of hops, however recently bred hops contain up to 19% (wt.) α-acids (**Roberts, 2016**). Three α-acids have been predominantly described, specifically n-humulone, ad-humulone and co-humulone (Fig. 1), however attention has also been focused on pre-humulone, post-humulone and adpre-humulone (**Caballero *et al.*, 2012; Knez Hrnčič *et al.*, 2019**). Based on the α-acid content, hops are referred to as either bitter hops containing more than 5% α-acids or aromatic hops with an α-acid content of up to 5%. Hops, which are rich in bitter α-acids and flavorings, are classified as double-acting hops and have a bitter aroma (**Machado *et al.*, 2019**).

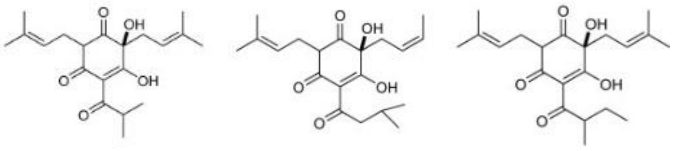

Figure 1 Chemical structure of co-humulone, n-humulone and ad-humulone **(Izawa *et al.*, 2010)**

Quantitative analysis of α-acids showed that their concentration in beer is much lower in comparison to wort. This is due to the thermal isomerization of α-acids to iso-α-acids during the brewing process (**Ayabe *et al.*, 2018**). **Jaskula *et al.* (2010)** consider the formation of stereoisomers of *trans*-iso-α-acids and *cis*-iso-α-acids to be the key isomerization process (Fig. 2). In addition to an intense bitter taste, these also affect the foam stability of the beer (**Kunimune and Shellhammer, 2008**) and inhibit the growth of Gram-positive bacteria (particularly *Lactobacillus*) in beer (**Simpson and Smith, 1992**). *Trans*-iso-humulone is the most predominant as it is the best soluble one in aqueous solutions (**Intelmann *et al.*, 2010**).

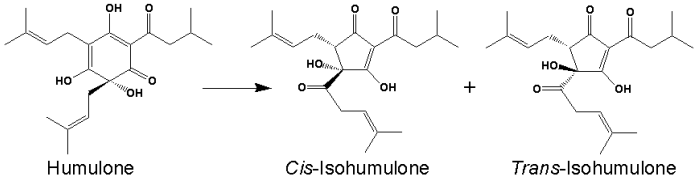

Figure 2 Isomerization of humulone to *cis*-isohumulone and *trans*-isohumulone (**De Keukeleire, 2000**)

Despite a successful elucidation of the mechanism of α-acid isomerization, there is still not enough information about β-acids as a source of bitter substances in beer. β-acids are triprenylated analogs of α-acids, representing about 10% (w/w) of hops. These are considered to act as antibacterial agents, however most of their activity is eliminated during the brewing process (**Mikyška *et al.*, 2019**). These acids could act as precursors to the bitter substances of beer, nevertheless they are still the main bitter components of iso-α-acid, constituting up to 80% of the beer bitterness. Within traditional beer brewing, where dried hops or hop granules are used, only 25-35% of α-acids are isomerized. In the case of more modern processes, in which hop extracts are used, isomerization is increased to 45%, while the utilization of isomerized hop extracts is achieved up to 85% (**Kostrzewa *et al.*, 2016**).

Hop essential oils

Essential oils represent 0.5% of the dry matter of the hop cone. These are a mixture of different fragrances, the content of which varies depending on the hop variety. They have a low boiling point, so it is advisable to add aromatic hops 15 to 30 minutes before the end of boiling or to use cold hopping instead (**Dietz *et al.*, 2020**). Hop essential oils include sesquiterpene α-humulene with a characteristic herbal aroma. It is a very volatile substance, which does not last during wort brewing, it remains in beer only due to a later hopping period or cold hopping (**Tan *et al.*, 2020**). Monoterpene myrcene makes up the largest proportion of hop essential oils, its aromas may vary between herbal, citrusy, earthy or pine. It is usually found in beer only in the case of a cold hopping process, while it is mostly contained in American hop varieties (40-60%). Noble hops such as Satu Mare contain less (25-30%) myrcene (**Dennenlohr *et al.*, 2019**). Caryophene is typical of English hop varieties. Its aromas are earthy, spicy or citrusy. During a long boiling period, it evaporates from the wort and is easily oxidized (**Zlochová *et al.*, 2020**). Farnesene has a light aroma in which floral, apple and citrusy tones predominate. It may be notable to state that it is either completely absent or predominant in different hop varieties. For example, it is completely absent in German hop varieties, however its content is up to 20% in Žatecký červeňák (**Nesvadba *et al.*, 2020**).

Polyphenolic compounds of hops

The most suitable classification of polyphenols lies in their distribution according to the chemical structure of aglycones (**Vollmannová *et al.*, 2018**). With this respect, they are divided into phenolic acids and simple phenols, coumarins, flavonoids, stilbenes, suberins and cutins, lignans and lignins, tannins, tocopherols and tocotrienols.

A large group of plant secondary metabolites are flavonoids, which may be found in every part of the plant and, subsequently are to be found in foods and beverages of plant origin. Flavonoids are natural antioxidants (**Wang *et al.*, 2020**), which act as scavengers of free reactive species (especially reactive oxygen species), are inhibitors of lipoxygenases and prevent their prooxidative

effect by chelating metal ions (**Karabín *et al.*, 2012**). The term natural antioxidant defines compounds with natural antioxidant properties, while their other biological properties are not excluded (**Reyes-Fermín *et al.*, 2020**). The disadvantage of several natural antioxidants is that their free forms are sensitive to chemical modifications caused by various physicochemical factors, thereby losing their antioxidant properties (**Sharma *et al.*, 2019**). The biological effect of flavonoids on human health has been demonstrated both *in vitro* and *in vivo*. Antibiotic, anticarcinogenic (chemoprotective), anti-inflammatory or estrogenic effects have been confirmed. These molecules may also act as free radical scavengers, metal chelating agents, modulators of enzymatic activities and inhibitors of cell proliferation (**Karabín *et al.*, 2015**).

