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Novel Approaches to the Measurement of flavour-related Properties: A Brief Overview and Future Prospects

The challenges of relating analytical data to sensory evaluation are well-known, and may be attributed to recognised sensory biases and interactions (matrix-effects) that influence the sensory activity of flavour attributes. One approach to obviating these difficulties is to attempt to mimic sensory detection mechanisms to determine the activity of flavour attributes. In this short paper, two examples are given. Firstly, the feasibility of determining beer bitterness by exploiting the lipophilicity of hop bitter acids and measuring changes in pH across a model membrane. Secondly, the derivation of a measure of astringency by observing the binding of beer polyphenol complements to an appropriate protein. Whilst such an approach needs much fine-tuning and benchmarking against existing analytical and sensory tests, it nonetheless offers an alternative strategy for enhancing the predictiveness of beer flavour based on analytical measures.

Descriptors: hops, bitterness, astringency, iso-α-acids, polyphenols, lipophilicity

1 Introduction

The detection and quantification of flavour-active compounds can be deduced either by sensory or analytical means. Both approaches have their merits and limitations (*Hughes*, 2009, Table 1) and indeed these disciplines have steadily developed as discrete scientific disciplines. Nonetheless, sensory analysis is inherently prone to well-known limitations of bias (*Poulton*, 1968), assessor fatigue and, from an analytical perspective, inconsistencies of assessment. Meanwhile it has become ever-more apparent that analysis is at best a modest predictor of all but the most basic of sensory properties.

It is worth exploring this in more detail. With the exception of GC-olfactometry, analytical detection and quantification is dependent on one or more physicochemical properties of the analyte (Table 2). These properties are exploited for their appropriate combination of sensitivity and selectivity. Perhaps an extreme example of this is the development and widespread application of sulphur-specific detectors, such as the Sievers' Chemiluminescence Detector (SCD) and the pulsed flame photometric detector (PFPD), for the determination of sulphur volatiles in beer. The low flavour thresholds of many sulphur volatiles mean that their determination, if it is to be meaningful, must be responsive at such orders of magnitude. Both of these detectors are both sensitive, and highly selective, so that there is minimal interference due to the presence of background "noise". Conversely, "universal" or mass detectors, such as the Evaporative Light Scattering Detector (ELSD), work effectively for compounds lacking useful physicochemical handles and occurring at relatively high concentrations, such as wort saccharides.

The difficulty though is that none of these detection handles reflect sensory performance, except in the broadest of terms. For instance, historically the iso-α-acids have been almost universally detected by the presence of their distinctive UV-chromophore (see, for instance, *Hughes*, 2001; Fig. 1), although other approaches have been employed, such as GC with FID detection, and liquid chromatography with mass spectrometric detection (LC-MS/MS; *Intelmann et al.*, 2009).

As all of the iso-α-acids bear this UV-active chromophore, and assuming relatively minor differences in the bitterness of the iso-α-acids, this has proved to be a convenient analytical handle. Put another way, the ratio of UV-absorbance/bitterness intensity is assumed to be constant for the iso-α-acids. From comparison of bitterness unit (BU; Brenner assay) and HPLC measurements, there are known systematic differences between the two analyses, not least because iso-octane used for the extraction of bitter substances from acidified beer is selective, but can also extract other substances that absorb at the 275 nm wavelength used for the analysis. These interferents include other hop degradation compounds such as the humulinic acids, phenolics, and aromatic heterocycles. However, the fact that the Brenner method is broadly successful indicates the utility of the method, and its relative ease of determination without the need to resort to chromatographic techniques ensures that this method has retained its popularity to this day.

There are, though, problems with the Brenner approach. Firstly, for a well-controlled brand or narrowly-defined category, BU or HPLC methods perform well in terms of ranking beers in order of sensory bitterness. However, it proves difficult to extend this

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to a broader range of products. Secondly, the approximation of a constant ratio between absorption of UV-light and bitterness intensity collapses when the chemically-modified iso-α-acids enter the fray. Thus the UV-absorbance/bitterness intensity ratio is dependent on molecular features beyond the chromophore, limiting the usefulness of applying quantification based on the UV-chromophore for the development of correlations to analysis to sensory performance.

