Talanta 126 (2014) 46–53

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

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

J. Newman, T. Egan, N. Harbourne, D. O'Riordan, J.C. Jacquier, M. O'Sullivan *

Institute of Food and Health, School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland

ARTICLE INFO

Article history:
Received 7 October 2013
Received in revised form
7 March 2014
Accepted 17 March 2014
Available online 21 March 2014

Keywords:
Bitterness
Electronic tongue
HPLC
Sensory analysis
Dairy protein hydrolysates
Prediction

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

* Corresponding author. Tel.: +353 1 7162832; fax: +353 1 7161147.
E-mail address: michael.osullivan@ucd.ie (M. O'Sullivan).

http://dx.doi.org/10.1016/j.talanta.2014.03.036
0039-9140/

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

and control the panel's accuracy over time and allows the ranking of all attribute intensities on one universal 15 point scale. The panellists also trained specifically in assessing bitterness in real food samples.

All samples were rehydrated to 10% w/w concentration in bottled mineral water at room temperature and were presented to assessors in opaque, plastic lidded 100 ml sample cups which were labelled with a corresponding 3-digit code. No more than 4 samples were assessed in a day to avoid palate fatigue, water and crackers were supplied for palate cleansing between samples [24]. The panellists were asked to assign bitterness scores for each sample using the 15 point intensity scale; two bitter reference solutions of caffeine at intensities of 5 (4.11 mmol/l) and 15 (10.3 mmol/l) were supplied to panellists to aid scaling [27]. Panellists were required not to drink caffeinated beverages, smoke or wear perfumes or strong soaps prior to analysis. All samples were analysed by panellists in triplicate.

2.2.4. Electronic tongue (e-tongue)

The e-tongue employed in this study was the α-ASTREE II Liquid Taste Analyser (Alpha M.O.S., Toulouse, France). The e-tongue consists of an array of 7 potentiometric sensors with stirrer which is mounted around an Ag/AgCl reference electrode and a 48 position auto-sampler with 25 ml glass sample containers. The sensors used in this study were developed for food applications named as follows: ZZ, JE, BB, CA, GA, HA, JB. Each sensor has a different bi-polymer layered membrane, differences in sensor membrane composition affect its selectivity, making the sensor array cross selective to tastes [46]. The e-tongue is attached to an electronic unit and PC which records the difference in potential between the reference electrode and the sensors. Prior to analysis the e-tongue's sensors were conditioned and calibrated as per manufacturers guidelines [1].

The samples were reconstituted to 10% w/w concentration. Analysis of each sample consisted of the sensor array and reference electrode being immersed into 2 vials of milipore water, then into a 'pre-conditioning' sample (10 s each) before finally being immersed in the sample for analysis for 120 s where the last 20 s of the acquisition is used for data analysis. The acquisition was repeated 10 times, with first two acquisitions being discarded from statistical calculation due to fluctuating readings at the beginning of analysis session [1]. KCl (0.01 mol/l) was used as a reference sample in each analysis to allow comparison of results from different analysis session [45].

2.2.5. Statistical analysis

All data was processed using SAS® software (Version 9.3; SAS Institute Inc,Cary, USA). Sensory data was analysed using PROC GLM statement to calculate the analysis of variance with repeated means for the panel scores between replicates. Fishers least significant difference test was utilised for means separation with a confidence level of 95%. To interpret the data generated by the e-tongue, partial least square regression (PLS) was utilised. All PLS regressions were constructed using the PROC PLS statement. For model validation the *k*-fold random split cross validation and root mean predicted residual error sum of squares (PRESS) was used to compare the models predictive capabilities. The *k*-fold random split cross validation involves splitting 2/3 of the samples into the 'calibration set' and the other 1/3 into the 'test set'. This was repeated in SAS *K* times to ensure that all samples are featured in the test set and calibration set.

The values used to construct the PLS from RP-HPLC was to split each samples chromatogram into 24 segments by taking the sum of the absorbance readings taken every second over a 2.5 min intervals over a 60 min time frame. The SEC results were similarly processed by segmenting each samples chromatogram into 20 segments by calculating the sum of absorbance values every 1 kDa over a 20 kDa range. Prior to analysis with PLS the variables were scaled, by dividing by the square root of the sum of the variable squares [2].

3. Results

3.1. Bitterness and physicochemical characterisation

The 19 DPHs in this study had a protein content range from 75–90% and a broad range of DH values, from 5.5–60% (Table 1). The protein content of the DPHs varies as they were produced using different starting materials and from different manufacturers, nonetheless the protein levels are reflective of the whey and caseinates available commercially that are used as substrates [5,22,30] for the manufacture of DPHs. The NaCaHs samples have a more diverse DH range in comparison to the WPH samples with mean DH values of $17.19 \pm 17.19\%$ and $18.91 \pm 10.25\%$ respectively.

