

Identity Confirmation of a Branded, Fermented Cereal Product by UV Spectroscopy: A Feasibility Study Involving a Trappist Beer

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ABSTRACT

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Brand protection is important for a food processor; trust in brand identity is essential for consumer confidence. In this study, the combination of UV spectroscopy and multivariate mathematics was investigated to confirm the identity of a processed cereal product—a Trappist beer. Samples (52) of Rochefort 8, other Rochefort and non-Rochefort beers, diluted 1:100 with distilled water, were analysed by UV spectroscopy (220–400 nm). Partial least squares discriminant analysis (PLS-DA) and soft independent modelling of class analogy (SIMCA) multivariate methods were applied separately to confirm the identity of Rochefort 8 beer. Spectral data were analysed in both raw and standard normal variate (SNV) pre-treated forms. Using PLS-DA, a two-stage modelling procedure was applied involving initial classification as either Rochefort or non-Rochefort followed by classification within the Rochefort class as Rochefort 8 or non-Rochefort 8. Correct classification rates for these two steps were (a) 100 and 94.4% and (b) 90.9 and 100% respectively. Applying the 3 principal component SIMCA model to the 8 test Rochefort and all of the non-Rochefort ($n = 36$) samples, 6 of the 8 Rochefort beers were correctly classified (75%; $p = 0.05$) but only two of the 36 non-Rochefort beers were wrongly classified as Rochefort (correct classification rate of 94.4%).

Key words: beer, chemometrics, identification, Trappist, UV spectroscopy.

INTRODUCTION

Consumer confidence in food depends to a significant degree on trust in declarations and claims made on food labels. To assure consumers that purchased products meet all declared claims regarding characteristics and properties, such as variety of food and its origin, there is a requirement for confirmatory analytical procedures addressing authenticity issues. Problems relating to food authenticity may arise accidentally by mis-labelling or by deliberate food or ingredient substitution which is a form of economic fraud. Many analytical techniques are available for food authenticity confirmation and quality control; among these are spectroscopic methods (UV, NIR,

MIR, visible, Raman, fluorescence, atomic), mass spectrometry (IRMS, ICP-MS, PTR-MS, GC-MS), separation techniques (GC, HPLC, CE), electronic nose, DNA-based technology (PCR), immunological technology (ELISA) and thermal analysis^{8,11}. Among these techniques, only very few examples of UV spectroscopy applications in food authenticity and quality control have been published^{2,4,7}; these examples have involved tequila, broccoli and olive oil authentication and have revealed the technique's potential in addressing such problems. UV spectroscopy covers the wavelength ranges 190–380 nm; it possesses the practical advantages of other vibrational spectroscopic techniques which include ease of sample presentation, non-destructive nature of the analysis, simplicity of use and speed. In common also, however, is the requirement for multivariate data analysis to extract useful information from spectra that often tend to be limited in specific features.

Many reports exist in the literature concerning detection of food adulteration and confirmation of geographic origin of foodstuffs^{6,13}. However, in relation to processed foods, the verification of claimed brand identity represents a continued challenge for analysts; a counterpart to such analysis is the use of similar techniques by branded food manufacturers to verify conformance to specification of finished goods. Beer is a fermented cereal product which is marketed heavily on a brand basis and therefore requires analytical techniques to confirm such identity claims.

The main ingredients of brewing are grains (mainly barley), water, hops and yeast. Additionally, some other components, such as sugar cane, honey, wheat, spices, herbs, fruits, etc. may be used to add distinctive characteristics while types and varieties of the ingredients also influence final product quality¹. Beers can be classified based on (i) fermentation type (ales or lagers), (ii) alcohol content, (iii) principal grist ingredients (wheat and sorghum beers), (iv) production origins (Pilsen, Burton ale, Irish stout) and (v) technological influences (dry beer, light beer and ice beer)⁵. Moreover, in Europe, some specific beers are brewed within Trappist monasteries, the so-called Trappist beers; these are entitled to carry a logo which is designed to reassure the consumer that the beer is produced in one of these breweries according to traditional recipes and techniques; this branding therefore carries implicit messages of quality and tradition. Only seven beers brewed under the control of Trappist monks may legally claim to be “Authentic Trappist” products—six are

