Screening of acrylamide contents in potato crisps using process variable settings and near-infrared spectroscopy

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In order to study the formation of acrylamide in potato crisps during processing, an experimental design was set up. The design variables were drying time (6 levels), frying temperature (2 levels) and frying time (8 levels). The design contained 36 samples, which were analysed for acrylamide contents using LC high-resolution mass spectroscopy (LC-HRMS), and fat contents using the Soxhlet apparatus. Prior to analysis, all potato crisp samples were ground and analysed on an NIRSystems 6500 near-infrared (NIR) spectrometer. The acrylamide contents were modelled by: (i) design variables using multiple linear regression, (ii) NIR spectra using partial least squares regression (PLSR) and (iii) design variables and NIR spectra in combination using a novel technique combining least squares regression on the former, and PLSR on the latter. The results showed that the NIR spectra alone or in combination with the design variables gave better prediction models for acrylamide than the design variables alone. This implies that the spectra contain chemical information that is not purely a result of the processing variables that were investigated in this experiment. NIR spectroscopy is proposed as a possible tool for screening and identification of potato crisps with a high acrylamide content.

Keywords: Acrylamide / NIR spectroscopy / Potato crisp / Rapid methods

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1 Introduction

Acrylamide (AA) is considered to be a potential carcinogen and is present at elevated concentrations in different types of heat-treated foods. It is formed during baking, frying and roasting of raw materials from plant origin, particularly potatoes and cereals. Acrylamide is one of the reaction products in the Maillard reaction between the acrylamide precursors; amino acids and reducing sugars [1, 2]. Asparagine is a crucial reactant and since it is more abundant than glucose and fructose [3, 4], it is most probably the content of sugars that is partly responsible for the variable acrylamide formation in fried potatoes [5, 6]. An additional challenge related to potatoes is the accumulation of sugars during storage, especially below 8 – 10°C, since elevated storage temperatures require the use of sprouting inhibitors. Thus, this natural high level of acrylamide precursors and the specific processing conditions, mainly short frying at high temperatures between 160 and 190°C, leave potato crisps in the group of food products with the highest level of acrylamide.

There is a focus on how to obtain potato products with low content of acrylamide and to minimise the formation given a certain raw material. Some propositions include improved storage conditions or means of decreasing acrylamide precursor contents by blanching or soaking in different solutions, changing the heat processing conditions or combinations [4, 7 – 9].

An important part of these investigations is monitoring the acrylamide content during processing or in the finished products. The established analytical methods are expensive and time consuming. Finding a rapid method that could be used for routine analysis, and preferentially online monitoring during processing to identify certain samples exceeding a preset value, would be very useful. Traditionally the colour of fried potato products has been an important param-

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Abbreviations: EMSC, extended multiplicative signal correction; **LS,** least squares; **NIR**, near-infrared; **PLS**, partial least squares; **PLSR**, partial least squares regression; **RMSECV**, root mean squared error of prediction by cross validation

eter during processing. Before the acrylamide problem was defined, it was known that colour formation during frying was a result of Maillard reaction. It depends on the content of amino acids, reducing sugar and the heating of the system [10]. It is therefore not surprising that there is a correlation between acrylamide and colour formation, and that the latter might be used as an indicator of acrylamide content [11]. In the L*a*b* system for colour measurements, it has been shown that a* (from green to red) shows the strongest correlation. However, extensive frying causes dark fries and an actual loss in acrylamide content, which breaks the correlation somewhat [12].

Near-Infrared (NIR) spectroscopy has been established as a technique for the analysis of proximate composition of complex foods, mainly due to its speed and ability to perform contact-free measurements. NIR spectroscopy is a nonspecific technique that utilises several wavelengths (often hundreds) in order to predict one or more chemical components. The wavelengths are strongly colinear, which requires regression techniques that can handle colinearity without overfitting the data. Principle component regression (PCR) and partial least squares regression (PLSR) are probably the most used regression techniques for multivariate calibration of rapid spectroscopic instruments.