The DH level is an indicator of how much the native proteins have been hydrolysed, for example, NaCaH 1 with a DH of 5.5% is likely to contain a number of intact proteins and large peptides, whereas NaCaH 12 having a DH of 60%, will be composed mostly of free amino acids and small peptides which will effect the DPH's sensory character [23].

NaCaHs were significantly more bitter than WPH, with mean bitterness intensities of 9.7 ± 2.8 and 5.8 ± 2.7 respectivly ($p=0.0085$). The intense bitter taste elicited by NaCaHs has been previously reported in numerous studies [41,34,25]. It is suggested that the less intense bitter taste of WPHs is because in whey proteins the hydrophobic amino acids are buried in the interior of the globular molecule [42] and would need extensive hydrolysis in order to expose these hydrophobic amino acids to the tongue in comparison to the casein which have relatively little tertiary structure and requires less extensive hydrolysis to expose hydrophobic amino acids [26]. Another possible explanation for NaCaHs eliciting higher bitterness intensity than WPHs is that NaCaH have a relatively higher concentration of hydrophobic amino acids including proline. Proline is a hydrophobic amino acid which has

Table 1
Protein content, degree of hydrolysis (DH) and bitterness intensity of DPHs.

Sample	Total protein (%)	DH (%)	Bitter intensity assigned by sensory panel
NaCaH 1	88	5.1	3.27
NaCaH 2	89.91	5.2	8.77
NaCaH 3	86.62	5.5	12.88
NaCaH 4	87.53	7.2	11.08
NaCaH 5	89.62	9	12.06
NaCaH 6	89.62	9.6	11.13
NaCaH 7	86.34	9.6	7.46
NaCaH 8	90.6	10.3	11.5
NaCaH 9	87.23	15.2	12.25
NaCaH 10	89.32	25.5	10.33
NaCaH 11	82.94	42.4	8.04
NaCaH 12	76.56	60	6.88
WPH 1	80	7	1.56
WPH 2	90	7.8	7.33
WPH 3	75	15.6	4.09
WPH 4	90	18.4	5.04
WPH 5	80	19.5	6.02
WPH 6	80	30	6.03
WPH 7	90.28	34.1	10.3

been established to cause intense bitterness due to its alteration of the peptide chain in such a way as to give the ideal conformation to stimulate bitter taste receptors [19].

3.1.1. Correlation of bitterness with degree of Hydrolysis

The relationship between the bitterness of DPH's and DH has often been reported [7,42,22]. The bitterness scores and DH values of each DPH (Table 1) were plotted against each other as shown in Fig. 1. It is clear that for the collection of DPHs analysed in this study there is no correlation between DH and bitterness with an overall R^2 value of <0.01 and individual R^2 of 0.08 and 0.43 for the NaCaH and WPH sample sets respectively. In the case of WPH the two units of measurement correlate well for the least and most hydrolysed WPHs samples that are also the least (WPH1) and most (WPH7) bitter (Table 1). However, there is poor correlation for the intermediate samples e.g. WPH5 and 6, WPH6 is more extensivly hydrolysed than WPH5 with a difference in percentage DH of 10.5% however there was no significant difference in bitterness as perceived by the sensory panel. There appears to be little correlation between DH and bitterness intensity in the NaCaH's. Furthermore, there was no significant difference between the mean DH values of the two groups of hydrolysates ($p=0.64$), although the sensory panel found the NaCaH's to be significantly more bitter than WPHs.

Ney [28] hypothesised that only small to medium peptides elicit a bitter taste. Therefore, it had been reasoned that DH may act as an indicator to the reduction of molecular weight of peptides in a DPH as a result of enzymatic hydrolysis and ergo bitterness intensity. However, there are a few factors which could contribute to a lack of correlation between DH and the molecular weight profile of a DPH. Firstly, extensive hydrolysis may lead to de-bittering of the peptides [39,12], as when a DPH is extensively hydrolysed degradation of bitter peptides to non-bitter free amino acids may occur. Secondly, the hydrolysates were produced using different starting materials, manufacturing processes and starter enzymes/cultures which will greatly affect the types of peptides produced and therefore the taste profile of the DPHs. The enzymes used for hydrolysis will also affect the types of peptides produced i.e. exopeptidases or endopeptidases. Exopeptidases, will cleave peptide bonds at the N or C termini, creating free amino acids with minimal alteration to the residual peptide molecular weights resulting in peptides which are too large to bind with the bitter taste receptor. Alternatively, hydrolysis to the same DH% with endopeptidases, which cleave peptide bonds in the interior of the peptide chain, is likely to result in an overall reduction in molecular weight profile, potentially giving rise to small and medium sized peptide chain which can be intensely bitter [32].