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brewed in Belgium (branded as Achel, Chimay, Orval, Rochefort, Westmalle and Westvleteren) and the other in the Netherlands (branded as La Trappe)¹². They are all ales, bottle-conditioned and mostly high in alcohol content. The characteristics of Trappist beers are closely matched by Abbey beers, which are not Trappist, yet have many of the features of Trappist beers¹⁰.

This report has focused on one specific food authenticity issue involving beer, namely the use of UV-VIS spectroscopy to confirm a claimed brand identity. In particular, work has focussed on whether this technique can confirm the claimed identity of a specific Trappist beer—Rochefort 8. During the investigation, several discriminant models were developed to achieve this goal. In order to make this discrimination, UV spectral data were processed by a number of advanced multivariate mathematical (chemometric) tools.

MATERIALS AND METHODS

Beer samples

Fifty-two (52) bottles of both Trappist and non-Trappist beers were analysed in this feasibility study. The samples were sourced by staff at a Belgian laboratory (CRA-W, Gembloux) as part of an EU-funded research project (www.trace.eu.org) and transported to AFRC, Dublin by air. Of the Trappist beers collected, 47 were produced in Belgium while 5 were sourced from a single brewery in The Netherlands; Trappist beers included Rochefort 8 (n = 11) and other Rochefort (n = 5). A summary of the details

of beer types, country of origin and numbers of samples is shown in Table I. Samples were collected from different production batches of each beer; all samples were ales and their colour ranged from dark to light. Bottles were stored in the dark at 3–4°C between receipt and spectroscopic analysis, a period of several months; prior to analysis, samples were removed from the cold and equilibrated to room temperature (20°C approximately) overnight. Beer bottles were carefully uncapped and allowed to settle undisturbed for 5 min before a sample was withdrawn, from approximately halfway down each bottle, using a disposable Pasteur pipette. For collection of UV spectra, all samples were diluted to 1:100 with distilled water to avoid detector saturation.

UV spectroscopy

Spectra in the wavelength range 190–1,100 nm were collected using a Shimadzu Model UV-1700 instrument (Shimadzu Corporation, Japan); this double-beam instrument used both a deuterium (190–364 nm) and tungsten halogen (364–1,100 nm) light source. Baseline correction and wavelength accuracy checks were performed prior to each scanning session according to the manufacturer's instructions. Instrument control and spectral collection was performed using UVProbe software supplied by the instrument manufacturer. Spectra of diluted beer were referenced against a water blank at room temperature. Only data in the ultraviolet range (220–400 nm) were studied in this work. Spectra were recorded in triplicate with re-sampling from the diluted extract and averaged before data analysis.

Table I. Summary table of beer brands, type, number of samples and country of origin.

