



Research Letter from ... a German Beer Garden

# The 'Bitter Dozen'

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**P**rost, zum Wohl! Where would Germany be without its proud tradition of beer-drinking? Enshrined in the world's oldest food quality regulation, the Reinheitsgebot (Beer Purity Law) of 1516, German beer's composition has been under scrutiny for almost 500 years. Traditional German beer is made from just barley, hops and water, with the addition of yeast.

One quality that clearly distinguishes beer from other drinks is the distinct bitterness conferred upon it by the hops. Bavarian food scientist, Thomas Hofmann, from the Technische Universität in Munich has set out to quantify exactly what makes beer bitter. In his group's recent article, "Three TAS2R Bitter Taste Receptors Mediate the Psychophysical Responses to Bitter Compounds of Hops (*Humulus lupulus* L.) and Beer" (*Chemosensory Perception*, publ. online 3/6/09), he has identified the taste receptors that recognise bitter substances in beer and has developed transfected receptor cell lines that are up to a thousand times more sensitive than trained human tasters!

There are 25 human bitter taste receptors, all members of the Taste Receptor, type 2, family (the TAS2Rs). Each TAS2R receptor can respond to various bitter compounds. Hofmann generated human embryonic kidney cells expressing the 25 TAS2Rs and their signalling partner, the G-protein, gustducin. In this way, activation of the TAS2Rs was coupled to the release of calcium ions from intracellular stores, in turn, detected with a calcium-sensitive fluorescent dye. But rather than getting his cells to "taste" beer directly, Hofmann gave them 15 purified, hop-derived, bitter compounds, including all the major hop acids, the  $\alpha$ -acids ("humulones") and  $\beta$ -acids ("lupulones"). His kidney cells show that all our bitter beer taste passes through just three of the TAS2R receptors – numbers 1, 14 and 40.

However, Hofmann wanted to be sure that his cells were giving physiologically meaningful data. Indeed, he complains at the lack of studies that correlate *in vitro* cell line data with *in vivo* human "psychophysical" experiments. Hence, he presents a range of comparable "psychophysical" taste tests with "real" beer drinkers.

## Two years of 'taste language' training

His "sensory panel" consisted of twelve assessors (five women, seven men, aged 25-40), who had given "informed consent to participate in the sensory tests and had no history of known taste disorders". We aren't told if the assessors were lab staff or research students, or if they were paid for doing it, but Hofmann certainly wanted them to be in shape when they competed with his cell lines – they all underwent extensive training "for at least 2 years in weekly training sessions with reference solutions in order to familiarize them with the taste language and to get them trained in recognizing and distinguishing dif-

ferent qualities of oral sensations in analytical sensory experiments"!

As references in their classification of bitter taste, the assessors were provided with magnesium sulphate (166 millimolar solution) "representing a short-lasting, metallic bitter taste quality perceived mainly at the anterior part of the tongue", salicin (1.4 millimolar) "imparting a long-lasting bitter taste sensation, perceived mainly in the back of the tongue as well as the throat" and caffeine (8 millimolar) "providing a long-lasting bitterness, perceived all over in the oral cavity".

## Kidney cells better than human tasters

Sensory analyses were performed "at 22-25°C" in three independent sessions for each test. "In order to minimize cross-modal interactions with olfactory responses, nose clips were used." Oh, and rest assured, under "Precautions Taken for Sensory Analysis of Purified Bitter Compounds", we are told, "In order to minimize the uptake of any toxic compound, analyses were performed using the sip-and-spit method by which the test materials were not swallowed but rather expectorated." The 'Bitter Dozen' highly-trained assessors tasted different concentrations of the 15 bitter beer compounds, until Hofmann had his recognition threshold concentrations and concentration-response curves.

So, how do humans compare to cells? Well, unfortunately, the threshold concentrations and EC<sub>50</sub> values from the cell taste receptor assays were much more sensitive than those determined by his human "psychophysical" experiments. Hofmann laments, they "perceived the bitterness of the investigated compounds at higher concentrations (up to 1,000-fold higher) than those predicted by the results of the *in vitro* experiments". So much for the fickle palate of beer drinkers! But he refused to accept the lost honour of his trained human tasters – there had to be an explanation! What do humans have that cell cultures lack? Why, of course, "the oral cavity"! His bitter compounds must be getting side-tracked from his tasters' taste buds by thoroughly unwarranted interactions with saliva and the oral epithelium. Carefully collecting his tasters' spit and analysing it by HPLC-MS, he showed that, sure enough, some of the bitter compounds remained stuck in his tasters' oral cavities.

So, where does this leave bitterness? In response to taste-driven consumer demand, the food industry is making food less bitter by removing bitter compounds. Beer is no exception, with a clear trend towards milder beers. Ironically though, hops are fighting back! Originally, hops were added to beer as a preservative – they possess complex antibiotic properties. Now, research has found that beer constituents and, in particular, hop bitter acids can protect us from cancer! To your good health!