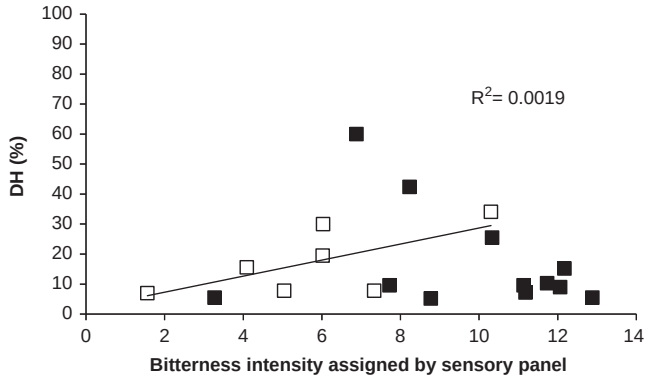

Fig. 1. Degree of hydrolysis (DH) and bitterness intensity scores of NaCaH (■) and WPH (□) on a 15 point intensity scale assigned by a trained sensory panel.

With these factors considered, DH alone is a poor predictor of bitterness intensity in DPHs.

3.1.2. Bitterness prediction model using SEC-HPLC

DPHs were analysed by SEC-HPLC to determine the molecular weight distributions of peptides present. The chromatograms shown (Fig. 2) are examples of the peptide molecular weight profiles of NaCaHs (Fig. 2A and B) and WPHs (Fig. 2C and D) that have relatively high and low bitter values. As discussed in the previous section, there was some correlation between DH and bitterness of selected samples and it can be observed from the chromatograms that with the increase bitterness values (Table 1) there is also a shift to a lower molecular weight range, this was most pronounced for WPH1 and WPH7 as they differ the most in bitterness and their also DH.

Intact caseins have molecular weights in the range of 19 to 24 kDa, in the case of the least bitter NaCaH sample, NaCaH1 with a bitterness score of 3.27 (Fig. 2.A), (Table 1), $>80\%$ of the eluting species have molecular weights within the size range of 2.75–17.5 kDa. Comparing this to the more bitter NaCaH9 sample (bitterness score of 12.25) the largest peak area in the sample, accounting for 49% of the total area, eluted in the size range of 2–5 kDa indicating that the native casein proteins have been further reduced in size by extended hydrolysis. A similar trend is seen in the WPH's (Fig. 2C and D), WPH1 and WPH7 are the least and most bitter and also have low and high molecular size ranges. Intact whey proteins are comprised of 60% β-lactoglobulin, 18 kDa, and 20% α-lactalbumin, 14 kDa [4]. For the least bitter WPH sample, the largest peak in the samples chromatogram, accounting for 61.5% of the total peak area, elutes within the size range of 6.8–17.65 kDa with some much smaller peaks having masses less than 6.8 kDa (Fig. 2C) also being observed. As was observed for the NaCaH, in the case of the more bitter WPH the overall molecular weight profile of the hydrolysate was decreased relative to the les bitter sample. In WPH7 which had a bitterness score of 10.3 and a DH of 34.1% over 45% of the eluting species have a molecular weight of <4.6 kDa (Fig. 2D) indicating that the intact proteins have been substantially degraded. The selected chromatograms suggest that with an increase of bitterness there is association with a decrease in molecular weight. It was therefore hypothesised that SEC-HPLC might be used to predict the bitterness of DPHs using PLS regression.

It can be observed from Fig. 2., there is such a broad range of free amino acids and peptides of differing sizes in the DPHs that to take an average molecular weight would be misrepresentative of the sample. Therefore PLS regression was conducted by using the sum of the absorbance eluting in each 1 kDa interval as independent variables and the bitterness scores assigned from the sensory panel. The resultant PLS regression is shown in Fig. 3, there is a trend between the actual and predicted bitterness values and the correlation is reasonable with an $R^2=0.73$ but a relatively high root mean PRESS of 0.69. SEC-HPLC data has not been used previously correlated with the bitterness of DPHs, the moderate correlation and prediction shows the potential of using the size distribution of peptides of DPHs as predictors of bitterness may have some potential for future analysis.

