Importance of Medical Effects of Xanthohumol, Hop (*Humulus lupulus* L.) Bioflavonoid in Restructuring of World Hop Industry

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Summary

Nowadays, the world hop industry is faced with one of the biggest crisis in the history. Permanent decrease of hopping rates in the world beer industry during last 20 years caused many problems to hop producers all around the world. It resulted in the huge surpluses of hop stocks between years 2008 and 2010, the ages known as "years of famine" for hop farmers. The hop contains some compounds having medical, pharmaceutical or biological activities, and xanthohumol is one of them. Xanthohumol is a bioflavonoid whose positive effect is confirmed both *in vitro* and *in vivo* experiments. It is successfully used in medical treatment of patients having diseases such as prostate and breasts cancer, osteoporosis, menopausal problems, and even HIV. However, such attributes of hop are strong argument to consider the restructuring of the world hop industry and forming a new supply chain, primarily oriented to the pharmaceutical industry.

Key words

xanthohumol, hop bioflavonoid, hop sector restructuring, prenylated flavonoids, pharmaceutical industry

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Introduction

The 23rd of April 1516 was surely the most important date in the history for every brewer and hop producer, because it was the date when Bavarian Duke Wilhelm IV declared the Purity Law (Barth et al., 1994; Moir, 2000). The sweet marriage between brewers and hop producers lasts for centuries. But from the beginning of nineties of the 20th century the hop dosing rates per hL of beer wort is constantly decreasing because of global tendency of beer producers to satisfy organoleptic preferences of younger generations. Suddenly, bitterness and hoppy aroma of beer became less desirable; flavour less suitable for the younger population that was estimated as the most important target buyers/consumers by the marketing experts of the beer industry. Consequently, the world hops areas are decreasing year after year with exceptions of some years, mostly because of temporary price fluctuations (Barth and Meier, 2010).

In order to satisfy more demandable requests of beer industry, the research programs in hop genetics and breeding, hop physiology and processing into hop products, used to be intensified during the last twenty years. However, in spite of many improvements such as development of new hop cultivars, modern growing techniques, implementation of new plant protection measures, nowadays even some of the biggest and the most respectable hop research organizations are faced with the simple survival and share the destiny of hop farmers. Both i.e. hop producers and researchers are faced with the most important question: - How to sustain?

The aim of this paper is to give a critical overview of position of hops in the world beer industry. The intention is to point out the importance of hop secondary metabolites, in order to establish the new supply chain to the pharmaceutical industry. It could improve the economical sustainability of the world hop sector.

Decrease of beer bitterness – problem for hop producers and beer technologists

It is already well known that invention of hop adding into wort was probably one of the most important innovations in history of brewing (Barth et al., 1994; Moir, 2000). The reason for that was primarily to decrease the sweetness of wort and to achieve a desirable beer bitterness after isomerisation of α-acids (Bamforth, 2003; Briggs et al., 2004; Jaskula et al., 2007; Jaskula et al., 2008; Srečec et al., 2008a; Hanke, 2009; Hartmeier and Reiss, 2010; Jaskula et al., 2010) and, on the other hand, to prevent the beer spoilage, caused mostly by lactic acid bacteria of *Lactobacillus* genera (Back, 1994; Kaltner et al., 2001; Sakamoto, 2002; Sakamoto and Konings, 2003; Suzuki et al., 2004; Suzuki et al., 2006). According to Košir (1996), in the year of 1888 Haydukca used the term α-acids for hop substances forming a bitter resin of acid reaction with ions of lead. In 1925 Wöllmer developed the first analytical method for their quantitative detection (Howard and Tatchell, 1956; Forster, 1993) and α-acids became name for the most important hop chemical compound in the world beer industry.

Low hopping rates caused surpluses of hops and made hop production unstable

rom the beginning of 1990 (Fig. 1) the hop dosing rates constantly decreased ($r = -0.99$; $P < 0.0001$) and consequently, the world hop acreage rose as well ($r = -0.85$; $P = 0.00001$, Fig. 1) (Anon., 2010; Barth and Meier, 2010; Pavlovič, 2010). Moreover, in spite of constant growth of the world beer production, consumption of α-acids constantly decreases ($r = -0.73$; $P = 0.008$, Fig. 2) due to decreasing of hop dosing rates (Fig. 2). Finally, in order to achieve some acceptable prices on the world hop market, which could guarantee their sustainability, the world hop producers decreased the hop acreage, but, because of implementation of new technologies and planting of hop cultivars with higher yields of α-acids per hectare, the hop yields increased as well as production of α-acids ($r = 0.13$; $P > 0.05$, Fig. 3).

