M. Kippenberger, S. Hanke, M. Biendl, G. Stettner and A. Lagemann

Transfer of Nitrate and various Pesticides into Beer during Dry Hopping

We describe the transfer of nitrate and the pesticides azoxystrobin, dimethomorph, myclobutanil and quinoxyfen from contaminated hops to the beer during wort production and dry hopping. The experiments reported comprise of two experiment series conducted in a 20 hL pilot plant. First, the substance transfer was investigated by dry hopping different base beers with different amounts of various hop varieties and products. Secondly, a beer was brewed and dry hopped exclusively with a hop that had a high load of the four aforementioned pesticides. The results are presented in terms of concentrations and processing factors. In a market survey, we also analyzed 14 commercial beer samples of different brands and types as well as different hop samples in regard to their nitrate concentrations which were in the range of 3.0 to 86.5 mg/L.
The six India Pale Ales of the commercial beer samples were also analyzed for a selected number of pesticides. None of the samples contained detectable amounts of those pesticides which were quantified after extraction using LC-MS/MS. Transfer of nitrate from hops to beer was nearly quantitative and scaled linearly with hop addition. Extraction of pesticides was strongly dependent on temperature and primarily limited by the water solubility of the compounds at the cold temperatures of maturation and fermentation. The dimensionless processing factors, defined as the residue level in the processed product in relation to the residue level in the raw product, had maximum values of 0.002.

Descriptors: hops, pesticide, nitrate, dry hopping, processing factor

1 Introduction

Dry hopping was originally intended as a technique to increase the microbiological stability and therefore the shelf life of beer. The technique was predominantly employed in Anglo-Saxon countries.

The recent boom in craft brewing especially in the United States and the developing craft brewer communities in England, Italy and Australia brought new attention to the technique of dry hopping.

Dry hopping means the addition of hops during the last step of beer production after main fermentation, mainly during lagering, which not only increases shelf live but also changes dramatically the flavor of the final product. The extraction of volatile hop compounds at the low temperatures during storage that are normally evaporated during wort boiling gives the beer a distinct aroma. The predominant compounds in the hop oil are α-humulene, myrcene and ß-caryophyllene. While these compounds comprise up to 90 % of the total hop oil [1], there are hundreds of further substances which with their low taste threshold contribute to the complex flavor of dry hopped beer.

Examples are linalool (flowery), trans-4,5-epoxy-(E)-2-decenal (metallic) or 4-mercapto-4-methylpentan-2-one which gives the beer a blackcurrant-like flavor [2, 3]. Little research on dry hopping in general has been conducted so far and was focused mainly on the transfer of wanted (aroma active) compounds.

However the transfer of unwanted substances like nitrate or pesticides has not been scientifically investigated so far [4]. Especially nitrate poses a potential problem due to its high water solubility. While it is only weakly toxic itself it can be reduced to nitrite in the human digestive system. The nitrite then reacts with secondary amines and amides to form carcinogenic nitrosamines and nitrosamides [5].

In this paper we present to our knowledge the first scientific study of pollutant transfer during dry hopping carried out on a semi-technical scale in the Bitburg pilot plant.

The aim of this study was to investigate the fates of nitrate and the hop pesticides azoxystrobin, dimethomorph, myclobutanil and quinoxyfen during the processes of wort production and dry hopping. Those substances are applied during hop cultivation against downy and powdery mildew and are frequently found during residue analysis. For example, in a study conducted by the Bavarian regional authority for health and food safety azoxystrobin was found in 80 %, dimethomorph in 51 %, myclobutanil in 49 % and quinoxyfen in 13 % of hop samples taken in Bavaria in 2007 [6].

Authors

Dr. Matthias Kippenberger, Dr. Stefan Hanke, Dr. Georg Stettner and Dr. Annika Lagemann; Bitburger Braugruppe GmbH, Bitburg, Germany; Dr. Martin Biendl, Hallertauer Hopfenveredelungsges.m.b.H. (Hopsteiner), Mainburg, Germany; corresponding author: matthias.kippenberger@bitburger-braugruppe.de

The transfer of substances from the hops to the aqueous medium is governed by the polarities of the two phases, the water solubility of the pollutants and temperature.

Navarro and *Vela* [7] classified pesticides according to their physical-chemical properties. They propose that brewers should especially monitor pesticides with a log $K_{ow} < 4$ which are comparatively water soluble and thus are more likely to be extracted from hops during brewing.

Substances with higher log K_{ow} values will stay predominately in the hop matrix which has an nonpolar character due to its waxes and oils which together account for up to 5 % of the mass of hops [8]. Three of the four pesticides whose partitioning behavior between hop and beer was analyzed possess log K_{ow} values between 2.5 and 2.9, while quinoxyfen as an example of a very hydrophobic compound has a value of 4.66 [9].

