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Computer vision-based image analysis for the estimation of acrylamide concentrations of potato chips and french fries

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Abstract

In this study, digital colour images of fried potato chips and french fries were analyzed to estimate acrylamide levels based on the correlation with analyses using liquid chromatography-mass spectrometry. In fried potato images, bright yellow (Region 1), yellowish brown (Region 2) and darker brown (Region 3) regions were clearly visible, having different kinds of image pixels with characteristic mean values of red, green and blue components. Pixels of the fried potato image were classified into three sets (Set 1, Set 2 and Set 3) by means of semi-automatic and automatic segmentation. There was a strong correlation between acrylamide concentration and NA2 value, which is defined as the number of pixels in Set 2 divided by the total number of pixels of the entire fried potato image. To verify the applicability of this approach, a linear regression equation was used to estimate the acrylamide concentrations of a number of commercial potato chips and home-made french fries. Mean differences between the measured and predicted acrylamide concentrations were found to be $+4 \pm 14\%$ and $-14 \pm 24\%$ for commercial potato chips and home-made french fries, respectively.
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1. Introduction

Acrylamide formation was found to occur during the browning process, by Maillard reaction, of reducing sugars with asparagine at temperatures above 120 °C (Friedman, 2003; Mottram, Wedzicha, & Dodson, 2002; Stadler et al., 2002; Yaylayan, Wnorowski, & Locas, 2003). Coloured products are also formed in foods during heating as a result of the Maillard reaction and melanoidins are known to be the main end-products of the reaction (Márquez & Aňón, 1986; Pedrechi, Moyano, Kaack, & Granby, 2005; Şenyuva & Gökmen, 2005). These brown polymers have significant effects on the quality of food, since colour is an important food attribute and a key factor in consumer acceptance. The mechanism of formation of brown colour

is not fully understood and the structure of melanoidins is largely unknown (Martins & van Boekel, 2003).

Since colour can easily be measured, it may be used as an indicator of other Maillard reaction products, such as acrylamide. Colour of foods is usually measured in by the $L^*a^*b^*$ system, which is an international standard for colour measurements, adopted by the Commission Internationale d'Eclairage (CIE) in 1976. L^* is the luminance or lightness component (black to white), and parameters a^* (from green to red) and b^* (from blue to yellow) are the two chromatic components (Papadakis, Abdul-Malek, Kamdem, & Yam, 2000). Amrein, Schönbächler, Escher, and Amado (2004) reported a significant correlation between the L^* values and the acrylamide content during baking at 180 °C. Surdyk, Rosén, Andersson, and Åman (2004) also reported a highly significant correlation between colour and acrylamide content in bread crust during baking. Pedrechi et al. (2005) reported that L^* and b^*

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values did not show as extensive changes as those shown by a^* during frying of potato chips. They found a linear correlation between the acrylamide concentration and the colour of potato chips, represented by the redness component a^* at temperatures of 120, 150 and 180 °C for up to 5 min of frying. Şenyuva and Gökmen (2005) reported that the changes in acrylamide levels and CIE a^* followed almost the same trend during heating of coffee at 150, 200 and 225 °C.

Although the previous findings profoundly suggest that surface colour may be correlated with acrylamide concentration in thermally processed foods, the measurement of surface image and its colour properties need to be investigated in more detail to establish a useful correlation. This paper describes a computer vision-based image analysis for the estimation of acrylamide levels of fried potatoes.

2. Materials and methods

2.1. Chemicals and consumables

Acrylamide (99+%) and $^{13}C_3$-labelled acrylamide (99% isotopic purity) were obtained from Sigma (Diesenhofen, Germany) and Cambridge Isotope Laboratories (Andover, MA, USA), respectively. Methanol, potassium hexacyanoferrate, zinc sulfate, formic acid (98%) and acetic acid (glacial) were of analytical grade and obtained from Merck (Darmstadt, Germany). Ultra pure water was used throughout the experiments (MilliQ system, Millipore, Bedford, MA, USA). Oasis HLB (1 ml, 30 mg) SPE cartridges were supplied by Waters (Milford, MA, USA). Glass vials with septum screw caps were supplied by Agilent Technologies (Wilmington, DE, USA). The analytical column (Inertsil ODS-3, 250 × 4.6 mm, 5 μm) was supplied by HiChrom (Berkshire, England).

