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Correlation of sensory bitterness in dairy protein hydrolysates: Comparison of prediction models built using sensory, chromatographic and electronic tongue data

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ABSTRACT

Sensory evaluation can be problematic for ingredients with a bitter taste during research and development phase of new food products. In this study, 19 dairy protein hydrolysates (DPH) were analysed by an electronic tongue and their physicochemical characteristics, the data obtained from these methods were correlated with their bitterness intensity as scored by a trained sensory panel and each model was also assessed by its predictive capabilities. The physiochemical characteristics of the DPHs investigated were degree of hydrolysis (DH%), and data relating to peptide size and relative hydrophobicity from size exclusion chromatography (SEC) and reverse phase (RP) HPLC. Partial least square regression (PLS) was used to construct the prediction models. All PLS regressions had good correlations (0.78 to 0.93) with the strongest being the combination of data obtained from SEC and RP HPLC. However, the PLS with the strongest predictive power was based on the e-tongue which had the PLS regression with the lowest root mean predicted residual error sum of squares (PRESS) in the study. The results show that the PLS models constructed with the e-tongue and the combination of SEC and RP-HPLC has potential to be used for prediction of bitterness and thus reducing the reliance on sensory analysis in DPHs for future food research.

1. Introduction

The incorporation of dairy protein hydrolysates (DPH) into foods has numerous benefits over non hydrolysed protein as they have improved functionality in the food matrix and have also been shown to be a rich source of bioactive peptides [17]. However, the addition of DPHs into food has been somewhat restricted due to the bitterness that can develop as a result of the hydrolysis process [42,13]. The traditional method of evaluating the bitterness of a food or food ingredient is by sensory analysis using a human taste panel. Sensory analysis is currently the only method which directly measures the perceived intensity of an attribute of interest [3] but it can present difficulties in implementation during the research and development phase of DPH products. Issues include the need for a large quantity of food grade sample which can be difficult in the early stage of laboratory development, in addition, there may be a risk or microbial or chemical contamination at lab production level. Analysis with a human sensory panel can also be very time consuming as the panel needs to be trained and no more than 3–4 samples can be analysed at a time as the human palate is easily saturated or fatigued. If more was known about the taste profile of a DPH at an earlier stage in the R&D phase then strategies to mask or otherwise ameliorate the negative taste defect could be applied earlier in the development phase. Thus there is an interest in using physicochemical characteristics as useful predictors for sensory defects, which may then reduce the reliance on sensory analysis in product development.

Physicochemical characteristics have been used previously as predictors for bitterness in various foods, such as measuring polyphenol of content in olive oil by HPLC analysis [14] or by measuring peptide size and hydrophobicity using Urea-PAGE and RP-HPLC respectively in Ragusano cheese [12]. In DPHs the physiochemical characteristics that may act as predictors for bitterness intensity are the extent to which they have been hydrolysed [42], molecular weight range and hydrophobicity of the peptides they contain. The relationship between a peptide size, hydrophobicity and bitterness was extensively researched by Ney [28]. Ney hypothesised that small to medium peptides consisting of a relatively high proportion of hydrophobic amino acids would be bitter and developed a method of predicting the bitterness, Neys rule. Neys rule allowed the estimation of a so called 'Q value' for peptides which was calculated using hydrophobicity and size of a peptide. Peptides with Q values greater than 1400 and molecular weight less than 6 kDa were assumed to be bitter. However, it should be noted that Neys rule is not without

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exception as it does not take into account spatial arrangement or structure of peptides, which has been shown to impact strongly on bitterness [25]. As DPHs contain a large number of peptides and free amino acids, to separate each individual constituent, calculate its Q value and then relate it to bitterness is not feasible as a rapid method of assessment. Alternatively, high performance liquid chromatography (HPLC) analysis can be employed to determine the molecular weight distribution using size exclusion chromatography (SEC) and relative hydrophobicity using reverse phase (RP) of each DPH for correlation with bitterness intensity. RP-HPLC has been previously used successfully to correlate the ratio of hydrophobic to hydrophilic peptides and amino acids with the bitterness of cheese made from raw and pasteurized milk [15].

Technologies which mimic the human sensory response to foods, beverages and pharmaceuticals, such as the electronic tongue (e-tongue) are under increasing interest from industry. The advantage of the e-tongue over traditional sensory analysis is that a small sample size is required; it can be used to assess non-food grade samples and can provide a more rapid analysis. Recent studies have shown that e-tongues have the potential to assess bitterness in an array of samples such as the bitter standard quinine, beverages such as beer and pharmaceuticals such as berberine hydrochloride [33]; Rudnitskaya et al., 2013; [40,43]. However, to date there have been relatively few studies on the analysis of protein rich samples using e-tongue devices [44,6,18,11,35,47].