3.1.3. Bitterness prediction model using RP-HPLC

RP-HPLC was conducted to characterise the relative hydrophobicity of each of the DPHs. Shown are the chromatograms of the same samples shown in the SEC prediction section i.e. NaCaH and WPH with high and low bitterness values, NaCaH 1 and 9 (Fig. 4A and B), and WPH 1 and 7 (Fig. 4C and D). In the case of the more bitter samples, NaCaH9 and WPH7, a substantial number of peaks eluted within the first ten minutes. In comparison, in the case of the less bitter samples, NaCaH1 and WPH1, the samples elute gradually over the analysis time with the largest peaks eluting late

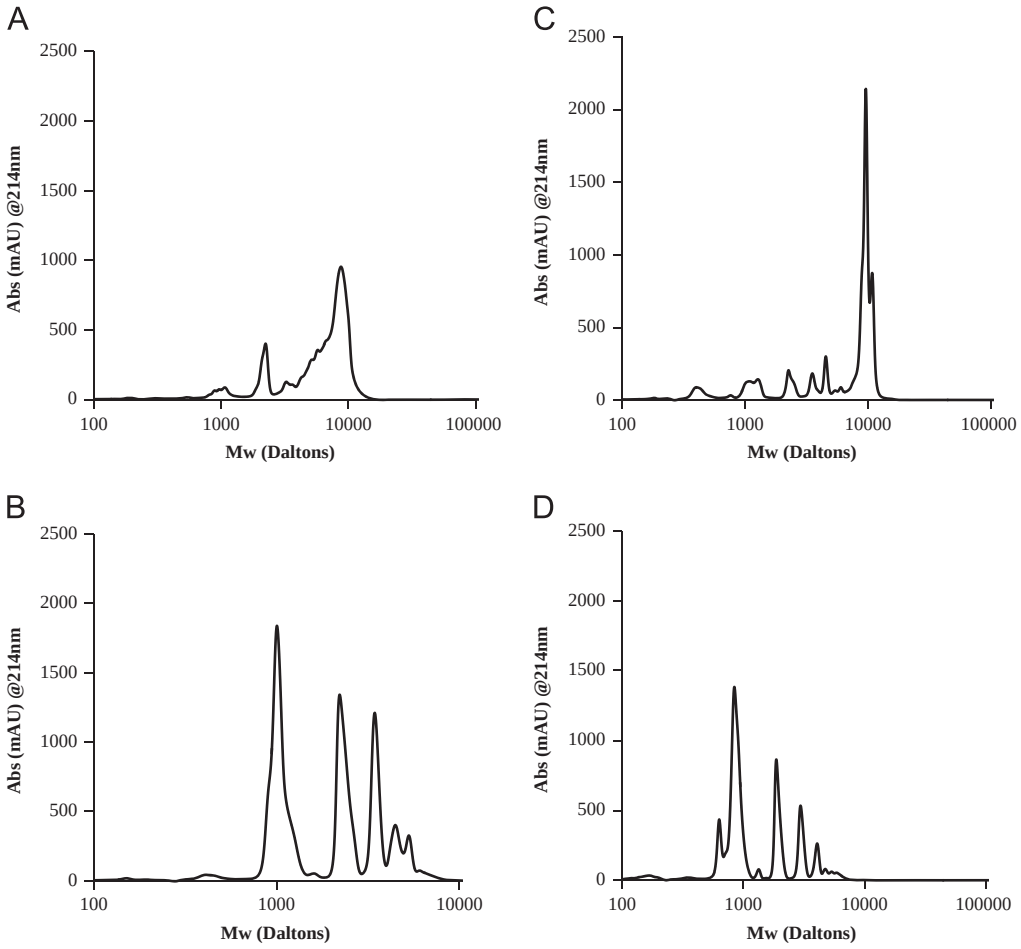

Fig. 2. Selected SEC-HPLC chromatogram of (A) NaCaH 1. (B) NaCaH 9 (C) WPH 1 and (D) WPH 7 as examples of the molecular weight profiles of DPHs of high and low bitterness intensities.

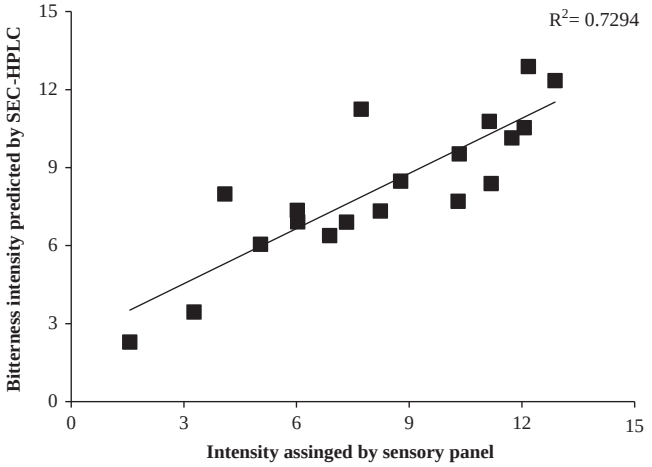

Fig. 3. PLS regression constructed using bitterness intensity values from a trained sensory panel correlated with SEC-HPLC data.

in the run e.g. the largest percentage peak area of WPH 1, which had bitterness intensity of 1.56, eluted between 50 min and 60 min. The elution profile of the most bitter samples suggests that they contain a higher proportion of hydrophilic peptides relative to the less bitter samples. It may be that these early eluting peaks are mainly free amino acids and dipeptides that are not retained by the stationary phase [15].