The basic structure of flavonoids is represented by a dibenzo-γ-pyrone skeleton and individual subclasses are defined by the degree of saturation and oxidation of pyran (**Santos *et al.*, 2020**). **Ferreira *et al.* (2018)** describe the structure of flavonoids as two aromatic rings connected through a three-carbon chain of a heterocyclic pyran (Fig. 3). Each of the rings may be substituted with -OH groups, which are most common in the 3′, 4′, 5′, 5 and 7 position. Prenyl, isoprenyl, methyl and methoxyl functional groups may also occur in the rest of the positions (**Zhao *et al.* 2020**). Due to one or more chelating sites in their structure, flavonoids with hydroxyl groups can form flavonoid-metal cation complexes (**Wang *et al.*, 2019**). **Ghosh *et al.* (2015)** found that because of this chemical property, the quercetin molecule reacts with Mg(II), thereby increasing the scavenging activity of free radicals-quercetin derivates.

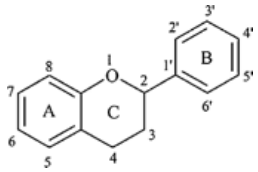

Figure 3 General chemical structure of flavonoids (**Vollmannová *et al.*, 2018**)

The more glycoside bonds and hydroxyl groups are attached to the basic structure of flavonoids, the greater is their solubility in water. Conversely, the more methyl, prenyl or isoprenyl functional groups are attached, the lower is their water solubility (**Slimane *et al.*, 2018**).

Biosynthesis of flavonoids

The primary substrate for flavonoid biosynthesis is *p*-coumaroyl-CoA, which reacts with malonyl-CoA to form naringin chalcone. The reaction is catalyzed by chalcone synthetase (CHS) (Fig. 4). Chalcone isomerase (CHI) intervention results in a specific cyclization to form naringenin (**Yonekura-Sakakibara *et al.*, 2019**). This is the basic compound for a two-step synthesis of isoflavones by isoflavone synthase (IFS), leading to 2-hydroxyisoflavanones which dehydrate to isoflavones (e. g. genistein) by dehydratase (HID) (**Wieslaw *et al.*, 2017**). Naringenin is also a basic compound for flavones, which can be synthesized either directly from naringenin by flavone synthase I (FNS I) or flavone synthase II (FNS II), or by flavanone-2-hydroxylase (F2H) to form 2-hydroxy-flavanone, which is converted to flavone (e. g. apigenin) via a yet unidentified dehydratase (**Gao *et al.*, 2020**). Flavanone-3-hydroxylase (F3H) produces dihydro-flavonols-dihydrokaempferol, which acts as a substrate for the synthesis of dihydromyricetin by flavonoid-F3′, 5′H-hydrolysis (F3 F5′H), or dihydroquercetin by flavonoid-3′-hydroxylase (F3′H). In general, flavonols are created from dihydroflavonols by flavonol synthase (FLS) (**Yonekura-Sakakibara *et al.*, 2019**). Figure 4 furthermore shows that anthocyanidins are created from dihydroflavonols by dihydroflavonol-4-reductase (DFR) to form leucoanthocyanidins, which are converted to anthocyanidins by leucoanthocyanidin dioxygenase/anthocyanidin synthase (LDOX/ANS) (**Mahmood *et al.*, 2016**). According to **Shi *et al.* (2018)** this synthesis of flavonoids ends with the formation of proanthocyanidins by reducing leucoanthocyanidins and anthocyanins to flavan-3-ols (e. g. catechin and epicatechin) by either leucoanthocyanidin reductase (LAR) or anthocyanidin reductase (ANR).

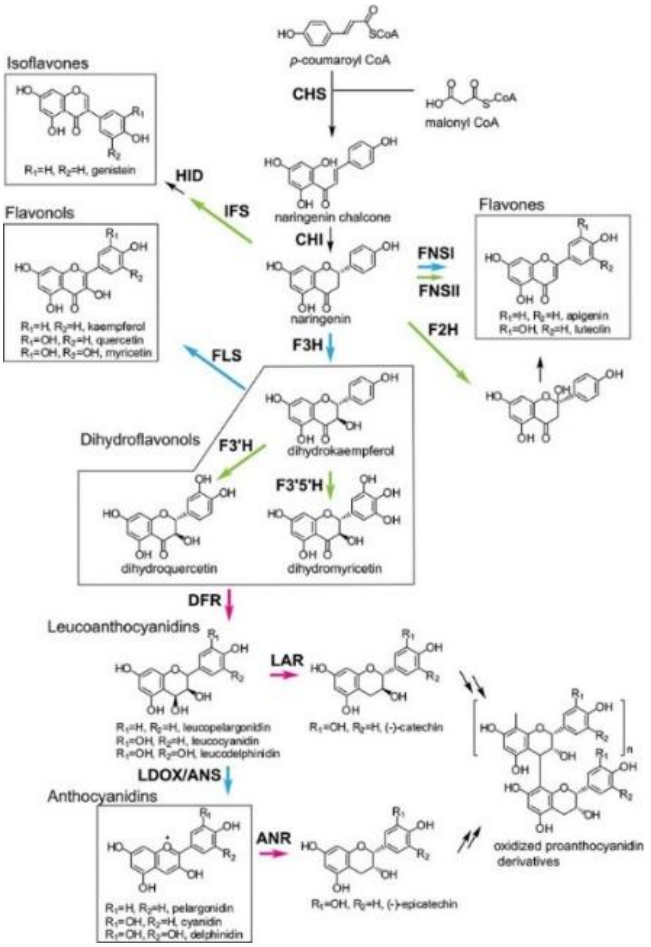

Figure 4 Flavonoid biosynthesis (**Yonekura-Sakakibara *et al.*, 2019**)

Dostalek *et al.* (2017) report that flavonoids are divided into flavones, flavonols, flavan-3-ol (catechins), isoflavones, flavanones and anthocyanidins (Fig. 5).