Brand name	Type	Number of samples	Origin
Chimay (Blue)	Trappist	2	Belgium
La Trappe (Triple)	Trappist	1	Netherlands
Orval	Trappist	1	Belgium
Westvleteren 12	Trappist	1	Belgium
Gueuze Girardin 1882	Non-Trappist	2	Belgium
Duvel	Non-Trappist	1	Belgium
Hapkin	Non-Trappist	1	Belgium
Hoegarden Grand Cru	Non-Trappist	2	Belgium
Hotteuse Grand Cru	Non-Trappist	1	Belgium
Lefte 9'	Non-Trappist	1	Belgium
Moinette Bruin	Non-Trappist	1	Belgium
St Feuillien (triple)	Non-Trappist	1	Belgium
Vondel	Non-Trappist	1	Belgium
Achel (Blond)	Trappist	2	Belgium
Chimay (Rouge)	Trappist	1	Belgium
Brugges Tripel	Non-Trappist	1	Belgium
Grimbergen Doree	Non-Trappist	1	Belgium
Gouden Corolus Tripel	Non-Trappist	1	Belgium
St Bernardus Prior 8	Non-Trappist	1	Belgium
Kwak	Non-Trappist	1	Belgium
Triple Karmeliet	Non-Trappist	2	Belgium
Lefte Bruin	Non-Trappist	2	Belgium
Val-Dieu (Triple)	Non-Trappist	1	Belgium
Charles Quint	Non-Trappist	1	Belgium
Moinette Blonde	Non-Trappist	1	Belgium
Jupiler	Non-Trappist	1	Belgium
La Trappe (Blanche)/Witte Trappist	Trappist	2	Netherlands
La Trappe (double)	Trappist	1	Netherlands
La Trappe (Quadruple)	Trappist	1	Netherlands
Rochefort 8	Trappist	11	Belgium
Rochefort 10	Trappist	5	Belgium

Multivariate data analysis

The Unscrambler software (version 9.7; CAMO, Norway) was used for all chemometric data analyses. Spectral files were exported from UVProbe software in ASCII format for importation into The Unscrambler. Spectral collections were examined using principal component analysis (PCA) to identify any sample clustering or possible outlying spectra. One spectral pre-treatment was examined for its effect on calibration accuracy—standard normal variate (SNV). Standard normal variate³ centres and scales individual spectra without any external reference point; this has the effect of removing complications arising from light scatter or other interfering phenomena. Two-class discrimination was studied using discriminant partial least squares regression (D-PLS). In this case, each sample was assigned a dummy Y-variable given a value of either 0 or 1 for each of the two classes involved; PLS

calibrations were developed to predict this dummy Y-variable and samples with predicted values of <0.5 were ascribed to the class 0; correspondingly, samples with a predicted value ≥ 0.5 were ascribed to the class 1. SIMCA (soft independent modelling of class analogy) was investigated as a class-modelling method for identity confirmation; classifications were made at a 5% confidence level. In view of the limited number of samples available, all calibrations were developed and validated using a leave-one-out cross-validation procedure. For further details on these chemometric methods, see Naes et al.⁹

RESULTS AND DISCUSSION

UV spectra

Absorption spectra of all 52 beer samples over the wavelength range 190–1,100 nm (911 variables) collected

