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Determination of stilbenes contents in various hop cultivars and conditionings

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SUMMARY

The discovery of resveratrol in pellets again highlights the key role of hop in improving health on moderate beer consumption. The aim of the present work was to investigate various hop cultivars and conditionings. We have compared the stilbene contents of seven hop varieties (leaf hop and pellets). Their resistance to pelletisation has been investigated.

INTRODUCTION

Nowadays, hop is almost exclusively used in brewery as sources of bitterness and flavours (1,2). Although hop polyphenols have been widely studied in the last decade for their antioxidant activity in the boiling kettle (3), very few is still known about its real impact on health. De Keukeleire et al. (4) have isolated very interesting phytoestrogenic prenylated flavonoids from hop. The recent discovery of resveratrol in pellets (5) again highlights the key role of hop in improving health on moderate beer consumption. Resveratrol (trans-3,4',5-trihydroxystilbene), already identified in wine and other food matrixes, seems to be linked to anti-carcinogenic, anti-viral, anti-oxidant, anti-inflammatory and estrogenic activities. The new method developed by Callemien et al. (6) to determine trans-resveratrol and its glycosides in hop pellets indicated a total stilbene concentration close to 3.5 ppm in Tomahawk pellets (T90, harvest 2002). Concentrations up to 7 ppm have been recently quantified in the same cultivars harvested in 2003 (7).

The aim of the present work was to investigate various hop cultivars and conditionings from harvest 2004. We have compared the stilbene contents of seven hop varieties (leaf hop and derived pellets).

ANALYTICAL METHODS

Materials

All cones and pellets T90 were a kind gift from Yakima Chief (Louvain-la-Neuve, Belgium). All samples were stored at 4 °C until needed.

Chemicals

Ethanol (97 %) was obtained from Belgaco (Gent, Belgium). Acetonitrile (99.99 %), toluene (97 %), and cyclohexane (99.96 %) were supplied by Fisher Scientific (UK). Formic acid (pa) was obtained from Aldrich (Germany). Methanol (99.9 %) and diethyl ether (99 %) were supplied by Romil (Cambridge, UK). Aqueous solutions were made with Milli-Q (Millipore, Bedford, MA, USA) water. *trans*-Resveratrol (99 %) and *trans*-piceid (97 %) were supplied by Sigma-Aldrich (Bornem, Belgium).

Extraction of stilbenes from hop

This method has been developed in our laboratory (6) to analyse stilbenes in hop. All extraction steps have been done with protection against day light, in duplicate. Hop cones or pellets were crushed in a mortar. Ground samples (2.5 g) were extracted, in successive 10 min steps at room temperature under gentle stirring, three times with 50 ml toluene and three times with 50 ml cyclohexane, in order to remove hydrophobic compounds. At the end of each step, the sample was centrifuged for 10 min at 3000 g. At the last step, hop powder was dried under vacuum to get rid of residual solvent. Delipidated hop powder was extracted three times with 40 ml ethanol:water (80:20, v/v); each time for 10 min under gentle stirring at 60 °C. After each extraction, the sample was centrifuged for 10 min at 3000 g and the supernatant collected. After filtration to remove residual particles, the combined supernatants were concentrated by rotary evaporation (35 °C) to dryness. The residue was solubilized in 2 ml of 50:50 (v/v) mixture of ethanol:water.

RP-HPLC-APCI (+)-MS/MS analysis of stilbenes

Quantifications were performed on a C18 Prevail column (150 x 2.1 mm, 2 µm) (Alltech, Deerfield, IL, USA) eluted with a linear gradient from water (containing 1 % acetonitrile and 0.1 % formic acid) to acetonitrile. Gradient elution was as follows: from 95 % water to 55 % in 23 min, 55 % to 0 % in 7 min, and isocratic for 10 min at a flow rate of 200 µl/min. Ten microliters sample were injected into the column kept at 30 °C. A SpectraSystem equipped with an AS3000 autosampler and a P4000 quaternary pump was used. The system was controlled with the Xcalibur software version 1.2 (Finnigan Mat). Mass spectra were acquired using a LCQ mass spectrometer equipped with an APCI source (Finnigan Mat). The following APCI inlet conditions in positive mode were applied: vaporization temperature, 470 °C; capillary voltage, 3V; capillary temperature, 175 °C; sheath gas, 40 psi; auxiliary gas, 7 psi; discharge current 5 µA. After the first monitoring on the m/z = 229, collision-induced dissociation spectra were recorded at 37 % relative collision energy.

Determination of α-acids in hop

The α-acids content of hop cones and pellets was determined by HPLC-UV according to Analytica-EBC (1987).

RESULTS AND DISCUSSION

The stilbene contents (*trans*-piceid, *cis*-piceid, and *trans*-resveratrol) of leaf hop from seven varieties (cones, harvest 2004) were determined by RP-HPLC-APCI(+)-MS/MS (6). As depicted in figure 1, concentrations ranging from 4.5 to 11 mg/kg *trans*-piceid, from 2.5 to 6 mg/kg *cis*-piceid, and up to 1 mg/kg *trans*-resveratrol were measured in hop cones.