2 Materials and Methods

2.1 Brewing and dry hopping

2.2.1 Nitrate transfer

All brewing trials were done in the pilot plant of the Bitburger Braugruppe GmbH (Bitburg, Germany). For experiment series 1 of this study different beers, brewed in the pilot plant (Lager and Pilsner type), were dry hopped with different amounts of various hop varieties. The main focus was on the investigation on nitrate transfer during dry hopping.

The dry hopped brews were allmalt brews with original gravities in the range of 11.3–14.5 %mas. Dry hopping was done with the hop varieties and the amounts shown in table 1. For dry hopping the hops were dissolved in the corresponding beer and were added from the top of the storage vessel. Contact time was three to ten days at 0 °C. After 2 days of contact time CO_2 was blown from the bottom to re-suspend the hops again. After the storage time the beers were bottled and analyzed.

2.1.2 Pesticide transfer

In experiment series 2 of this study the transfer of pesticides and nitrate during every step of beer production was quantified. A brew exclusively hopped with variety Hallertau Tradition which showed elevated levels of some pesticides (see below) was produced on a 20-hL-scale. This trial was done to simulate a “worst case scenario” when a hop product containing higher pesticide levels is solely used for hopping. For experiment 2, 100 % Pilsner malt were mashed in a decoction mashing regime for 94 min and mash separation was done with a lauter tun. Wort was boiled for 1 hour with low over pressure with trub separation in a whirlpool afterwards. Finished wort was cooled to 9.5 °C and fermented until final degree of fermentation. The brew was separated prior to fermentation into two vessels (10 hL each) and fermentation was done simultaneously. When diacetyl was below the threshold of 0.1 ppm the green beer was cooled down to 0–1 °C and dry hopping was applied for 10 days in one vessel. The other fermentation vessel served as non dry hopped reference. Dry hopping was done in a static way (no circulation) for 10 days at 0°C with 500 g/hL hop dosage. The applied pellets type 90 of the variety Hallertau Tradition (2012 harvest) contained 7.1 mg/kg azoxystrobin, 7.4 mg/kg

Table 1 Transfer of nitrate during dry hopping of beer

Beer type	Nitrate base beer / mg/L	Hop variety	Hop product	Hop addition / g/hL	Potential nitrate load / mg/L	Actual concentration in dry hopped beer / mg/L	Extraction efficiency/%
Pale Ale	13.6	Hallertau Mandarina Bavaria	Pellets	192	21.9	31.6	82
	13.6		Pellets	383	43.7	54.8	94
	15.2		Pellets	280	31.9	48.3	104
Lager	2.4	Nelson Sauvin	Pellets	100	3.4	5.0	76
	2.6	Hallertau Blanc	Pellets	150	4.7	5.9	70
	14.7	Hallertau Tradition	Pellets	500	23.0	39.0	106
	1.4		Pellets	250*	11.5	12.6	97
	1.4		Pellets	500*	23.0	25.3	104
	6.3	BL A	Powder	75	4.5	8.9	58
	4.7	BL B	Powder	75	6.9	7.9	46
	3.5	BL C	Powder	75	2.2	5.2	78
	1.4	BL D	Powder	75	6.7	5.8	66
	25.7	BL (US)	Pellets	150	20.8	47.1	103
	11.3	Styrian Golding	Pellets	100	8.6	17.4	71
	44.6		Pellets	100	8.6	50.0	63

BL = breeding line
* = also analyzed for pesticides

dimethomorph, 1.6 mg/kg myclobutanil and 0.5 mg/kg quinoxyfen. After the contact time the beer was filtered with diatomaceous earth (no stabilization) and bottled. The hop addition was as follows. At the beginning of boiling 10 g alpha/hl (153 g hops/hL) were added and boiled for 1 hour, a second hop addition of the same amount (153 g hops/hL; 10 g alpha/hl) was given into the whirlpool. For the reference no more hop addition was applied; the dry hopped beer was treated as mentioned above (see Table 1).

Wort and beer samples were taken at different stages of production at the end of boiling, mid of cooling, after maturation, as well as before and after filtration.

2.2 Liquid chromatography and tandem mass spectrometry

The LC system consisted of a Shimadzu (Kyoto, Japan) HPLC system equipped with autosampler SIL-20AC Prominence, degasser DGU-20A3 Prominence and column oven CTO-20AC Prominence. The system was coupled to a 4000 QTRAP triple quadrupol mass spectrometer (AB Sciex, Toronto, Canada), equipped with a Turbo V Ion Spray source operated in positive ESI mode. LC separation was performed on a Gemini RP 18, 150 mm x 4,6 mm, 5 µm column (Phenomenex, Aschaffenburg, Germany) at 30 °C.

