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Physiological significance of polyphenols and hop bitters in beer

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The physiological significance of phenolic substances and hop bitter substances has been the subject of in-depth investigations, and a great many new facts have emerged in the last five years. This contribution provides an overview of the current state of knowledge.

The term polyphenols refers to substances which are made up of phenol derivatives. They primarily include phenolic acids and monomeric and oligomeric flavanols. In terms of volatility, phenolic substances in beer can be subdivided into monomeric monophenols, monomeric polyphenols and polymeric polyphenols.

In the order of 153 mg of polyphenols are present in one litre of German Pilsener lager. They are of basic significance for chemico-physical stability, foam formation, bitterness, taste, smell and resistance to staling of beer.

In physiological terms, polyphenols, carotinoids, phytosterols, saponins, glucosinolates, monoterpenes, phytoestrogens and lectins have recently been classified as belonging to the group of "secondary plant substances". Plants use them, inter alia, as deterrents against plant pests, as growth regulators and as colourings. As they can have a multiplicity of health-promoting effects, they are also known as "phytochemicals" in American English and as "phytoprotectants" in British English (*Watzl*, 1996).

Hop bitter substances

Beer is the only alcoholic beverage containing hops. German Pilsener lager has an average of 33.5 mg of isohumulone/l. In addition, a number of derivatives of bitter substances, as well as a number of hop oils and hop oil derivatives, are known. Hence up to 400 mg of hop substances – in the widest sense – may consequently be present in one litre of beer.

Apart from their significance for bitterness and aroma as well as formation of foam, the hop contents contribute to inhibiting growth of pathogenic micro-organisms in beer. Thus, beer complies with essential requirements applicable to this food by the imperatives of hygiene. Since the twenties, the sedative, hypnogenic, bacteriostatic, antibiotic, tuberculostatic, hormonal and amarum-aromaticum effects have been identified in many investigations (*Piendl* and *Schneider*, 1981).

Recent findings

General properties

Polyphenols have a wide spectrum of health properties. There are many indications that they have effects such as

- anticarcinogenic,
- antimicrobial,
- antioxidative,
- antithrombotic,
- immunomodulating,
- antiphlogistic,
- blood pressure regulating,
- blood glucose regulating

(Watzl, 1996).

Many secondary plant substances may have an inhibitory effect at almost any stage in the emergence of cancer. It has been shown in vitro that some phenolic acids can form bonds with activated carcinogenic substances and can "occlude" binding sites for carcinogens in deoxyribonucleinic acid (DNA), this may inhibit cancer-ation as a result of damage to DNA. The body is also provided with various protective mechanisms to deactivate reactive oxygen molecules and free radicals. In terms of quantity, polyphenols are the most common and most effective antioxidants in food of plant origin (*Ho et al.*, 1992, *Huang et al.*, 1992 and *Papas*, 1999).

Low-density lipoproteins

A polyphenol extract (from red wine) or the quercetin flavonoid provides in vitro more effective protection against oxidation than a comparable quantity of vitamin E (*Frankel et al.*, 1993). "In in vitro studies with phenolic substances in red wine and normal human low-density lipoprotein (LDL) we found that red wine inhibits the copper-catalysed oxidation of LDL. Wine diluted 1000-fold containing 10µmol total phenolics/liter inhibited LDL oxidation significantly more than alpha-tocopherol. Our findings show that the non-alcoholic components of red wine have potent antioxidant properties toward oxidation of human LDL … If potent antioxidant phenolic components are routinely ingested by the regular consumption of red wine they may collectively reduce thrombotic phenomena and thereby contribute to the amelioration of atherosclerosis and morbidity and mortality from coronary artery disease."