Predictive solutions operating in industry today are usually based on process variable models, or in some cases on a rapid spectroscopic instrument, *e.g.* an NIR spectrometer. Very often, the instrumental readings and the process variables represent different parts of the picture. Thus, combining these two sources of information could potentially improve the relations between the raw materials or intermediate product, the process and the quality of the finished product. However, such a combination of data with very different characteristics is not trivial. A regression approach designed for this type of situation was recently presented by Jørgensen *et al.* [13]. The technique, termed LS-PLS regression, uses ordinary least squares (LS) regression to model the responses based on the process or design variables, followed by multivariate modelling of the remaining response variance using partial least squares (PLS) regression. The process is iterative, optimising the information output from both data sources.

Most NIR applications focus on bulk components like fat, water, carbohydrate and protein [14], but several studies have shown that the technique is also capable of separating and quantifying different species and states within these main classes. In particular, water, which is the strongest NIR absorber in food systems, has shown interesting features that can reflect small changes in other molecules that get attached to the water molecules through hydrogen bonding. Because of such relations, it is also possible that acrylamide formation can be monitored by NIR spectroscopy through other accompanying molecular changes.

2 Materials and methods

2.1 Samples and reference analyses

Potato tubers (Saturna), stored for 1 – 2 months at 8°C, were used for crisps production. Storage was conducted in a storage room with controlled temperature and humidity. Potato tubers were washed, peeled (carborundum peeler, Millert B.V., Ulft, Holland) and sliced (1.5 mm). The potato slices were fried in a 10 L electric frying pan (Elframo) for 3–7 min at 160°C and for 2 – 5 min at 185°C. Palm oil was used at both temperatures. After frying, potato slices were dried in a hot air oven (WTB Binder) at 105°C for 30–120 min. The postdrying step was introduced in order to reduce the water content to a desired level. A study related to this process will be published elsewhere. After frying and postdrying, fat and acrylamide contents were measured. At each of the two temperatures (160 and 185°C), a 13-sample central composite design (CCD) was run with frying time and drying time as variables. In addition, ten samples with the same frying time levels and no postdrying were run, giving totally 36 samples. The frying time levels were not the same for the two temperatures. Acrylamide was analysed by NILU (NILU, Norway) using LC-HRMS as described elsewhere [8]. Fat content was measured using Soxhlet method [15].

2.2 NIR spectroscopy

Prior to analysis, the potato crisps were coarsely ground. A rectangular cell with a quartz window (165 mm × 35 mm) was filled with crisps and scanned in the diffuse reflectance mode (400 – 2500 nm) using a NIRSystems 6500 spectrometer (Foss NIRSystems, MD, USA). Each spectrum was the average of 32 successive scans. Reflectance data were stored as $\log(1/R)$, where R is the reflectance.

2.3 Data analysis

For analysis of the design variables and their relation to the AA content, classical LS regression and analysis of variance (ANOVA) were utilised. Spectral prediction models were built using PLSR [16]. Design variables and spectra were combined using LS-PLS regression [13]. All models were validated using full crossvalidation [16], with and without the removal of nonsignificant variables using jackknifing [17]. The following spectral preprocessing techniques were tested in the spectral modelling step: extended multiplicative signal correction (EMSC) [18], second deri-

Table 1. Summary of the acrylamide data

Temperature (°C)	Number of samples	Mean AA (μg/kg)	Minimum AA (μg/kg)	Maximum AA (μg/kg)	S_{total} (μg/kg)	S_{cp} (μg/kg)
160	18	941	169	1620	475.7	260.8
185	18	2106	764	2987	623.3	203.9
160 + 185	36	1523	169	2987	804.9	232.4

vatization (Savitzky-Golay, 9 pts, 2nd order polynomial) and standardisation (1/SD). ANOVA was performed in Minitab (version 14.20). Spectral preprocessing, LS regression and PLSR were performed using The Unscrambler (v. 9.2, CAMO Process AS, Oslo, Norway). The LS-PLS script was written in Matlab (v. 7.0.4, The Mathworks, Nattick, USA).

