Scope for improvement in the sensomics approach

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Abstract

Sensomics is a stepwise approach for determining the compounds responsible for food odour. In this work, meta-analysis was carried out on a selection of sensomics publications and previously unpublished research, identifying two steps with scope for improvement. Firstly, it was found that Flavour Dilution (FD) factors, as calculated for odourants using Gas Chromatography – Olfactometry (GC-O), are very poor predictors of an odorants' Odour Activity Value (OAV). FD factors are used to prioritize odorants for quantitation and, following this work, it is recommended that all odorants are quantitated, regardless of FD factor, or other measures are considered in odorant prioritization. Secondly, from the statistical information available, it was found that Flavor Profiling®, used to compare the odour simulation to original material (OM), is able to test for significant difference in specific odour attributes but not overall odour. It is therefore recommended that simulations are assessed by additional methods such as Napping®. All sensory methods should be powered to meet the criteria of the statistical testing to be performed.

Introduction

Sensomics is an accepted approach to identify the key odorants in food with more than 100 publications determining odorants in over 200 different foods [1]. Sensomics is an established technique, however there is limited literature available for its validation. The sensomics approach to odour analysis is stepwise, combining: (1) The bioactivity guided detection of key odorants using GC-O, where the method of Aroma Extract Dilution Analysis generates FD factors, which are used to prioritise odorants for quantitation. (2) Accurate quantitation, which is used to calculate an odorants' Odour Activity Value (OAV), the ratio of the concentration of an odorant in the food and its odour detection threshold in a suitable matrix. OAV is assumed to relate to an odorants' overall importance in a food. (3) Accurate reconstitution, using odorants with OAV > 1, to create an odour simulation. This simulation is then validated by comparison to the original material (OM) using the human sensory method of Flavour Profiling®. (4) Sensory omission studies to reduce the simulation to the smallest number of odorants.

There is a wealth of literature validating methods for the quantitation of odorants [2] and odour detection thresholds [3]. There are previous publications on the applicability of sensory omission studies [4]. However, there is limited information on the ability of FD factors to determine and prioritise importance of odorants and the ability of Flavour Profiling® to validate odour simulations. Here, a meta-analysis is conducted on results from a sample of sensomics publications focusing on the prediction of an odorant's OAV from the FD factor, and the use of Flavour Profiling® to compare odour simulations to the OM.

Experimental

Meta-analysis was conducted on a selection of sensomics publications [5-19]. Covering a period from 1993 to 2017, the selection included analysis of fish, meat, coffee,

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nuts and fruit, using the sample preparation techniques of Solvent Assisted Flavour Evaporation, High Vacuum Distillation and Static Headspace. For each publication the odorant data was tabulated with other available information on the odorant and food product [20-22]. Statistical analysis was carried out using R version 3.3.3 and the *ranger* library.

Two statistical modelling methods were used to assess the ability of FD factors to predict OAV. The first was a simple linear model with OAV as response and FD factor as fixed effect, both on the $\log_{10}$ scale. The second was a random forest approach, with 200 trees and 4 variables selected per tree. Additional predictors (variables) were used for the random forest model, including odorants' vapour pressure (VP), air/water partition coefficient (K_{aw}) and odour detection threshold. The models were fitted to 70% of the data. The remaining data was then predicted, and used to calculate root mean square error (RMSE), as a measure of fit. Within the reviewed publications there was little statistical information (e.g. variance) available for the comparison of the odour simulation to the OM. Therefore, the data used to first assess Flavour Profiling® and then investigate an alternative approach, Napping®, is from unpublished work on liver and tuna.

Results and discussion

Results for the prediction of OAV from FD factor using a simple linear model are displayed in Fig. 1A. The figure shows that prediction of OAV from FD factor alone is very poor, RMSE 1.14. It is therefore recommended that FD factors alone are not used to select or prioritise odorants for quantitation. Previous publications have noted that there are differences between FD factors and OAVs. The reasoning was two-fold. Firstly, that FD factors are not corrected for losses in sample preparation [23]. Secondly, that in GC-O the whole aroma extract is vaporised, whereas OAVs are calculated using odour thresholds in a matrix, i.e. considering only the amount of an odorant in the headspace. The example given is that polar compounds are often overestimated by AEDA, because they are quite soluble in water, and thus their vapour pressure is comparatively low [11].

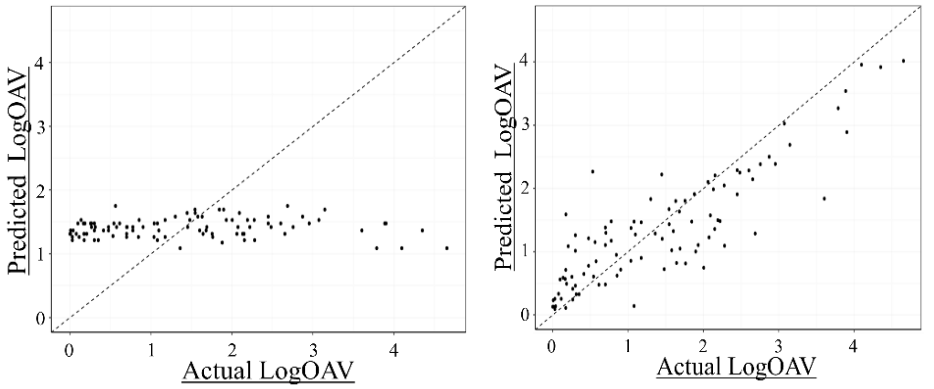

Figure 1: A, left, prediction of OAV from FD factor alone, using a simple linear model. B, right, prediction of OAV from FD factor along with additional measures