As the hydrolysis process can lead to the release of hydrophobic peptides from the native protein with strong bitter tastes (that would otherwise be buried inside the internal structure of the native protein) it was hypothesised that the relative hydrophobicity assessed by RP-HPLC of a DPH may be used as an indicator for bitterness. However, as it can be seen from Fig. 4. the chromatograms contain a plethora of free amino acids and peptide chains with a range of relative hydrophobicities. Therefore a relative hydrophobicity value was not calculated as it would not be a true reflection of the relative hydrophobicity of the assortment of free amino acids and peptides present in each sample. Accordingly the data obtained from RP-HPLC was analysed similarly to the SEC-HPLC data, by segmenting the chromatograms and treating them as separate variables, this data was then correlated to bitterness scores from the trained sensory panel (Table 1) using a PLS regression (Fig. 5).

The correlation between the two methods of analysis was good with an $R^2=0.76$ and a root mean PRESS of 0.55. As the correlation and root mean PRESS of the model built with RP-HPLC data is moderately stronger than SEC-HPLC it can be concluded that using the data pertaining to the relative hydrophobicity of DPHs gives a slightly better prediction of bitterness than that based on size. RP-HPLC has previously been utilized to characterise the hydrophobicity of dairy peptides for profiling of cheese extracts for authenticity and process optimisation [12,31]. Gomez et al., [15] has previously established a relationship between the hydrophobicity of peptides in cheese extracts, as quantified by RP-HPLC, and the bitterness of cheese using linear regression and found hydrophobicity to be a reliable indicator of bitterness.

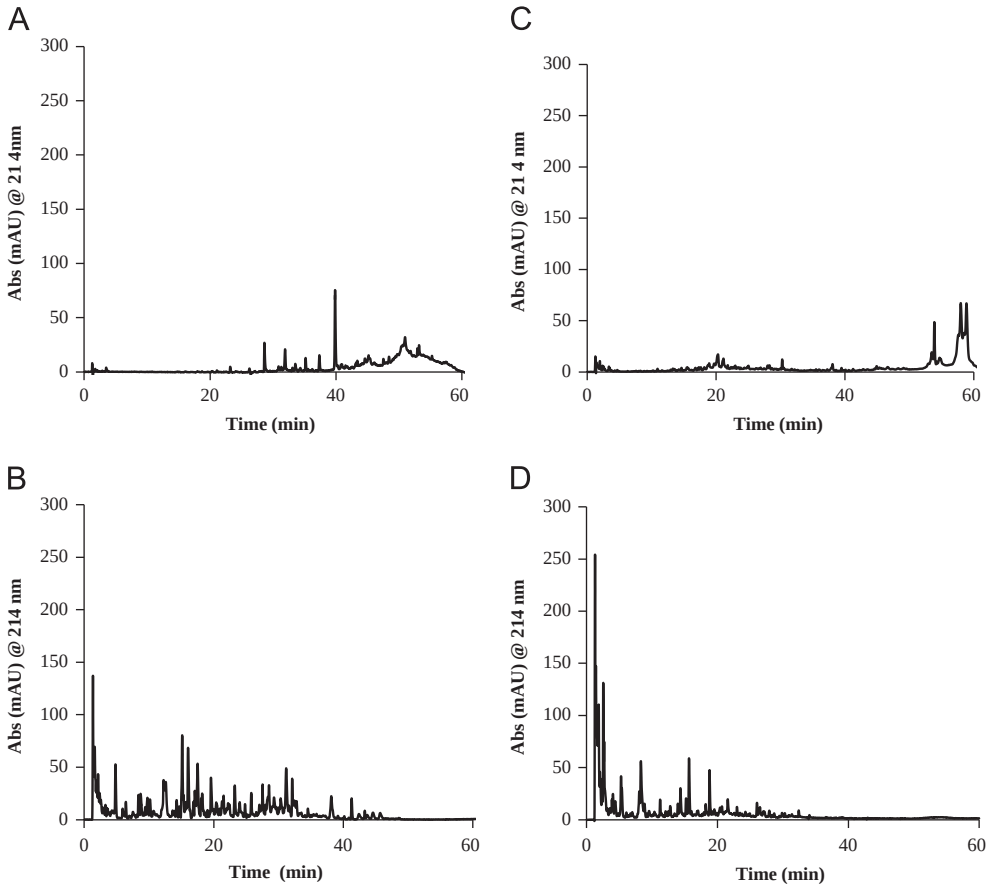

Fig. 4. RP-HPLC chromatogram of (A) NaCaH 1 (B) NaCaH 9 (C) Whey 1 and (D) Whey 7 as examples of the relative hydrophobicity of DPHs of high and low bitterness intensities.