The mobile phase A was 5 mM/L ammonium acetate (Fractopur, Merck, Darmstadt, Germany) in high-purity water and mobile phase B was 5 mM/L ammonium acetate in methanol (hypergrade, Merck). High purity water was generated using Synergy UV ultrapure water purification system (Millipore, Schwalbach, Germany). The following gradient with a flow of 0.7 mL/min was used: 0–0.5 min 80 % B; 0.5–14 min 80–95 % B, 14–17 min 95–80 % B; 17–20 min 80 % B. The injection volume was 5 µL.

The mass spectrometer was operated in positive electrospray ionization mode at a temperature of 400 °C, a spray voltage of 5500 V, a curtain gas pressure of 20 psi and nebulizer gas pressures 1 and 2 of 50 and 60 psi respectively. Nitrogen served as collision gas in the collision cell of the mass spectrometer which was operated in MRM (multiple reaction monitoring) mode. The selected fragmentations for all investigated pesticides along the relevant potentials are given in table 2.

2.3 Ion chromatography

The nitrate concentration in beer was determined by ion chromatography. The system consisted of the Smartline Pump 1000, Smartline Manager 5000, the sample injector S5200 and the column oven Jetstream 2 Plus (all Goebel, Au, Germany), the conductivity detector 819 IC, the IC Separation Center 820 and the IC Liquid Handling Unit 833 (all Metrohm, Filderstadt, Germany). Separation was performed on a Metrosep A Supp 5 column (250 x 4,6 mm) with pre-columns Metrosep A Supp 2 Guard (35 x 16 mm) and Metrosep RP Guard (all Metrohm).

For the eluent stock solutions of sodium carbonate and sodium bicarbonate (both p.a., Merck) in high-purity water with a concentration of 1 mol/L each were prepared as follows.

In a 2 L volumetric flask, 6.4 mL of sodium carbonate solution and 2.0 mL sodium bicarbonate solution and 150 mL of Aceton (hypergrade, Merck) were diluted with approximately 1 L high-purity water. The solution was then degassed in an ultrasonic bath and filled to the calibration mark with water.

Separation was performed at a flow rate of 0.7 mL/min, a column temperature of 35 °C, with high-purity water and sulfuric acid (100 mmol/L) as suppressor solutions 1 and 2 respectively. The runtime of one separation was 25 min. Calibration standards were prepared by dilution of a commercial nitrate stock solution (CertiPur, Merck) with a concentration of 1000 mg NO_3-/L. Three calibration standards with concentrations of 1, 5 and 10 mg/L were used.

2.4 Sample preparation

For pesticide quantification, hop pellets were finely milled and 5.0 g were weighted into a 50 mL centrifuge tube and extracted for 5 min with 50.0 mL methanol in an ultrasonic bath. Subsequently, the sample was centrifuged at 3000 rpm for 5 minutes and the supernatant was filtered off and placed in the refrigerator at 6 °C for 30 min. The still cool supernatant was filtered. Hot trub and fermentation trub samples were dried at 90 °C for 1 hour and extracted in the same way as the pellets.

Beer samples were vigorously shaken to remove the carbon dioxide. The wort and beer samples were filtered through a filter paper and then through a membrane filter (pore size 0.45 µm, Chromafil CA-45/25, Machery Nagel, Düren, Germany).

For nitrate analysis, 100 mL of high-purity water were added to 5 g of hop powder or milled hop pellets. The resulting suspension was vigorously shaken for 30 minutes and subsequently heated to 70 °C in a water bath. The still hot suspension was then shaken for another 30 minutes, passed through a fluted filter and then through a membrane filter (0.45 µm). The resulting solution was then diluted 1:100 and injected into the HPLC system.

Beer and wort samples were passed trough a 0.45 mm membrane filter, diluted 1:10 with high purity water and injected.

2.5 Calibration standards and Quantification

The seven analytical pesticide standards (Pestanal, see Table 2) were all purchased from Sigma-Aldrich (Selze, Germany). Dimethomorph was a mixture of (E)- and (Z)-isomers.

For preparing calibration solutions, 5.0 mg of each analyte were weighted out (with the exception of folpet for which 30 mg were weighted out) in a 50 mL volumetric flask and dissolved in acetonitrile. This stock solution was then diluted with acetonitrile to yield calibration standards of 0.1 mg/mL, 0.2 mg/mL, 0.5 mg/mL, 1.0 mg/mL and 4.0 mg/mL.

The substances were quantified by standard addition. In a sample vial 100 µL of standard solution or purified water (for the blank sample) were added to 900 µL of methanolic extract, beer and wort samples. The solutions were then vortexed for 5 s and injected into the LC system.