Given the typical case where analytical measurements poorly correlate with sensory performance, are there opportunities to rethink analytical strategies so that they might be more relevant flavour predictors? This is becoming an increasingly relevant question as research moves towards the understanding of sensory interactions: phenomena that generally are only accessible by sensory evaluation[1]. In this paper, we explore two examples of such an approach: the determination of bitterness and of astringency. The key feature of what are termed here functional detection systems is that they attempt to approximate specific features of the sensory detection mechanisms for their detection and quantification by applying novel instrumental approaches.

2 Functional detection of bitterness

To construct a functional bitterness detector, we need to understand how bitterness is perceived in the first place. Fortunately, recent advances in understanding tnhe mechanism of bitterness perception leave us with a clear, albeit incomplete model of bitterness perception (*Adler, et al.*, 2000; *Chandrasekar et al.*, 2000). Essentially, specific receptor proteins (so-called T2Rs), are embedded in the cell membranes of taste cells. Critically, parts of the protein are exposed to both the extracellular and intracellular regions of the taste cell (Fig. 2). The hop bitter acids bind to certain of these receptor proteins, causing a subtle conformational change. This is sufficient to trigger a cascade of intracellular reactions that ultimately leads to signal transduction to the brain's cortex and the perception of bitterness. Currently there are an estimated 30 distinct receptor proteins that bind bitter compounds (Adler *et al.*, 2000; *Matsunami et al.*, 2000), which may go some way to explaining why bitterness is elicited by such a diverse range of chemical entities. Thus bitterness perception is predicated on the ability of bitter compounds to bind to receptor proteins and induce the conformational change necessary to propagate the taste signal.

The mode of binding of bitter acids to their receptor proteins is not clear, but most bitter compounds exhibit some degree of hydrophobicity, so circumstantially hydrophobic interactions might be expected to play a role in such binding. Indeed, an additional parallel might be drawn with the proposed antibacterial mechanism of the hop acids, whereby the hydrophobicity of the hop acids, together with their outstanding metal chelating properties, allows the partitioning of the hop acid into the cell membranes of bacteria and essentially destroy pH gradients across these membranes by proton – metal cation exchange. Once the pH gradient is suppressed or eliminated, a key mechanism for the bacterial cell to acquire nutrition is lost and the cell dies (*Simpson and Smith*, 1992; *Simpson*, 1993). The implication then is that hop acids allow the bacterial membranes to become permeable to protons.

In our laboratory, we have been applying these concepts to develop an analytical device that exploits these hydrophobicity features. There is some similarity here with the approach taken by *Kaneda and Takashio* (2005), where they employed a quartz crystal microbalance with a lipid coated crystal to detect sorption of hop acids on to the lipid layer. In our case, rather than relying on mass detection, we sought to monitor pH to detect the presence of hop bitter acids. Details of the probe developed will be reported fully elsewhere. However, essentially we have been able to demonstrate that, for model solutions, synthetic membranes can reliably distinguish between concentrations of iso-α-acids and their chemically-modified counterparts, and indeed the dynamic form the pH/time response seems to indicate a sensitivity to the degrees of reduction of the iso-α-acid. Further development is required to indicate whether the dynamics of the probe's responses and its sensitivity in the presence of a beer matrix are suitably robust for widespread industrial uptake.

3 Functional detection of astringency

It is known that astringency is elicited by a number of chemical entities, not least of which are the polyphenols. These are a chemically diverse set of chemical species in beer that originate, either as is or in a modified form, from both malted barley and hops. They are significant in terms of brewing not only for their ability to exhibit astringency, but also induce haze formation in beer. Indeed this latter has spawned a plethora of literature (for a dated but thorough review of the colloidal stability of beer, see *Moll*, 1987).

