End-of-the-line packaging in shrink films on trays is handled by a Variopac packer. "The Variopac enables us to run both loose tray goods and shrink films", says Wolf Gabriel in explanation. Like the can packaging, the 18- and 24-bottle trays have a rim height of 100 mm, but are made of sturdier cardboard.

For the palletiser, Holsten opted for its first flexible robot solution, in the shape of the Robot 3A, which is able to position an enormous range of different package and pallet shapes. In all, a mere three operators handle the entire line, including the dry end.

Minimum shelf-life of 6 months

Holsten is currently looking to fill only the 0.5-litre bottle, though in two variants: monolayer with UV stabilisers for beer-based mixed drinks and multilayer for beer, both of them in the light-barrier colours of brown and green. One new feature is the bottle base: to ensure the requisite stability and pressure withstand capability, a bottle with a petaloid base was chosen, but with eight recesses to retain the familiar beer-bottle look and ensure thermal stability during the process. The result is an elegant and stable mixed shape midway between the petaloid base and the champagne base more usual for beer.

The bottle shape itself, designed as a long-neck container, had already been determined by the existing Holsten PET bottle. Fine-tuning of the new bottle shape was also a contribution from Krones AG. Only plastic screw caps are used as the closure, with a scavenger admixture for beer, without one for beer-based mixed drinks. With the totality of its quality-boosting measures, Holsten is able to guarantee a minimum shelf-life of six months both for beer and for beer-based mixed drinks in PET. ■

Brewing trials with a xanthohumol-enriched hop product

Recently, the presence of unusually high levels of xanthohumol were reported in Stouts and Porters (Brauwelt international II/2004, p. 100). Further increases in these levels have been made possible by the use of a xanthohumol-enriched hop product. This hop product was introduced at the last EBC Congress in Dublin where it was used in the production of a Pilsner. In the following article, these results are compared with those from the production of a stout beer.

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The hop compound, xanthohumol, has been attracting the attention of those outside the brewing industry due to its wide range of potential positive health effects. Most recently, a report of a potential anti-viral effect has been added to the list of the numerous properties of xanthohumol that are already known (1). Especially promising seems to be the cancer chemopreventive activity of xanthohumol (2), and investigations are at the stage of animal tests. These tests will yield results on the metabolism and bioavailability of this compound (3) and provide the necessary basis for carrying out clinical studies. Only then will it be possible to ascertain the effective dosage in humans.

Xanthohumol is isomerised to isoxanthohumol during wort boiling. Commercial hopping results in levels of up to about 2.5 mg/l isoxanthohumol in filtered beers whereas the xanthohumol content is generally lower than 0.2 mg/l. However, an exception to this are the stout and porters in which the isomerisation process is partly inhibited. In this case, despite conventional processing, isoxanthohumol as well as xanthohumol are present (4). For example, 0.4 mg/l xanthohumol was detected in a German porter, which was brewed according to the purity laws. Similarly, a highly hopped Danish porter had xanthohumol levels of 1.2 mg/l. Experiments on the laboratory scale suggested that the inhibitory effect could be due to the presence of roasted barley (which is required for stouts) or roasted malt. Further information is not yet available.

Isoxanthohumol also shows positive health effects although it seems to be less effective than xanthohumol. Increasing the concentration of isoxanthohumol in beer could potentially compensate for this disadvantage. In addition, pharmaceutical investigations are ongoing and it may be that other new positive effects will be discovered in which isoxanthohumol could be more active than xanthohumol. In fact, in a recent patent application the anti-inflammatory effect of isoxanthohumol and its 'anti-aging' effect were rated higher than that of xanthohumol (5). The isomerised form was therefore suggested as an additive for various foodstuffs.

Hops contain up to 1% xanthohumol, the content depending upon both the variety and year. The hop harvest of 2003 clearly

showed lower xanthohumol contents than the years before. However, the ratio of xanthohumol to alpha acids is constant, and varies between 0.03 and 0.09 depending upon variety (6).

It is known that xanthohumol fractionates with the pure resin during ethanol extraction. In contrast, it is not extracted by liquid or super critical carbon dioxide (at pressures under 300 bar). Xanthohumol can therefore be separated from the alpha acids by combining both of these established industrial scale processes. Such a xanthohumol-enriched (relative to alpha acids) hop product has already been successfully tested during the production of a Pilsner beer. These results were presented at the EBC congress in Dublin (7). The beer had a very high isoxanthohumol content, due to the isomerisation of xanthohumol to isoxanthohumol during wort boiling. Because of the partial inhibition of this isomerisation during the production of stouts/porters, it was of interest to investigate the xanthohumol-enriched hop product in the context of this technology and to compare it to that in the production of a Pilsner beer.