Results for the prediction of OAV from FD factor and additional variables, using the machine learning model, is shown in Fig. 1B. The figure shows a great improvement in prediction, RMSE 0.58, showing that by incorporating other variables odorants can be selected or prioritised for quantitation. The variables that have highest importance in the

model are displayed in Table 1. Additional variables with their importance were: the amount of water (19.7), protein (15.8), fat (14.8) in the food matrix; the physical chemical constants, VP (23.0), LogP (14.1), exact mass (12.1); Linear Retention Index (LRI) on a standard non-polar column (21.0); the percentage abundance of the odorant in studied food (12.8) as defined by Dunkel et al [1]; FD factor (23.3). The results show that within the model, FD factor is not the best predictor of OAV, even when normalised by taking into account the amount of food used for sample preparation. In fact, LRI on a normal polar column is the best single predictor of OAV. A possible explanation is that LRI is a good correlator of odour release from foods. Whereas, VP and K_{aw} are calculated within systems at equilibria, LRI is calculated within a dynamic system, as is odour release. GC-O can identify odorants, but multiple additional measures are required to predict an odorant's importance.

Table 1: Highest importance score of variables used with the machine learnt random forest model.

Variable	*Importance*
LRI Standard Polar Column	52.3
K_{aw}	39.8
Carbohydrate (% wt.)	37.6
Normalised FD factor (g^{-1})	34.17
Odour Detection Threshold (mg /L)	33.05
LRI Semi-Standard Non-Polar Column	24.57

In the absence of statistical data from published work using Flavour Profiling®, the data reviewed is from previously unpublished work on liver (Fig. 2a). In statistical testing, by analysis of variance (ANOVA), there was no significant difference between simulation and OM for each odour attribute. Power analysis of the data showed that, for each attribute, a difference of 0.5 would be detected 80% of the time (if present), at a significance level of 5% with 25 assessors. The results therefore show that Flavour Profiling® is able to test odour attribute differences of 0.5 between the simulation and OM. Within Flavour Profiling® this is equivalent to half way between a moderate to strong odour attribute. But what about differences in overall odour? Using a sensory discrimination test, Triangle testing, a significant difference in overall odour was observed ($p<0.01$) with 60 participants. In sensomics publications simulations are described as characteristic (and not similar) to the overall odour of the OM. Indeed, a previous review commented that there are difficulties in producing flavour simulations for solid foods as it is not possible to recreate the composition and distribution on the non-volatiles components in a suitable odourless matrix [23]. In effect, the simulation matrix causes a difference. Since Flavour Profiling® does not assess overall odour the assessment of the overall odour of a canned skipjack tuna odour simulation was carried out by the human sensory method of Napping®. The odour simulation was compared to the OM (Skipjack 1) and other tuna samples of different species (Albacore), manufacturer (Skipjack 2) and samples that had been opened and left in a fridge for 24 h (Aged). The results show that the overall odour of the simulation clusters with tuna samples from the same manufacturer and tuna species. The overall odour is characteristic of a specific manufacturer's canned skipjack tuna product. However, the overall odour is not similar as Triangle testing showed a significant difference ($p<0.01$). In addition to the previous explanation on why simulations are not similar, the Napping® shows that the overall odour of canned tuna changes over time from opening. Food does not have a constant

stable odour. Reviewing the results, it is recommended that for odour simulation assessment Flavour Profiling®, with additional methods such as Napping®, are used. All sensory methods should be powered to meet the criteria of the statistical testing to be performed.

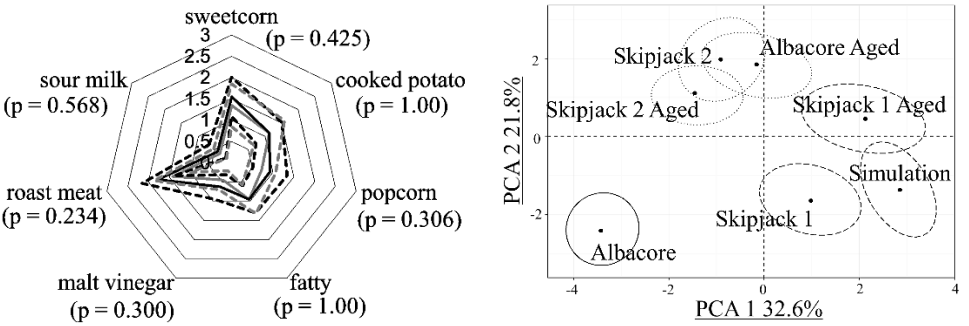

Figure 2: A, left, radar plot showing Flavour Profiling® comparison of odour attributes for liver (black) to liver odour simulation (grey). Solid line represent mean, dashed lines 95% confidence limits, n = 20, p-value from testing significant difference between each odour attribute for liver and liver odour simulation. B, right, PCA of Napping® result for the comparison of a canned skipjack tuna odour simulation to skipjack tuna from different manufacturer and species (albacore). All tuna samples were analysed freshly opened and after aging in a fridge for 24 hours. n= 10, ellipsoids represent 95% confidence intervals, line style represents clustering determined by hierarchical cluster analysis: solid black cluster 1, dotted grey cluster 2, dashed cluster 3.

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