The anti-oxidant properties of low-density lipoproteins has been recently also established in the case of beer (*Vinson et al.*, 1999). "The order of antioxidant quality for the beverages was black tea > coffee > prune juice = beer > green tea > orange juice > red wine …"

Mutagenicity

Alcoholic beverages, in particular beer and its phenolic compounds, may inhibit mutagenic effects (= spontaneously or artificially induced changes in hereditary structures) brought about by heterocyclic amines (*Arimoto-Kobayashi et al.*, 1999). "The possibility that beer and other alcoholic beverages could be antimutagenic

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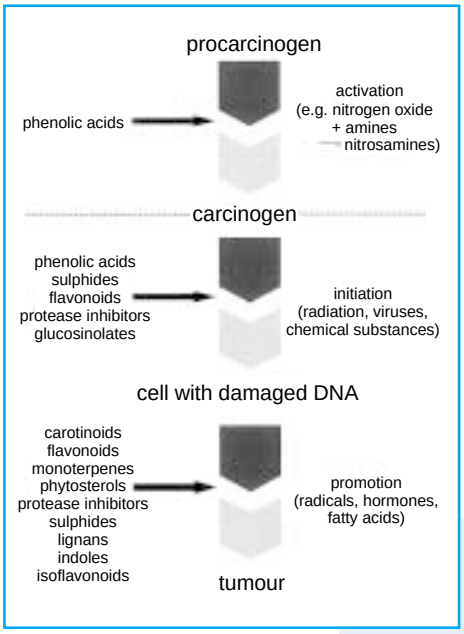

Potential for secondary plant substances to inhibit cancer formation (according to Caragay, 1992, and Wattenberg, 1993; graphic representation according to Watzl and Leitzmann, 1999)

against the heterocyclic amines, a group of carcinogens produced on cooking proteinaceous foods, has been explored … Beer showed inhibitory effects against several heterocyclic amines. Because ethanol itself has no effect at its concentration in beer and because non-volatile (f.e. phenolic substances) of beer show antimutagenic effects, substances derived from the fermentation processes and/or from the raw materials of beer must be the cause of these antimutagenic effects … Possibly there are several antimutagenic factors in the various beers, and the efficacy of them for the inhibition of the mutagenicity of individual mutagens may be different … Because an extract of hops inhibited the mutagenicity of Trp-P-2(NHOH), the antimutagenic components in beer may be of plant origin … Because of the widespread consumption of beer in human populations, its potential protective effect against mutagens are worthy of further studies."

Caries

Hop polyphenols may delay the onset of dental caries (*Tagashira et al.*, 1997). "The inhibitory effet of hop bract polyphenols (= HBP) on cariogenic streptococci was investigated. It was found that the high molecular weight polyphenol (estimated about 36,000 – 40,000) inhibited the cellular adherence of *Streptococcus mutans* … and *Streptococcus sobrinus* … at much smaller concentrations than the polyphenols extracted from oolong tea or green tea leaves. Furthermore, HBP also inhibited the action of glucan synthesis, but did not suppress the growth and the acid production of the bacteria. These results suggest that HBP would be a candidate to act against dental caries caused by *Mutans Streptococci* … Although hop bract parts had been thought to be useless, they might yield a new candidate for drugs or food materials against dental caries."

Cancer

Various flavonoids of hops may inhibit proliferation of human cancer cells (*Miranda et al.*, 1999). "Six flavanoids from hops (Humulus lupulus) were tested for their antiproliferative activity in human breast cancer, colon cancer and ovarian cancer cells in vitro. Xanthohumol, dehydrocycloxanthohumol and isoxanthohumol caused a dose-dependent (0.1 to 100 µM) decrease in growth of all cancer cells. Xanthohumol and isoxanthohumol may have potential chemopreventive activity against breast and ovarian cancer in humans."

Botulism

Hop extract may combat getting botulism (*Johnson* and *Haas*, 1999). Botulism is a poisoning, mostly food poisoning, induced by toxins of *Clostridium botulinum*. A patent application has been filed for the use of hop extracts containing beta-acids as antibacterial additives in various foods and beverages against two highly dangerous food-borne pathogenic bacteria, *Clostridium botulinum* … and its close relative *Clostridium difficile*".

Fungal growth

Components of hard resins of hops as well as humulone and lupolone may inhibit growth of various fungi (e.g. *Trichophyton*, *Candida*, *Fusarium*, *Staphylococcus* and *Escherichia coli*) (*Mizobuchi* and *Sato*, 1985 a and 1985 b). "6-isopentenylnaringenin, xanthohohumol and isoxanthohumol from hard resins of hops (*Humulus lupulus L.*) were found to have antifungal activities" … "Humulone, lupulone and related compounds were found to have antifungal activities."