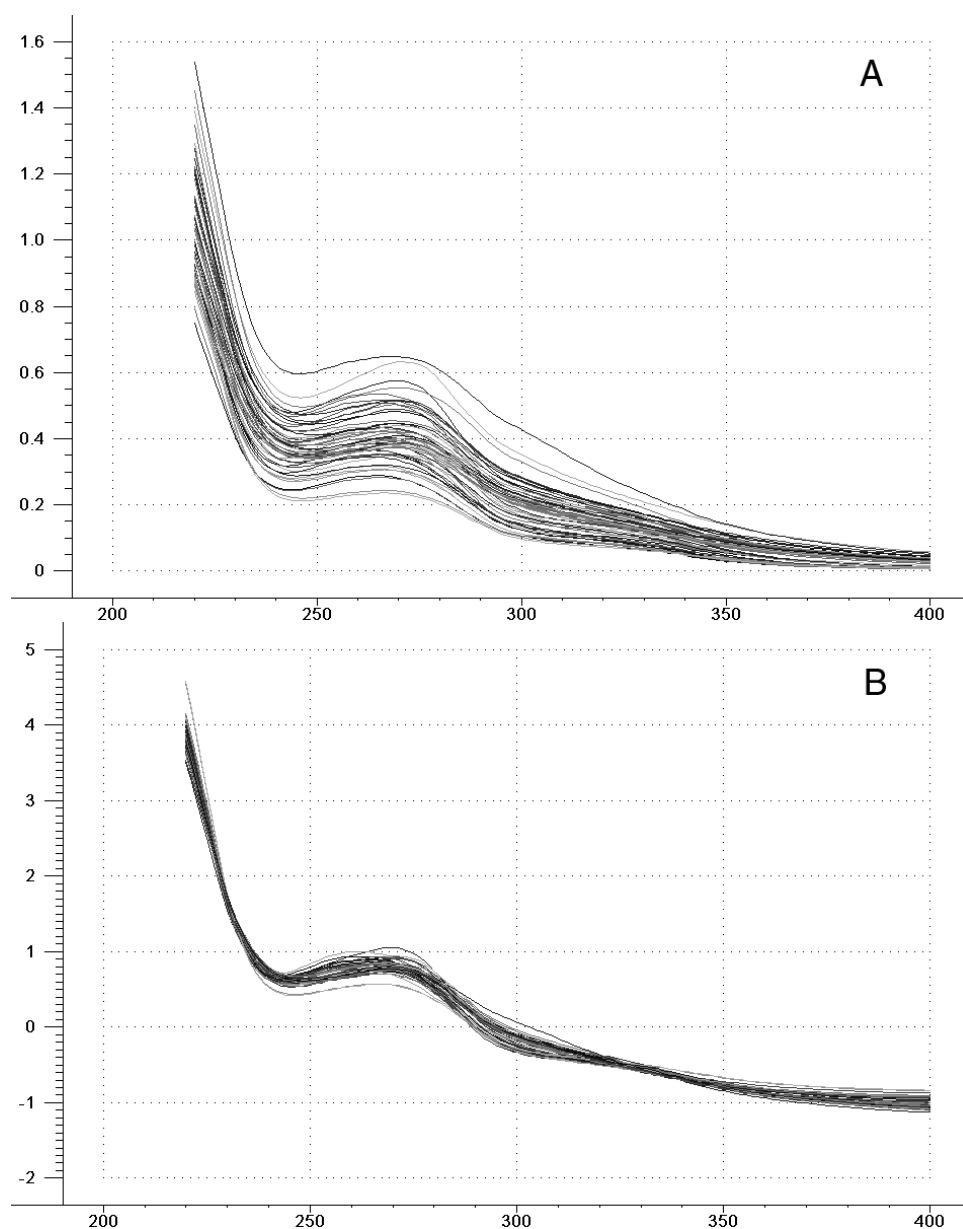


Fig. 1. Spectra of all beer samples (A) raw spectra 220–400 nm; (B) SNV-treated spectra 220–400 nm.

at different dilutions (1:50, 1:100 and 1:200) were studied in order to select an appropriate dilution for beer analysis. In this case, a 1:100 dilution was chosen since this produced UV spectra with a maximum absorption below approximately 1.5, thereby maximising the likelihood of detector response linearity in this spectral range. A consequence of this dilution was that little meaningful absorbance was detected in the visible wavelength range. During chemometric analysis therefore, to avoid regions of high noise and low absorbance, the wavelength range studied was attenuated to 220–400 nm, the ultraviolet range (Fig. 1a). A standard normal variate (SNV) pre-treatment was also applied to the raw spectra in order to remove interfering spectral effects arising from factors such as light scatter—this resulted in the spectra shown in Fig. 1b.

These spectra reveal little in the way of discrete features; apart from a rapidly descending absorbance from below 200 nm, the main feature is a broad maximum centred around 270 nm. A reduction in overall variance in the spectral dataset is apparent after SNV-treatment while the presence of some structure is apparent after data transformation by a second derivative pre-treatment. The only molecular moieties likely to absorb light in the 200 to 800 nm region are π -electron functions and heteroatoms having non-bonding valence-shell electron pairs, commonly referred to as chromophores. They include groups such as C=C, C \equiv C, C=O, N=O and C-X, where X = Br or I.

Multivariate data analysis

Prior to the discriminant modelling process, the spectral dataset was explored using principal component analysis (PCA). The results of this exploration are described below on the basis of a number of possible segregations by simply labelling samples in the relevant plots separately.