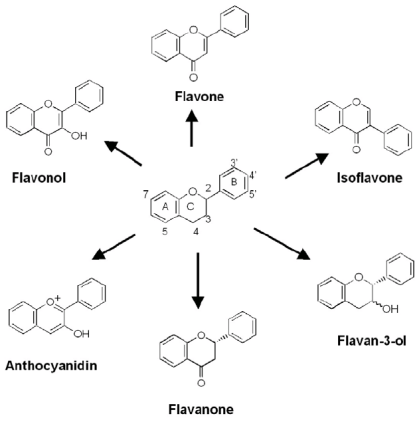

Figure 5 Structure of major subgroups of flavonoids (**Nishiumi, 2011**)

Hop flavan-3-ols

Flavan-3-ols are colorless monomers (catechins) or polygomers (proanthocyanidins) which contain two chiral centers. They may occur in four isomers, as aglycone monomers, oligomers, galocatechins and epigallocatechins (Fig. 6) (**Das *et al.*, 2019**). **Mena *et al.* (2019)** explain that catechins and epicatechin are epimers and at the same time (-)-epicatechin and (+)-catechin are the most common optical isomers in nature. Epigallocatechins and gallocatechins have one additional phenolic hydroxyl group as compared to epicatechin and catechin. **Collin *et al.* (2013)** state that hops are an exceptional source of catechins. Although hops are added to the beer in smaller amounts when compared to malt, the content of total polyphenols in beer derived from hops is up to 30%. In comparison, the content of (+)-catechin in dried hop cones is about 2821 mg/kg, while (-)-epicatechin constitutes around 1483 mg/kg and (+)-catechin from malt represents only 10 to 100 mg/kg.

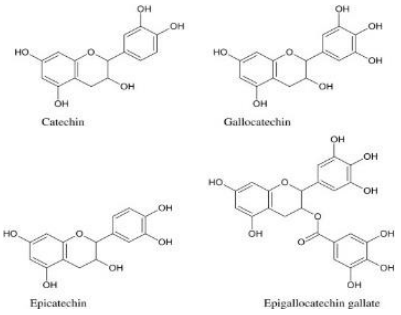

Figure 6 Structure of flavan-3-ol isomers (**Nishiumi, 2011**)

Hop proanthocyanidins

Proanthocyanidins are also called condensed tannins. These are oligomeric or polymeric forms of flavan-3-ol that are created as a final product of flavonoid biosynthesis (**Ruiz-Ruiz *et al.*, 2020**). The main building blocks are catechins and epicatechins (Fig. 7). Their origin is associated with the action of leucoanthocyanidin reductase, which catalyzes the production of catechins, serving as the first step in the synthesis of proanthocyanidin (**Rauf *et al.*, 2019**). Their chemical structures differ depending on their stereochemistry, the degree of polymerization, esterification of the 3-hydroxyl group, or the type of bond between the monomer units. They are divided into procyanidins, prodelfinidines and propelargonidines (**Wannenmacher *et al.*, 2018**).

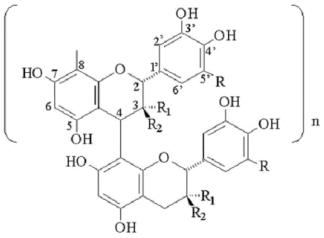

Figure 7 Structure of proanthocyanidins (**Hurst *et al.*, 2009**)
Legend: R = H is a procyanidin: catechin (R_1 = H and R_2 = OH) and epicatechin (R_1 = OH and R_2 = H)

Proanthocyanidins is a group of phenolic compounds that exhibit antioxidant, anti-inflammatory, antihypertensive and hypocholesterolemic effects in the human body (**Dong *et al.*, 2020**). **Zhao *et al*. (2017)** report that regular consumption of proanthocyanidins is beneficial for health and leads to a reduced risk of cardiovascular and neurodegenerative diseases. An increased concentration of proanthocyanidins in beer has been linked to a better beer stability.

Hop flavonols

Flavonols usually occur in plants, foods or beverages of plant origin in their glycosylated forms. While these are predominantly found in red wine, they may also occur in beer. Their structure consists of a flavone in which a double bond is conjugated to hydroxyl groups between carbon 2 and 3 **(Ramos-Pineda *et al.*, 2018).** Important flavonols include quercetin (Fig. 8), which is formed by the attachment of an -OH group to the R_1 position and hydrogen to the R_2 position; myricetin, which has an -OH functional group attached to both the R_1 and R_2 positions; and kaempferol, which has one hydrogen attached to the R_1 position and one -OH group attached to R_2 **(Shervington *et al*., 2018).**

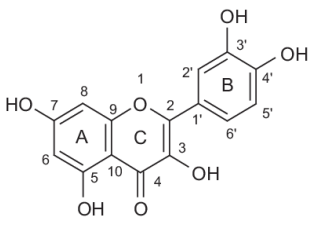

Figure 8 Structure of quercetin (**Zheng *et al.*, 2009**)

Quercetin and kaempferol are present in wort and beer in their glycoside forms. Hops are their only source, in contrast to myricetin, the only source of which is malt (**Mikyška *et al.*, 2019**). **Kim *et al.* (2018)** summarized recent studies particularly evaluating the biological and pharmaceutical effects of quercetin. The authors describe a wide range of its antioxidant, anti-inflammatory, antimicrobial, gastroprotective and immunomodulatory activities. **Jandera *et al.* (2005)** set the average content of quercetin in beer at 0.004 mg/L, while interestingly, out of the analyzed European beers (France, Czech Republic, Italy, Austria, Germany, the Netherlands) the molecule was present in Czech beers exclusively.

Flavonoid glycosides naturally occur most often bound to one or more carbohydrate molecules, either D-glucopyranose or 6-*O*-α-L-rhamnosyl-D-glucose. Out of this group of molecules, quercetin-5-*O*-glucoside-3-*O*-rutinoside and kaempferol-5-*O*-glucoside-3-*O*-rutinoside may be found in hops **(Karabín *et al.*, 2015).**

Hop flavonones

Flavonones are derived from a flavone similar to flavonols, but they do not have a double bond between carbon 2 and 3. Therefore, a chiral center is formed on the second carbon to which the ketone is attached (Fig. 9). They are highly reactive and undergo glycosidation, hydroxylation and *O*-methylation reactions (**Cassidy *et al.*, 2013**).

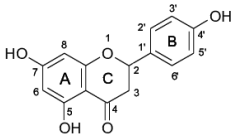

Figure 9 Structure of naringenin (**Uivarosi *et al.*, 2016**)

Naringenin is one of the most important hop flavanones. It is attributed to contribute to the bitterness of beer (**Salehi *et al.*, 2019**). The molecule also possesses pharmacological and therapeutic effects. Several authors have pointed out that naringenin is effective in the treatment of inflammation and can also regulate lipoprotein metabolism (**Zeng *et al.*, 2018**). Naringenin occurs in nature in two available forms, the more biologically available aglycosylated naringin and the less available glycosylated naringenin or naringenin-7-*O*-glucoside (**Moghaddam *et al.*, 2020**).

