APPLICATION OF NEAR-INFRARED REFLECTANCE SPECTROSCOPY FOR DETECTION OF ACRYLAMIDE CONTENT IN POTATO CRISPS AND BREAD

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Acrylamide is a chemical compound that is produced naturally in food products as a result of high-heat cooking. Acrylamide is a neurotoxin and possible carcinogen, which upon oral intake is absorbed from the gastrointestinal tract and distributed to all organs. The analytical potential of near-infrared spectroscopy (NIRS) for detection of acrylamide in the potato crisps and bread was evaluated. Acrylamide content in 24 samples of potato crisps and 16 rye bread samples was biochemically analysed. The average acrylamide content in potato crisps was 3329.5 $\mu g\ kg^{-1}$, the minimum was in crisps made from potato variety 'Lady Clair' (495.0 $\mu g\ kg^{-1}$), the maximum in crisps from breeding clone '19514.20' (7460.0 $\mu g\ kg^{-1}$). The average acrylamide content in rye bread was 100.8 $\mu g\ kg^{-1}$, the minimum was 18.5, and the maximum level was 270.0 $\mu g\ kg^{-1}$. Near-infrared spectra of chopped potato crisps and bread were recorded with NIRS instrument. Laboratory results of the same samples (reference values) were associated with the corresponding spectra. NIR calibration models for acrylamide in potato crisps and bread had been developed with WinISI software (version WinISI 4.6.8.14739). Calculations were carried out with a modified partial least squares' (MPLS) procedure using an interior cross validation by groups, mathematical treatment 1, 4, 4, 1, and SNV and detrend scatter correction of the spectra. The content of acrylamide in potato crisps and bread could be predicted successfully by NIR scans of the chopped products. The coefficient of determination (RSQ) for potato crisps calibration equation was 0.808, but RSQ for bread calibration equation was 0.869. Statistical parameters indicate good accuracy of calibration equations, which can be improved by supplementing the equation with new samples with known reference values.

Key words: acrylamide, potato crisps, bread, near-infrared reflectance spectroscopy.

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INTRODUCTION

Acrylamide is undesirable compound in several food categories including fried and baked potato products and bread (Haase 2008). This compound is formed during the Maillard reaction and is probably carcinogenic. Maillard reaction occurs between reducing sugars and amino acids in the presence of heat treatment (Mottram et al. 2002).

The potential to form acrylamide in fried potatoes correlates well with the concentration of reducing sugars and asparagine in tubers. The concentration of these compounds is mainly determined by cultivar, fertilisation, meteorological conditions during the growing and conditions during the storage (Halford et al. 2007, Pedreschi 2007).

Asparagine content is the main factor affecting acrylamide formation during the processing of rye (Curtis et al. 2010) and wheat (Halford et al. 2007) flour. However, significant correlation between the content of asparagine and reducing sugars and subsequent acrylamide content has been found in the study on rye (Curtis et al. 2010). This relationship was not confirmed by Postles et al. (2013). Different findings are partially explained by the cultivars with different content of reducing sugars. Wheat flour is not as rich in reducing sugars as rye flour is (Curtis et al. 2010), therefore the contents of asparagine is the main factor driving the acrylamide formation (Muttucumaru et al. 2006, Halford et al. 2007).

Level of acrylamide in produced potato crisps as well as the bread consumed by large number of society members, including children, should be closely monitored. Near-infrared reflectance (NIR) is a spectroscopic method based on the absorption of light in the wavelength region between 700 and 2500 nm due to vibrations of molecular functional groups (e.g., C-C, C-O-C, C-H, N-H, or O-H) in the sample (Dahm & Dahm 2001, Miller 2001). The method can be applied for detection of wide range quality parameters of agricultural raw materials and processed products.

The aim of this study was to develop NIRS calibration for detection of acrylamide content in potato crisps and bread.

MATERIAL AND METHODS

Samples and reference analyses

Potatoes

In the study twelve potato (*Solanum tuberosum* L.) varieties and breeding clones ('Verdi', 'Brasla', 'Imanta', 'Lenora', 'Gundega', 'Magdalena', S01085-21, 19514.20, 2000-49.82, 'Blue Congo', 'Lady Claire', S04009-37) were evaluated. Potato tubers harvested in 2015 and 2016 were stored at an air temperature of 4 °C and at a relative air humidity of 80 ± 5%. Before frying tubers were kept in warm room (18 - 20 °C) for two weeks. Potato tubers were washed peeled and sliced (1 mm). The potato slices were washed in water and dried on paper towel. After that slices were fried in canola oil at 180 °C. Potato crisp samples were crushed with the pestle until homogeneous consistency. Acrylamide content was measured of prepared samples using HPLC-MS/MS detection.