Stock solutions of acrylamide (1 mg/ml) and $^{13}C_3$-labelled acrylamide (0.1 mg/ml) were prepared by dissolving in distilled water. Working standards were prepared by diluting the stock solution of acrylamide to concentrations of 0.1, 0.2, 0.3, 0.5, 1.0 and 2.0 μg/ml with 0.01 mM acetic acid. Stock solutions and working standards were kept at 4 °C for one month. Carrez I solution was prepared by dissolving 15 g of potassium hexacyanoferrate in 100 ml of water, and Carrez II solution by dissolving 30 g of zinc sulfate in 100 ml of water.

2.2. Preparation of fried potatoes

Ten types of potato tubers, obtained from local markets, were used to prepare potato chips or french fries. Potatoes were washed and peeled before cutting. One type of potato tuber was used to prepare potato chips (2 mm), using a slicer. Frying was performed in an oil bath set at 170 °C with sampling at 1, 3, 5, 8, 10, 15, 30, 45 and 60 min for potato chips. Formations of acrylamide and colour were monitored in potato chips in a time-dependent manner. The data were used to build correlations between acrylamide levels and surface image properties for fried potatoes. In order to test the applicability of the computer vision-based approach, french fries made of 10 types of tubers were analyzed. Potatoes cut into strips (8.5 × 8.5 × 70.0 mm) were fried at 170 °C for 10 min in an oil bath.

2.3. Measurement of acrylamide

2.3.1. Sample preparation

A sample preparation procedure previously, described by us elsewhere was used (Gökmen, Şenyuva, Acar, & Sarıoğlu, 2005). Finely ground potato chips or french fries were weighed (1 g) into a 10 ml glass centrifuge tube with cap. The sample was suspended in 5 ml of methanol and extracted for 2 min in a vortex mixer. The suspension was centrifuged at 5000 rpm for 10 min. The clear supernatant was transferred into a centrifuge tube and treated with Carrez I and II solutions (25 μl each) to precipitate the co-extractives. Following centrifugation at 5000 rpm for 5 min, 1.0 ml of clear supernatant (0.2 g sample) was quantitatively transferred into a conical bottom glass test tube placed in a water bath at 40 °C and evaporated to dryness under nitrogen at 3 psig. The remaining residue was immediately redissolved in 1 ml of water by mixing in a vortex mixer for 1 min. For the SPE cleanup, an Oasis HLB cartridge was preconditioned, consequently, with 1 ml of methanol and 1 ml of water at a rate of two drops per second, using a syringe. Then, 1 ml of the extract was passed through the cartridge at a rate of one drop per second using a syringe. The first 10 drops of the effluent were discarded to prevent any dilution of sample by replacing water hold in the sorbent void fraction with the sample effluent. The subsequent drops were collected and filtered through a 0.45 μm syringe filter and 20 μl of the final test solution were injected onto the LC column for quantitation by LC–MS.

2.3.2. LC–MS analysis

LC–MS analyses were performed by an Agilent 1100 HPLC system, consisting of a binary pump, an autosampler and a temperature-controlled column oven, coupled to an Agilent 1100 MS detector equipped with atmospheric pressure chemical ionization interface. The analytical separation was performed on a Inertsil ODS-3 column (250 × 4.6 mm, 5 μm), using an isocratic mixture of 0.01 mM acetic acid in 0.2% aqueous solution of formic acid at a flow rate of 0.6 ml/min at 25 °C. The LC eluent was directed to the MS system after a delay time of 6.5 min, using MSD software. Data acquisition was performed in selected ion monitoring mode, using the interface parameters: drying gas (N_2, 100 psig) flow of 4 l/min, nebulizer pressure of 60 psig, drying gas temperature of 325 °C, vaporizer temperature of 425 °C, capillary voltage of 4 kV, corona current of 4 μA, fragmentor voltage of 55 eV. Ions monitored were m/z 72 and 55 for acrylamide and m/z 75 and 58 for $^{13}C_3$-labelled acrylamide for the quantification of acrylamide in the samples.