Materials and methods

Hop products

Alpha and beta acids were removed by extraction of the ethanol pure resin extract with carbon dioxide at a pressure of 280 bar at 50°C. The xanthohumol remained in the unextracted material together with numerous unspecific components of the soft resin and above all of the hard resin fraction. The iso-alpha acids, which were found in lower amounts in the ethanol pure resin extract, were also not extracted and remained in this residue. The pasty ethanol pure resin extract was mixed with Kieselguhr to enable trouble-free handling of the product. This carrier material is of course insoluble. This xanthohumol-enriched hop product, which resulted from the extraction residue, was used for the pilot brewing trials. The composition of two different production batches of this xanthohumol product (which were isolated from the hop variety Hallertauer Taurus) are shown in Table 1. Beers were also brewed with hop pellets and ethanol extracts as controls.

Table 1 Composition of xanthohumol-enriched hop products

	Xanthohumol product (batch 1)	Xanthohumol product (batch 2)
Xanthohumol	2.0%	1.8%
Alpha acids	0.8%	0.3%
Iso-alpha acids	1.4%	1.3%
Beta acids	0.1%	<0.1%
Non specific resins	16.7%	16.6%
Kieselguhr	79%	80%

Pilot Brewing.

The Pilsner beer was brewed at the 20 hl pilot brewery at Bitburg (300 kg grist) and was described in the EBC poster. Hop addition was immediately after the beginning of wort boiling. Wort boiling was for 75 min followed by a 20 min whirlpool rest. Fermentation and maturation were carried out at 10°C until the diacetyl concentration dropped below 0.1 mg/l (approximately 13 days). The beer was then held at –1.5°C for 8 days and filtered over Kieselguhr before bottling.

The stout beers were brewed in the 1 hl pilot brewery at Brewing Research International (BRi Nutfield, England). The grist was: 17 kg Fanfare malt, 1.5 kg crystal malt, 1.2 kg chocolate malt, 0.8 kg roasted barley and 0.5 kg wheat flour. Hop addition was divided into two: 80% was added 5 min after the beginning of wort boiling and 20% after 50 min. Boiling itself was for a total of 60 min, followed by a whirlpool rest of 30 min.. Fermentation was carried out at 18°C for 6 days and the beer was held for 14 days at 3°C. The beer was filtered (prefilter XE5, polishing filter XE200) before bottling and then pasteurised (60°C, 15 min). The finished beer had a diacetyl concentration below 0.1 mg/l. The brews with the xanthohumol product were carried out in duplicate, whereas the control brews were single trials. Table 2 shows the dosage of the hop products. For the commercial hop products, 10g alpha acids/hl were added. The target with the dosage of the xanthohumol product was to produce a beer with a bitterness of about 35 EBC bitterness units. The relatively high ratio of xanthohumol to alpha acids in this product (compared to ethanol extracts and pellets) allowed at least a 6 fold higher amount of xanthohumol to be dosed wile keeping the bitterness at the target level.

Sensory analysis

Tasting was carried out according to the DLG tasting scheme. The panel was composed of 11-13 tasters. A special emphasis was placed on the bitterness and estimation of the EBC bitterness units.

Results.

The results of the wort and beer analysis from duplicate brews showed that the xanthohumol product can be used reproducibly. The results are not shown in detail, rather Table 3 shows results for a few selected parameters in wort and beer.

In the Pilsner beer brewed with the xanthohumol product, levels of 8.1 mg/l isoxanthohumol were found (average of both brews) whereas the stout contained 9.0 mg/l isoxanthohumol as well as 3.3 mg/l xanthohumol. This was once again indicative of the isomerisation-inhibiting effect of

Table 2 Hop product dosage

	Hop product	Dosage (g/hl)	Alpha acids (mg/l)	Iso-alpha acids (mg/l)	Isoxanthohumol (mg/l)	Xanthohumol (mg/l)
Pilsner – trial1	Xanthohumol product 1	250	20	35	35	50
Pilsner – trial 2	Xanthohumol product 1	250	20	35	35	50
Pilsner – control (extract and pellets)	Ethanol extract / bitter hops Pellet Type 90 / aroma hops	392 647	71 25	3.0 –	3.0 –	4.0 2.0
Stout – trial 1	Xanthohumol product 2	300	9.0	39	39	54
Stout – trial 2	Xanthohumol product 2	300	9.0	39	39	54
Stout – control (extract)	Ethanol extract/ Select	50,5	97	3.0	3.0	9.0
Stout – control (pellets)	Pellet Type 90/ Select	370	100	–	–	9.6