Table 2 List of pesticides with retention times and MS/MS parameters

Analyt	Parent ion	Fragment	Declustering potential/V	Cell entrance potential/V	Cell exit potential/V	Retention time/min
azoxystrobin	404.1	372.0	51	21	24	2.9
	404.1	344.1	51	35	10	
dimethomorph	388.1	301.1	131	31	52	3.1
	388.1	165.2	131	45	8	
myclobutanil	289.1	70.1	56	37	12	3.0
	289.1	125.0	56	43	20	
quinoxyfen	308.0	197.0	111	47	16	4.8
	308.0	162.0	111	65	8	
tolylfluanid	367.0	149.0	61	21	10	3.3
	367.0	110.0	61	31	10	
trifloxystrobin	410.0	185.9	81	27	16	3.5
	410.0	187.0	81	23	16	
folpet	315.0	130.1	66	33	22	3.3
	315.0	101.7	66	69	16	

For quantification, the peak area was then plotted as a function of added concentration and the concentration of the sample solution was calculated from the intercept with the x-axis.

3 Results and discussion

3.1 Pesticides

3.1.1 Dry hopping of an unfiltered beer – experimental series 1

During the "dry hopping only" experiments an uncontaminated beer was dry hopped with the same batch of Hallertau Tradition used in experiment 2. This batch was contaminated with pesticides during cultivation thus representing a "real" sample compared to an artificially spiked one. The hop additions were 250 g/hL and 500 g/hL. The results are summarized in figure 1. At 250 g/hL, between 18 and 26 % of pesticides were transferred from hop into the beer.

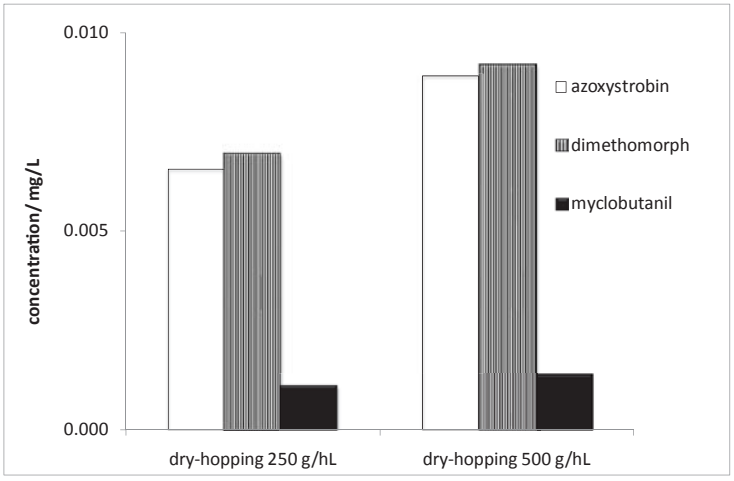

Fig. 1 Concentration of different pesticides after dry hopping the same base beer with 250 and 500 g/hL Hallertau Tradition

The dry hopping resulted in almost identical concentrations of azoxystrobin and dimethomorph in the beer while the concentration of myclobutanil, although being extracted as efficiently as dimethomorph (18 and 19 %), was close to the detection limit due to its lower concentration in the hops.

Upon doubling the amount of hops the concentration of azoxystrobin and dimethomorph increased only by 30 % and by 25 % for myclobutanil. The extraction efficiencies decreased to values of 13 to 20 % while processing factors increased by 36 to 56 %.

Contrary to nitrate (see section 3.2), the concentrations do not scale linearly with the amount of hops added probably because the low water solubility limits the extraction at the cold temperatures of storage and an increased adsorption of unpolar substances on the surface of the hop pellets, especially at the low temperatures during storage.

Slow and inefficient diffusional distribution of extracted pesticides from the bottom to the top of the storage vessel may additionally have hindered extraction.

The processing factors were approximately 0.0005 (250 g/hL) and between 0.0006 and 0.001 (500 g/hL). Due to the inefficient extraction at the low temperatures concentrations and processing factors were only about 50 % of the corresponding values during wort production (see section 3.1.2 for details). But experiment series 1 demonstrates that dry hopping can increase pesticide levels in beer if the latter is uncontaminated in the beginning. However the contaminant levels resulting from dry hopping were (even at the highest dosages) lower than the levels found if the hops was used for wort production. This was the case even if the hop dosage in the brewhouse was only half of the dosage used for dry hopping.

3.1.2 Wort production and dry hopping with contaminated hops – experiment 2

Figure 2 summarizes the results of experimental series 2 where a beer was exclusively brewed and dry hopped with the hop batch that had elevated pesticide levels. The concentration increased after the first and second addition and scaled almost linear with hop addition (90 % extracted) for dimethomorph, but less so for azoxystrobin (62 %). Myclobutanil concentration was below the detection limit of 0.001 mg/L after the first addition but increased after the second addition (mid of cooling) to 0.003 mg/L. It may have been degraded by reaction with wort compounds or evaporated during boiling since its vapor pressure is 200 times that of dimethomorph and 2 x 10^6 times that of azoxystrobin [9] (see table 5 for details).