Representing the sample scores as Trappist (symbol 1) or non-Trappist (symbol 0) beers, results based on PC1 and PC2 are illustrated in Fig. 2. In this model, it can be seen from the score plot (Fig. 2a) that the two groups of samples could not be clearly distinguished from each other; it is also apparent that samples belonging to each category do not cluster neatly together and there is some suggestion that sub-clusters within each class may exist. In general, however, samples of class 0 tend to inhabit the top left-hand section of the PC1 and 2 score space with class 1 samples being mainly found in the opposite section. The loading plots of PC1 (Fig. 2b) and PC2 (Fig. 2c) illustrate a number of features. Separation of samples from left to right along PC1 is effected mainly on the basis of the overall absorbance level of the beers because the pattern of the PC1 loading resembles an average beer spectrum; the main separation on PC2 arises from competing spectral features which may be seen in Fig. 2c. Here, spectral data <250 nm produce positive influences on sample scores while absorbances >250 nm tend to have

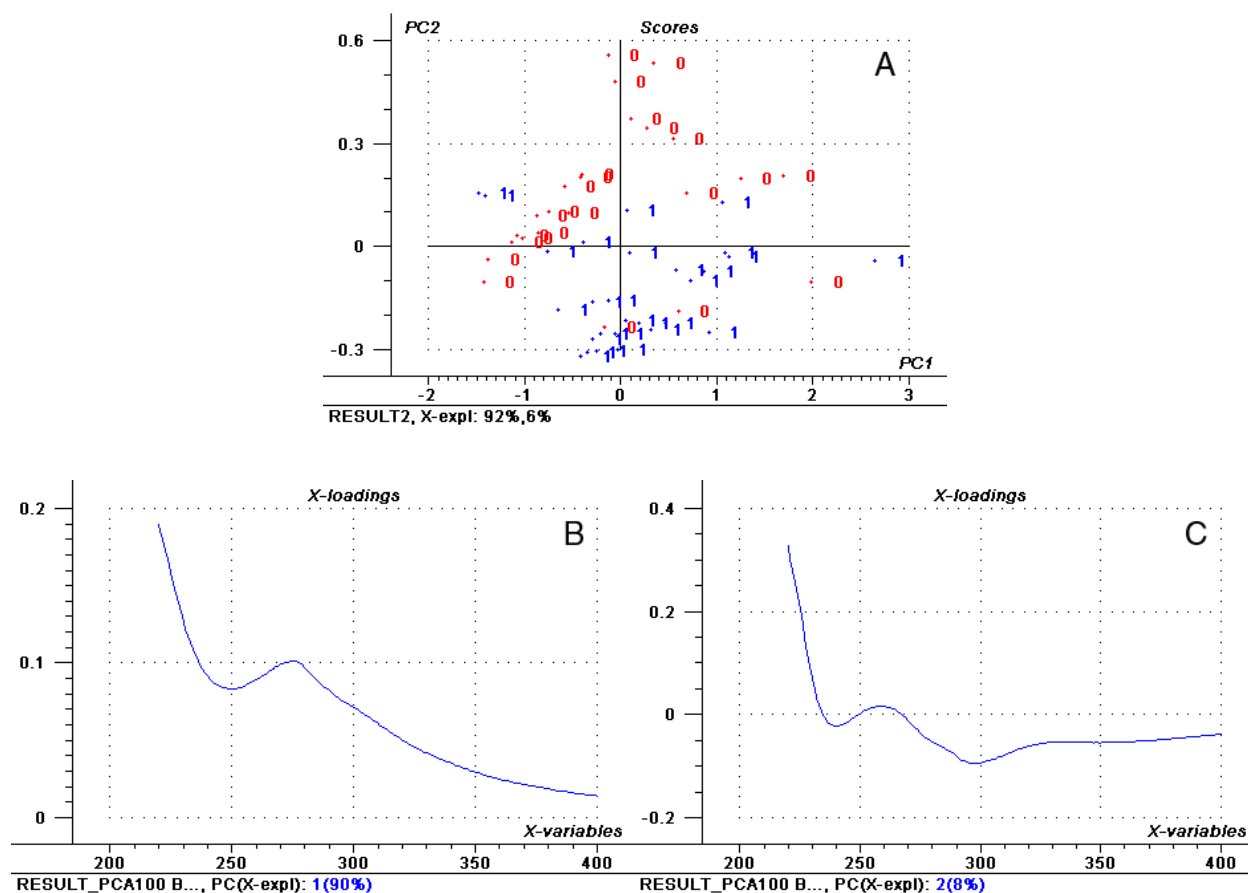


Fig. 2. PC 1 and 2 scores (A) 1 = Trappist, 0 = non-Trappist) and loadings (B, C) for raw spectral data (220–400 nm).