Hop prenylflavonoids

Prenylflavonoids are produced in the lupulin glands, similarly to the essential oils and hop resins. Their content in hop cones is variable and depends on the variety, agro-ecological conditions of cultivation and storage. In general, their content oscillates around 5 g per 100 g of dry hop matter (**Ciriminna *et al.*, 2018**). **Mukai (2018)** states that a characteristic feature of prenylflavonoids is the side chain of either the prenyl (Pn) or geranyl (Gn) type at position 8 in the basic structure of flavonoids (Fig. 10). This chain gives them nonpolar properties.

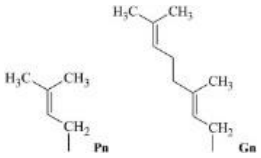

Figure 10 Types of prenylflavonoid side chains **(http 1)**
Legend: Pn prenyl, Gn geranyl

Prenylation generally means the addition of hydrophobic molecules to a chemical compound. These groups are thought to facilitate the adhesion of substances to the cell membranes (**Storck *et al.*, 2019**). *O*-prenylation is less common and results from the substitution of a hydroxyl group directly on the flavonoid skeleton. In contrast, *C*-prenylation is more frequent and takes place on the A ring at position 6 or 8 and on the B ring at positions 3′ and 5′, which is arranged perpendicular to the phenolic hydroxyl group (Fig. 11) (**Yang *et al.*, 2015**). **Jeong *et al.* (2019)** state that the prenylation of flavonoids increases their antimicrobial and chemoprotective effects. Prenylation also increases the bioaccumulation of flavonoids in tissues by inhibiting the efflux of these substances from cells (**Terao and Mukai, 2014**).

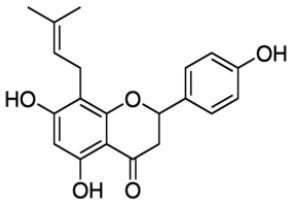

Figure 11 Basic structure of flavonoids and prenylflavonoids **(http 2)**

The most common type of attachment of a prenyl group to a flavonoid is on its 3,3-dimethylallyl chain. However, 1,1-dimethylallyl, farnesyl, levandulyl or geranyl may also be bound (Fig. 12) (**Santos *et al.*, 2020**).

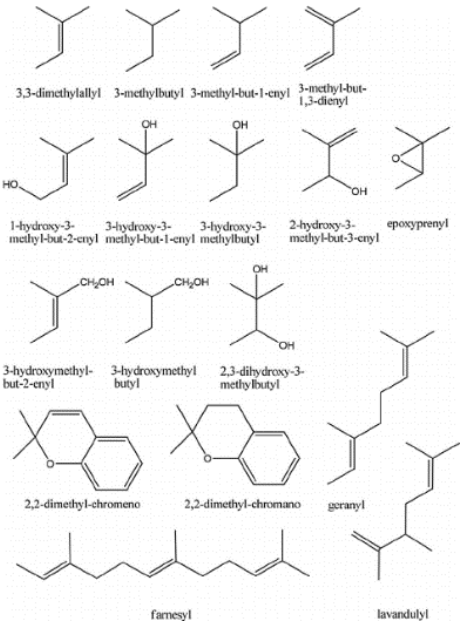

Figure 12 Types of skeletal prenylation of flavonoids (**Yang *et al.*, 2015**)

Prenylated flavonoids act as xenobiotics for the human body and their absorption is based on the principle of conjugation with carbohydrates in the form of glycosides. These are converted in the small intestine by β-glycosidases to aglycones, which are absorbed by the intestinal walls. Interestingly, when prenylflavonoids come directly from beer, they are usually absorbed spontaneously and stay unchanged. During their passage through the wall of the small intestine, they do not conjugate with carbohydrates, but they do combine with glucuronic acid in the liver. Bile conjugates pass back into the small intestine and are metabolized in the large intestine by the intestinal microbiome. In addition, prenylflavonoids, particularly their prenyl chain, can be oxidized under the catalytic activity of cytochrome P450 enzymes (**Karabín *et al.*, 2012**). Nevertheless, prenylated flavonoids have a relatively high metabolic stability. Based on the results collected from an experimental animal study, **Nookandeh (2004)** reported that up to 89% of xanthohumol was excreted unchanged from the body. The authors hypothesize that this phenomenon was observed due to the presence of prenyl, which reduced the polar nature of the molecule.

Prenylflavonoids are a special group of flavonoids, which are divided according to their chemical structure into two main groups, namely prenylated chalcones and prenylated flavanones and their derivatives (**Bartmańska *et al.*, 2018**). Chalcone derivatives represent the most numerous class of prenylated flavonoids, including hop substances that contain flavonoid chains with an α, β-unsaturated carbonyl group (**Cai *et al.*, 2017**). They contain a large number of hydrogen atoms that can be substituted to form numerous derivatives (**Klósek *et al.*, 2017**). According to **Xu *et al.* (2019)** chalcones and their derivatives exhibit antimicrobial, anticancer, antimalarial, anti-inflammatory, antioxidant, antiprotozoal, anti-HIV, antiulcer and antileishmanial effects. In the case of chlorine derivatives, prenylation on the A ring in position 3 is the most common, as most molecules are already hydroxylated in positions 2′, 4, 4′ and methylated in positions 6′, 4′ and 2′ (Fig. 13) (**Santos *et al.*, 2020**).

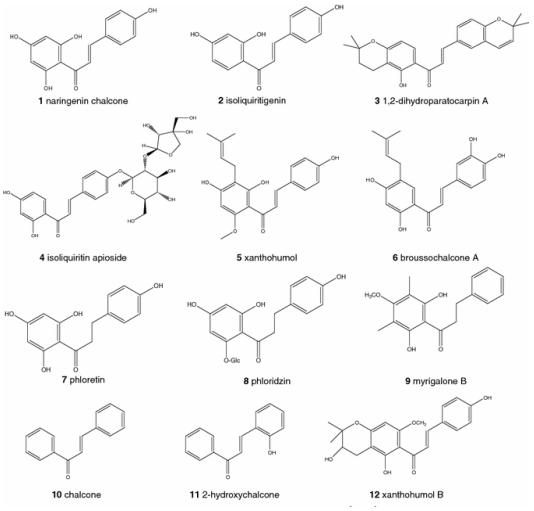

Figure 13 Structure of the best known chalcone derivatives (**Orlikova *et al.*, 2011**)