2.4. Measurement of CIE colour parameters

Colour measurements ($L^*a^*b^*$ parameters) were performed using a Minolta CM-3600d model spectrophotometer. Potato chips and french fries were aligned to sample measurement hole, manually, to measure the reflectance from both front and rear sides.

3. Results and discussion

3.1. Formation of colour and acrylamide in potato chips during frying

In potato chips, measured acrylamide concentration increased rapidly at the onset of frying, reaching an apparent maximum concentration of 5482 ng/g at 8 min. Then the acrylamide concentration in potato chips tended to decrease exponentially. These results suggest that acrylamide forms as an intermediate product during Maillard reaction and its concentration begins to decrease as the rate of degradation exceeds the rate of formation during heating. As illustrated in Fig. 1, change of colour in potato chips is a dynamic process in which certain colour transitions occur as the frying proceeds. The initial pale soft yellow colour of potato first turned to bright yellow, then to brownish yellow during 8–10 min of frying at 170 °C. After 10 min, browning in the surface became clearer, reaching a dark-brown at the end of frying for 60 min. CIE colour space parameters ($L^*a^*b^*$) were determined to describe the changes occurring in the colour of potato chips during frying. CIE L^* and b^* values decreased exponentially during frying at 170 °C while CIE a^* value increased rapidly during the first 5–8 min of frying reaching a plateau at ca. 15 min of frying. It tended to decrease as the frying proceeded.

As seen in Fig. 1, the change of redness parameter a^* during frying was somewhat similar to that of acrylamide concentration in potato chips. However, these two variables could not be linearly correlated with each other. Here, acrylamide concentrations were translated to a dimension-less form by dividing each concentration by the apparent maximum concentration of acrylamide (5482 ng/g) measured in potato chips during the frying process.

Pedrechi et al. (2005) reported that CIE L^* and b^* parameters did not show as extensive changes as those shown by the CIE a^* parameter during frying of potato chips. A linear correlation was found between the acrylamide concentration and the colour of potato chips, represented by the redness component a^* at temperatures of 120, 150 and 180 °C for up to 5 min of frying. However, the effect of prolonged frying on acrylamide concentration and colour was not mentioned by these researchers. Taubert, Harlfinger, Henkes, Berkels, and Schömig (2004) investigated the relation between the level of surface browning and acrylamide concentration of french fries by linear regression. They reported that there could be a close correlation for small-surface material being fried. A somewhat less close correlation was observed for intermediate-surface material, while no correlation was observed for large-surface material.

Since the acrylamide concentrations were lower in darker potato chips, CIE $L^*a^*b^*$ parameters used to measure non-homogeneous surface colour may not be a reliable predictor of acrylamide concentration in potato chips. Image analysis, instead of colour analysis, may provide a solution to estimate the acrylamide level in a given potato chip image.

3.2. Computer vision-based analysis of fried potato images

It is experimentally observed that a machine vision based system can be designed to remove fried potatoes having high acrylamide levels from a packaging line. It may not be possible to define a specific range of colours for acrylamide level estimation in the CIE $L^*a^*b^*$ colour space. However, after the frying process, three different kinds of image pixels appear in a typical potato image, as can be observed in Fig. 2a. Digital image pixel values of a potato chip can be used to estimate the acrylamide levels in a fried potato chip. In this section, a relationship between the acrylamide concentration in a fried potato chip and its image is established.