Table 3 Beer and wort analysis

Parameter	Pilsner trial 1	Pilsner trial 2	Pilsner extract/ pellets	Stout trial 1	Stout trial 2	Stout ethanol extract	Stout pellets
Wort pH	5.53	5.54	5.49	4.98	4.97	4.95	5,0
Fermentability	62.3 %	62.7 %	62.6 %	63 %	60 %	63 %	62 %
Bottled beer:							
pH	4.48	4.57	4.49	4.18	4.15	4.21	4.23
Colour (EBC)	6.8	6.8	6.1	150	150	150	150
Bitterness (EBC)	31	31	37	37	39	27	26
Iso-alpha acids (mg/l) Utilisation	18.8 34 %	18.7 34 %	36.1 36 %	11.9 25 %	12.8 27 %	22.4 22 %	20.2 20 %
Xanthohumol (mg/l) Isoxanthohumol (mg/l) Utilisation (Xn + Ix)	< 0.1 8.6 17 %	< 0.1 7.5 15 %	< 0.1 1.7 28 %	3.2 9.3 23 %	3.4 8.6 22 %	0.8 1.7 28 %	0.7 1.5 23 %

Table 4 Intensity (estimated bitterness) and quality (1 = unpleasant, 5 = pleasant) of bitterness (tasting of fresh beer)

Bitterness	Pilsner trial 1	Pilsner trial 2	Pilsner extract/ pellets	Stout trial 1	Stout trial 2	Stout ethanol extract	Stout pellets
Intensity	30	29	31	32	32	26	26
Quality	3.1	3.0	2.7	2.9	2.7	3.1	3.1

the stout production process, which resulted in surprisingly high levels of xanthohumol in the beer. Xanthohumol concentrations of this magnitude have never been reported before in filtered beer.

In both the Pilsener and stout hopped with commercial products, the isoxanthohumol levels were between 1.5 and 1.7 mg/l. However, the xanthohumol content was 0.8 and 0.7mg/l for stouts made with ethanol extracts and pellets respectively, and below 0.1 mg/l in the Pilsner. These levels of xanthohumol in the stouts were relatively high despite conventional hopping and their bitterness being below 30 EBC bitterness units. The reason for this is certainly due mainly to the hop variety, which was identical in the extract and pellets. This variety Hallertauer Spater Select was purposely chosen as it has the maximum ratio of xanthohumol to alpha acids (0.09). The somewhat reduced utilisation in the pellet brew relative to the ethanol extract could be indicative of a matrix effect.

The lower utilisation of iso-alpha acids in all the stout brews is striking. In the first place, this could be due to the very low pH value of the wort, which is typical for this beer type. Other factors which could have an influence were the divided hop addition, the shorter boiling time and the more intensive precipitation process taking place in the 1hl pilot brewery (the Pilsner trial was on the 20 hectolitre scale). Equally striking is the relatively high utilisation of xanthohumol and isoxanthohumol in the stouts.

It is not surprising that there are differences in the levels of specific iso-alpha acids between the trial and control brews, since there were clearly less alpha or iso-alpha acids dosed into the trial than that brewed with ethanol extract or pellets. The EBC bitter units in the beer brewed with the xanthohumol product were clearly higher than would be expected from the level of iso-alpha acids. This implies that there are undefined hop components present, which are making a considerable contribution to the EBC bitterness units measured. It is currently not known which substances are involved and appropriate investigations to identify these compounds are planned.

Table 4 summarises the most important results from the beer tasting. The estimated bitterness clearly correlated more closely with the measured EBC bitterness units rather than with the iso-alpha acid content in all the beers brewed with the xanthohumol product. This indicates that these currently unidentified components actually give rise to a bitter taste.

The tasting panel could differentiate the Pilsner brewed with the xanthohumol product from the control beer. The quality of the bitterness was placed as somewhat higher and described as more rounded. The situation was reversed in the stouts, although the comparison was made more difficult by the considerable differences in bitterness. In addition, this assessment was made more difficult by the typical astringency of the stouts. An aging test has only been carried out with the Pilsner. After 2 months storage at 28°C, the beer brewed with the xanthohumol product could be distinguished from the control beer (results not shown). Although the commercially hopped beer was assessed as having a less rounded bitterness, this difference was less than that seen in the fresh beer.

Conclusions

Various beer types have been brewed in a reproducible manner by the use of a xanthohumol-enriched hop product combined with conventional brewing technologies. In a Pilsner brewing process, the bottled beer had an isoxanthohumol content of 8.1 mg/l and a xanthohumol content below 0.1 mg/l. In the stout beer, the concentrations of isoxanthohumol and xanthohumol were 9.0 and 3.3 mg/l respectively. These values show that it is possible to considerably increase the maximum concentrations of isoxanthohumol and xanthohumol compared to what is possible with conventional hop products (pellets, extract). The concentrations of these compounds that would be required for a health-promoting effect are not known and will depend upon the outcome of further pharmaceutical experiments.

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