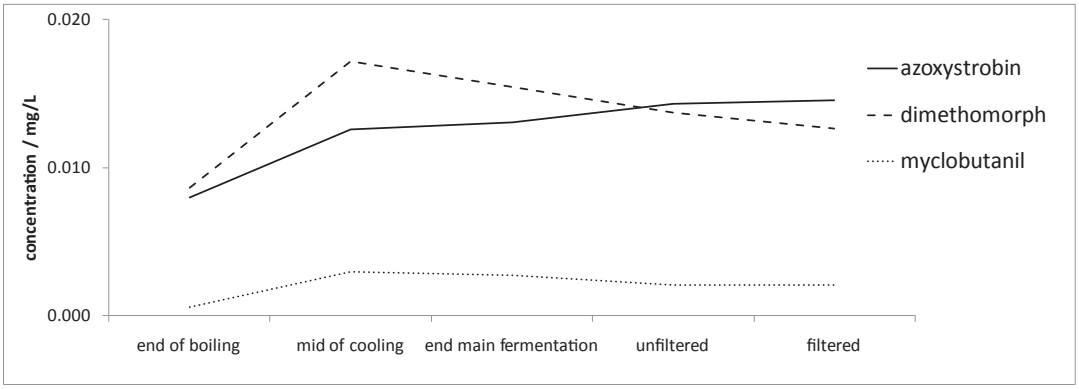

Fig. 2 Pesticide concentration in wort and beer during the different production stages. Dry hopping with 500 g/hL was done after the ending of main fermentation

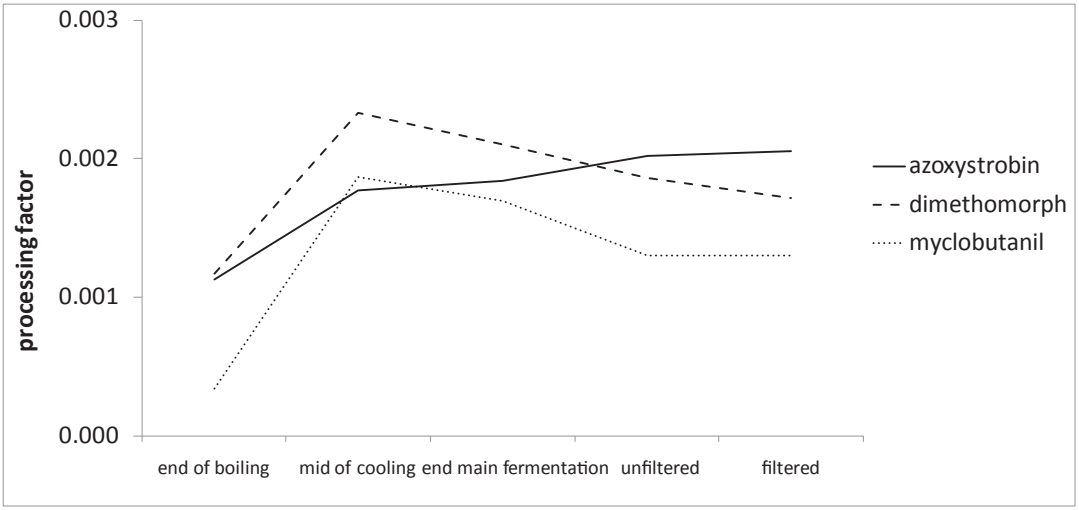

Fig.3 Pesticide processing factors in wort and beer during the different production stages. Dry hopping with 500 g/hL was done after the ending of main fermentation

Although the contaminated hop batch also contained 0.5 mg quinoxyfen per kilogram this substance could not be detected in wort or beer at any stage of production.

A trub sample taken from the whirlpool after end of cooling contained 0.295 mg quinoxyfen per kg which equals an extraction of 40 %. However the resulting concentration would have been $6x10^{-4}$ mg/L and thus below the detection limit of the employed analytical method.

Additionally, this concentration has to be considered an upper limit because some quinoxyfen in the hot wort might precipitate again upon cooling. Another route of loss, proposed by *Hengel* and *Shibamoto* [10], could be escape of quinoxyfen to the atmosphere due to its high Henry constant (compared to the other three pesticides), comparatively high water solubility, which result in a high volatility [11]. The pesticide concentrations hardly changed during fermentation and maturation although dimethomorph decreased by roughly 20%.

Dry hopping with 500 g/hL after maturation also had only very little effect on the concentration of all pesticides, also compared to the non dry hopped reference. The final filtration of the beer had no effect on pesticide concentrations, as was to be expected, given the highly polar character of filter materials.

These findings concerning myclobutanil are in agreement with the experimental results of *Schmidt* et al. [12] who did not observe a significant change in concentration after fermentation, although filtration removed azoxystrobin and dimethomorph to some extent, while myclobutanil remained unchanged.