Otitis

Humulone may inhibit inflammation of the ear (in mice) (*Yasukawa et al.*, 1993). "The methanol extract of hop … markedly inhibited the inflammatory activity induced by TPA-induced inflammation seem to be roughly in parellel with their inhibitory activities of tumor promotion. Since flavonoids, tannins and triterpenoids are widely distributed in the plant kingdom in fruits and vegetables, it is important to determine their potential as antitumor-promoting food additives … The 50% inhibitory dose of humulone for TPA-induced inflammation was 0.2 mg/ear."

Antioxidants

Humulone and lupolone have a high antioxidant potential (*Tagashira et al.*, 1995). "Hop bitter acids, humulones and lupulones, were shown to have potent radical scavenging activity (= RSA) and lipid peroxidation inhibitory activity (= LIA). Furthermore 5-acetyl lupulones and 4-methyl lupulones had more potent LIA than notive lupulones but no RSA … The RSA of humulone and lupulone are nearly equivalent to those of two natural antioxidants, alpha-tocopherol and ascorbic acid. As for LIA, humulone and lupulone are superior to natural antioxidants by about 10 to 100 times … These results suggest two important facts. One is that hop bitter acids have potent antioxidantive activity in vitro and the other is that chemical modification of lupulone can distinguish between the two kinds of antioxidative activity (RSA and LIA)."

Cancer of the skin

Humulone may inhibit growth of skin tumours in mice (*Yasukawa et al.*, 1995). "Humulon, one of the bitters in the hop, was

Table 1 Total polyphenols, bitter substances and isoxanthohumol in German beers

	Duckstein	Gentner Fränkisch Dunkel	Bitburger Premium Pils	Märkischer Landmann	Hoepfner Porter
Original gravity (g/100 g)	11.6	13.3	11.6	12.5	15.8
Alcohol (ml/100 ml)	5.12	5.56	4.89	5.05	5.77
Extract real (g/100 g)	3.77	4.98	4.12	4.89	7.32
Degree of attenuation apparent (A_a, TA %)	83.6	78.3	80.0	76.3	67.6
Colour (EBC)	29	49	6.3	113	92
Total polyphenols (EBC) (mg/l)	212	252	195	248	344
Bitter substances (EBC) (mg/l)	26.5	25.0	32.0	22.0	38.5
Isoxanthohumol (mg/l)	1.7	2.1	2.1	1.3	1.9

isolated from the female flowers of Humulus lupulus. This component has inhibitory activity against 12-0-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation. At 1 mg/mouse, humulon inhibited markedly the tumor-promoting effect of TPA on skin tumor formation. Furthermore, humulon inhibited arachidonic acid-induced inflammatory ear edema in mice."

Leukaemia

Humulone may inhibit growth of leukaemia cells (*Honma et al.*, 1998). "In this study we examined the effect of humulone on the differentiation of human myelogenous leukemia cells. Humulone alone inhibited the growth of monoblastic leukaemia U 9317 cells … humulone effectively enhanced the differentiation-inducing action of VD_3, the active form of vitamin D … The combination of humulone and VD_3 may be useful in differentiation therapy of myelomonocystic leukemia."

Duodenititis

Lupolone may inhibit growth of *Helicobacter pylori*, a micro-organism responsible for duodenal ulcers and stomach cancer (*Ohsugi et al.*, 1997). "Twenty-seven natural medicines, which have been traditionally used in China, Indonesia, Vietnam or Japan, were examined for in vitro antibacterial activity against *Helicobacter pylori*. Among these, hop … significantly ingibited the growth of *Helicobacter pylori* … Lupolone is the active constituent of hop against *Helicobacter pylori*, as lupulone showed very strong antibacterial activity against three clinically isolated strains including an erythromycin resistant strain, as well as two standard strains of *Helicobacter pylori*."