A typical image captured by a digital camera consists of an array of vectors called pixels. Each pixel has red, green and blue colour values:

$$x[n,m] = \begin{bmatrix} x_r(n,m) \\ x_g(n,m) \\ x_b(n,m) \end{bmatrix}$$

where $x_r(n,m)$, $x_g(n,m)$ and $x_b(n,m)$ are values of the red, green and blue components of the (m,n)th pixel, respectively. In digital images, x_r, x_g and x_b colour components are represented in 8 bits, i.e., they are allowed to take integer values between 0 and $255(=2^8-1)$ (Gonzales & Woods, 2002). Digital and analogue cameras have built-in white-balancing systems modifying actual colour values, therefore, pixel values in an image, captured by a camera of a machine vision system or a consumer camera, may not correspond to true colours of imaged objects. In addition,
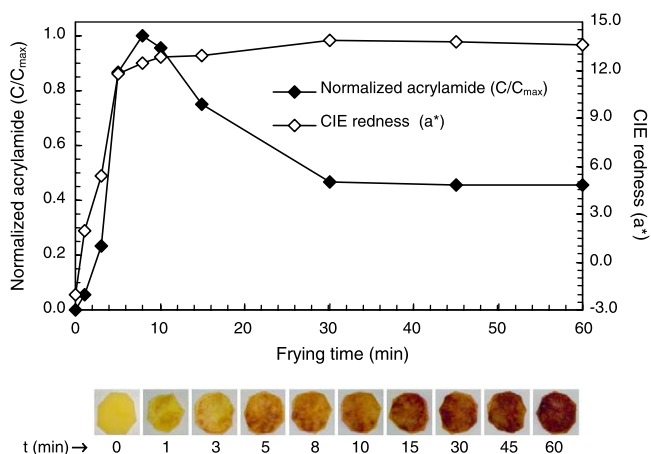

Fig. 1. Change of acrylamide concentration and CIE redness parameter a* in potato chips during frying at 170 °C.
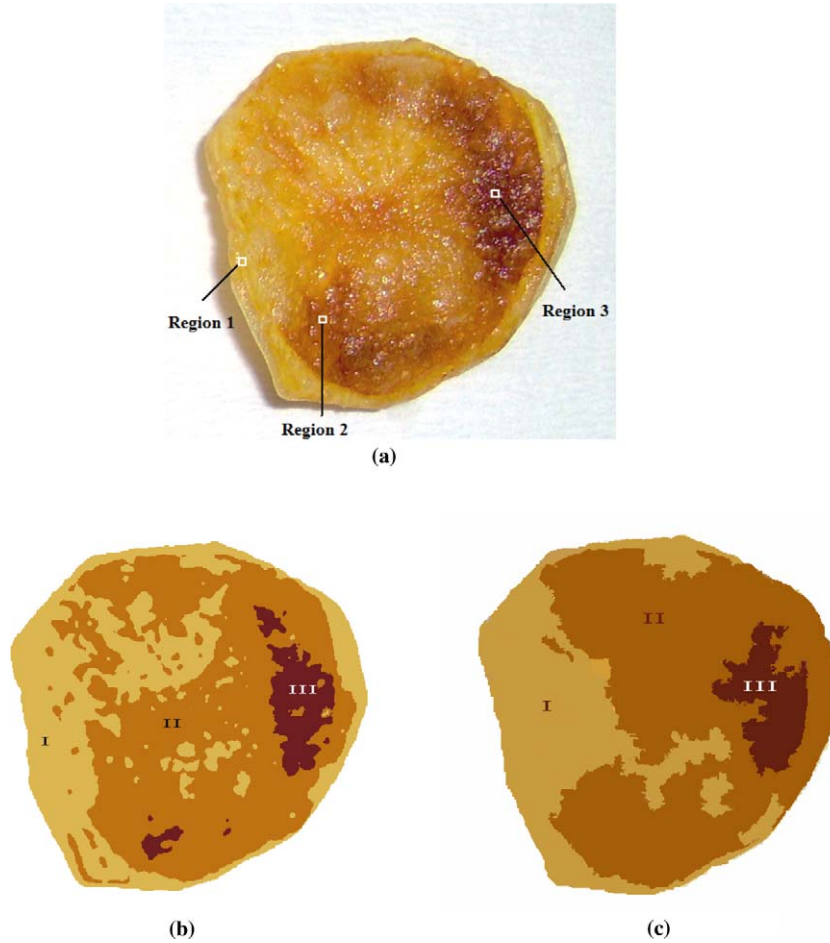
Fig. 2. (a) Original fried potato chip image with cropped windows shown; (b) result of semi-automatic segmentation algorithm; and (c) result of automatic mean shift segmentation algorithm.