The processing factors of the different stages are shown in figure 3. Factors increased from first to second hop addition and then stayed comparatively constant throughout the remaining steps of beer production with slight loses of dimethomorph and myclobutanil. The insignificant increase in azoxystrobin can be attributed to the analytical error or small inhomogenities during sampling. The processing factors are in agreement with values published by the German Federal Institute for Risk Assessment (BfR, [13]) which quotes a factor of 0.002 for dimethomorph (this study: 0.002) and <0.009 for myclobutanil (this study: 0.001). For azoxystrobin, we calculated a factor of 0.002 (no BfR value for reference). Since dry hopping did not change pesticide concentrations the processing factors are applicable to dry hopped and non dry hopped beers.

The beer brewed exclusively with the contaminated hops exhibited a significantly higher concentrations compared to the dry hopped beer of experiment series 1. However, this contamination did not stem from the dry hopping itself but exclusively from the hop additions during wort boiling.

The processing factors of experiment series 1 were at least a factor of 2 below the ones of series 2 where beer was exclusively brewed and dry hopped from the contaminated hop batch. Analogous to the increase of the pesticide concentrations during the second hop addition in the whirlpool there was also an increase in the processing factors for this production step. The factors are about 0.002 for all pesticides (except quinoxyfen) after completion of wort production.

A direct proportionality between the solubility of the substances and the processing factors is not evident, since the solubility limit is not reached at temperatures above 20 °C.

The extraction efficiencies during experiment 1 (dry hopping only) were in the range of roughly 15 to 25 % for azoxystrobin and dimethomorph which is considerably lower than the 75 % during wort boiling. Although the solubility of myclobutanil is the largest of all

pesticides investigated, its apparent extraction of about 20 % during boiling was below the values of the aforementioned compounds.

This study further shows that the usage of a contaminated but still marketable hop batch for the purpose of dry hopping does not necessarily lead to elevated pesticide levels in the final beer.

The maximal concentration is not solely dependent on the hop amount and contamination of hops but also on the solubility of the respective pesticide at the low temperature in the storage vessel. If the same contaminated hops is already used for wort production then dry hopping will not lead to an increased contamination of the final product. However if an unpolluted beer is dry hopped with such hops the pesticide concentrations will be increased but stay below the concentrations which would normally arise from a usual hop addition in the course of wort production. Hence the processing factors for the pesticide transfer calculated from the results of experiment 2 can serve as an upper limit to calculate the expected pesticide concentration in the dry hopped beer (if levels in the base beer are negligible).

In summary, dry hopping did not lead to a significantly elevated pesticide contamination compared to non dry hopped beer. Therefore the concentration of plant protecting substances to assess the marketability of a beer has to be considered in the same way it would have to be without dry hopping.

The extraction of the pesticides is in general particularly efficient at the high temperatures of wort boiling and in the whirlpool. The concentration of azoxystrobin and dimethomorph scaled approximately linearly with the amount of hops added (0.013 and 0.017 mg/L respectively). In contrast the extraction of myclobutanil seemed to be relatively inefficient during wort boiling (20 %) while being quantitative during halftime of cooling.

Of the four pesticides found in the Hallertau Tradition pellets, all posses an log K_{ow} < 4 with the exception of quinoxyfen whose log K_{ow} is 4.66. Hence quinoxyfen is only of minor importance when assessing the pesticide contamination of beer by hop addition in agreement with the rule proposed by Navarro and Vela [7].

Also dry hopping did not change significantly the concentration of any pesticide anymore. Likely the beer was saturated at the low fermentation and storage temperatures so no net transfer into the beer occurred.

The overall extraction efficiency related to the whole amount of added hops therefore decreased again during the brewing as did corresponding processing factors.

The concentrations of all pesticides in the unfiltered beer differ between the dry hopped and the non hopped beer only by 0.001 mg/L and were therefore constant within the margin of the analytical error. Since all monitored pesticides possess a rather nonpolar character they were not retained by PVPP filters and remained in unchanged concentration in the filtrate.

None of the 6 India Pale Ales and Pale Ales analyzed contained any of the pesticides of interest. This is in agreement with Schmidt et al. [12], who could not detect any pesticides in beer (c < 0.002 mg/L) that was brewed with hops, which contained typical levels of the four pesticides ranging from 0.3 to 0.8 ppm.

Table 3 **Nitrate concentration in different hop samples (BL = Breeding line, NZ = New Zealand, FR = France, GER = Germany, USA = United States of America, SLO = Slovenia). The hops marked with asterisk was used in experiment series 1 and 2 and had higher pesticide levels. Beer and wort samples hopped with that variety were also analyzed for pesticides**

	Nitrate content / g/kg
Nelson Sauvin (NZ)	3.4
Hallertau Blanc (GER)	3.1
Aramis (FR)	9.1
Mandarina Bavaria (GER)	11.4
Chinook (USA)	10.9
Hallertau Tradition (GER)*	4.6
BL A (GER)	6.0
BL B (GER)	9.2
BL C (GER)	2.9
BL D (GER)	8.9
BL E (GER)	5.2
BL (USA)	13.9
Golding (SLO)	8.6
Hersbrucker Pure (GER)	2.7

*= also analyzed for pesticides

3.2 Nitrate

The analysis of different hops samples revealed that the hop plant is a nitrate enriching organism with concentration ranging from 2.7 to 13.9 g/kg (Table 3).