Osteoporosis

Xanthohumol, especially humulone, may inhibit bone resorption in humans and thus prevent the onset of osteoporosis (= brittleness of the bones) (*Tobe et al*, 1997). "We searched hop extract for active compound(s) that inhibited bone resorption, and isolated xanthohumol and humulone as active ingredients. Especially humulone has extraordinary strong inhibitory activity … We think that xanthohumol and humulone may be candidates for the therapeutic drugs for osteoporosis."

Cytochrome P-450 enzymes

Xanthohumol and a number of related prenylflavonoids may inhibit enzymes of the cytochrome P-450 type which transfers procarcinogens into carcinogenic compounds (*Stevens et al.*, 1998). "The findings suggest that xanthohumol and some other related prenylflavanoids from hops could be effective as new cancer chemopreventive agents by blocking the cytochrome P450-mediated activation of procarcinogens and by inducing the carcinogen-detoxifying enzyme, quinone reductase."

Table 2 (continuation of Table 1)

	Mahr's Bräu, non–bunged – yeast haze	Uerige Dat leckere Dröpke	Uerige Sticke	Aecht Schlenkerla Rauchbier Märzen	Aecht Schlenkerla Rauchbier Urbock
Original gravity (g/100 g)	12.0	11.5	14.5	13.4	17.1
Alcohol (ml/100 ml)	5.21	5.05	6.53	4.95	6.64
Extract real (g/100 g)	4.09	3.81	4.71	5.99	7.37
Degree of attenuation apparent (A_a, TA %)	82.2	83.3	84.2	69.2	71.4
Colour (EBC)	15	30	36	53	73
Total polyphenols (EBC) (mg/l)	204	279	326	226	232
Bitter substances (EBC) (mg/l)	42.5	51.5	50.5	33.5	37.0
Isoxanthohumol (mg/l)	2.6	2.5	2.5	2.0	2.7

Table 3 Total polyphenols, bitter substances and isoxanthohumol in international beers

	Guinness Draught Stout (IRL)	Guinness Extra Stout (IRL)	Guinness Traditional Brewed Stout (IRL)	Guinness Foreign Extra Stout (IRL)	Guinness Special Export Stout (IRL)
Original gravity (g/100 g)	10.2	10.1	11.8	17.5	17.9
Alcohol (ml/100 ml)	4.39	4.32	5.25	7.78	7.94
Extract real (g/100 g)	3.41	3.47	3.79	6.04	6.14
Degree of attenuation apparent (A_a, TA %)	82.7	81.8	84.4	82.7	82.9
Colour (EBC)	147	148	157	175	168
Total polyphenols (EBC) (mg/l)	247	273	265	446	402
Bitter substances (EBC) (mg/l)	34.0	36.5	38.5	60.0	38.0
Isoxanthohumol (mg/l)	1.6	1.8	1.7	2.4	1.9

Table 4 (continuation of Table 3)

	Beamish Genuine Stout (IRL)	Murphy's Irish Stout (IRL)	Tennent's Stout (UK)	Imperial Russian Stout (UK)	Coopers Best Extra Stout (AUS)
Original gravity (g/100 g)	10.6	9.2	15.5	24.5	14.9
Alcohol (ml/100 ml)	4.41	3.79	6.62	10.84	7.05
Extract real (g/100 g)	3.82	3.31	5.72	9.40	4.34
Degree of attenuation apparent (A_a, TA %)	79.7	79.6	78.8	78.9	88.0
Colour (EBC)	169	152	173	197	110
Total polyphenols (EBC) (mg/l)	240	242	302	354	384
Bitter substances (EBC) (mg/l)	36.0	28.0	39.5	33.0	26.5
Isoxanthohumol (mg/l)	2.5	1.0	2.0	2.4	1.2

Atherosclerosis

Xanthohumol and xanthohumol B may have a highly inhibitory effect on the diacylglyceroltransferase enzyme (in rat liver

Table 5 (continuation of Table 3)