CCD or CMOS imaging sensors of some cameras may not be calibrated during production. As mentioned above, even though it may not be possible to define a specific range of colours for acrylamide estimation in the CIE $L^* a^* b^*$ colour space, one can clearly visualize three different colours (or equivalently three different kinds of pixels) in a fried potato chip image as shown in Fig. 2a.

Three small rectangular windows were manually marked in Fig. 2a. Pixel values corresponding to small rectangular windows are given in Table 1. It was experimentally observed that Region 2 had a high probability of containing the highest level of acrylamide. These windows were cropped from the image, as shown in Fig. 2a, using the "imcrop" command of MATLAB.

Image pixel values corresponding to Region 1 have a mean value of [198.43 159.53 65.12], i.e., the mean value of the red, green and blue components are 198.43, 159.53, and 65.12, respectively. Average image pixel values corresponding to Region 2 and Region 3 are [189.61 114.6 1.4] and [103.56 41.75 26.18], respectively. Pixels of the fried potato image shown in Fig. 2a can be classified into three sets, based on their Euclidian distances to the above three representative mean values. Members of Set 2 in Fig. 2b are those pixels having the property:

$$\left\{ (x_r(n,m) - 189.61)^2 + (x_g(n,m) - 114.6)^2 + (x_b(n,m) - 1.4)^2 \right\}$$
$$\leqslant \left\{ (x_r(n,m) - 198.43)^2 + (x_g(n,m) - 159.53)^2 \right.$$
$$\left. + (x_b(n,m) - 65.12)^2 \right\}$$

and

$$\left\{ (x_r(n,m) - 189.61)^2 + (x_g(n,m) - 114.6)^2 + (x_b(n,m) - 1.4)^2 \right\}$$
$$\leqslant \left\{ (x_r(n,m) - 103.56)^2 + (x_g(n,m) - 41.75)^2 \right.$$
$$\left. + (x_b(n,m) - 26.18)^2 \right\}$$

or in vector form:

$$\left\| \begin{bmatrix} x_r(n,m) \\ x_g(n,m) \\ x_b(n,m) \end{bmatrix} - \begin{bmatrix} 189.61 \\ 114.6 \\ 1.4 \end{bmatrix} \right\|^2 \leqslant \left\| \begin{bmatrix} x_r(n,m) \\ x_g(n,m) \\ x_b(n,m) \end{bmatrix} - \begin{bmatrix} 198.43 \\ 159.53 \\ 65.12 \end{bmatrix} \right\|^2$$

and

$$\left\| \begin{bmatrix} x_r(n,m) \\ x_g(n,m) \\ x_b(n,m) \end{bmatrix} - \begin{bmatrix} 189.61 \\ 114.6 \\ 1.4 \end{bmatrix} \right\|^2 \leqslant \left\| \begin{bmatrix} x_r(n,m) \\ x_g(n,m) \\ x_b(n,m) \end{bmatrix} - \begin{bmatrix} 103.56 \\ 41.75 \\ 26.18 \end{bmatrix} \right\|^2$$

Members of Set 1 and Set 3 are defined in a similar manner.