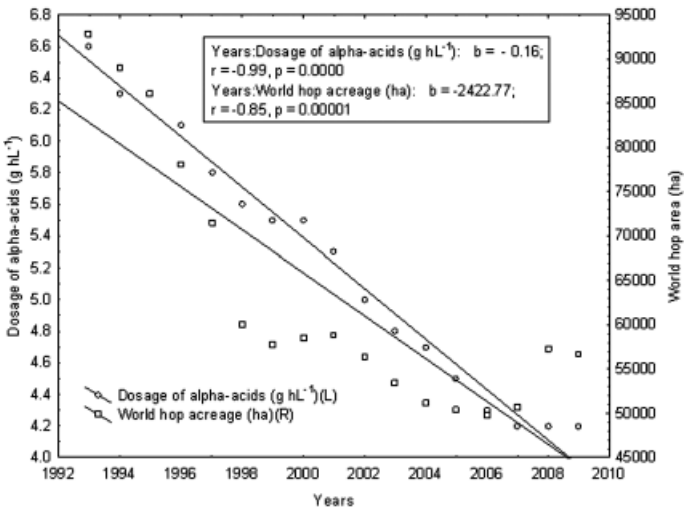

Figure 1. Decreasing of hop dosing rates and world hop area since 1992 till 2009

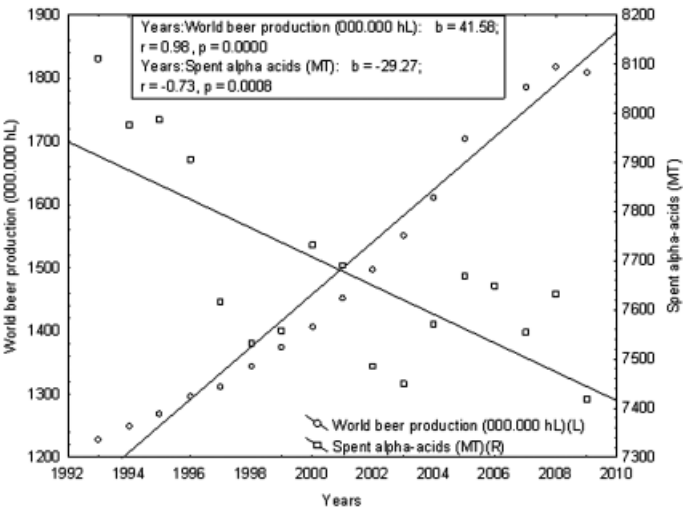

Figure 2. Increasing of world beer production and decrease of α-acids consumption since 1992 till 2009

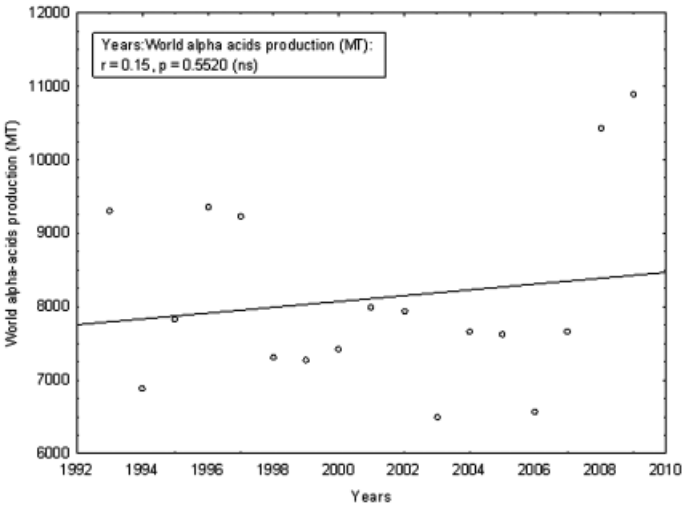

Figure 3. World production of α-acids since 1992 till 2009

During the years of 2008 and 2009, the world production of α-acids increased (Fig. 3), but this was a short term change caused by the speculative prices on the world hop market in year of 2007. Currently, there is among 3 481 tones of α-acids surplus on the world hop market. At the same time the hopping rate of 4.1 g of pure α-acids per hL of wort is the lowest in history (Barth and Meier, 2010).