In both cases, the primary mechanisms are based on polyphenol-protein interactions. In particular, astringency appears to be elicited by the interaction and subsequent precipitation of polyphenol-salivary protein complexes, which presumably thickens up the salivary layer coating the oral cavity. The salivary proteins in human parotid saliva contain about 70 % proline-rich proteins (PRPs), which have been shown to have a high affinity with polyphenols (*Kauffman and Keller,* 1979; *Bennick*, 1987). *Baxter et al.* (1997) have demonstrated that the relatively open structure of proline-rich protein domains allows the partial intercalation of polyphenols, particularly oligomeric polyphenols, so that there are multiple binding points between protein and polyphenol. The differences in the expression of astringency between the various polyphenols is substantial. *Delcour et al.* (1984) derived astringency thresholds for tannic acid, (+)-catechin, procyanidin B-3 and mixtures of trimeric and tetrameric proanthocyanidins in deionized water. The thresholds determined – between 4.1 and 46.1 mg/l – suggest that an overall polyphenol measurement for assessing astringency would have limited value.

Here, we have taken advantage of the ability of astringent polyphenols to bind proline-rich proteins to establish the losses of individual polyphenols after treatment with such a protein sorbent. Any aggregates formed are removed by nanofiltration, for instance using Centriprep tubes and centrifugation (*Millipore*, accessed June 2010). The results will be reported elsewhere, but the comparison of chromatograms before and after protein treatment and nanofiltration indicate which chromatographic bands are likely to

elicit an astringent response. Nevertheless, it is far from trivial to identify some of these polyphenolic components.

4 Conclusion

Analytical methods, hardware and software all contribute to enable analysts to identify and quantify ever decreasing concentrations of analytes, usually much more quickly than in the past. The proposal here is that, for sensorially-relevant analytes, we apply the rapidly developing knowledge of sensory transduction mechanisms to reconsider the properties of specific molecules that should be measured. Of the two examples given here – those of hop-derived bitterness and polyphenol-induced astringency – neither are likely to be exact renditions of the actual sensory mechanisms. Nonetheless, movement in this direction provides opportunities to better determine flavour activity rather than measuring a molecular property that may have little or no direct link to sensory properties.

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Footnote

1 An exception might be when one flavour component has a physicochemical interaction with another, such as the retention of diacetyl in the presence of high concentrations of dextrins (*Harrison and Hills*, 1997)

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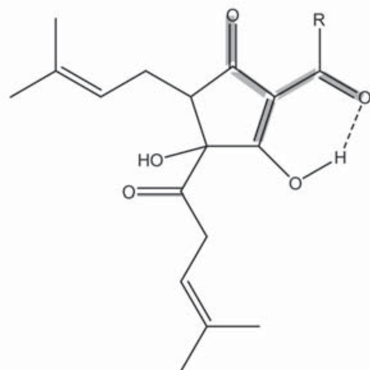
Appendix

Table 1 A comparison of sensory and analytical data attributes (Hughes, 2009)

Instrumental analysis	Sensory evaluation
Simplify: eliminate matrix	Holistic; full matrix
Sequential measurements	Virtually instantaneous assessment
All components considered singly	All components can interact
Aim is to measure concentration	Aim is to determine sensory activity
Instrumentation can be calibrated	Time-consuming to “calibrate” tasters
Output: hard numbers	Output: subjective values

Table 2 **Chromatographic detection systems commonly used for the identification and quantification of flavour-active components in beer (Hughes, 2009)**