Table 1
Cropped window pixel values of Regions 1, 2 and 3 shown in Fig. 2a

Red component										Green component										Blue component									
Region 1																													
198	201	202	201	201	201	199	199	198	198	156	157	162	159	160	160	157	156	155	154	24	38	49	60	65	65	65	65	69	70
197	198	202	202	202	201	199	199	198	198	159	159	161	161	160	160	157	157	155	155	26	46	58	64	72	72	72	71	71	71
199	199	202	202	204	201	199	199	199	199	159	159	163	165	165	160	160	159	157	157	39	51	61	68	72	72	74	74	76	77
200	199	199	201	203	201	199	199	198	200	157	157	160	165	165	165	162	159	159	159	39	46	60	66	68	71	71	74	78	78
200	200	198	197	198	199	199	201	199	198	158	158	159	160	162	164	164	164	162	162	43	54	56	60	64	68	71	75	78	78
200	200	195	195	195	196	199	197	197	198	157	157	157	157	160	160	164	162	161	161	45	54	57	59	60	64	71	73	76	78
201	200	195	194	190	189	195	195	196	196	159	159	157	156	152	153	159	159	161	161	57	59	59	59	59	63	71	73	76	79
204	202	198	192	189	189	193	195	196	196	164	163	157	154	152	153	157	159	159	159	64	66	62	61	59	62	69	75	79	79
207	205	200	193	192	192	198	198	197	195	165	164	160	156	154	154	161	161	160	157	67	67	66	63	61	61	67	76	79	79
206	205	203	198	193	196	202	203	203	195	167	165	163	157	156	161	165	168	168	156	71	72	63	61	61	66	69	79	79	79
Region 2																													
189	188	188	189	189	192	198	199	203	199	114	112	111	112	112	1113	116	117	116	115	3	3	3	3	3	1	4	9	10	3
189	188	188	188	189	191	192	197	199	197	114	114	114	115	115	115	115	115	115	115	3	3	3	3	1	0	0	1	3	3
189	188	188	188	188	190	190	193	197	196	117	114	114	115	116	115	115	113	115	115	3	1	1	1	1	0	0	0	3	4
189	187	187	188	188	188	188	188	190	194	117	116	116	117	117	115	114	112	112	116	1	0	1	1	1	0	0	0	0	4
188	187	187	187	188	188	187	188	188	189	117	117	117	118	116	115	112	112	112	114	0	0	0	1	1	0	0	0	0	1
190	187	188	188	188	187	187	187	187	188	120	118	119	118	117	114	111	111	111	113	0	0	0	2	2	0	0	0	0	0
190	189	188	187	188	187	186	186	187	189	120	118	118	118	114	113	110	110	111	114	0	0	0	0	3	1	1	1	1	1
191	189	188	187	187	187	187	187	189	190	118	116	116	114	113	112	111	111	113	114	0	0	0	0	4	1	1	1	1	3
191	189	188	188	188	188	188	189	190	191	116	116	114	113	112	112	112	113	114	116	0	0	0	0	3	4	3	1	1	3
195	193	189	189	189	190	193	192	191	191	115	115	114	114	113	113	116	117	116	117	1	0	0	2	3	3	3	1	0	0
Region 3																													
125	125	112	97	97	102	106	106	106	107	53	53	48	48	45	51	44	44	44	50	29	29	29	29	31	27	22	22	23	30
129	129	112	102	97	97	105	108	114	114	71	78	63	55	43	43	43	47	50	52	59	59	69	58	43	31	27	27	26	25
141	153	153	105	90	90	92	114	115	115	73	109	109	55	33	31	31	47	54	55	62	89	89	63	46	27	27	28	28	26
113	118	100	90	85	92	102	115	115	115	65	78	39	33	32	32	37	52	55	59	17	43	43	47	43	22	22	22	24	25
97	100	96	90	90	102	117	115	117	117	38	39	38	32	32	37	52	52	56	62	10	18	23	44	47	47	24	22	24	25
96	96	96	92	92	102	113	115	117	119	35	38	38	34	34	37	44	52	60	75	8	18	22	23	22	19	24	24	30	30
96	97	96	90	90	90	96	97	118	132	35	42	38	29	16	18	20	35	62	81	12	18	20	12	12	7	13	20	31	55
97	101	94	90	90	92	96	96	97	132	42	43	34	18	15	16	20	26	35	75	12	20	20	7	2	7	12	12	20	55
102	104	94	90	92	95	96	92	97	97	43	43	30	17	16	18	20	25	26	31	20	28	18	7	5	7	7	12	12	15
105	105	94	90	90	95	92	87	79	92	43	43	30	17	13	17	17	19	9	9	32	32	32	7	4	7	4	7	4	9

The potato chip image was processed, pixel by pixel, to determine the entries of Sets 1, 2 and 3. This process is also called vector quantization (Cetin & Weerackody, 1988; Rabiner & Juang, 1993) and the corresponding MATLAB code is given in Appendix A. The segmented image is shown in Fig. 2b. In this image, Set 2 pixels occupy 55% of the area of the entire image. The normalized area of Set 2 (NA2) was defined as the number of pixels in Set 2 divided by the number of pixels in the entire potato chip image. It was experimentally observed that there was a strong correlation between NA2 values and the measured acrylamide levels. As shown in Fig. 3, both the normalized area of Set 2 and the normalized acrylamide concentration of potato chips follow the same pattern during the frying process.