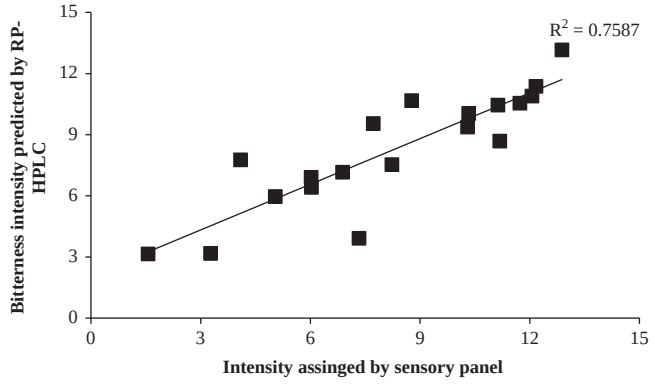

Fig. 5. PLS prediction model constructed from bitterness intensity values from a trained sensory panel correlated with RP-HPLC data.

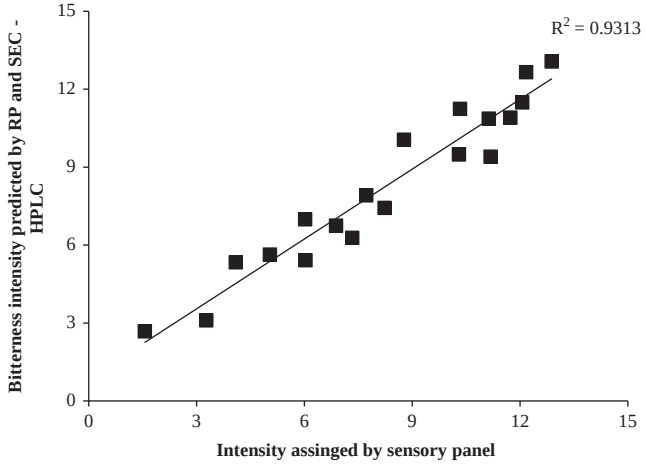

Fig. 6. PLS prediction model constructed with bitterness intensity values from a trained sensory panel correlated with data from RP-HPLC and SEC-HPLC.

3.1.4. Bitterness prediction model using combined SEC- HPLC and RP-HPLC data sets

The data obtained by SEC and RP-HPLC was combined into a larger data set for analysis with PLS regression, the resultant PLS regression had a stronger correlation than previously observed using SEC or RP alone with an R^2 of 0.93 and a relatively low root mean PRESS of 0.55 (Fig. 6). The advantage of the model constructed with both sets of data is that it encompasses two physiochemical characteristics which are thought to be linked to bitterness, both size and relative hydrophobicity [28]. The high R^2 value and the results of the *K*-fold random split cross validation test indicates that the combination of size and hydrophobicity provides a good method of predicting bitterness in DPHs.

3.2. Bitterness prediction model using a commercially available e-tongue

The bitterness intensity scores generated by the trained sensory panel, shown in Table 1, were also to construct a PLS regression with the output from the e-tongue, a device specifically developed to mimic the human taste response. The resultant PLS is shown in Fig. 7 and shows a strong correlation between the e-tongues transformed data and the bitterness scores from the sensory panel with an R^2 of 0.81 and root mean PRESS of 0.43. While the model with the correlation of the combined SEC and RP-HPLC data had the

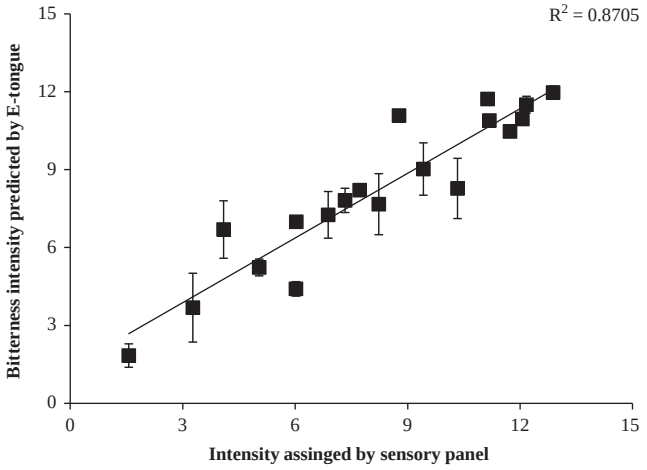

Fig. 7. PLS prediction model constructed with bitterness intensity values from a trained sensory panel correlated with the response of the e-tongues sensors.

highest R^2 in the study, the e-tongues lower root mean PRESS indicates it has the best predictive capabilities [10].