3 Results and discussion

The data from the two temperature series will be treated both separately and in combination throughout this paper. The acrylamide data are summarised in Table 1. S_{total} gives the SD for the dataset, quantifying the variation around the mean value. S_{cp} gives the SD between the AA values for the five design center points for each temperature series. S_{total} and S_{cp} can be used to evaluate the performance of the AA prediction models to be presented. Mean prediction errors should be lower than S_{total}, but cannot be expected to go below S_{cp}.

3.1 Process variables and AA formation

ANOVA of the results in Table 1 showed that AA levels were significantly higher at 185°C compared to 160°C. It should be noted that the frying times were shorter at 185°C, which means that we cannot compare the AA values directly. The fact that higher frying temperatures accelerate the acrylamide formation has been reported in previous works [19, 20]. The second most important factor affecting acrylamide creation in fried products was time of frying, which caused increased AA accumulation at increased levels [21]. However, upon prolonged heating, the acrylamide contents in both model systems and real food products are reduced to a varying degree [1, 12, 22, 23]. It should be noted that excess heating is not a general solution since this most often induces dark brown or burned products and was not aimed in our investigation.

The ANOVA showed that the effect of postdrying time was nonsignificant on the levels of AA ($p = 0.48$). For modelling purposes, this variable is thus not very interesting. This can be seen in Table 2, where prediction results for regression models based on the design variables are summarised

Table 2. Prediction results for design models

Parameters	Temperature (°C)	Response	RMSECV (μg/kg)	R
$F + D + FD + F^2 + D^2$	160	AA	254.1	0.851
$F + F^2$	160	AA	215.2	0.887
$F + F^2$	160	Log(AA)	168.7	0.935
$F + D + FD + F^2 + D^2$	185	AA	468.0	0.678
$F + F^2$	185	AA	325.8	0.843
$F + F^2$	185	Log(AA)	325.3	0.853
$T + F + F^2$	160 + 185	AA	279.9	0.938

(F = frying time, D = drying time, T = frying temperature). In practice, however, this is a positive result, implying that the frying time can be shortened. Potato crisps can thus be exposed to a lower frying load when combined with postdrying, giving a product with correct moisture contents (below 2%) and reduced AA levels. An extra benefit of such a combination is a simultaneous reduction in the fat content, as illustrated in Fig. 1. R is the correlation between predicted values using the model and reference values. The mean prediction error (root mean Square error of crossvalidation) is defined as

$$\mathrm{RMSECV} = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(y_i - \hat{y}_i)^2}$$

where i denotes the samples from 1 to N.

Table 2 shows the prediction performance of full and reduced models for the two temperature sets, and the optimised model for the combined set (both temperatures included). Table 2 shows that for the individual frying temperatures, the best prediction performance was achieved using a model with linear and square terms for frying time only, modelled against the logarithm of the AA contents. The logarithm of the AA contents was tested in the modelling due to the nonlinear development of AA. The drying time and the interaction between the two variables were found to be nonsignificant for both the temperature sets. For the 160°C set, the correlation between measured and predicted log(AA) values was 0.935, which means that 87.4% of the log(AA) variation is explained by the model (explained variance = $R^2 \times 100\%$). This corresponds to an average prediction error (RMSECV) of 168.7 μg/kg. For

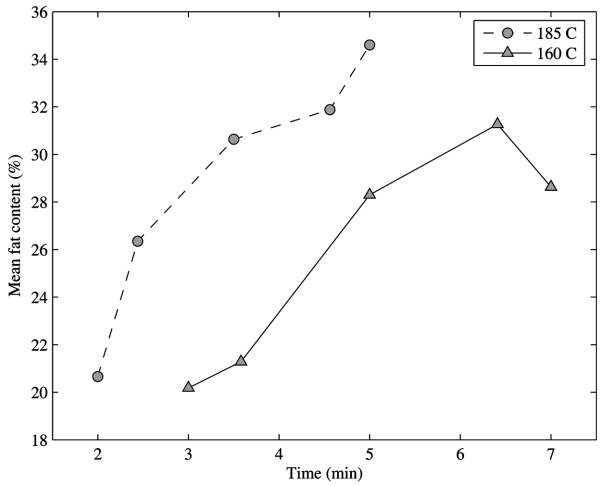

Figure 1. Frying time *versus* mean fat content for potato crisps. Frying temperature: 160°C (solid line) and 185°C (dashed line).