Image segmentation method, mentioned above, is semi-automatic and depends on the selection of representative RGB means of three regions before the segmentation process starts. Automatic segmentation can also be carried out using a public domain mean shift-based segmentation tool (Comaniciu & Meer, 2002). The mean shift segmentation algorithm was initialized with the following parameters: Spatial bandwidth, 10 pixels and Colour bandwidth, 15 pixels. Using the mean shift segmentation algorithm, similar results were obtained, as shown in Fig. 2c. In this image, Set 2 pixels occupy 59% of the area of the entire image. Segmentation results of automatic or semi-automatic methods were close to each other.

In order to predict the acrylamide concentration of a given potato chip sample, a calibration curve of the NA2 parameter versus the measured acrylamide concentration was plotted, using the kinetic data obtained for potato chips prepared by frying at 170 °C. As can be seen in this plot, shown in Fig. 4, there is a clear linear correlation between the acrylamide levels and the NA2 parameter.

3.3. Test of the applicability of computer vision-based analysis approach

The applicability of this approach for the prediction of acrylamide level in fried potatoes was verified by analyzing a number of commercial and home-made fried potato sam-
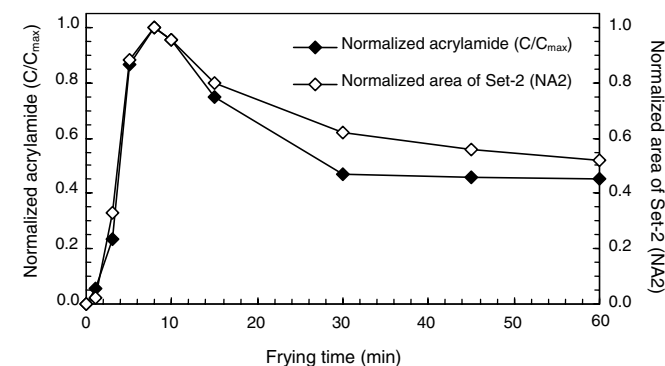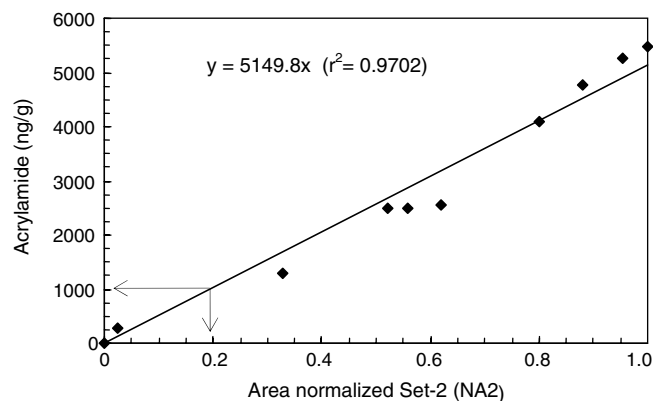

Fig. 4. Correlation between the NA2 parameter and the acrylamide level in fried potato chips.

ples. Measured and predicted acrylamide concentrations of commercial potato chips ($n = 3$) and home-made french fries ($n = 10$) are given in Table 2. The linear regression equation ($y = 5149.8x$) was used to calculate the acrylamide concentration of a potato sample from the NA2 parameter determined by computer vision-based image analysis. Measured and predicted values of acrylamide concentrations compare well with each other. Mean difference between the measured and the predicted acrylamide concentrations were $+4 \pm 14\%$ and $-14 \pm 24\%$ for commercial potato chips and home-made french fries, respectively. In both potato chips and french fries, only a randomly selected single face of a given sample was used to estimate the NA2 parameter, and thus to estimate acrylamide concentration. Although it is more reliable to determine a volumetric parameter for the estimation of acrylamide level, this is not possible from two dimensional digital images. It would be more accurate to analyze every face of a given french fry but in a computerized machine vision classification system; it is also not practical to image six faces of a fried potato.