Flavone derivatives are also classified as prenylated flavonoids, however these do not occur in hops. Within these, *C*-prenylated flavones exhibit antioxidant characteristics (**Silbermann *et al.*, 2019**). Flavanone derivatives have been isolated from hops as well. In terms of antioxidant activity, these represent the second most widespread group of prenylated flavonoids. More often, prenylation occurs on the A ring, where both mono- and di-prenylated substitutions may arise, as well as on the B ring in the form of monoprenylated derivatives (**Finali *et al.*, 2016**). Isoflavonoid derivatives were isolated only from the *Fabaceae*, *Moraceae* and *Apiaceae* families. These compounds are prenylated on the A ring at positions 6 or 8. The best known of these is erynone, which is substituted with multiple prenylated units (**Liu *et al.*, 2019**). The uniqueness of xanthone-type derivatives lies in their structure which contains at least four fused rings, which are formed by the cyclization of prenyl in the basic flavone molecule, creating a pyran ring or xanthone nuclei can be attached to the main nucleus. The resulting product could end up containing up to 6 fused rings (**Santos *et al.*, 2020**).

Prenylated chalcones include xanthohumol, desmethylxanthohumol, xanthogalenol, and isoxanthohumol, while prenylated flavanones comprise 6-prenylnaringenin, 8-prenylnaringenin, 8-geranylnaringenin (**Nespor *et al.*, 2017**). **Karabín *et al.* (2012)** report that the content of xanthohumol is 0.2 - 1.1 g/100 g dry matter of hop cones. According to **Miligan (2009)**, 8-prenylnaringenin (a demethylated isoxanthohumol derivative), the most effective isolated phytoestrogen in hops, is present only at very low concentrations up to 100 ppm. Xanthohumol represents the largest part of hard hop resins (**Steenackers *et al.*, 2015**). Due to its chemical structure, the molecule represents a transition between hard resins and polyphenols. It is believed to possess a high chemoprotective potential and, together with 8-prenylnaringenin, which has a high phytoestrogenic effect (**Schaefer *et al.*, 2003; Stevens *et al.*, 2004**), may be a suitable nutritional supplement in a chemoprotective diet. Due to the fact that prenylflavonoids tend to isomerize following heat treatment (Fig. 14), their accurate qualitative and quantitative analysis is complicated. According to **Yang *et al.* (2015)** there are several methods to quantify the content of individual prenylated flavonoids, however liquid chromatography-mass spectrometry detection has proved to be the most suitable.

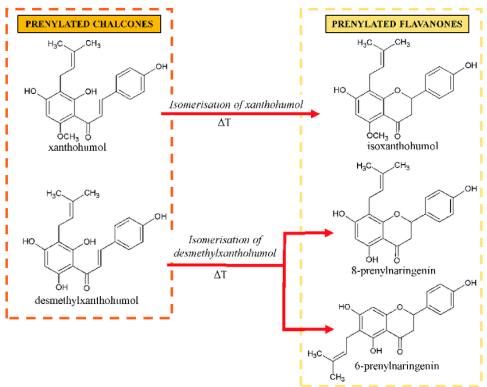

Figure 14 Isomerization of prenylated flavonoids (**Štulíková *et al.*, 2018**)

Xanthohumol

Xanthohumol (E)-1-[2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-enyl)phenyl]-3-(4-hydroxyphenyl)prop-2-en-1-one) is a chalcone derived from hops, which has gained scientific attention because of its antioxidant, anticancer and chemoprotective effects (**Liu *et al.*, 2019**). Even 1 μM xanthohumol was shown to be 8.9 times more effective in scavenging hydroxyl radicals and 2.9 times more active in neutralizing peroxyl radicals than TROLOX (a standard substance used in the determination of antioxidant activity by the ORAC method). Xanthohumol is also able to scavenge superoxide radicals generated by xanthine oxidase (**Gerhauser *et al.*, 2006**). According to **Kroft (2010)**, these effects manifest themselves already following the intake of 0.32 mg xanthohumol/kg of body weight. With this respect, **Loureiro *et al.* (2019)** found that there is about 0.1 mg of xanthohumol/L of beer. The action of xanthohumol in cells is gradually being studied by various scientific teams. For instance, **Lv *et al.* (2017)** describe its involvement in a positive regulation of AMP-protein kinase activity, which contributes to the activation of the signaling antioxidant pathways of the NRF2 transcription factor. The role of NRF2 is to increase the expression of cytoprotective genes, thereby protecting the cell from oxidative stress induced by toxic or carcinogenic substances (**Swamy *et al.*, 2016**).

Xanthohumol is a thermosensitive substance with a low solubility in polar solvents, and a high adhesion to the yeast surface (**Jelínek *et al.*, 2013**). The chemical structure of xanthohumol is composed of an open-chain flavonoid that contains two aromatic rings in the *trans*-configuration. These are linked by an α, ß-unsaturated ketone to a tricarbon chain and one prenylated unit. Substitution of the α-ketone unit with a prenylated moiety or -OCH_3 may occur in the structure, leading to an increased lipophilicity and a strong affinity towards biological membranes (**Kamiński *et al.*, 2017**).

The biosynthesis of xanthohumol (Fig. 15) starts with phenylalanine (L-Phe), which is converted to cinnamic acid by phenylalanine ammonia lyase. This is then oxidized by cinnamate-4-hydroxylase and subsequently binds to coenzyme A using 4-coumarate-CoA ligase to give 4-hydroxy-cinnamoyl-CoA (**Nagel *et al.*, 2008**). This compound is further extended by three malonyl-CoA units. Claisen condensation and tautomerization lead to the production of chalconaringenin (**Dewick, 2010**). In the penultimate step, prenylation occurs, with prenyltransferase 1 binding a dimethylallyl diphosphate molecule from the DXP pathway (**Tsurumaru *et al.*, 2012**). The DPX pathway represents one of the options for the biosynthesis of isoprenoids. The intermediate is deoxyxylulose-5-phosphate. Methylation with *S*-adenosylmethionine is the final step in the synthesis of xanthohumol.