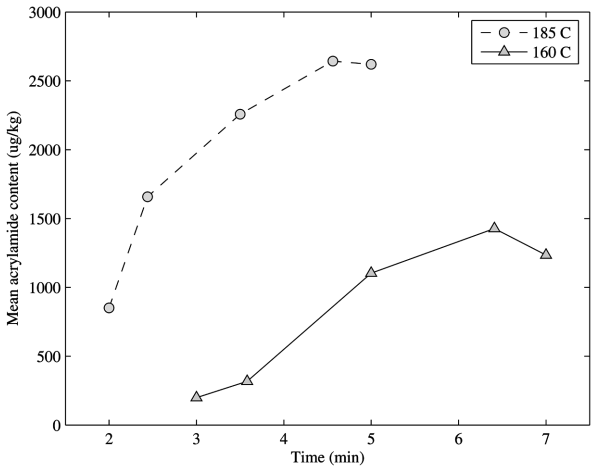

Figure 2. Frying time *versus* mean acrylamide content for potato crisp. Frying temperature: 160°C (solid line) and 185°C (dashed line).

the 185°C set, the performance was slightly poorer, with a correlation of 0.853 for the best model (RMSECV = 325.3 µg/kg). For the 185°C set, the gain of using log(AA) instead of AA directly was nonsignificant. The effects of frying time and frying temperature on the AA formation are illustrated in Fig. 2. The figure clearly indicates a nonlinear relationship between frying time and AA formation.

Eliminating the drying time from the analysis allows us to study square and interaction effects of frying time and frying temperature. The last row in Table 2 shows that using the significant terms in a combined model ($T + F + F^2$) gives a mean prediction error of 279.9 and a correlation of 0.938. The prediction error should now be compared to the S_{total} and S_{cp} values given in the last row of Table 1. Modelling against log(AA) values gave poorer models than AA, directly for the combined dataset. It should be noted that because of the shorter frying times that were used at the highest temperature, these two variables are not orthogonal. There is a correlation of −0.56 between frying time and frying temperature.

3.2 NIR spectra and AA formation

In general, chemical contents in the order of 100 µg/kg (*i.e.* 0.00001%) will not be detectable in NIR spectra. However, as already shown, the AA formation is accompanied by changes in the bulk chemical composition of the potato crisps. Thus, the spectral analysis will be focused on bands originating from water, carbohydrates and fat rather than amide bands.

3.2.1 Spectral features

Figure 3 shows the EMSC-transformed 400–2500 nm spectral range for the 36 samples. The most pronounced differences that can be related to AA levels are found in the 1900–2200 nm range, containing O–H, C–O and N–H vibrations. The dominating feature in this region is the band centred at 1934 nm, which is very different for the different sample spectra. In general, the intensities at 1934 nm are oppositely correlated with the AA levels. The 1934 nm band is most likely representing starch, *i.e.* O–H stretch and deformation vibrations, since water is fairly absent in these samples. It is oppositely correlated to the intensity of the 2100 nm feature, which also appears to be a typical starch band ($2 \times$ O–H deformation + $2 \times$ C=O stretch vibrations). However, second derivatives of the original spectra (Fig. 4) show that the apparent band at 2100 is really

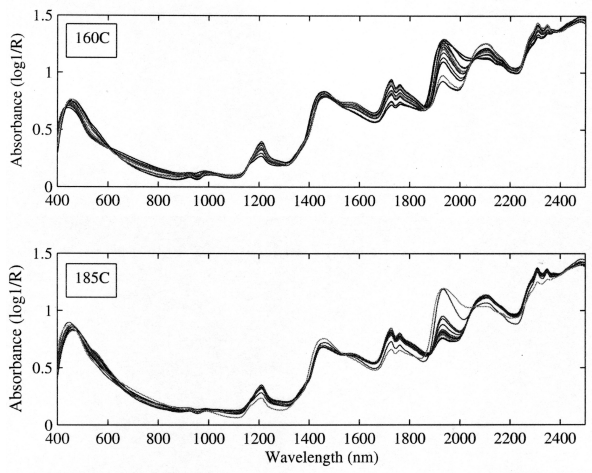

Figure 3. Reflectance spectra in the spectral region 400–2500 nm. Upper panel: frying temperature 160°C; lower panel: frying temperature 185°C.