Chromatography	Detector	Application
HPLC	UV-visible	Compounds with absorption from 190–800 nm
	Fluorescence	Selective for compounds with defined excitation and emission wavelengths
	Refractive index	Universal detector, relying only on the ubiquitous property of refractive index
	Evaporative light scattering	Universal detector; useful for compounds with few analytical "handles", such as sugars
	Mass spectrometry	Almost universal detector; often complex fragmentation patterns with few rules for spectral interpretation
	Electrochemical	Can be used in oxidation or reduction modes as a selective detector
	Chemiluminescence	Highly selective for chemiluminescent compounds
GC	Flame ionization	General purpose; detects carbon
	Electron capture	Sensitive to specific compounds such as halogen- and nitro-containing species and 1,2-diketones
	Atomic emission	Can be set to detect a wide range of elements
	(Pulsed) flame photometric	Used mainly for sulphur-containing compounds
	Sievers chemiluminescence	Used mainly for sulphur-containing compounds
	Nitrogen phosphorus	Specific for nitrogen- or phosphorus-containing species
	Mass spectrometry	Very common, general-purpose detector
	Olfactometry	Used extensively for flavour volatile research and troubleshooting



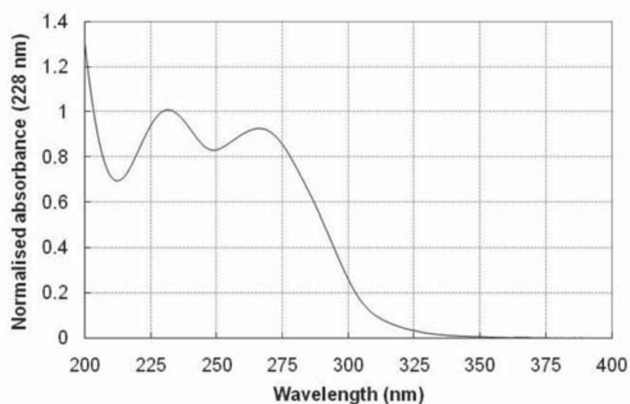


Fig. 1 **The UV-chromophore of the iso-α-acids and a typical UV spectrum of an iso-α-acid, *trans*-isohumulone. (60/40/1 (w/w/w) acetonitrile/water/acetic acid)**

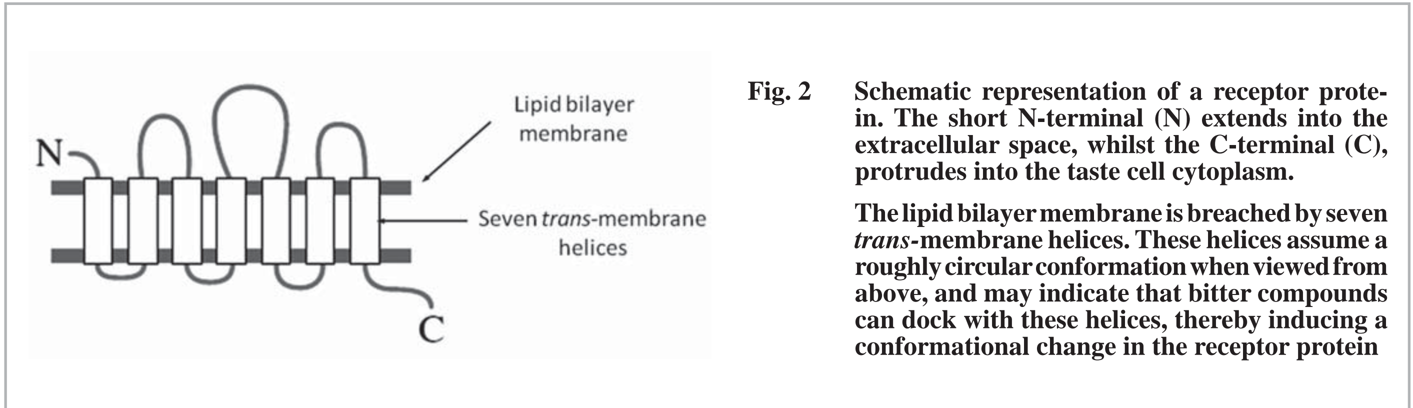


Fig. 2 **Schematic representation of a receptor protein. The short N-terminal (N) extends into the extracellular space, whilst the C-terminal (C), protrudes into the taste cell cytoplasm.**