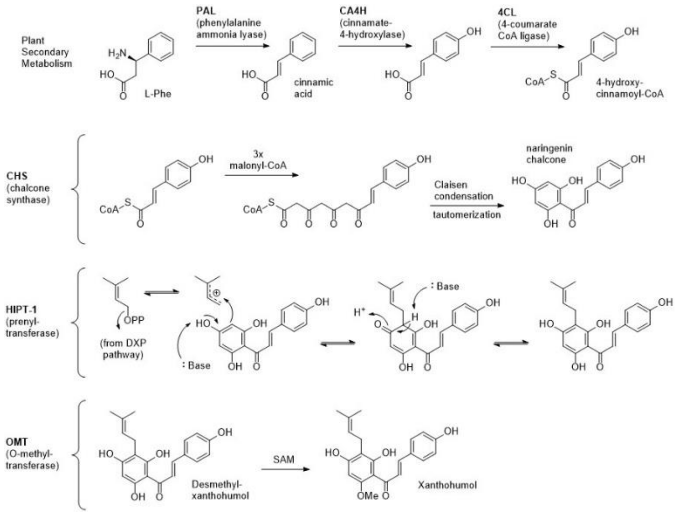

Figure 15 Biosynthesis of xanthohumol from phenylalanine **(http 3)**

Within the human body, xanthohumol is metabolized by a non-enzymatic cyclization to isoxanthohumol, from which 8-prenylnaringenin is produced by *O*-demethylation by the enzymes of the intestinal microorganisms as well as liver enzymes (**Seliger *et al.*, 2018**) (Fig. 16). 8-prenylnaringenin is considered to be the most powerful natural phytoestrogen (**Miranda *et al.*, 2018**). Another possibility is the conversion of xanthohumol to desmethylxanthohumol, which undergoes cyclization to produce 6-prenylnaringenin or 8-prenylnaringenin (**Legette *et al.*, 2014**).

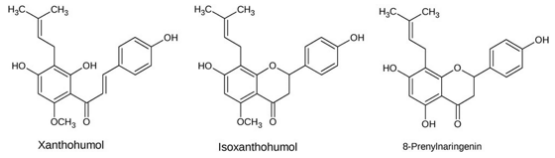

Figure 16 Structure of xanthohumol, isoxanthohumol and 8-prenylnaringenin (**Seliger *et al.*, 2018**)

When beer is brewed, xanthohumol is converted to isoxanthohumol, which exhibits a lower antioxidant activity (**Rancán *et al.*, 2017**). The frequency of isomerization increases with an increasing temperature and pH. Isoxanthohumol is more soluble in water than xanthohumol (5.0 mg/L and 1.3 mg/L, respectively) (**Żolnierczyk *et al.*, 2015**).

Several compounds from the group of prenylated chalcones have a more intensive action than the basic xanthohumol. These are e. g. xanthohumol H, xanthohumol C and 3'-geranyl-6'-*O*-methyl-chalconarenin (**Nuti *et al.*, 2017**). In addition to the antioxidant, antiproliferative an cytotoxic effect, xanthohumol C has been shown to have an inhibitory effect on the growth of MCF-7 breast cancer cells (**Forino *et al.*, 2016**). Furthermore, positive effects of xanthohumol in the prevention and treatment of prostate, breast and lung adenocarcinoma have been demonstrated and explained by a mechanism of inhibition of the cytochrome P450 oxidative enzymes. The chemoprotective effect of prenylflavonoids lies in their ability to inhibit the metabolic activation of procarcinogens, to induce the activity of carcinogen-detoxification enzymes, and to cease tumor growth as shown by an initial developmental study (**Stevens *et al.*, 2004**). Xanthohumol, isoxanthohumol, 8-prenylnaringenin and nine other hop prenylflavonoids present with a capability to inhibit the synthesis of cytochrome P450 enzymes, monooxygenases Cyp1A1, Cyp1B1, Cyp1A2, and thus affect the metabolism of potential carcinogens such as heterocyclic amines, benzopyrene and other potentially toxic molecules occurring in food production (**Henderson *et al.*, 2000**). The antiproliferative and cytotoxic effect of xanthohumol and five other prenylated hop flavonoids was tested on breast cancer (MCF-7), colon cancer (HT-29) and ovarian cancer (A-2780) cells (**Miranda *et al.*, 2000**). Xantohumol inhibited MCF-7 and A-2780 cell proliferation at an IC50 dose (half maximal inhibitor concentration) of 13 and 0.52 μM following only two days of administration. Even in advanced stages of cancer, prenylflavonoids were able to inhibit DNA synthesis and angiogenesis as well as to induce apoptosis of tumor cells (**Karabín *et al.*, 2012**). The antitumor effects of xanthohumol are also associated with the inhibition of the regulatory nuclear factor κB, which promotes the transcription of cyclooxygenases COX-1 (IC50 17 μM) and COX-2 (IC50 42μM) required for prostaglandin synthesis. Uncontrolled proliferation of tumor cells is associated with an inflammatory response and overproduction of prostaglandins, which are involved in the development of chronic inflammation, and initiate angiogenesis and cell proliferation. The activity of factor κB depends on the oxidative state of cells, which may be significantly affected by the antioxidant activity of xantohumol (**Ramos, 2007; Ramos, 2008**). Xanthohumol may also present with an inhibitory effect on tumor growth as an inhibitor of nitric oxide synthase (iNOS) gene expression (**Zhao *et al.*, 2003**). This vasodilator is involved in providing blood supply to the tumor; however, it tends to interact with free radicals, leading to the production persulfate ions, which have strong oxidizing effects and cause significant damage to the cell membranes (**Karabín *et al.*, 2012**). Xantohumol has been shown to inhibit DNA polymerase α activity *in vitro*, slowing down the replication and a subsequent division of tumor cells (**Gerhauser *et al.*, 2006**). Prenylated flavonoids are also able to inhibit DNA topoisomerase (**Ramos, 2007**).

The antimicrobial activity of xanthohumol against viruses, bacteria and yeast has been investigated as well. The molecule exhibits antibacterial effects, particularly against *Staphylococcus aureus* or *Streptococcus mutans*, as well as an antifungal activity against *Trichophyton* spp. (**Liu *et al.*, 2015**).