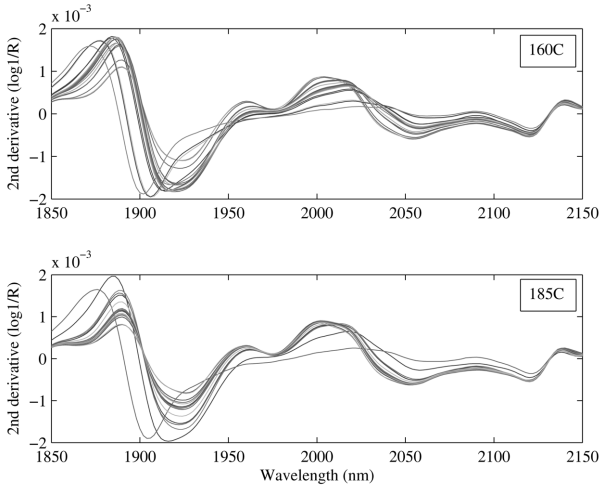

Figure 4. Second derivatives of reflectance spectra in the spectral region 1850 – 2150 nm. Upper panel: frying temperature 160°C; lower panel: frying temperature 185°C.

a combination of two bands (original bands are now negative peaks): one centred around 2058 nm and the other at 2120 nm. The 2058 nm band increases in intensity with increased AA contents, while the intensity at 2120 nm is fairly unchanged. Both these bands are probably N – H vibrations originating from the protein fractions of the samples. It can also be noted that an intensity increase at the 2058 nm band is accompanied by a shift from 2062 to 2052 nm. The same tendencies are seen around 1930 nm, where increased intensities result in a shift from 1926 to 1916 nm. Four samples stand out from the others at this band, *i.e.* their bands are centred between 1902 and 1906 nm as opposed to the 1916 – 1926 nm region. Three of these samples have been subjected to the shortest frying times at the lowest temperature, while the fourth sample represents the shortest frying time at the highest frying temperature (no postdrying).

3.2.2 NIR prediction models

Prediction results for a selection of NIR models are summarised in Table 3. Columns 3 – 5 indicate the quality of the models. The number of PLS components should be as low as possible in order to assure robustness, the mean prediction error (RMSECV) limits the precision of the model and should thus be as low as possible, and the correlation between the predicted (NIR) and the measured (HPLC) AA values (*R*) should be as close to 1 as possible.

All models presented in Table 3 show relatively good prediction performance. Compared to the predictions based on design variable settings, the NIR spectra show similar or slightly better performance. *R* values ranging from 0.928 to 0.952 means that the prediction models explain between

Table 3. Prediction results for NIR spectral models, all samples included

Wavelength Regions	Pretreatment	No. of PLSComp.	RMSECV (μg/kg)	*R*
1100 – 2498	No	4	284.7	0.935
400 – 780	EMSC	3	299.2	0.928
1100 – 2350	EMSC	3	289.6	0.933
1100 – 2498	2.der (9pt)	3	267.5	0.943
400 – 2498	EMSC, jackknife	2	256.6	0.948
400 – 498	EMSC, 1/SD, jackknife	3	246.8	0.952