	Dark Horse Porter (UK)	Neame Original Porter (UK)	Neame Bishops Finger (UK)	Young's Old Nick (UK)	Farsons Strong Ale (GBY)
Original gravity (g/100 g)	12.9	12.4	12.9	18.6	15.0
Alcohol (ml/100 ml)	5.01	5.25	5.72	6.33	6.27
Extract real (g/100 g)	5.32	4.41	4.27	9.48	5.64
Degree of attenuation apparent (A_g, TA %)	73.6	80.3	83.4	62.4	78.2
Colour (EBC)	175	95	41	90	34
Total polyphenols (EBC) (mg/l)	280	225	202	415	244
Bitter substances (EBC) (mg/l)	33.0	28.5	33.5	37.5	40.0
Isoxanthohumol (mg/l)	1.7	1.5	1.3	1.8	1.1

microsomes) which transforms diacylglycerol into triacylglycerol, and may thus be effective against atherosclerosis (*Tabata et al*, 1997). "Too much accumulation of triacylglycerol in certain organs and tissues of the body causes high risk conditions of fatty liver, obesity, and hypertriglyceridemia, leading to serious diseases of atherosclerosis, diabetes, metabolic disorders and functional depression of some organs."

In a review paper, *Biendl* (1999 a and b) presented the biochemical and anticarcinogenic properties of polyphenols and hop contents. He stated expressly that most studies are still in their infancies. It remains to be seen if the effects which were mostly obtained in tests with isolated cells (in vitro) will also manifest themselves in the human organism.

Mechanisms of development and prevention of cancer (Watzl and Leitzmann, 1999)

Development of cancer is a multi-facetted process which evolves in at least three stages, i.e.

- initiation,
- promotion and
- progression.

Although this classification no longer reflects state-of-the-art, it has the advantage that the possibilities of secondary plant substances exerting an influence can be explained relatively easily (see illustration).

Initiators are inter alia radiation, viruses or chemical substances such as e.g. nitrosamines, polycyclic aromatic hydrocarbons and heterocyclic amines. Promoters are non-genotoxic substances such as e.g. radicals which can promote cancer formation. They induce cells with a changed deoxyribonucleinic acid to propagate increasingly, passing on their genetic information, eventually leading to formation of tumours.

Certain secondary plant substances in particular, in addition to various nutrients such as antioxidative vitamins, act as anticarcinogenics and antipromoters. They intervene in the various stages of cancer development.

- On the one hand, they may inhibit activation of cancerous precursors to actual carcinogenic compounds;
- on the other hand, they may inhibit initiation i.e. triggering of cancer formation;
- finally, they may inhibit promotion of growth of cancer, and
- possibly also progression i.e. growth of tumours and/or formation of metastases.

Effective prevention of cancer would be to identify protective compounds in food and increasingly consume such food. At the same time, consumption of food containing initiators and promoters must be restricted (Watzl and Leitzmann, 1999).

Polyphenols in beer

It is general practice to go for a low polyphenol content (together with various protein substances) in beer so as to achieve a long physical-chemical stability of this food. Should this notion be maintained in future (*Piendl*, 1998 a and b)?

Gromus and *Lustig* (1999) demonstrated in a recent study that beers containing very different levels of polyphenols and reducing substances can be brewed, depending on the selection of raw materials and the brewing processes employed. A high poly-phenol content must not necessarily be accompanied by lower colloidal stability.

Modern HPLC methods facilitate selection of suitable raw materials, e.g. fresh aroma hops from moderate growing regions. Medium and short wort boiling times should be given preference to long ones. They do not damage colour or foam stability of beers, the reductive capacity rises, taste and taste stability can be influenced positively. Brewery technicians wishing to develop a "polyphenol philosophy" have ample opportunities for implementing same (*Forster et al.*, 1999).

Wilson et al. (1998) provided evidence that xanthohumol, isoxanthohumol, isoadand isoprehumulone can improve foam stability of beer in a lasting manner.

Kammhuber et al. (1998) tested hop varieties and bred strains for xanthohumol. The xanthohumol content of hops was surprisingly high – from 0.2 to 1.0 % by weight, the "Hallertauer Taurus" variety having the highest value. When hop cones are pro-cessed to pellets, xanthohumol remains intact. In the case of extract, a clear differentiation can be made between ethanol and carbon dioxide extracts. The recovery rate of xanthohumol in ethanol extracts is almost 100% whereas only traces are contained in carbon dioxide extracts (*Forster* and *Köberlein*, 1998, and *Kammhuber et al.*, 1998).