Gushing – consequence of low hopping rates

Gushing is a phenomenon in which beer spontaneously, without agitation, vigorously overfoams out from the bottle (or some other packaging) immediately after opening. It has a negative influence on beer image and also causes big economic losses. The fungi developed on the kernels (caryopsis) of barley (Petr and Capouchová, 2001) are causing gushing and the most frequent among them are primarily of genera *Fusarium*, but also *Aspergillus*, *Nigrospora*, *Penicillium* and *Stemphylium*. Additionally, these fungi produce mycotoxins, like deoxynivalenol (DON) that is connected with the development of gushing potential. So, screening of mycotoxins in barley and malt may offer a means of reducing beer gushing problems. The predictors of gushing activity are hydrophobins, small, moderately hydrophobic proteins produced also by filamentous fungi. They have been found on the cell walls of hyphae and on spore surfaces and can also be secreted in the culture media (Sarlin et al., 2005; Stewart, 2006; Zapf et al., 2007).

Humulones and linalool are hop constituents that prevent beer from gushing (Hanke et al., 2009), especially when hops are added at late stages of wort boiling. Linalool significantly reduces the gushing volume and also decreases the gushing tendency of beer.

Unfortunately, there is no available data about financial losses caused by gushing. It would be very interesting to find out the influence of so called "savings" in hops to compare the financial consequences of returning the whole beer contingents from the market to the price of hops and hop products.

Xanthohumol "rediscovered" hop secondary metabolites

The first literature sources about medical effects of hops was book "Physica", the first known pharmacopoeia, written by Abbess Hildegard von Bingen in 12th century (Schattenhofer, 1989). Xanthohumol, a secondary metabolite, is a structurally simple prenylated chalcone that occurs only in the hop plant, where it is the principal prenylflavonoid of the female hop cones (Stevens and Page, 2004). Dried hops contain up to 1.0% of xanthohumol, which is found in the lupulin glands together with α- and β-acids and essential oils (De Keukeleire et al., 2003). Flavonoids are known to have antiallergenic, antiinflammatory, antiviral, antifungal, antibacterial, antioxidative, antiproliferative, and anticancerogenic effects (Middleton and Kandaswami, 1994). Practically all hop secondary metabolites exhibit more or less pronounced bioactive effects (Kondo, 2003). Nowadays, in the world scientific databases there are more than 250 references of published scientific papers considering the biosynthesis, accumulation, medical effects and metabolism of xanthohumol. This compound is one of the most examined prenylated flavonoids in the world. Unfortunately, the results of brewing tests showed that 80 to 90% of the added xanthohumol is eliminated during the brewing process. The effective dosages of xanthohumol or isoxanthohumol are minimally 0.35 mg per kg of body weight. It corresponds to approximately 30 mg per person per day or an average of 30 L of beer per day (Forster and Köberlein, 1998). Therefore, it is not possible to attribute physiological effect to a small amount that is consumed by drinking beer. According to Stevens and Page (2004), one way to achieve higher level of xanthohumol is addition of the pure compound during the brewing process (Biendl et al., 2001) which requires extraction and purification of large amounts of this compound. However, depending on addition of roasted malt or special roasted malt extracts enriched with xanthohumol, dark beers with more than 10 mg of xanthohumol per liter were achieved as well as wheat beers with more than 1 mg of xanthohumol per liter (Wunderlich et al., 2005; Magalhães et al., 2008). There are some commercial products of xanthohumol pills and beer enriched with xanthohumol on market (Biendl, 2007; Dhooghe et al., 2010). Consequently, the higher content of xanthohumol is also a goal in different breeding programs all over the world (Darby et al., 2003).

Biosynthesis and accumulation of hop secondary metabolites

Biosynthesis of hop secondary metabolites was not completely understood, until recently. It was known that biosynthesis of hop secondary metabolites starts in leaf cells (Čeh et al., 2007) and then they accumulated in hop glandular trichomes (Srečec et al., 2010b and 2011; Fig. 4).