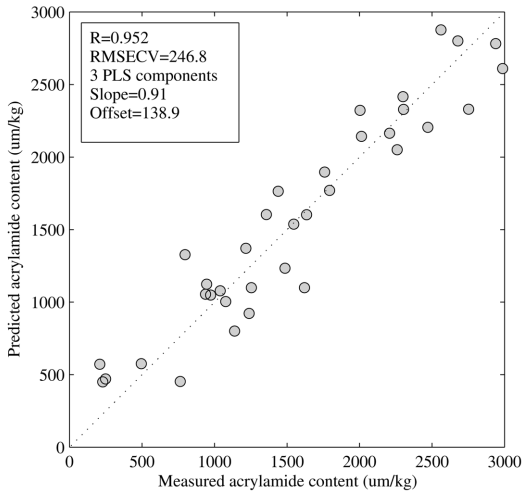

Figure 5. Predicted acrylamide values *versus* reference values. The prediction model is built using spectral variables only.

86.1 and 90.6% of the AA variation. The best performance was achieved using the EMSC-transformed full spectral region (400 – 2498 nm), including the visible part. Jackknifing was used to select the most significant wavelengths. This two-component model gave a mean prediction error of 256.6 μg/kg and an *R* value of 0.948. The spectral variables utilised in this model were spread over the whole spectral region. The prediction performance was apparently slightly improved when standardising the spectra, *i.e.* by dividing each spectral variable by its own SD. However, one more PLS component was needed. The performance of this model is illustrated in Fig. 5. The regression vector for this model (prior to the removal of nonsignificant spectral variables) is shown in Fig. 6, where filled circles indicate the significant variables. For comparison with the original spectral features, the mean spectrum is shown in Fig. 7, with the same spectral variables highlighted. Figs. 6, 7 show that a large part of the visible region, *i.e.* between 400 and 600 nm, is significantly related to the AA contents. In addition, both fat regions (1700 – 1800 and 2270 – 2370 nm) and the starch bands (at 1456 and 1934 nm) are highlighted as significant. The regression vector in Fig. 6 indicates that

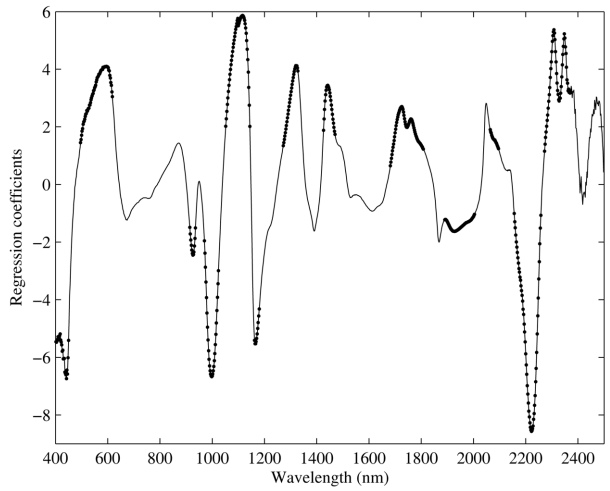

Figure 6. Regression coefficients for the model presented in Fig. 6. Significant spectral variables are highlighted.

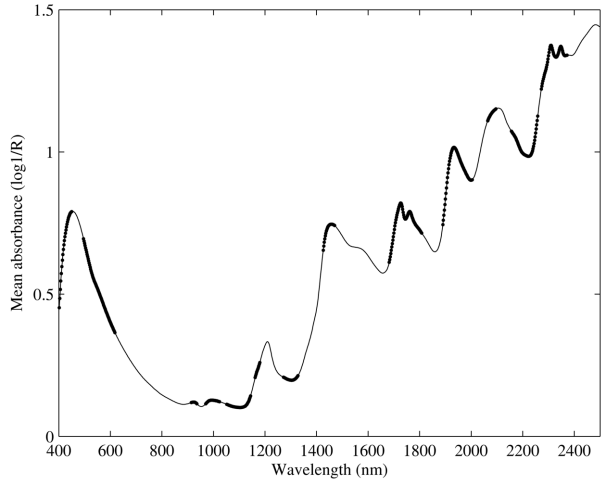

Figure 7. Mean spectrum with significant spectral variables highlighted.

all these regions appear to be positively correlated to the AA content. In the higher frequency NIR region (900–1300 nm), five spectral regions are highlighted. The most important negative features of the regression vector are centred around 926, 1000, 1162 and 2222 nm.