Using PLS regression to correlate the sensory information generated by a trained panel and the output of an e-tongue has been demonstrated before in complex food samples to successfully predict the sensory attributes. [38] used PLS to correlate an in house constructed e-tongue with a trained sensory panel for comprehensive sensory profiling of beer sensory attributes including bitterness for which an R^2 value of 0.95was obtained. Until recently, the few e-tongue studies that feature the analysis of protein rich food samples have been focused on the technologies discriminatory capabilities rather than correlation with a sensory panel in the assessment of a specific attribute or to predict the intensity of that attribute. Such studies include applications such as identifying samples of goats milk that have been adulterated with bovine milk [8] or discrimination between different peptide isolates from puffer fish [47]. There is one quantitative study by Hruskar et al., [18] that correlates the overall sensory quality of probiotic fruit-flavoured fermented milks as rated by a trained sensory panel and the output of the same e-tongue as employed in the current study; a high correlation between the e-tongue and the scores of the sensory panel for a number of sensory attributes in that matrix was observed. The results of the current study demonstrate the ability of a number of objective analytical methods to predict bitterness of protein rich, complex food samples. Further refinement of such predictive models based on instrumental data may afford the opportunity to further reduce dependence on subjective analysis using sensory panels for evaluation of bitterness in foods.

However, it should also be noted that while bitterness is a key sensory defect in DPHs that needs to be monitored, there are many off tastes and flavours which can also develop during manufacture. In order to develop predictive model for the full sensory profiling of DPHs both the e-tongue and HPLC methods would need to be used in cooperation with other methods which focus on the volatile aspect of flavour, such as gas chromatography, electronic nose and further sensory analysis.

4. Conclusion

Data obtained from an electronic tongue and the HPLC analyses were transformed and correlated with the bitterness intensity scores of DPHs. The PLS regression model constructed with the combination of SEC and RP-HPLC data had the best correlation with the bitterness intensities as scored by a sensory panel but the model based on the e-tongue was shown to have the strongest predictive capabilities. As a technique the e-tongue has the advantage over analysis by HPLC that it requires less laborious sample preparation and shorter analysis time. However, the two technologies have comparable costs and HPLC has the convenience of having a more flexible range of application over the electronic tongue. Both methods of analysis have potential to be used as a means of evaluating bitterness in dairy protein hydrolysates.

