

## Hop as a determinant nutrition key for health?

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*Descriptors:*

*Antioxidant, beer consumption, health, hop constituent, polyphenol*

**SUMMARY**

Nowadays, hop is almost exclusively used in brewery for bitterness and flavours properties. Although hop polyphenols have been widely studied for their antioxidant activity, little is known on their impact on health. Especially, resveratrol, responsible for "French paradox", has never been investigated in hop. We have optimized an extraction procedure for recovering flavonoids, phenolic acids and resveratrol. The resulting extracts were concentrated and analyzed by HPLC-UV/-MS-ESI-SIM. We have detected for the first time the resveratrol in hop. Resveratrol found in hop might be a key element for the nutritional properties of beer.

**Hopfen als bestimmender Ernährungsfaktor für die Gesundheit?**

*Deskriptoren:*

*Antioxidantium, Bierverbrauch, Gesundheit, Hopfenbestandteil, Polyphenol*

**ZUSAMMENFASSUNG**

Heutzutage wird Hopfen in der Brauerei meist ausschließlich für die Bittere und für geschmackliche Eigenschaften eingesetzt. Obwohl Hopfenpolyphenole aufgrund ihrer antioxidativen Aktivität weitreichend untersucht worden sind, ist wenig über ihren Einfluss auf die Gesundheit bekannt. Wir optimierten einen Extraktionsprozess zur Ausbeute von Flavonoiden, Phenolsäuren und Resveratrol. Die daraus resultierenden Extrakte wurden aufkonzentriert und mittels HPLC-UV/-MS-ESI-SIM analysiert. Wir haben erstmalig Resveratrol in Hopfen mit einer Konzentration zwischen 42 und 170 ppm nachgewiesen. Das in Hopfen vorkommende Resveratrol könnte ein Schlüsselement für die Nährstoffeigenschaften von Bier sein.

## HOP AS A DETERMINANT NUTRITION KEY FOR HEALTH?

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### ABSTRACT

*Descriptors:* hop, resveratrol, polyphenols, health

Nowadays, hop is almost exclusively used in brewery for bitterness and flavours properties. Although hop polyphenols have been widely studied for their antioxidant activity, little is known on their impact on health. Especially, resveratrol, responsible for “French paradox”, has never been investigated in hop. We have optimized an extraction procedure for recovering flavonoïds, phenolic acids and resveratrol. The resulting extracts were concentrated and analyzed by HPLC-UV/-MS-ESI-SIM. We have detected for the first time the resveratrol in hop. Resveratrol found in hop might be a key element for the nutritional properties of beer.

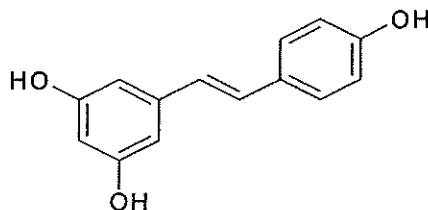
### Le houblon : élément nutritionnel déterminant pour la santé?

*Descripteurs:* houblon, resvératrol, polyphénols, santé

Actuellement, le houblon est employé en brasserie pour son amertume et ses arômes. Bien que les polyphénols du houblon furent largement étudiés pour leur activité antioxydante, leur impact sur la santé est peu connu. En particulier, le resvératrol, responsable du “French paradox”, n’avait jamais été recherché dans le houblon. Une méthode d’extraction pour récupérer les flavonoïdes, les acides phénoliques et le resvératrol a été mise au point. Les extraits obtenus ont été concentrés et analysés par HPLC-UV/-MS-ESI-SIM. Pour la première fois, le resvératrol a été détecté dans le houblon. Il pourrait être un élément clé pour les propriétés nutritionnelles de la bière.