The application of supervised pattern recognition techniques such as linear discriminate analysis (LDA) artificial neural networks (ANN) and partial least square regression (PLS) are being increasingly applied in food science [2]. These techniques can be used to process a large amount of data and group or order samples based on the pattern of measurements in the data set [2]. PLS has been employed previously to chromatographic data in the analysis of foods such as in the determination of anthocyanins in wine using HPLC-DAD and infrared spectroscopy [37], adulteration of olive oil using fluorescence spectroscopy [16] and prediction of the sensory attributes of wine with an e-tongue [21]. PLS regression has been used previously for constructing models for numerical predictions in foods and beverages ([36,38,21,37]). In order to estimate the predictive power of a model, it must be validated [2]. The *k*-fold cross validation method involves splitting the data set randomly into training and test sets, the test sets comprised of a third of the samples, this results in less data to construct the model but more to test the quality of the model, preventing over fitting [2,21].

The objective of this study was to compare the correlation and the predictive capabilities of models pertaining to the bitterness intensity of DPHs constructed with data from physiochemical characterisation and analysis with an electronic tongue. For this, a collection of DPHs of sodium caseinate (NaCaH) or whey (WPH) were characterised by composition, degree of hydrolysis, SEC-HPLC, RP-HPLC and analysed by an electronic tongue. The data obtained by these methods was then correlated using PLS regression with bitterness scores for the samples obtained using a trained sensory panel.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

HPLC grade acetonitrile was purchased from Fisher Scientific (Pittsburgh, PA, USA). Trifluoroacetic acid (TFA), protein standards used for HPLC-SEC & all chemicals used for the sensory panel were of USP grade and were obtained from Sigma-Aldrich (Poole, Dorset, United Kingdom). Standard chemicals utilised for electronic tongue start up were supplied by manufacturer (Alpha M.O.S., Toulouse, France). Ultra pure Mili Q water for use with the HPLC and the electronic tongue was obtained using a Synergy UV Millipore system (Merck, Darmstadt, Germany).

2.1.2. Dairy protein hydrolysates

Eleven commercially available, spray dried and shelf-stable DPH were obtained from 3 international manufacturers. A further 8 DPH powders were produced to food grade specifications by a research partner (Moorepark technology Ltd., Teagasc). The dry samples were stored at 20 °C prior to analyses.

2.2. Methods

2.2.1. Compositional analysis

The protein composition of all samples was determined by Kjeldahl analysis [20]. the degree of hydrolysis was ascertained using the o-phthaldialdehyde (OPA) method [29] and all analyses were conducted in triplicate.

2.2.2. HPLC

All HPLC analysis was performed on Agilent 1200 HPLC system with a diode array detector (Agilent Technologies, Palo Alto, CA, USA).

2.2.2.1. Sample preparation. DPHs were solubilised to a concentration of 2% w/w in Mili Q H_2O and pure molecular weight standards (99% pure) used in SEC-HPLC were made to a concentration of 0.5% W/V. All samples were filtered through a 0.45 μm membrane filter (Whatman, GE Healthcare UK Limited, Buckinghamshire, United Kingdom) prior to injection.

2.2.2.2. Reverse phase HPLC (RP-HPLC) analysis. RP-HPLC analysis was performed using an Aeris widepore XB-C18 column (4.6 mm × 150 mm, particle size 3.6 μm,) connected to a C18 wide pore guard column (Phenomenex, Cheshire, UK). A binary solvent system was used Solvent A) 90% acetonitrile containing 0.1% w/w TFA and solvent B) Mili Q H_2O containing 0.1% w/w TFA. The separations were performed at 30 °C by gradient elution at a flow rate of 1 ml/min and an injection volume of 5 μl. The following mobile phase timed gradient schedule was applied: 0–5 min, held at 8% A; 5–60 min, 8 to 50% A; 60–65 min, 65% A; 65–70 min; 65 to 8% A. Eluting peaks were detected at 214 nm.

2.2.2.3. HPLC analysis—Size Exclusion Chromatography (SEC). SEC of the DPH samples were performed on a BioSep-SEC-S2000 (300 mm × 7.8 mm, particle size 5 μm) column with a Gel Filtration Chromatography guard column 4 × 3 mm (Phenomenex, Cheshire, UK) by isocratic elution at 30 °C and a flow rate of 1 ml/min, injection volume was 5 μl and detection was at 214 nm. The mobile phase was 0.1% w/w in TFA in acetonitrile/ Mili Q H_2O (45:55). A calibration curve was constructed for peptides within the range of 700–17,000 Da. The standards used were thyroglobulin, aprotinin, cyctochrome C, insulin, uridine, sodium azide, angiotensin I and II.

2.2.3. Sensory analysis

A sensory panel ($n=8$), with over 300 h of training and experience was used in this study, the advantage of the highly trained panel is that it allows the use of reduced numbers of panellists while maintaining panel accuracy [9]. Panel training included exercises using the 15 point spectrum intensity scale as outlined by Meilgaard et al., [27], where 5 corresponds to a weak and 15 to a very strong intensity. This method of training serves to hone the panellist's skills, acts as calibrating technique to check