Bread

From harvests 2015 and 2016 sixteen rye samples were used for bread baking purposes. Bread was baked at 200 °C for 40 minutes. After baking each loaf of bread (including cortex) was cut into about 1x1 cm cubes and placed in an oven (Memmert, Schwabach, Germany) for 16 hours at 50 °C. Dried bread samples were ground with mills MICROTRON MB 550 (Kinematica, Luzern, Switzerland) until smooth homogeneous consistency. Acrylamide content was measured of dried bread samples using HPLC- MS/MS detection.

NIR spectroscopy

NIRS scanning

Each potato crisps and bread sample was well homogenized and scanned in triplicate on a near infra-red (NIR) spectrometer (Rapid Analyzer XDS, FOSS, Hillerød, Denmark) with a spectral range from 400 to 2498 nm.

Development of calibration model

Obtained spectral data were exported to WinISI 4 software (FOSS, Hillerød, Denmark). The spectra were first examined visually to eliminate abnormal scans before development of calibration equations.

Calibration equations for acrylamide content of potato crisps and bread were developed by modified partial least square regression (MPLS-R), using the results from the analytical reference methods. Cross validation process was used. Global regression equations with full spectrum were used for the development of the calibration model. The global calibration is designed to analyze all reasonable samples of a given product. Spectra were pre-treated using moving average smoothing (1,4,4,1), scatter correction, standard normal variate (SNV) conversion, and detrending.

Statistical evaluation of acquired calibration equations

The calibrations were evaluated using standard error of calibration (SEC), coefficient of determination (RSQ), standard error of cross validation (SECV), and variance or 1 minus the variance ratio (1-VR). The statistics used for comparison of predicted and reference values (experimental values obtained by classical determinations) were standard error of prediction (SEP), bias, and slope.

SEC is the standard deviation of difference between reference values and predicted values. **RSQ** describes the proportion of variance in the reference set explained by the calibration model. **SECV** is the difference between the reference and predicted values when samples are sequentially removed from the calibration equation during model development. It is an estimation of prediction accuracy. **1-VR** is the term from cross validation that describes how much of constituent variance is explained by the calibration equation. A 1-VR equal to 1 means that 100% of the constituent variance in the calibration samples (reference values) is explained by the calibration equation during the cross validation process. **SEP** is the standard error of differences between predicted and reference values. Low SEP values are desired. Values of SEC and SEP should be within 20% of each other. **Bias** is the measurement of centrality. Bias is the special case of the mean, where Bias is the difference between two means. The limit used for Bias was calculated as: 0.6 x SECV. **Slope** is the steepness of the line, measured by the increase in Y for each unit increase in X. Slope of the regression line, relating predicted values to reference values, should be close to 1 (LVS EN ISO 12099; NIRS white paper; Hruschka, 2001).

Spectral data corresponding to calibration set were analyzed by principal component analysis (PCA). Anomalous spectra were detected by applying Mahalanobis distance (H-statistics). Samples with H-values greater than 3 may be considered as not belonging to the population from which the equations were developed and in this case, the equations should not be used to make any prediction (Martin et al., 2014).

RESULTS AND DISCUSSION

Acrylamide content in potato crisps was found in range between 495.0 (potato variety 'Lady Clair') and 7460.0 µg kg^{-1} (breeding clone 19514.20) (Table 1), which was higher comparing to other research results. For example, Ayvaz & Rodriguez-Saona (2015) indicated acrylamide range of approximately 200 to 1100 µg kg^{-1} for regular potato crisps, and 920 to 2450 µg kg^{-1} for sweet potato crisps.