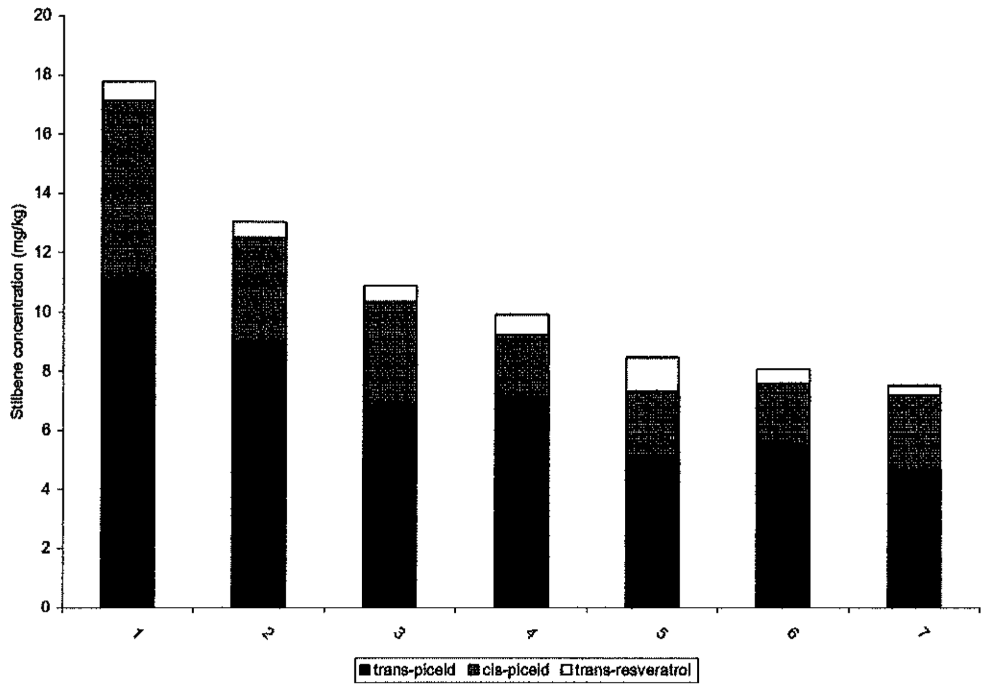

Figure 1: Stilbene concentration in hop cones.

As the hopping rate in the boiling kettle is calculated according to the hop α-acid content, total stilbene contents have been compared for a same bitterness potential (figure 2). The low-bitterness cultivars clearly emerge as the most interesting.

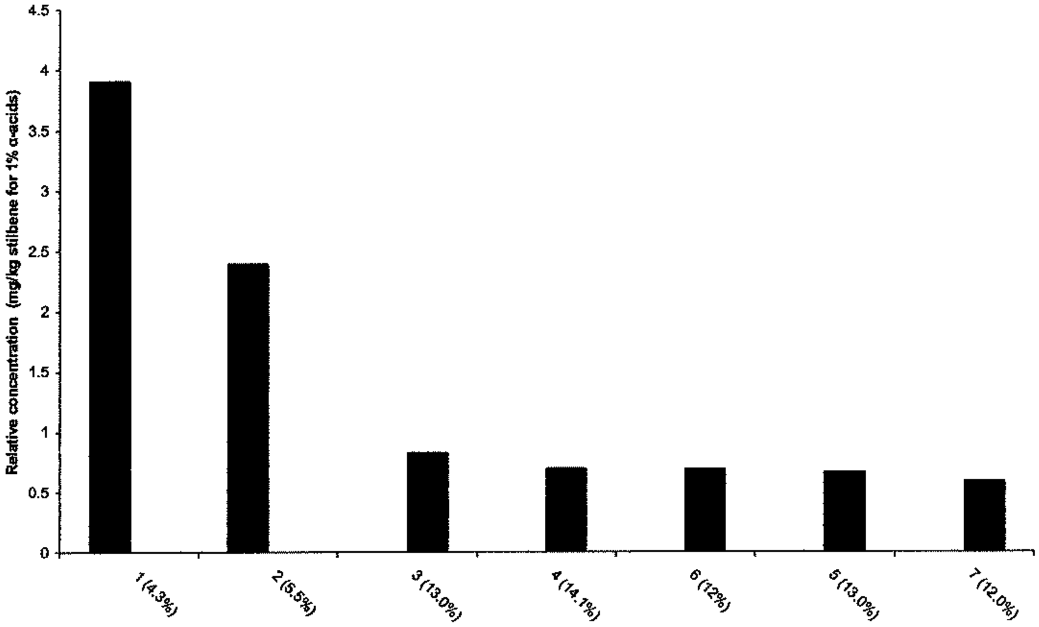

Figure 2: Relative concentration in hop cones (α-acids %).

Figures 3 and 4 present stilbene concentration in pellets (T90, harvest 2004) derived from the here-above described cones. Concentrations are very different from leaf hop; they range in hop pellets from 1.5 to 5 mg/kg *trans*-piceid, from 1 to 4.5 mg/kg *cis*-piceid, and up to 1 mg/kg *trans*-resveratrol. Low α-acids cultivars emerged as the most concentrated before pelletisation. Pelletisation induced strong stilbene degradation (> 56 %) in some cultivars (1, 2, 4, 6) while other varieties (3 or 5) revealed much more resistant (no degradation).