Individual spectral models for the two temperatures (not shown) showed the opposite behaviour compared to the individual design models, *i.e.* the design model gave the best performance at 160°C, while spectral models performed better at 185°C.

It should be noted that changing process parameters like frying and drying times will affect the bulk composition of the samples, and consequently the NIR spectra. Additionally, changes in the raw materials, *e.g.* the sugar and water contents of the potatoes, will be reflected in the NIR spectra. This illustrates both the strengths and the weaknesses of NIR spectroscopy: chemical changes will be picked up by the NIR spectra, relevant or not in terms of AA formation. In comparison, using process variables only for AA formation control will never pick up variations caused by raw material differences. Combining process variables and NIR spectra may thus give valuable information and stabilisation to the prediction models.

3.3 Combination of process variables and spectral variables

Information from the design variables and the spectra was combined using LS-PLS regression [13]. The design variables were modelled against the AA contents using ordinary LS regression, followed by PLS modelling of the spectra against the remaining spectral variance from the LS models. This regression technique allows utilisation of spectral information that is not already covered by the process variables. In Table 4, the performance of three prediction models is summarised.

The best result is achieved by simply combining the spectral and design variables in the input matrix, and running a PLSR model against the AA levels. The jackknifing procedure includes the frying temperature in the model, but not the frying or drying time. This model is very similar to the pure spectral model, and the inclusion of the frying temperature into the model does not improve the prediction performance significantly. The LS-PLS approach is presented for two datasets: The second row in Table 4 represents all three design variables and all EMSC-transformed spectral variables. The third row represents the same variables as in the PLSR model, *i.e.* the frying temperature and the spectral variables highlighted in Figs. 6, 7. This was done to ensure fair comparison between the different modelling approaches.

Table 4 shows that the prediction results were not improved by combining the design data and the spectral data. The most likely reason for this is that the design matrix and the spectral matrix contain overlapping information. An easy way to test if this is so is to model the design variables using the spectra. Performing this test, however, gave models with significantly poorer performance compared to the AA models (not shown). This, combined with the fact that the spectra gave better prediction models for the full dataset than the design variables, may indicate that the spectra give a more precise representation of the process load than the design variables do.

In summary, NIR spectral models appear to give a good indirect representation of the AA formation of potato

Table 4. Prediction results for combined input data

Regression technique	Spectral pretreatment	Design parameter included	No. of PLS components	RMSECV (μg/kg)	*R*
PLSR	EMSC, 1/SD, jackknife	T	3	245.4	0.952
LS-PLSR	EMSC, 1/SD	T, F, D	3	258.7	0.947
LS-PLSR	EMSC, 1/SD, jackknife	T	3	251.8	0.950

crisps. It should be stressed, however, that this is a feasibility study. The results are valid for these samples only, and it is likely that the models would be somewhat different for different potato cultivars and different processes. NIR applications always need to be tested and normally also recalibrated for each new situation.

4 Concluding remarks

It has been found that the frying time significantly affects the formation of acrylamide in potato crisps, and the relationship is nonlinear at both frying temperatures studied, *i. e.* 160 and 185°C. Drying time was not found to be significant at either of the two frying temperatures. Acrylamide formation was significantly higher at 185°C compared to 160°C. VIS and NIR spectra acquired from the processed samples were found to give a slightly better correlation with the acrylamide content. The best prediction model was found using the whole spectral region (400 – 2500 nm), using EMSC transformation, standardisation and jackknifing for removal of nonsignificant variables. This model gave an average prediction error of 247 μg/kg and a correlation of 0.952 between predicted values and reference values. The best process variable model, including the main terms for frying temperature and frying time, and the square term for the latter, gave a mean prediction error of 280 μg/kg and a correlation of 0.936. The combination of spectral and design variables did not improve the prediction results. The spectral models are accurate enough to suggest that VIS/NIR spectroscopy can be used for screening of acrylamide contents in processed potato crisps. However, the method needs to be tested and calibrated for each specific production process.

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