### INTRODUCTION

For centuries, hop has been used in traditional medicine (e.g.: antibacterial, sedative, estrogenic and anticarcinogenic activities,...). Nowadays, the plant is almost exclusively used in brewery. Besides the two most important hop properties for brewers, namely bitterness and flavours, its beneficial effect on human health should be more considered (6;7;29). Although hop polyphenols have been widely studied for their antioxidant activity in the boiling kettle (16), little is known about their impact on health. Especially, resveratrol or *trans*-3,4',5-trihydroxystilbene (Figure 1), believed to be at the origin of the “French Paradox” (9;22;26), has never been quantified in hop.



**Figure 1.** Structure of *trans*-resveratrol.

Resveratrol is a stilbene phytoalexin which has been first reported in the skins of grapes. Present in red and white wine (1;15;19-21;26;28), it could be responsible for the decrease in coronary heart disease observed among wine drinkers (3;5;18). Resveratrol could also exhibit anti-carcinogenic (4;12;14;25), anti-viral (8), anti-oxidant (2;10;23), anti-inflammatory (17;30) and estrogenic activities (11). Resveratrol has also been identified in grape berries, cranberries juice (31), peanuts or peanut butter (13;24;27).

The aim of this work was to investigate the potential presence of resveratrol in hops and beers.

## **ANALYTICAL METHODS**

### **Hop pellets extraction procedure**

We tested two extraction procedures on 5 g of moulded hop pellets. Both methods were carried out in absence of light under nitrogen atmosphere. For the former<sup>1</sup> successive washes with petroleum ether and hexane under reflux allowed to get rid of hydrophobic compounds. Polyphenol extraction was then carried out with methanol:water (75:25, v/v) under reflux. In the second method<sup>2</sup>, all steps were carried out at room temperature. The extract was finally concentrated under vacuum and diluted in 100mL of methanol:water (50:50, v/v).

### **Standard addition method**

A recovery factor of 78% was obtained by spiking the Tomahawk hop sample with increasing amounts of *trans*-resveratrol (0, 100, 200 ppm in hop).

### **Instrumentation**

**High Performance Liquid Chromatography analyses.** A SpectraSystem (Finnigan Mat, San Jose, CA, USA) equipped with a SCM degasser, an AS3000 autosampler, a P4000 quaternary pump and a diode array detector UV6000LP was used. The system was controlled with the Xcalibur software version 1.2 (Finnigan Mat). The compounds were separated on a Phenomenex normal phase Luna silica column (250 x 4.6 mm i.d., 5 µm) (Bester, Holland) at 25°C. Separations were carried out at a flow rate of 1 mL/min with a linear gradient from A (dichloromethane) to B (methanol) and a constant 4 % level of C (acetic acid and water, 50:50, v/v). Gradient elution was 14-28% B, 0-30 min; 28-50% B, 30-60 min; 50-86% B, 60-65 min and isocratic, 65-70 min. The system was re-equilibrated with the initial conditions for 15min. The volume injected was 20 µl.