Table 1 summarizes the results of the dry hopping experiments conducted with the different beer types, hop products and hop

Table 4 **Nitrate concentration in several commercial beer samples**

	Nitrate concentration / mg/L
IPA Brand 1 (UK)*	30.9
IPA Brand 2 (UK)*	3.0
IPA (NL)*	34.2
IPA Brand 1 (GER)*	86.5
IPA Brand 2 (GER)	31.7
Pale Ale Brand 1 (USA)*	14.9
Pale Ale Brand 2 (USA)*	31.7
Pale Ale (GER)	21.1
Pilsner Beer Brand 1 (GER)	11.6
Pilsner Beer Brand 2 (GER)	6.9
Pilsner Beer Brand 3 (GER)	4.7
Pilsner Beer Brand 4 (GER)	4.9
Pilsner Beer Brand 5 (GER)	6.2
Pilsner Beer Brand 6 (GER)	13.8

*= also analyzed for pesticides

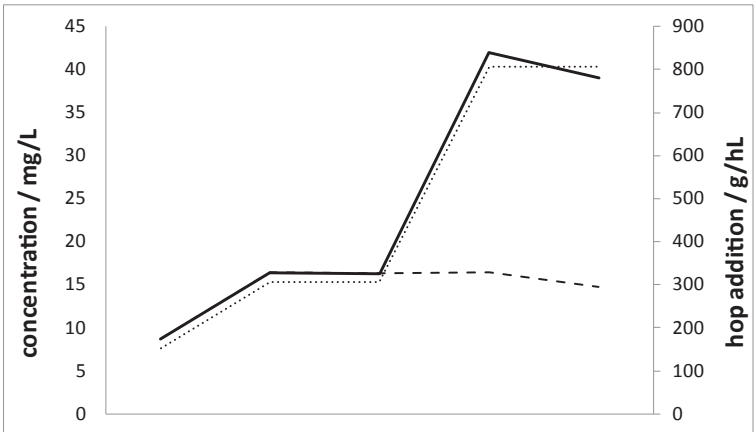

Fig. 4 **Nitrate concentration in beer and wort (experiment series 2) at different stages of production of the dry hopped beer (solid line) and the non dry hopped reference (dashed line). Hop additions to the wort were 153 g/hL at the end of boiling and in the mid of cooling. The base beer was then divided into a dry hopped portion and a non dry hopped portion (reference). Dry hopping with 500 g/hL was done after the ending of main fermentation. The dotted line shows the total amount of hop added per hL during beer production (dry hopped beer, secondary y-axis)**

amounts. On average 81 % of nitrate in hops was extracted, the range of transfer efficiencies being 46 to 106 %. Extraction seemed to be generally less efficient if powder was used instead of pellets (62 % to 88 % on average). However these differences could also arise from non-optimized dry hopping practices for powder.

The concentrations in the final beer were in several samples close to or above the current maximum residue level (MRL) values for nitrate in drinking water in the EU of 50 mg/L [15]. No official MRL exists for beer but the value for drinking water is often used as reference instead. With 88 % extraction from pellets, brewers probably have to assume quantitative extraction to be on the safe side when estimating the nitrate content in the finished beer.

In the commercially available beers nitrate concentrations between 3.0 and 86.5 mg/L were found (Table 4) with the median being 14.4 mg/L. The medians of concentrations in the three beer types exhibit a tendency towards higher values in the generally stronger hoped beer: 6.6 mg/L (Pilsner), 21.1 mg/L (Pale Ale) and 31.7 mg/L (India Pale Ale). Almost all samples were below the limit of 50 mg/L. Only one IPA from Germany (Brand 1) exceeded it, containing 87 mg/L.

The results from dry hopping the same base beer with 2 different amounts of Hallertau Tradition are summarized in table 1. Extraction was quantitative and scaled linearly with the amount of hop added in both trials. The results from experiment 2 regarding the nitrate levels at the different stages of beer production are shown in figure 4.

In contrast to pesticides the extraction of nitrate was quantitative and linearly dependent on the amount of hops added, regardless of temperature. Since the Hallertau Tradition sample had a comparatively low nitrate content (see Table 3) the concentration in the finished beer stayed below the limit for drinking water of the EU of 50 mg/L. Filtration removed only 8 % and 12 % of the nitrate in the dry hopped beer and the reference respectively.

With regard to nitrate, dry hopping led to significantly elevated levels which can be up to several times above the non dry hopped counterparts. Especially when hops with high nitrate content in high dosages were used, nitrate concentrations in beer reached problematic levels.