According to Nagel et al. (2008) and Wang et al. (2008), there are three independent biosynthetic pathways for terpene-derived natural products found in hop trichomes (Fig. 5). In order to understand the molecular basis for terpene accumulation in hop trichomes, Wang et al. (2008) constructed a trichome cDNA library and 9 816 cleansed expressed sequence tag (EST) sequences were obtained from random sequencing of 16 152 cDNA clones. The ESTs were assembled into 3 619 unigenes (1 101 contigs and 2 518 singletons). Biosynthetic pathways for terpene-derived nat-

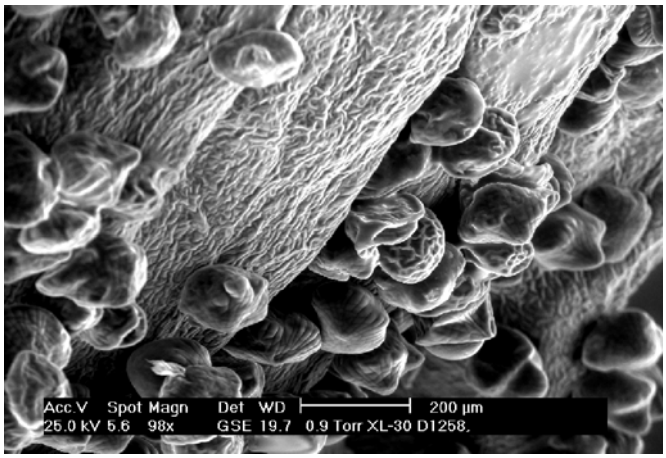

Figure 4. ESEM (environmental scan electron microscope photography) of hop peltate glandular trichomes in different stages of morphogenesis, developed from the epidermal cells of cone bract, Srečec et al., 2011 (magnification 113×, bar=200 µm)

ural products found in hop trichomes, showing EST abundance in the hop glandular trichome cDNA library.

The same authors found that accumulation of monoterpene and sesquiterpene derived hop secondary metabolites in hop glandular trichomes is more intensive during the third and fourth week after beginning of hop flowering, which generally corresponds with the results of Hirosawa et al. (1995) and Čeh et al. (2007). Accumulation of hop secondary metabolites is under the strong influence of external or climatic factors (Forster, 2001a, b; Srečec et al., 2004; Srečec et al., 2008b; Kučera and Krofta, 2009; Mozny et al., 2009).

However, there is a large variability in accumulation of some most important hop secondary metabolites, such as α-acids and β-acids, xanthohumol, essential oils, total polyphenols and flavonoids. Such variability depends primarily on genotype of hop cultivars, external factors (the influence of crop year) and also on the level of processing the natural hop cones into hop products (Forster, 2001a,b; Krofta et al., 2008; Čerenak et al., 2009; Srečec et al., 2009; Jelínek et al., 2010). Moreover, there is a variability in content of hop secondary metabolites even in wild hop