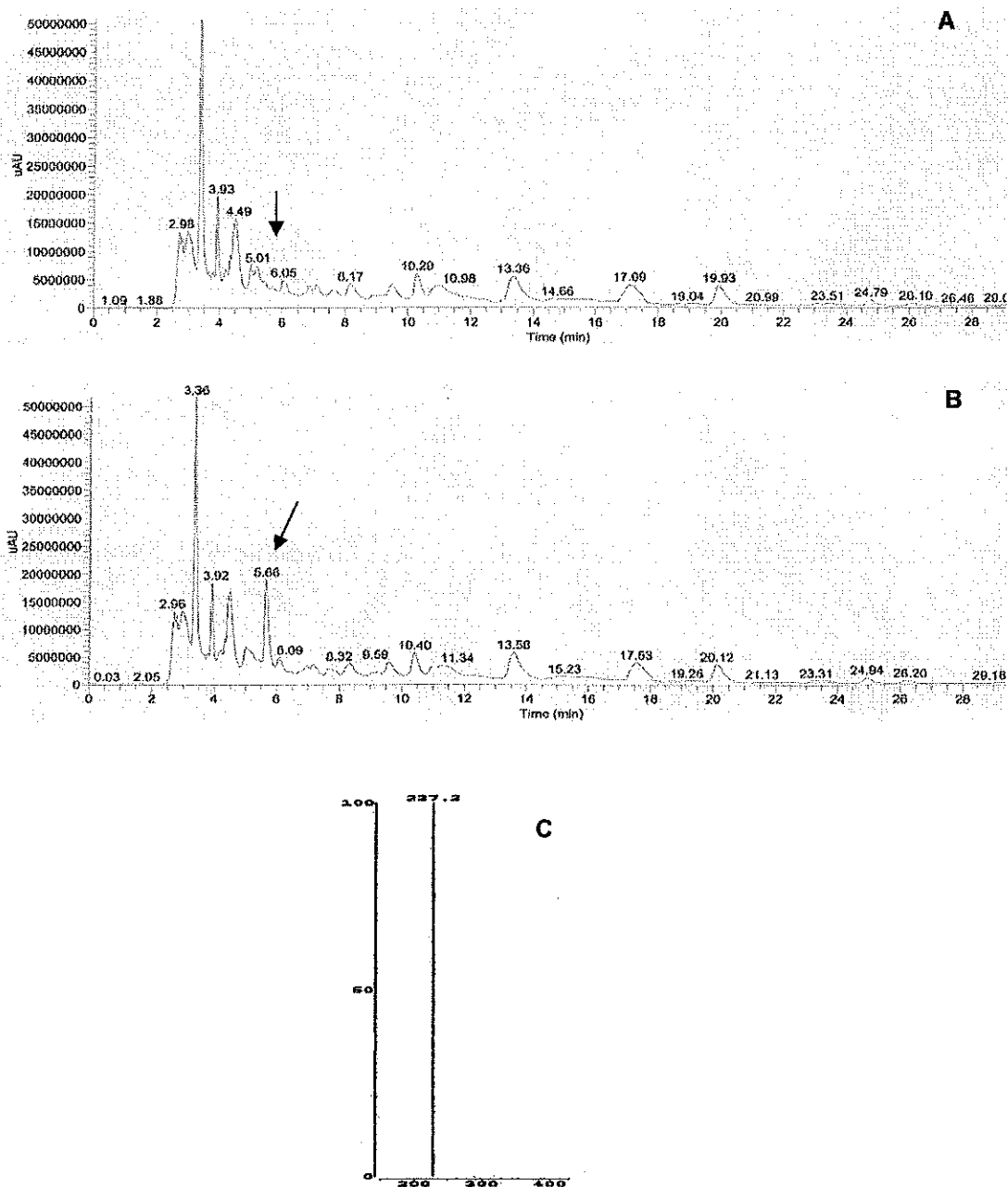
**UV analyses for quantification.** Resveratrol was monitored at 306 nm, rutin and syringic acid at 270 nm, procianidins (P1, P2, P3, P4, P5, P6) at 280 nm, p-coumaric acid and ferulic acid at 320 nm and myricetine, quercetin and ellagic acid at 370 nm.

**Mass Spectrometry analyses for structure confirmation.** MS analyses were carried out using a LCQ Duo (Finnigan Mat) multipole mass spectrometer equipped with an ESI interface. The negative ion mode with a 4,5 kV source voltage, a - 20 kV capillary voltage, a 225°C capillary temperature and a shear gas (nitrogen) of 50 arb (arbitrary units) was selected for resveratrol analysis. Only 1:10 of HPLC flow was led to the ESI interface of the mass analyzer. Data were collected on a computer (Xcalibur software) using Selective Ion Monitoring (SIM) mode (226,1-228,1 m/z for resveratrol).

## RESULTS AND DISCUSSION

### Resveratrol

As depicted in Figure 2A, *trans*-resveratrol has been detected for the first time in hop. The same hop extract spiked with 5 ppm of *trans*-resveratrol (Figure 2B) allowed to confirm the identification. Moreover, mass spectrometry used in SIM mode between 226,1 and 228,2 m/z led to a M-H= 227,2 peak at the same retention time (Figure 2C).