The effect of xanthohumol has also been reported in the prevention of weight gain due to excessive food consumption, as it is involved in the process of inhibiting adipogenesis and promoting adipocyte apoptosis. Using 3T3-L1 cells (embryonic fibroblasts), both xanthohumol and xanthohumol-enriched hop extract were shown to inhibit adipogenesis by reducing peroxisome proliferator-activated receptor γ (PPARγ) and CCAAT/enhancer binding protein α (C/EBPα), which are key for preadipocyte differentiation. When experimental rats were fed a high-fat diet, xanthohumol supplementation resulted in an inhibition of weight gain, liver weight, and decreased blood triacylglycerol levels (**Liu *et al.* 2015**). Several reports describe the effect of xanthohumol, which is a lipophilic substance due to the prenyl group, on fat metabolism and its potential as a remedy in the prevention of e. g. hypertriglyceridemia, which is associated with a risk for coronary heart disease or atherosclerosis. Its antioxidant activity prevents low density lipoprotein (LDL)-induced oxidation of bivalent copper. **Miranda *et al.* (2000)** report that xanthohumol is a more effective antioxidant than α-tocopherol and the flavone genistein, but less effective than the flavanol quercetin. In HepG2 cells (hepatocellular carcinoma cells), xanthohumol inhibited the synthesis of triglycerols localized in the membrane of the endoplasmic reticulum as well as their transfer into the lumen of the organelle.

The molecule acted as a diacylglycerol inhibitor acyltransferase in the process of triglycerol synthesis. Furthermore, it inhibited the activity of cholesteryl ester transfer protein (CETP), which transports cholesterol between lipoproteins, thereby increasing HDL cholesterol levels and reducing the risk for atherosclerosis (**Stevens *et al*., 2004; Karabín *et al.*, 2012; Liu *et al*., 2015; Krofta *et al.*, 2015**). **Hirata *et al*. (2017)** found that xanthohumol presented with an antiatherogenic activity as it reduced apolipoprotein B secretion, inhibited triacylglycerol synthesis and prevented the oxidation of low-density lipoproteins (LDL). By monitoring the effect of xanthohumol on the metabolism of experimental rats with diabetes, **Liu *et al.* (2015)** revealed that the inhibition of oxidative stress reduced inflammatory responses and improved the healing of typical diabetic wounds. The hypoglycemic effect of xanthohumol was also present due to the inhibition of glucose absorption by the intestinal epithelium and α-glucosidase activity (**Liu *et al.* 2015**).

Xanthohumol may also exhibit protective effects against age-related brain degeneration. Aging is a process accompanied by structural and functional changes in the brain, and age is a major risk factor for the development of various pathological conditions, including memory loss and decreased cognitive functions that can lead to senile dementia or Alzheimer's disease. **Rancán *et al.* (2017)** designed an *in vivo* study on mice of different ages which were treated with xanthohumol at 1 mg/kg body weight per day and 5 mg/kg per day over a period of 30 days. The authors analyzed the expression of several pro-inflammatory parameters, markers of brain damage and apoptosis. They found that aging significantly increased the levels of pathological markers in animals not treated with xanthohumol, whereas no changes were observed in xanthohumol-treated mice. The effect was significantly higher correspondingly to a higher dose of xanthohumol. As such, xanthohumol, accompanied by its antioxidant, anti-inflammatory and anti-carcinogenic effects, may play an important role in protecting the brain during aging.

Toxicological studies were required for a potential use of xanthohumol in nutritional supplements or for the production of beer with its increased content. **Miranda *et al.* (2000)** showed that concentrations up to 10 μM xanthohumol showed no toxicity and did not cease oxidative phosphorylation in rat hepatocytes. However, such a concentration of xanthohumol cannot be achieved in the body by consuming ordinary beer. If this drink is to be a source of prenylated flavonoids, it would be necessary to brew beer with a content of min. 5 mg xanthohumol/L (**Steven *et al.*, 2004**). **Nuti *et al.* (2017)** demonstrated a very good tolerance of the animal organism to xanthohumol. Over a period of four weeks, xanthohumol was administered orally to experimental mice and no organ damage, alterations to the homeostasis, or changes in carbohydrate, lipid, or protein metabolism were found. A dose of 100 mg/kg body weight did not affect the reproductive performance of female mice. However, a weak hepatotoxic effect was demonstrated after 28 days when 1000 mg xanthohumol/kilogram of rat body weight was administered.

Xanthohumol-enriched beers

The concentration of xanthohumol is greatly reduced during a traditional brewing process. Its content oscillates on average around 0.2 mg/L, because its isomerization to isoxanthohumol during wort cooking. Xanthohumol concentration is also reduced by its absorption to the yeast surface, during filtration and stabilization of the beer (**Protsenko *et al.*, 2018**). It is possible to increase the content of xanthohumol in beer by using more hops, by performing extra beer hopping at low temperatures, leading to a content of xanthohumol up to 3.2 mg/L, as well as by taking advantage of hop products enriched with xanthohumol, adding pure xanthohumol, adding hops before the end of brewing and cooling the mixture to 80 °C, by adding roasted malt extracts, as they are able to form compounds with xanthohumol that are stable and locked in beer, that can thus contain up to 10 mg xantohumol/L (**Habschied *et al.*, 2020**).

Ciriminna *et al*. (2018) studied the effect of different types of malt on the conversion of xanthohumol to isoxanthohumol during beer brewing. The report demonstrated that up to 90% of xanthohumol was isomerized using pilsner malt, while only a 65-85% isomerization was found in the case of caramel malts and only 50% of xanthohumol was isomerized with respect to roasted malts. This may be explained by the fact that roasted (dark) malts contain products of the Maillard reaction (especially high concentrations of melanoidins), which have antioxidant properties or bind to SO_2 and metal ions. These act as an immobilization carrier for xanthohumol, thereby increasing its stability and inhibiting isomerization to isoxanthohumol (**Magalhães *et al.*, 2011**). **Carvalho *et al.* (2016)** found that when 10 mg/L xanthohumol was added to light wort containing Pilsner-type malt, oxygen uptake increased, indicating an increase in oxidation reactions, including the Fenton free radical reaction: (Fe (II) + H_2O_2 → Fe (III) + HO- + HO•