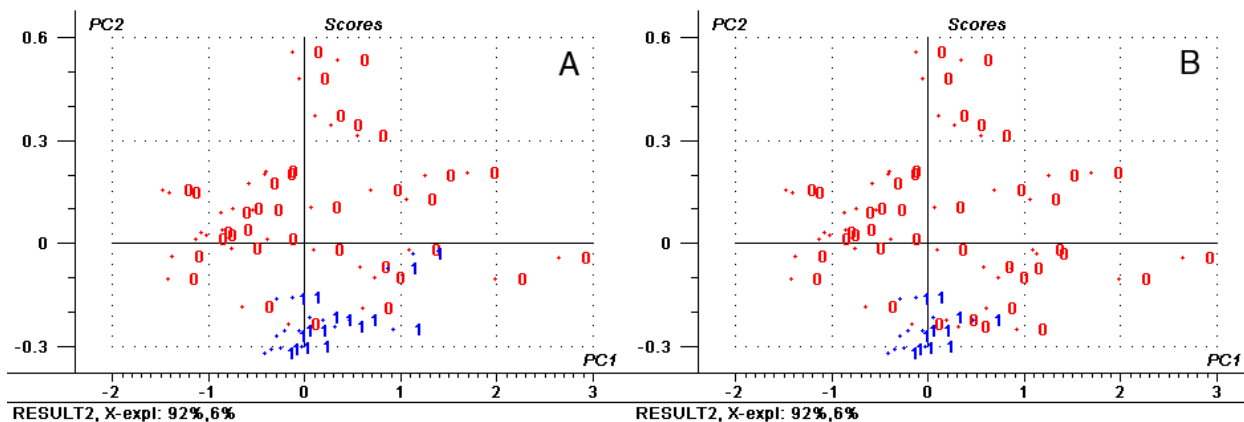


Fig. 3. (A) Rochefort (1) vs. non-Rochefort (0); (B) Rochefort 8 (1) vs. non-Rochefort 8 (0).

the opposite effect with a particularly important feature at 297 nm. That the samples are not separated strictly along either PC1 or 2 indicates that the observed separation does not occur along directions of maximum variance in the spectral dataset.

Labelling the above Fig. 2a on the basis of Rochefort versus non-Rochefort beers produced the score plot shown in Fig. 3a, while labelling on the basis of Rochefort 8 versus all other beer types produced the plot shown in Fig. 3b.

It is apparent that the Rochefort and Rochefort 8 beers cluster together but are not separated from other beers in the PC1 vs. PC2 multidimensional space; no greater separation was observed when higher order spaces were examined (data not shown). Thus, the challenge of segregating Rochefort 8 beers from the others appeared to be significant. PCA on SNV-treated spectral data revealed that, while the clusters observed were smaller than their counterparts shown above, no clear separation of the groups of interest from the other beer samples was achieved.