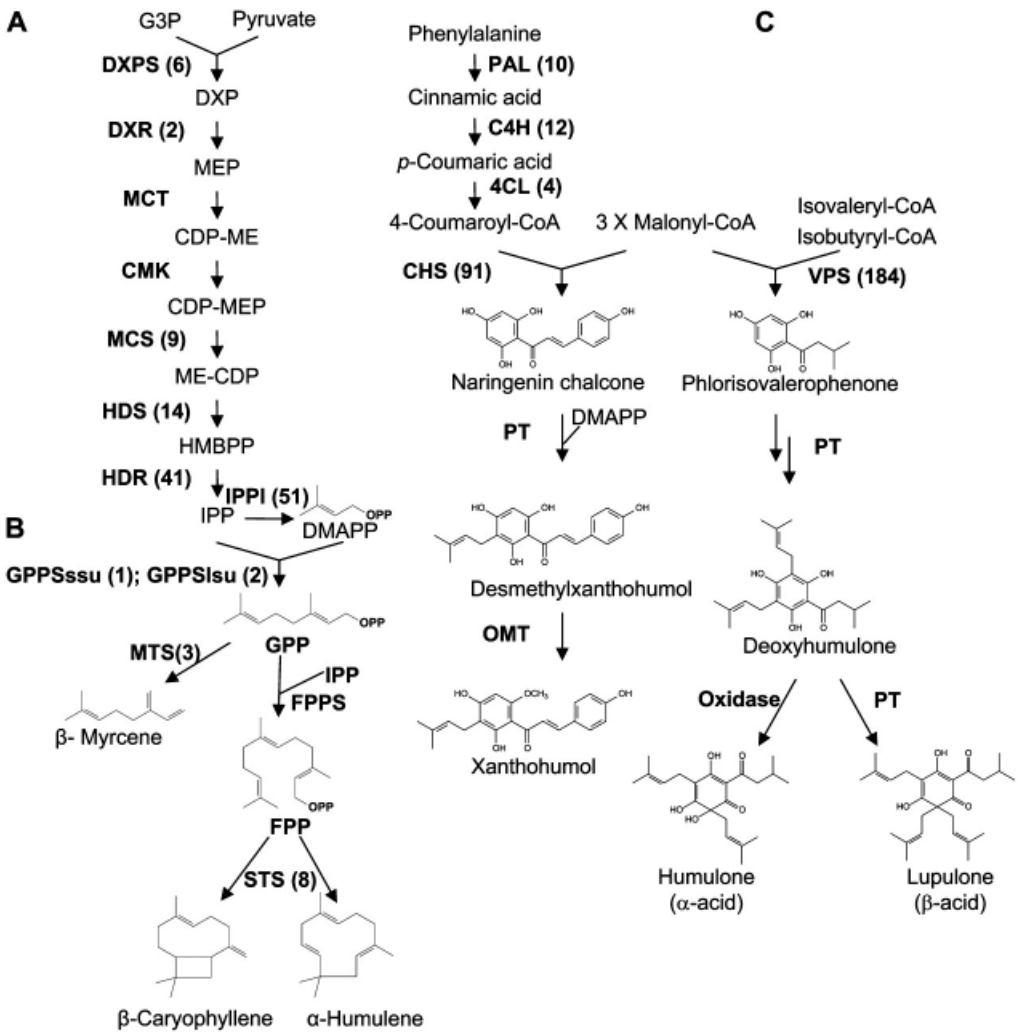

Figure 5. Biosynthetic pathways for terpene-derived natural products found in hop trichomes, showing EST abundance in the hop glandular trichome cDNA library (Wang et al. 2008)
A, The pathway leading to dimethylallil pyrophosphate (DMAPP) biosynthesis.
B, General reactions of mono- and sesquiterpene biosynthesis. C, Prenylflavonoid and bitter acid biosynthesis. DXPS, 1-Deoxy-D-xylulose-5-P synthase; DXR, 1-deoxy-D-xylulose-5-P reductoisomerase; MCT, 2-*C*-methyl-D-erythritol-4-P cytidylyltransferase; CMK, 4-diphosphocytidyl-2-*C*-methyl-D-erythritol kinase; MCS, 2-*C*-methyl-D-erythritol-2,4-cyclodiphosphate synthase; HDS, 1-hydroxy-2-methyl-2-(*E*)-butenyl-4-diphosphate synthase; HDR, 1-hydroxy-2-methyl-2-(*E*)-butenyl-4-diphosphate reductase; IPPI, isopentenyl diphosphate/dimethylallyl diphosphate isomerase; OMT, *O*-methyltransferase; PT, prenyltransferase.

populations, which is the consequence of their genetic diversity (Krofta et al., 1998; Hampton et al., 2002, Patzak et al., 2010a,b; Srečec et al., 2010a).

Medical effects of xanthohumol

It is well known that xanthohumol as much as other prenylflavonoids is cancer chemopreventive agent. Even low micromolar concentrations cause inhibition of metabolic activation of procarcinogens, induction of carcinogen-detoxifying enzymes, and inhibition of tumour growth by inhibiting inflammatory signals and angiogenesis (Stevens and Page, 2004). Xanthohumol is highly potent in suppressing the growth of human breast cancer cells (MCF-7) and ovarian cancer cells (Miranda et al., 1999). Albini et al. (2006) refer that xanthohumol even in low concentrations of only 5 μM, is very potent orally available antiangiogenic chemoprevention agent whose mechanism targets endothelial cell migration, invasion, and proliferation. Lust et al. (2005) cultured *in vitro* lymphocytes from patients with B-cell chronic lymphocytic leukemia (B-CLL) in presence of xanthohumol. Xantohumol induced killing of B-CLL cells at LD_{50} (24 h) at 24.4 ± 6.6 μM, independent of known adverse prognostic factors including functional loss of p53 (tumour protein 53). Colgate et al. (2007) found that xanthohumol at 2.5-50 μM decreases cell viability, induces apoptosis and inhibits NF-kappaB (protein complex that controls transcription of DNA) activation in prostate epithelial cells. Buckwold et al. (2004) found that ultra-pure preparations of xanthohumol (> 99 %) showed antiviral activity against bovine viral diarrhea virus (BVDV) and herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), and cytomegalovirus (CMV). Wang et al. (2004) found that xanthohumol in half of maximal effective concentration (EC_{50}) of 0.82, 1.28 and 0.50 μg mL^{-1}, inhibits HIV-1 induced cytopathic effects, production of p24 (capsid protein of HIV) antigen and reverse transcriptase in C8166 lymphocytes. Xanthohumol also inhibited HIV-1 replication in peripheral blood mononuclear cells (PBMC) with EC_{50} value of 20.74 μg mL^{-1}. However, the activity of recombinant HIV-1 reverse transcriptase and the HIV-1 entry were not inhibited by xanthohumol.