Table 1. Acrylamide content in potato crisps samples

Variety or breading clone	Acrylamide, µg kg-1		
	Year 2015	Year 2016	Average
'Lady Claire'	495.0	613.0	554.0[a*]
'Verdi'	718.0	501.0	609.5[a]
S 04009-37	1440.0	2180.0	1810.0[a]
S 01085-21	1920.0	2480.0	2200.0[a]
'Brasla'	1730.0	3250.0	2490.0[a]
'Blue Congo'	4960.0	2180.0	3570.0[b]
2000-49.82	3420.0	3860.0	3640.0[b]
'Gundega'	4540.0	3670.0	4105.0[b]
'Magda-lena'	5590.0	4140.0	4865.0[b]
19514.20	2990.0	7460.0	5225.0[b]
'Lenora'	5360.0	5430.0	5395.0[b]
'Imanta'	6080.0	4900.0	5490.0[b]
Average	**3270.3**	**3388.7**	**3329.5**
Least significant difference (LSD)	p>0.05		2785.0

*Different letters indicate significant differences within the acrylamide content in potato crisps made of potatoes from different varieties

The average acrylamide content in potato crisps from harvest 2015 and 2016 was more or less similar (3270.3, and 3388.7 µg kg-1, respectively). Statistically significant difference was not observed (p>0.05). Statistically significant difference (p≤0.05) in acrylamide content was observed in crisps made from different potato varieties. The lowest values of acrylamide content corresponded to potato crisps made from tubers of varieties 'Lady Claire' and 'Verdi' (554.0 and 609.5 in average, respectively), but the highest values of acrylamide content corresponded to potato crisps made from tubers of varieties 'Imanta', 'Lenora', and breeding clone '19514.20' (5490.0, 5395.0, 5225.0 in average, respectively) (Table 1). One of the factors, that contribute to acrylamide formation in the product, is a high concentration of reducing sugars and asparagine in raw material (Halford et al. 2007, Pedreschi 2007). Previous our studies showed that, for example, potato variety 'Lenora' and breeding clone '19514.20' has high content of amino acid asparagine, which could explain high acrylamide content in crisps (Konosonoka et al. 2016).

Table 2. Acrylamide content in rye bread samples

Sample No	Variety	Acrylamide content, µg kg-1
Year 2015		
R1	'Su Drive'F1	26.0
R2	'Su Drive'F1	42.9
R3	'Su Drive'F1	42.0
R4	'Su Drive'F1	**18.5**
R5	Matador	29.1
R6	Matador	43.5
R7	Matador	24.9
R8	Matador	47.4
	Average, 2015	**34.3***
Year 2016		
R9	'Su Drive'F1	**270**
R10	'Su Drive'F1	118
R11	'Su Drive'F1	214
R12	'Su Drive'F1	122
R13	Amilo	164
R14	Amilo	174
R15	Amilo	110
R16	Amilo	176
	Average, 2016	**168.5***

The significance symbol *** indicates significant differences between averages at $p < 0.05$.

The acrylamide content in rye bread was found to range between 18.5 and 270.0 µg kg^{-1}, which is significantly lower than in potato crisps. The average acrylamide content in rye bread from harvest 2015 was significantly lower ($p<0.05$) than in bread from harvest 2016 (34.3 and 168.5 µg kg^{-1}, respectively) (Table 2), indicating the impact of meteorological conditions during the

Table 3. Statistical parameters of developed calibration equations

Constituent	N	Mean	SD	Est min	Est max	SEC	RSQ	SECV	1-VR	SEP	Bias	Slope
Acrylamide in potato crisps, µg kg^{-1}	64	3329.5	1723.15	495.0	7460.0	823.87	0.808	957.71	0.686	763.34	-29.812	0.969
Acrylamide in bread, µg kg^{-1}	66	79.9	64.38	18.5	270.0	23.23	0.869	42.31	0.688	27.56	1.042	1.069

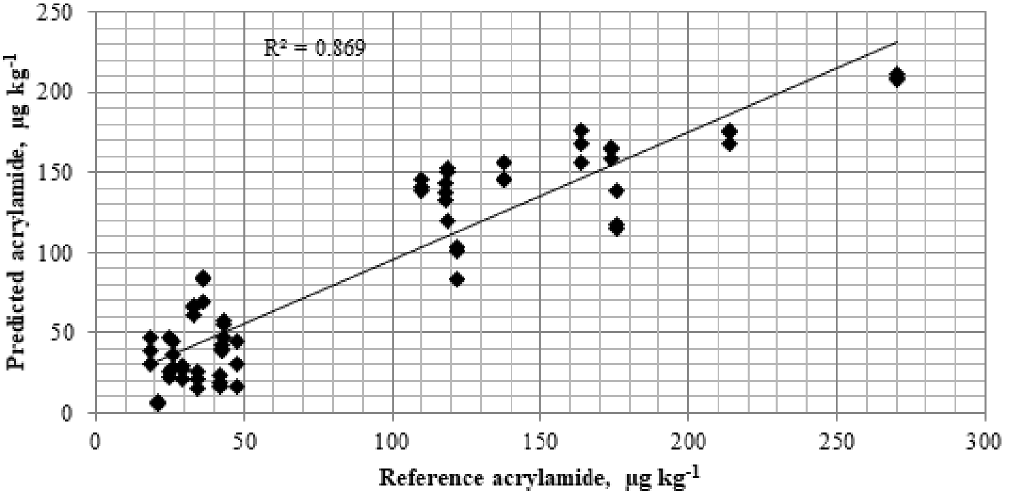

Fig.1. Predicted acrylamide vs reference acrylamide in bread samples.