Discriminant partial least squares (D-PLS) regression

Despite the difficulties implied by the results of PCA above, discriminant partial least squares regression (D-PLS) was investigated for discrimination of beers. Given the overlap of Rochefort 8 and the other beers, it was judged unlikely that a single-step discriminant model would achieve such discrimination and this proved to be the case; using raw spectral data, the correct classification rates of Rochefort 8 and other beers were 90.9 and 85.4% respectively, values which are too low to be of commercial utility. Even the discrimination between Trappist and non-Trappist by this technique produced very low correct classification rates of 78.6 and 83.3% respectively; this is a reflection of the broad nature of these classes as seen in the score plots shown in Figs. 2 and 3. Therefore, a hierarchical approach was investigated involving two decision points. Beer would initially be classified as Rochefort or non-Rochefort and secondly the Rochefort beers would be identified as Rochefort 8 or Rochefort 10.

The results from D-PLS are summarised in Table II; no samples were deleted from the dataset in the development of these models. Perhaps surprisingly in view of the sample score distribution in Figs. 2 and 3, Rochefort beers

Table II. Summary of D-PLS discriminant model performance.

Model	n ^a	Class 1	Class 2	#L ^b
T ^c vs. non-T ^d	52	T 78.6	Non-T 83.3	2
T ^c vs. non-T ^d (Belgian only)	47	T 95.7	Non-T 95.8	5
R ^e vs. non-R ^f	52	R 100	Non-R 94.4	5
R8 ^g vs. non-R8 ^h	52	R8 90.9	Non-R8 85.4	4
R8 ^g vs. non-R8 ^h (Rochefort only)	16	R8 90.9	Non-R8 100	2

^a Number of samples modelled.

^b Number of PLS loadings in the model.

^c Trappist.

^d Non-Trappist.

^e Rochefort.

^f Non-Rochefort.

^g Rochefort 8.

^h Non-Rochefort 8.

were clearly discriminated from non-Rochefort samples; Rochefort beers were 100% correctly classified while non-Rochefort beers reported a 94.4% correct classification rate (34 out of 36 samples correctly identified) by a model involving 5 PLS loadings. Therefore, this model was selected as the first discriminant step in the hierarchical process. Applying the discriminant process to segregate Rochefort 8 beers from all others was less successful with 90.9 and 85.4% correct classification for Rochefort 8 and non-Rochefort 8 beers respectively. However, using only Rochefort beers for this latter discriminant model, a 100% correct classification of Rochefort 8 versus non-Rochefort 8 beers was achieved after removal of a single Rochefort 8 sample which appeared to be unusual. With the inclusion of this sample, % correct classification rates for Rochefort 8 and non-Rochefort 8 beers were 90.9 (1 mis-classification) and 100%.

An interesting observation related to the country of origin of Trappist beers. Beers of one particular brand (La Trappe) were brewed in the Netherlands, while all the other Trappist beers originated in Belgium. Omitting the 5 samples of La Trappe beers from the sample set produced correct classification percentages for Trappist versus non-Trappist beers of 95.7 and 95.8 respectively using 5 PLS loadings. With SNV-treated spectral data, similar results

were obtained although correct classification rates were slightly lower at 95.7 (T) and 91.7 (non-T) % respectively.

Although these results overall were encouraging, some samples were frequently mis-classified as Trappist, the most frequent of these being Leffe Brune. In the past, Leffe Brune was a true Trappist beer, brewed by Trappist monks. However, its production was transferred to a commercial brewing company and it may not now be labelled as a Trappist beer, although beer production was sold to the company on condition that it used the traditional original recipes. It may therefore be expected to have similar characteristics to Trappist beers and mis-classification is not surprising, although it represents a real challenge to this authentication scheme

SIMCA

Two classes of sample were contained in this small sample collection; given the aim of the research, only the Rochefort samples were modelled initially. Using 50% of these samples for model development and applying this 3 principal component model to the remaining 8 Rochefort samples and all of the non-Rochefort ($n = 36$), 6 of the 8 Rochefort beers were correctly classified (75%; $p = 0.05$) but only two of the 36 non-Rochefort beers were wrongly classified as Rochefort (correct classification rate of 94.4%); interestingly, these were both Leffe Brune beers. Given the small number of samples involved in this exercise, it may only be stated that SIMCA appears to be a promising method for confirming the identity of Rochefort 8 beers. It has an advantage over the discriminant PLS approach in that it is a class-modelling method to which a probability level may be attached. When the non-Rochefort beers were modelled using SIMCA, all of the Rochefort beers were wrongly classified as non-Rochefort. This is a reflection of the dispersion of the non-Rochefort samples in multivariate space.