Xanthohumol has also estrogenic effects, because *in vitro* studies identified 8-prenylnaringenin (8-PN) as one of the most potent estrogens in hops. 8-PN belongs to the group of prenylated flavones, also containing isoxanthohumol, 6-prenylnaringenin, and a number of diprenylated analogues (Nikolić et al., 2006). Also, acne vulgaris is definitely one of the most common skin disease affecting children and adolescents caused by proliferation of bacteria such as *Propionibacterium acnes*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Kocuria rhizophila and Staphylococcus pyogenes*. According to the results of Yamaguchi et al. (2009) hop extracts of xanthohumol and the lupulones (*β*-acids) showed strong inhibitory activities against all of the bacteria strains causing acne vulgaris.

Nowadays, antioxidants are among popular over-the-counter and other nonprescription medications, because of the increasing awareness on their importance for human health. They are able to eliminate reactive oxygen and nitrogen radicals that irreversibly damage live tissues and induce serious diseases, e.g. degenerative diseases, such as cardiovascular disease and cancer. Since oxidative damage is also considered to be the main cause of ageing it opens another field of antioxidants application. Due to their antioxidant power, hop polyphenols may contribute positively to beer flavour stability (Mudura et al., 2010) and use of hop as nutraceutical with antioxidative properties (Krofta et al., 2008; Yamaguchi et al., 2009; Mudura et al., 2010) could be also expanded to gain an extra profit.

Dosage and side effects of xanthohumol

Hussong et al. (2005) referred weak hepatotoxicity noticed *in vivo* only at doses of 100-1000 mg of xanthohumol per kilogram of Sprague Dawley rats body weight during the 28 days of diet. However, these concentrations are extremely high. For instance, average body weight in US population is approximately 86 kg for males and 74 kg for females (Ogden et al., 2004), and according to these data, the daily input of 100 mg kg^{-1} of human body weight would be input of 8.6 kg of pure xanthohumol for males or 7.4 kg for females. The consumption of such high amounts of pure xanthohumol or beer containing such doses is not realistic.

Cancer disease statistics and hop surpluses

According to the WHO statistics, in 2007 different cancer diseases were cause of death for 7.6 million of people and some three quarters of them are in low- or middle-income countries (Anon., 2007). On the other hand, according to the statistics of American Cancer Society cancer caused 570 280 of deaths in 2005 and there were 1 372 910 estimated new cases (Anon., 2005). In male population the most often is prostate cancer and in female breasts cancer (33% and 32%, respectively). Most of the world hop cultivars synthesize and accumulate in hop cones approximately less than 1% of xanthohumol. It means that, in 1 tone of dried hop cones only 10 kg of xanthohumol is accumulated, which is, according to Albini et al. (2006) sufficient daily chemopreventive dose for 5 643 341 of people. However, this is just minimal quantity for chemoprevention of malignant disease of critical groups in the population, without medical treatments of patients with some cancer disease.

Particularly, hop preparations can be used in the most threatened part of population, such as workers in chemical industry, the people who live in polluted areas, possible recurrences, i.e. cured patients who get over some cancer disease, etc.

Conclusion

There is a large quantity of research done on xanthohumol. Its biological activity is a strong argument to consider the restructuring of the world hop industry and forming a new supply chain, primarily oriented towards pharmaceutical industry. It seems that using of nutraceuticals based on hop or functional food (*e.g.* beer enriched with xanthohumol) aimed primarily for chemoprevention and even for some medical treatments. Respecting the increasing number of some types of cancer such application of hops constituents could mean a strong opportunity for future restructuring and sustainability of the world hop sector.

In spite of a huge research work with encouraging results, which positive effects are confirmed *in vitro* and *in vivo* only a few drugs or nutritional supplements containing xanthohumol are registered. It seems that the world pharmaceutical industry and medical science still do not pay attention to possible applications of hop compounds.

Unfortunately, at the moment huge quantities of hops are stored in hop warehouses and waiting to be sold or destroyed.

Using of xanthohumol is opportunity to achieve sustainability of world hop industry. At the same time it is possible to make new economic profit from underutilized hop sources, because hop production is well established, experiential based and promoted by new genetic research and technological improvements.

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