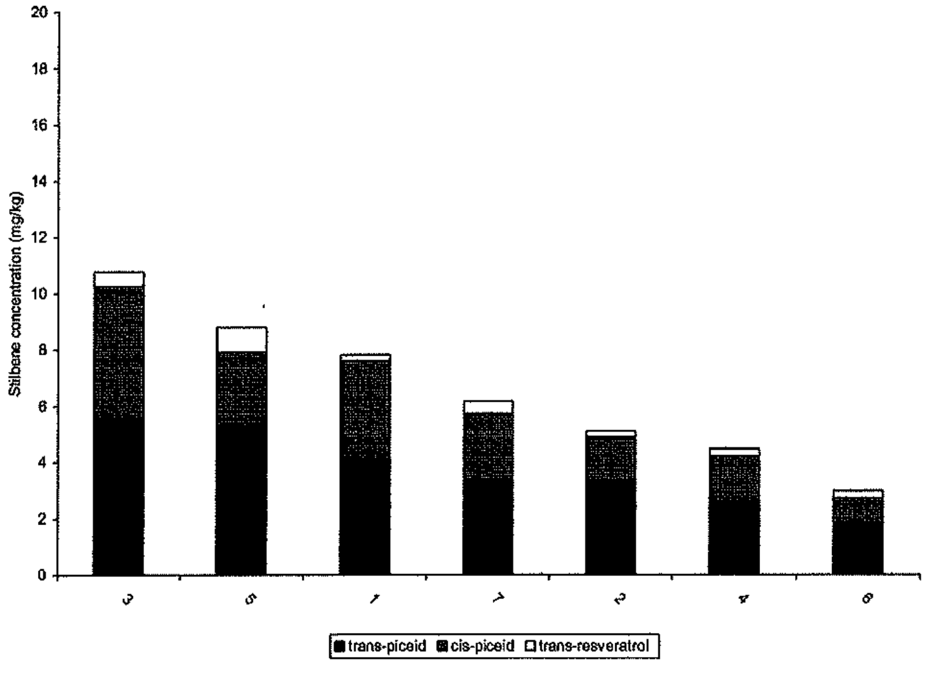

Figure 3: Stilbene concentration in hop pellets.

Moreover, sensitivity against oxidation seems very dependent on the harvest year, 1 and 2- 2003 pellets being much more concentrated (above 13 ppm total stilbene) compared to the here-investigated 2004 samples.

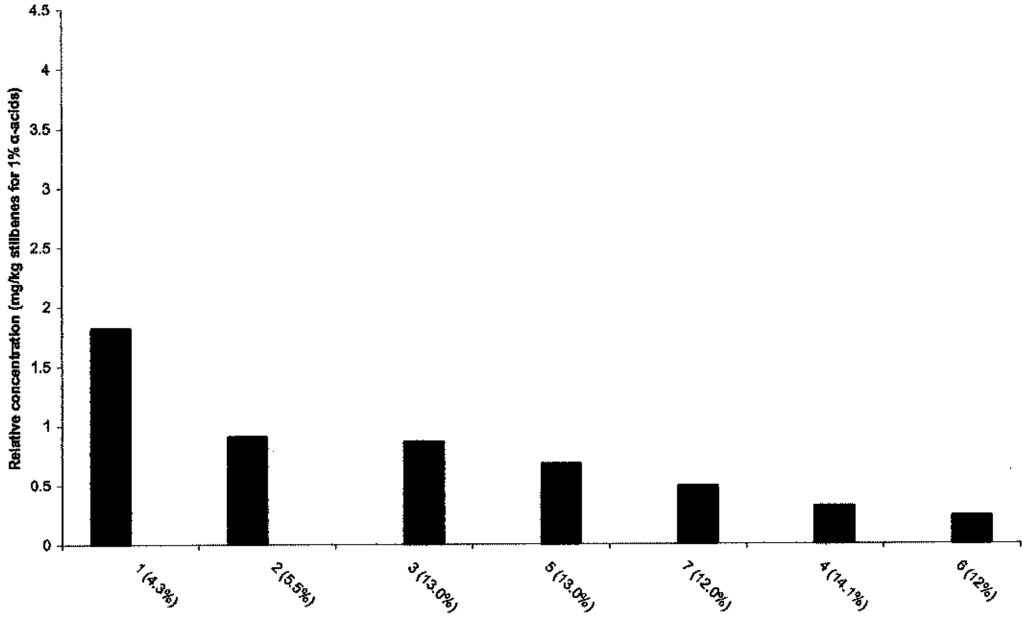

Figure 4: Relative concentration in hop pellets (α-acids %).

CONCLUSIONS AND PERSPECTIVES

Resveratrol and its glycosides were found in both conditionings. Low α-acids cultivars emerged as the most concentrated before pelletisation. Pelletisation induced strong stilbene degradation in some cultivars. Sensitivity against oxidation seems also very dependent on the harvest year.

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