Stevens et al. (1999 a and b) and Forster and Köberlein (1998) investigated the behaviour of xanthohumol in beer production. During wort boiling, xanthohumol is transformed into isoxanthohumol, with about 70% yield. About 40% of isoxanthohumol is removed with trub and yeast. Accordingly, about 30% of the xanthohumol introduced by hops is still present in the finished beer in the form of isoxanthohumol. The highest isoxanthohumol level ever detected in a beer, in this instance in an American beer, was 3.4 mg/l (a "strong ale" produced by an American microbrewery). A stout imported into the US had 2.1 mg/l and a Pilsener lager 1.1 mg per l.

Testing a range of beers for polyphenols, bitters and isoxanthohumol

A number of beers with high levels of polyphenol and bitter substances were selected from a larger range of national and international beer brands (1000 brands) and tested for isoxanthohumol.

A Skaba instrument was used to measure original gravity, alcohol and extract. Colour, total polyphenols and bitters were analysed using EBC. Analytical instruments and methods used for analysing isoxanthohumol comprised:

- *Solvents and chemicals:*
  – All solvents using EBC 7.8.
  – Solid-phase column Bakerbond spe Octadecyl (C18), 6 ml (1000 mg), article no. 7020-07.
  – Vakubox Baker spe-12-G.
- *Sample preparation:*
  – Degas sample by 20 transfers from one beaker to another and allow to stand overnight.
  – Mix 200 ml of degassed beer in beaker with 400 ml of 85% o-phosphoric acid.
- *Solid–phase extraction:*
  – Mount solid–phase column on Vakubox and set vacuum to 400 mbar.
  – Aspirate 20 ml of methanol through it.
  – Aspirate 10 ml of solution A (0.2 ml of o-phosphoric acid in 50 ml of water and 50 ml of methanol) through it.
  – Make sure that column does not dry out!
  – Aspirate immediately 50 ml of degassed, acidified beer through.
  – Dry column within 120 seconds (suck air through).
  – Aspirate 10 ml of solution B (0.2 ml of o-phosphoric acid in 100 ml of water) through.
  – Dry column for 120 seconds (Aspirate air).
  – Aspirate 10 ml of solution C (0.1 ml of o–phosphoric acid in 90 ml of methanol and 10 ml of water) through. Collect eluate in a 5 ml graduated flask and add solution C to make up 10 ml. Mix intensively. This solution is used for HPLC measurement.
- *HPLC determination:*
  – HPLC analysis using EBC 7.8 method (only difference: detection wavelength 290 nm instead of 270 nm in original method).
  – External calibration standard: Isoxanthohumol (purity > 95% with DAD-HPLC) supplied by Phytochem Referenzsubstanzen GbRmbH, 89335 Ichenhausen/ Germany.

Levels of polyphenols, bitters and isoxanthohumol in the beers fluctuate widely (see tables). Based on the – few – available findings, it is not possible to derive the isoxanthohumol content from any conventional beer characteristic. Further studies are urgently required.

Summary

- New findings relating to the physiological significance of polyphenols and hop bitters in beer are presented.
- Polyphenols may inhibit oxidation of low-density lipoproteins.
- Secondary plant substances, including polyphenols and hop bitters of beer, may be effective against formation and growth of cancer in all stages.
- Contents of polyphenols, bitter substances and isoxanthohumol fluctuate very widely in German and international ales and lagers.
- Further studies to investigate the behaviour of isoxanthohumol during beer production are necessary.

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CORRECTION

Microgramme and milligramme

In the article by C. Walker, **"Phytoestrogens in beer – good news of bad news"** published in "Brauwelt International" no. I, 2000, a printing error occurred on page 39, second column, in view of the correct measure, which should of course read microgramme (µg), instead of milligramme (mg): "… Milligan reports the detection of 8PNG in beer at levels up to 100 µg/litre…" ■