Fig. 3. Change of normalized acrylamide and NA2 values in potato chips during frying.

Table 2
Measured and predicted acrylamide concentrations of commercial potato chips and home-made french fries

	NA2	Acrylamide (ng/g)		Difference[a] (%)
		Predicted	Measured	
Potato chips	0.15	782	897	−13
	0.37	1883	1675	+12
	0.55	2834	2543	+11
French fries	0.57	2922	4849	−40
	0.28	1464	2445	−40
	0.66	3409	6151	−45
	0.24	1218	2063	−41
	0.49	2515	3636	−31
	0.18	931	684	+36
	0.05	245	332	−26
	0.21	1061	1392	−24
	0.04	183	200	−8
	0.18	941	1053	−11

[a] Difference $= \frac{\text{Predicted} - \text{Measured}}{\text{Measured}} \times 100$.

4. Conclusion

The computer vision-based image analysis seems to be a promising approach for the prediction of acrylamide level in fried potatoes. Since higher NA2 values indicate higher acrylamide levels, fried potatoes, exceeding a predefined critical value of NA2, may be simply sorted out in a processing line based on this principle. In such systems, cameras can be installed in the packaging lines and fried potato images can be analyzed in real time and those products with high NA2 values can be removed. For example, if a provisional maximum permitted concentration of acrylamide in the finished product is established as 1000 ng/g, the fried potatoes exceeding a NA2 value of 0.2 would be removed by the machine vision system.

It should be noted that the calibration would need to be developed using more replicates over a wider range of concentrations, potato cultivars and frying conditions to improve accuracy of the approach. The purpose of this report was to demonstrate the feasibility of the method, not to implement it.

Appendix A

Function VectorQuantize accepts two inputs: *im_seq* (image to be segmented) and *u*(reference means) Representative cluster centers for Region 1, Region 2 and Region 3 are held in vector *u* together with an additional cluster center for background. This extra fourthvalue is to separate the background from potato image. Segmentation is carried on the image held in *im_seq* by using a nearest mean classifier. AN2 value is printed to the command window at the program termination. Segmented image can be displayed using the following code.

```
seg_im = VectorQuantize(im_seq,u);
    imshow(seg_im);

% File VectorQuantize.m
% ====================
function [seg_im] = VectorQuantize(im_seq,u);
[r c h]=size(im_seq);
% median filter the segmentation image with a [3×3] win-
dow to remove tiny oil sparks
filt_im = cat(3,medfilt2(im_seq(:,:,1),  'symmetric'),med-
filt2(im_seq(:,:, 2),··· 'symmetric'),medfilt2(im_seq(:,:,3),
'symmetric'));
% reduce from 3 dimensions to 2 dimensions for easy
handling of data im = reshape(filt_im,r*c,h)';
% compute the distance from cluster centers for all
pixels
for i = 1:4
    dist(i,:) = sum((im-repmat(u(:,i),[1 r*c])).²);
end
% find and store the location of minimum distance clus-
ter for each pixel
[y loc] = min(dist); vseg_im = zeros(r*c,h);
% change pixels values with their representative cluster
means for displaying purposes
for i = 1:4
    pos = find(loc==i);
    seg_im(pos,:) = repmat(u(:,i)',[length(pos) 1]);
end
% restore the image back to its original dimensions
seg_im = reshape(seg_im,[r c h]);
% median filter the segmented image with a [7×7] win-
dow to fuse tiny unconnected
% regions
seg_im = cat(3,medfilt2(seg_im(:,:,1),[7    7],  'symmet-
ric'),medfilt2(seg_im(:,:,2),··· [7 7], 'symmetric'), med-
filt2(seg_im(:,:,3),[7 7],'symmetric'));
% compute AN2 ratio from segmented image
ratio = length(find(loc==2))/length(find(loc~ = 4));
% display this ratio in command prompt
disp(ratio);
```

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