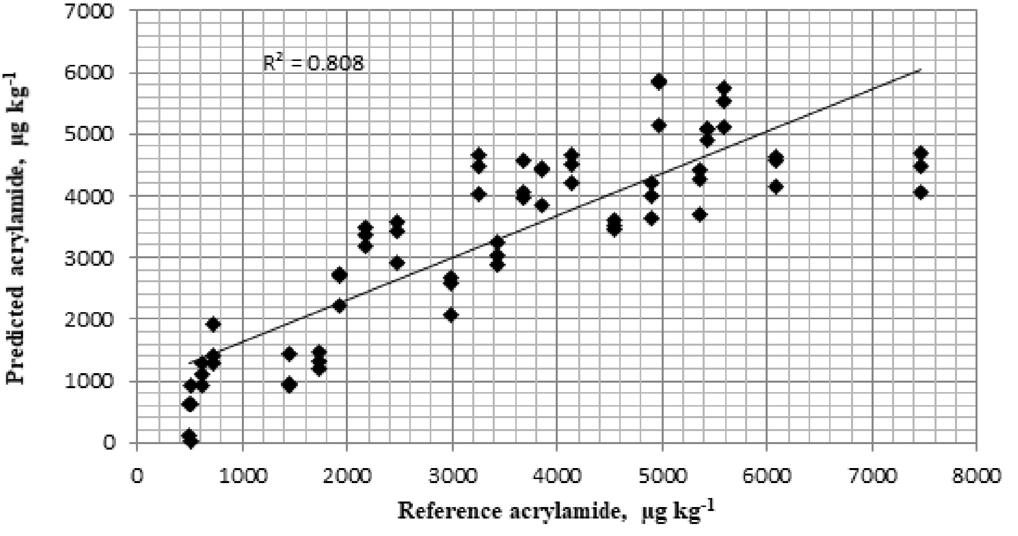

Fig.2. Predicted acrylamide vs reference acrylamide in potato crisp samples.

growing period to asparagine formation in grains. After scanning of samples multiple ranges of spectra cleaning and data pre-treatment were applied, and the optimum calibration models for acrylamide of potato crisps and bread were acquired. The SEC values obtained were 823.87 and 23.23 for potato crisps and bread, respectively, indicating the accuracy of the calibration model. In addition, the RSQ data (0.808 and 0.869 for potato crisps and bread, respectively) indicated that 80.8 % of the constituent acrylamide of potato crisps and 86.9 % of the constituent acrylamide of bread variances in the calibration samples is explained by the designed equation (Table 3, Fig. 1,2). Besides, SECV and SEC values were similar (957.71 and 823.87, respectively, for acrylamide of potato crisps, and 42.31 and 23.23, respectively for acrylamide of bread), indicating the sufficient accuracy of the prediction models. The values of 1-VR were 0.686 and 0.688 for acrylamide in potato crisps and acrylamide in bread, respectively, which means that 68.6 % of variance in acrylamide contents in potato crisps, and 68.8 % of variance in acrylamide contents in bread of calibration dataset were explained by the calibration equations during the cross validation process. The difference between means of the NIR predicted and reference values (Bias) for both models (potato crisps and bread) were significantly different from zero, and this indicates that the calibration is biased. Supplementation of calibration models with new samples is necessary for Bias correction (LVS EN ISO 12099; Williams, 2001; NIR White paper).

The results obtained indicate that the NIR spectrometry can be successfully used for acrylamide content determination in potato crisps and bread. Before use, developed calibration equations are necessary to expand with more samples and to validate with at least 20 independent samples with known reference values (LVS EN ISO 12099).

CONCLUSIONS

Acrylamide content of potato crisps and bread can be predicted by NIR scans. However, the calibration equations obtained unfit for acrylamide content prediction in unknown samples; it is necessary to expand calibration set with including more samples with known values in order to increase accuracy of calibration equations; after that validation of developed calibration equations on an independent sample set are necessary.

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