The lipid bilayer membrane is breached by seven *trans*-membrane helices. These helices assume a roughly circular conformation when viewed from above, and may indicate that bitter compounds can dock with these helices, thereby inducing a conformational change in the receptor protein

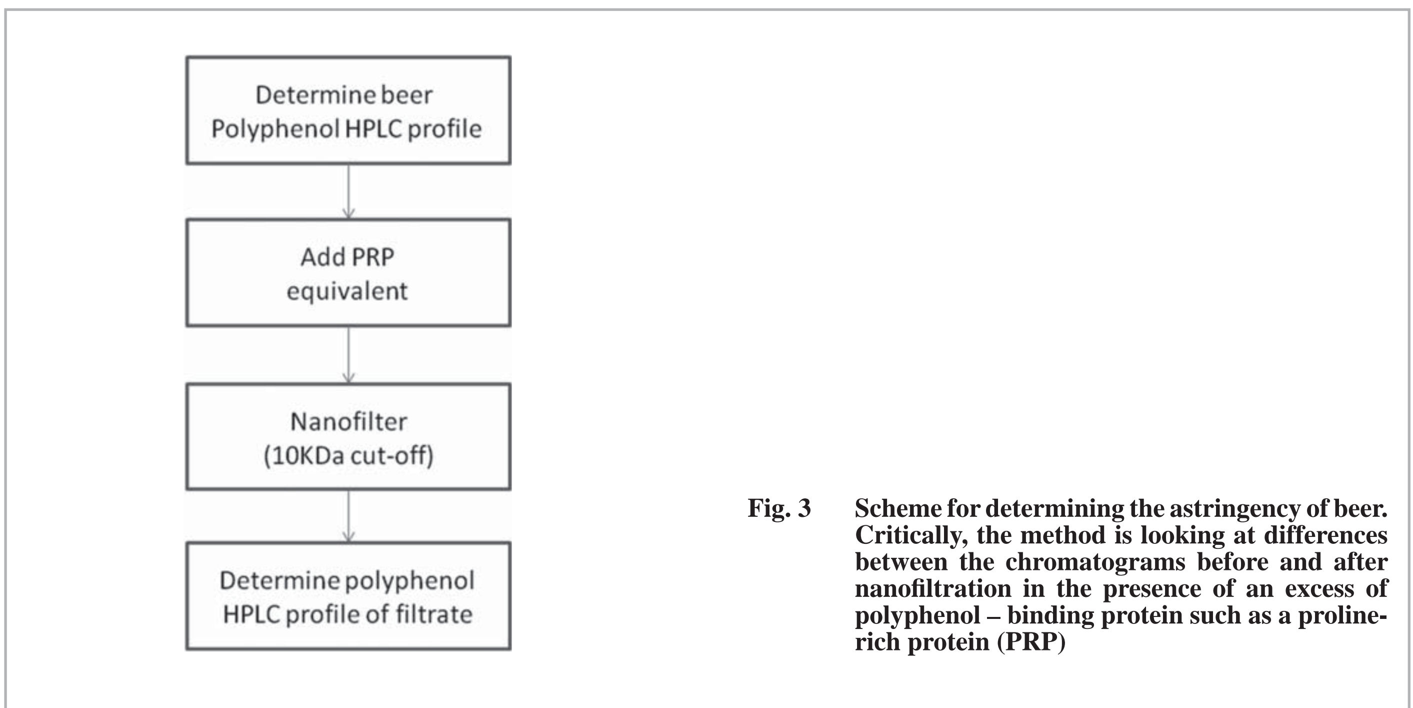


Fig. 3 **Scheme for determining the astringency of beer. Critically, the method is looking at differences between the chromatograms before and after nanofiltration in the presence of an excess of polyphenol – binding protein such as a proline-rich protein (PRP)**