**Figure 2. A & B.** HPLC-UV (306 nm) of Tomahawk hop pellets: **A.** hop extract, **B.** hop extract spiked with 5 ppm of *trans*-resveratrol. **C.** HPLC-MS-ESI-SIM (negative mode, M-H = 227,2).

Five hop cultivars were further analysed for their *trans*-resveratrol content (UV-quantification, Table 1).

**Table 1.** Concentration of *trans*-resveratrol (ppm) in various hop cultivars <sup>3</sup>.

Hop	Resveratrol concentration
Hallertau Taurus <sup>1</sup>	39,92
Wye Challenger <sup>1</sup>	5,15
Hallertau Magnum <sup>1</sup>	13,04
Galena <sup>1</sup>	21,91
Tomahawk <sup>2</sup>	27,87

} 0,01 – 0,08 ppm in beer

Extraction methods <sup>1</sup> or <sup>2</sup>. <sup>3</sup> Recovery factor used = 78%.

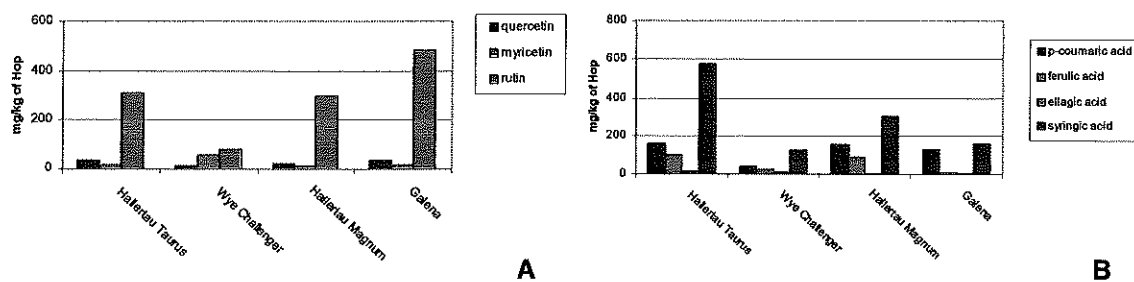
Up to 39,92 ppm *trans*-resveratrol has been quantified in the Hallertau Taurus sample while only 5,15 ppm were present in the Wye Challenger. If we take into account the dilution factor applied to hop, around 0,01 to 0,08 ppm *trans*-resveratrol could be expected in beer. These values are similar to the level found in white wines: 0,000456 to 0,547 ppm and not too far from those described for red wines: 0,3 to 11,9 ppm (1;15;19-21;26;28).

#### Phenolic acids, flavonols and procyanidins

As depicted in Table 2 and Figure 3, the same hop cultivars were analysed for their phenolic acid, flavonol and procyanidin contents (extraction<sup>1</sup> and UV-detection). Except for the Galena sample, the higher the procyanidin level, the higher the phytoalexin concentration.

**Table 2.** Concentrations (ppm) of procyanidins in hop pellets.

Procyanidins	Hallertau Taurus	Wye Challenger	Hallertau Magnum	Galena
P1	5310	4541	4447	3011
P2	2755	2339	3159	2163
P3	2800	1551	1893	1341
P4	864	1395	746	843
P5	784	309	325	390
P6	279	307	296	238
Total	12794	10444	10868	7988



**Figure 3.** Concentrations (ppm) of flavonols (A) and phenolic acids (B) in the same hop samples.

## CONCLUSIONS

Resveratrol might be a key element for the nutritional properties of beer. The identification of this compound opens a new door to an increased understanding of different health benefits potentially brought by beer. A more specific resveratrol extraction method is currently optimized in our laboratory for hop and beer analyses.

## ACKNOWLEDGEMENT

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