Isoxanthohumol and 8-prenylnaringenin

Isoxanthohumol is 5-*O*-methyl-8-naringenin(2,3-dihydro7-hydroxy-2-(4-hydroxyphenyl)-5-methoxy-8-(3-methyl-2-butenyl)-4H-1-benzopyran-4-one) belonging to prenylated flavanones. Its content in native hops is around 0.008% (wt.) (**Stevens *et al.*, 2004**), low concentration of isoxanthohumol is also found in hop products, however a higher concentration may be found in beer due to the isomerization of xanthohumol (**Magalhães *et al.*, 2007; Jurková *et al.*, 2013**). **Stevens *et al.* (1999)** analyzed the content of xanthohumol, isoxanthohumol and 8-prenylnaringenin in different beers and found that the content of isoxanthohumol varied from 0.04 to 3.44 mg/L, while the content of xanthohumol was only 0.001 - 0.11 mg/L and the amount of 8-prenylnaringenin oscillated between 0.002 and 0.69 mg/L. Isoxanthohumol has a significant phytoestrogen effect (**Żołnierczyk *et al.*, 2015**). The action of isoxanthohumol and 8-prenylnaringenin on the endocrine system lies in their ability to inhibit chorionic gonadotropin-stimulated androgen production in Leydig cells (**Izzo *et al.*, 2010**). The antiproliferative effect of xanthohumol and its derivatives was tested on human ovarian carcinoma (A-2780), breast carcinoma (MCF-7) and colon carcinoma (HT-29) cells. Prenylated flavonoids were administered at concentrations from 0.1 to 100 μM. Xanthohumol proved to be the most effective, however in the case of MCF-7 cells it was isoxanthohumol, to which HT-29 cells were resistant (**Żołnierczyk *et al.*, 2015**). The effect of isoxanthohumol on prostate adenocarcinoma has been assessed by monitoring prostate specific antigen (PSA) levels (**Vollmer *et al.*, 2012**). A visible result, inhibition of PSA production, was already recorded at a concentration of 10 μM. The activities of hop extract, and xanthohumol and isoxanthohumol alone, were also tested on colorectal cancer cells (HT-29 and SW620) and non-malignant cells (IEC-6). Prenylated flavonoids inhibited cell differentiation even at micromolar concentrations, and no effect of these substances was observed in the case of IEC-6 cells (**Hudcová *et al.*, 2014**). Isoxanthohumol inhibits cytochrome P450-catalyzed activation of the mutagenic carcinogen 2-amino-3-methylimidazo-(4,5-f) quinone and aflatoxin AFB1. All three major prenylated hop flavonoids have the ability to inhibit aromatase activity, which plays an important role in the development of breast cancer (SK-Br-3 cells). This enzyme is called estrogen synthase and it belongs to the monooxygenases of the cytochrome P450 group. The activities of xanthohumol and isoxanthohumol have been tested in several pathological processes. For example, **Karabín *et al.* (2015)** report that the molecules have also a positive effect in the treatment of dermatitis, including atopic eczema and pigmentation disorders, furthermore, they enhance osteoblast differentiation *in vitro*, inhibit the enzyme cyclooxygenase 2, which catalyzes prostaglandin synthesis Isoxanthohumol has a low antiviral activity against cytomegalovirus, Herpes simplex virus HSV1 and HSV2 and bovine viral diarrhea virus (**Żołnierczyk *et al.* 2015**).

Moreover, 8-prenylnaringenin has also been shown to possess a significant effect on the human body. Although its content in beer is much lower when compared to isoxanthohumol, and generally speaking, only a very small amount of this molecule enters the human body, dietary metabolism of microorganisms converts isoxanthohumol to 8-prenylnaringenin, probably in amounts of around 4 mg/L.

8-prenylnaringenin (5,7-dihydroxy-2-(4-hydroxyphenyl)-8-(3-methylbut-2-enyl)-2,3-dihydrochromen-4-one) has about a 100-fold higher activity as a phytoestrogen than genistein (**Karabín *et al.*, 2012**) and therefore it could be used in eliminating issues associated with menopause and post-menopause, including protecting bones from bone loss. The essence of action lies in the activation of estradiol receptors ERα and ERβ (**Shaefer *et al.* 2003**). The estrogenic effects of hop prenylflavonoids decrease in the following order: 8-prenylnaringenin > 6-prenylnaringenin > 8-geranylnaringenin > 6,8-diprenylnaringenin (**Milligan *et al.*, 2000**). Its inhibitory effect on tumor growth has been described as well (**Štulíková *et al.*, 2018**). Nevertheless, according to **Possemiers *et al.* (2005)**, the dose of these substances consumed in one conventional beer is 500-1000 times lower than that required to induce estrogenic activities *in vivo*. If 8-prenylnaringenin was a part of nutritional supplements, such flavonoids could selectively modulate estrogen receptors, which is why such a supplement could become an alternative to hormone replacement therapy in menopause (**Possemiers *et al*., 2006; Karabín *et al.*, 2012**). **Campillo *et al.* (2018)** describe xanthohumol, isoxanthohumol and 8-prenylnaringenin as components of a nutritional supplement for menopausal symptoms. Both stereoisomers, (2R)-8-prenylnaringenin and (2S)-8-prenylnaringenin, have a high affinity for the estrogen receptor ERα (**Stevens *et al.*, 2004**). **Mukai *et al.* (2012)** designed a muscular atrophy study using a denervated mouse model and showed that consumption of 8-prenylnaringenin prevented the loss of muscle mass (calf muscle gastrocnemius) by activating protein kinase B phosphorylation. As such, administration of 8-prenylnaringenin could be considered in the prevention of muscle atrophy.

CONCLUSION

This paper describes the most significant effects of prenylated hop flavonoids, particularly xanthohumol, isoxanthohumol and 8-prenylnaringenin. The source of these substances with interesting pharmacological activities is beer. Of course, the health benefits of beer must be balanced with the alcohol content of this beverage, however since prenylated flavonoids come from hops, they are also preserved in non-alcoholic beers. Xanthohumol, its isomerization and demethylation products - isoxanthohumol and 8-prenylnaringenin are very intricate compounds with antioxidant, estrogenic, and especially anticarcinogenic activity. A possible use of these substances could be regarded in cancer prevention, as they inhibit metabolic pathways of procarcinogen activation, act as

inducers of carcinogen detoxification enzymes and cease tumor growth by inhibiting inflammatory responses and angiogenesis. Furthermore, these molecules could be suitable as an alternative treatment for unpleasant side effects of (post)menopause, including the prevention of osteoporosis. It is possible to increase the intake of prenylated flavonoids by a targeted optimization of the beer production process in order to minimize the loss of biologically active prenylated flavonoids, by preparing extra hopped beer or beer enriched with xanthohumol. It is also possible to consume food supplements containing hop extracts and design new types of functional foods with a significant content of prenylated flavonoids. Genetic engineering also represents a possible way to produce new hop varieties with a high content of prenylated flavonoids, such as by overproducing enzymes responsible for the biosynthesis of these molecules in hops.

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