References

[1] Anon, Alpha M.O.S α-Astree e-Tongue Technical Notes 2010.
[2] L.A. Berrueta, R.M. Alonso-Salces, K. Heberger, J. Chromatogr. A 1158 (2007) 196–214.
[3] R.N. Bleibaum, H. Stone, T. Tan, S. Labreche, E. Saint-Martin, S. Isz, Food Qual. Pref. 13 (2002) 409–422.
[4] C.M. Bryant, D.J. McClements, Trends Food Sci. Technol. 9 (1998) 143–151.
[5] M.E. Carunchia-Whetstine, A.E. Croissant, M.A. Drake, J. Dairy Sci. 88 (2005) 3826–3839.
[6] P. Ciosek, Talanta 76 (2008) 548–556.
[7] K.M. Clegg, A.D. Mcmillan, Int. J. Food Sci. Technol. 9 (1974) 21–29.
[8] L.A. Dias, A.M. Peres, A.C.A. Veloso, F.S. Reis, M. Vilas-Boas, A.A.S.C. Machado, Sens. Actuators B: Chem. 136 (2009) 209–217.
[9] M.A. Drake, J. Dairy Sci. 90 (2007) 4925–4937.
[10] K.H. Esbensen, D. Guyot, F. Westad, L.P. Houmoller, Multivariate Data Analysis: In Practice: an Introduction to Multivariate Data Analysis and Experimental Design, Ed. 5, Camo Process AS, New Jersey, 2002.
[11] L. Escuder-Gilabertand, M. Peris, Anal. Chim. Acta 665 (2010) 15–25.
[12] V. Fallico, P.L.H. Mcsweeney, J. Horne, C. Pediliggieri, J.A. Hannon, S. Carpino, G. Licitra, J. Dairy Sci. 88 (2005) 1288–1300.
[13] C.S. Favaro-Trindade, A.S. Santana, E.S. Monterrey-Quintero, M.A. Trindade, F.M. Netto, Food Hydrocoll. 24 (2010) 336–340.
[14] F. Favati, N. Condelli, F. Galgano, M.C. Caruso, Food Chem. 139 (2012) 949–954.
[15] M.J. Gomez, S. Garde, P. Gaya, M. Medina, M. Nuňez, J. Dairy Res. 64 (1997) 289–297.
[16] F. Guimet, J. Ferre, R. Boque, Anal. Chim. Acta 544 (2005) 143–152.
[17] B. Hernández-Ledesma, M. Del Mar Contreras, I. Recio, Adv. Colloid Interface Sci. 165 (2011) 23–35.
[18] M. Hruskar, N. Major, M. Krpan, Talanta 81 (2010) 398–403.
[19] N. Ishbahhi, M. Chino, H. Fukui, I. Shinoda, E. Kikuchi, H. Okai, S. Fukui, Agric. Biol. Chem. 52 (1988) 95–98.
[20] ISO/DIS 8968-1, Milk and milk products—Determination of nitrogen content and crude protein calculation, Kjeldahl principle.
[21] D. Kirsanov, O. Mednova, V. Vietoris, P.A. Kilmartin, A. Legin, Talanta 90 (2012) 109–116.
[22] P.P. Leksrisompong, R.E. Miracle, M.A. Drake, J. Agric. Food Chem. 58 (2010) 6318–6327.
[23] G.A. Linde, A.J Laverde, E.V.D. Faria, N.B. Colauto, F.F.D. Moraes, G.M. Zanin, Food Res. Int. 42 (2009) 814–818.
[24] M.A.D. Listiyani, R.E. Campbell, R.E. Miracle, L.O. Dean, M.A. Drake, J. Dairy Sci. 94 (2011) 4347–4359.
[25] K. Maehashi, M. Matano, H. Wang, L.A. Vo, Y. Yamamoto, L. Huang, Biochem. Biophys. Res. Commun. 365 (2008) 851–855.
[26] D.J. McMahon, B.S. Oommen, J. Dairy Sc. 91 (2008) 1709–1721.
[27] M.C. Meilgaard, G.V. Civille, T. Carr, Sensory Evaluation Techniques, Ed. 4, CRC Press, Michigan, 2007.
[28] K.H. Ney, Bitterness of peptides: amino acid composition and chain length, in: C. Boudreau (Ed.), Journal of Chemistry, American Chemical Society Symposium Series, Food taste chemistry, Washington, 115, 1979, pp. 149–171.
[29] P.M. Nielsen, D. Petersen, C. Dambmann, J. Food Sci. 66 (2001) 642–646.
[30] J. O'Regan, D.M. Mulvihill, Food Res. Int. 43 (2010) 224–231.
[31] E. Parente, H. Patel, V. Caldeo, P. Piraino, P.L.H. McSweeney, Food Chem. 131 (2012) 1552–1560.
[32] O. Park, J.C. Allen, J. Dairy Sci. 81 (1998) 2858–2865.
[33] M. Pein, C. Eckert, M. Preis, J.R. Breitkreutz, J. Pharm. Biomed. Anal. 83 (2013) 157–163.
[34] J.P. Pélissier, P. Manchon, J. Food Sci. 41 (1976) 231–233.
[35] A.M. Peres, L.G. Dias, A.N.C. Veloso, S.G. Meirinho, J.S.A. Morais, A.A.S.C. Machado, Talanta 83 (2011) 857–864.
[36] J.M. Poveda, A. García, P.J. Martín-Alvarez, L. Cabezas, Food Chem. 84 (2004) 29–33.
[37] M. Romera-Fernández, L.A. Berrueta, S. Garmón-Lobato, B. Gallo, F. Vicente, J.M. Moreda, Talanta 88 (2012) 303–310.
[38] A. Rudnitskaya, E. Polshin, D. Kirsanov, J. Lammertyn, B. Nicolai, D. Saison, F.R. Delvaux, F. Delvaux, A. Legin, Anal. Chim. Acta 646 (2009) 111–118.
[39] B.C. Saha, K. Hayashi, Biotechnol. Adv. 19 (2001) 355–370.
[40] K.A. Saunders, A. Legin, Anal. Chim. Acta 770 (2013) 45–52.
[41] T. Shiba, K. Nunami, Structure of a bitter peptide in casein hydrolyzate by bacterial proteinase, Tetrahedron Letters 15 (1974) 509–512.
[42] D. Spellman, G. O'Cuinn, R.J. Fitzgerald, Food Chem. 114 (2009) 440–446.

[43] Y. Wang, Y. Feng, Y. Wu, S. Liang, D. Xu, Fitoterapia 86 (2013) 137–143.
[44] F. Winquist, C. Krantz-Rülcker, I. Lundström, K. Östergren, T. Skoglund, Sens. Actuators B: Chem. 111–112 (2005) 299–304.
[45] K. Woertz, C. Tissen, P. Kleinebudde, J. Breitkreutz, J. Pharm. Biomed. Anal. 51 (2010) 497–506.
[46] K. Woertz, C. Tissen, P. Kleinebudde, J. Breitkreutz, J. Pharm. Biomed. Anal. 55 (2011) 272–281.
[47] M.X. Zhang, X.C. Wang, Y. Liu, X.L Xu, G.H. Zhou, Food Chem. 135 (2012) 1463–1470.