3.3 Comparison with literature

Schmidt et al. [12] also observed a decrease of all pesticides during fermentation of 50 % or more. Their hop pellets were artificially spiked and pesticide levels in the pellets were 1.7 (myclobutanil) to 6.9 times (quinoxyfen) higher than in the Hallertau Tradition used in this study.

Initial pesticide concentrations in the wort were a factor 2 to 3 higher for azoxystrobin, myclobutanil and quinoxyfen than in our study. For dimethomorph the potential concentration was even approximately seven times higher. But despite these large differences in initial concentrations, the residues in the final beer were quite similar to this study which could indicate proximity to the solubility limit. Therefore the observed removal of pesticides during fermentation may have been partially caused by precipitation of the compounds during cooling.

Hengel and Shibamoto [10] used treated hops for brewing on a laboratory scale and monitored the pesticide concentrations during 38 days of fermentation. Of the pesticides of interest only dimethomorph was found in beer. Its concentration decreased by 20 % during fermentation, in very good agreement with our findings where a decrease of 18 % was observed.

The transfer of nitrate from hops to beer is generally considered to be quantitative [21, 22, 23, 24], although only non dry hopped beers were considered. The results of this study show that extraction

Table 5 **Physicochemical and legal limits of the pesticides found in the Hallertau Tradition used for dry hopping experiments**

	MRL (hops) US (ecfr.gov)/ppm	MRL (hops) EU	Processing factor (BfR)	water solubility / mg/L	vapour pressure/ Pa	log K_{ow}	Content in HT hop / mg/kg	Residues in beer (Schmidt et al. [12]) / mg/L
quinoxyfen	3.0	0.5[16]	–	0.047	1.2×10^{-2}	4.66	0.50	n.d.
myclobutanil	10.0	2.0[17]	0.002	132	1.98×10^{-1}	2.89	1.60	0.002
dimethomorph	60.0	50.0[18]	< 0.009	28.95	9.85×10^{-4}	2.68	7.36	0.033
azoxystrobin	20.0	30.0[19]	–	6.7	1.00×10^{-7}	2.5	7.10	0.018

n.d. = not detected

during dry hopping is also near quantitative. On average 81 % of nitrate was extracted which is well in line with a recently reported value of 75 % [14].

4 Summary and Conclusion

In a market survey we determined the concentration of nitrate and seven different pesticides in hops and commercial beer samples. The analysis yielded nitrate concentrations in beers that were partially close to or above the threshold value for drinking water safety in the EU of 50 mg/L. None of the seven pesticides screened for was detected in the commercial beer samples (limit of detection: 0.001 mg/L).

To investigate specifically the influence of dry hopping on beer quality different types of beer were dry hopped with various hop varieties, revealing differences in the extraction of nitrate between the different varieties. To assess the transfer of pesticides specifically in dry hopped beers, a pils type beer was dry hopped with 2 different dosages of a hop batch, that had higher levels of azoxystrobin, dimethomorph, myclobutanil and quinoxyfen (but was still marketable).

The pesticides whose transfer during dry hopping was investigated are representative of different families of pesticides that are allowed for hop cultivation in Germany [20].

Azoxystrobin belongs to the large group of strobilurin-based pesticides that also includes trifloxystrobin and pyraclostrobin. Dimethomorph is a morpholine derivative, like for example fenpropimorph. The triaziole derivative myclobutanil represents another common class of pesticides, to which also triadimenol belongs. The quinolone derivative quinoxyfen, with its log K_{ow} of 4.66, is a model substance for highly hydrophobic pesticides.

Furthermore, a beer was brewed and dry hopped exclusively with the contaminated hops and samples were taken at five stages of production to gain insights into the transfer mechanism and potential ramifications of pollutant extraction for product safety and consumer health.

The transfer of nitrate from hops into beer was very efficient and often almost quantitative regardless at which state of production (i.e wort boiling, fermentation, storage) the hops were added. While a moderate hop addition during wort boiling did normally not cause any problems, the large amounts often used in dry hopped beers caused severely increased nitrate concentration close or above 50 mg/L, especially if the hops themself exhibited already an elevated nitrate concentration.

The brewing and dry hopping experiments clearly showed that with 88 % extraction efficiency brewers must assume a quantitative transfer of nitrate from hops to beer when calculating the concentration in the final product. Product safety at high hop dosages can be compromised.

In contrast, dry hopping did not lead to a significantly increased contamination with azoxystrobin, dimethomorph, myclobutanil and quinoxyfen. Due to their low water solubility the beer saturated at very low concentrations and low hop dosages.

The calculated processing factors of dimethomorph and myclobutanil of 0.002 and 0.001 respectively were in very good agreement with the values published by the German Federal Institute for Risk Assessment of 0.002 and < 0.009.

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