CONCLUSION

In conclusion, UV spectral data (220–400 nm) may be collected simply and with only a dilution step required. Results from this preliminary study reveal that such data, when processed by D-PLS, has the potential to be applied as a tool for brand confirmation. Little difference in performance was found between raw spectral data and such data after SNV-transformation. The application of SIMCA also showed promise as a tool for confirming the identity of Rochefort beers. A difficulty with both types of modelling was encountered with a single beer brand (Leffe Brune), which was formerly a Trappist beer, but which is not now entitled to use the Trappist logo. Given that the raw materials and production methods are believed to have remained unchanged after transfer of ownership, the reason for this classification problem is obvious. UV spectroscopy coupled with chemometric data analysis

may have a useful role to play in beer brand authentication. Furthermore, the technique should have a valuable role to play in routine quality control within breweries as it has shown itself to be sensitive to differences in production methods which may include raw material quality and brewing techniques.

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REFERENCES

1. Bamforth, C. W., *Scientific Principles of Malting and Brewing*. American Society of Brewing Chemists: St Paul, MN, 2006.
2. Barbosa-García, O., Ramos-Ortiz, G., Maldonado, J. L., Pichardo, J. L. and Meneses-Nava, M. A., UV-vis absorption spectroscopy and multivariate analysis as a method to discriminate tequila. *Spectrochim. Acta A*, 2007, **66**, 129-134.
3. Barnes, R. J., Dhanoa, M. S. and Lister, S. J., Standard normal variate transformation and de-trending of near infrared diffuse reflectance spectra. *Appl. Spectrosc.*, 1989, **43**, 772-777.
4. Casale, M., Armanino, C., Casolino, C. and Forina, M., Combining information from headspace mass spectrometry and visible spectroscopy in the classification of the Liguarian olive oils. *Anal. Chim. Acta*, 2007, **589**, 89-95.
5. Glover, B., *The Complete Handbook of Beers and Brewing*. Southwater: London, 2003.
6. Lees, M., *Food Authenticity: Issues and Methodologies*. Eurofins Scientific: Nantes, France, 1998.
7. Luthria, D. L., Mukhopadhyay, S., Robbins, R. J., Finley, J. W., Banuelos, G. S. and Harnly, J. M., UV spectral fingerprinting and analysis of variance-principal component analysis: a useful tool for characterizing sources of variance in plant materials. *J. Agr. Food Chem.*, 2008, **56**, 5457-5462.
8. Luykx, D. M. A. M. and van Ruth, S. M., An overview of analytical methods for determining the geographical origin of food products. *Food Chem.*, 2008, **107**, 897-911.
9. Naes, T., Isaksson, T., Fearn, T. and Davies, T., *A User-Friendly Guide to Multivariate Calibration and Classification*. NIR Publications: Chichester, UK, 2002.
10. Protz, R., *The World Beer Guide*. Carlton Books: Italy, 2000.
11. Reid, L. M., O'Donnell, C. P. and Downey, G., Recent technological advances for the determination of food authenticity. *Trends Food Sci. Technol.*, 2006, **17**, 344-353.
12. The International Trappist Association. <http://www.trappist.be/index.js.cfm?v=01&taal=en> (last accessed Jan 2010).
13. Woodcock, T., Downey, G. and O'Donnell, C. Better quality food and beverages: the role of near infrared spectroscopy. *J. Near Infrared Spectrosc.*, 2008, **16**, 1-29.

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