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Computer vision-based image analysis for rapid detection of acrylamide in heated foods

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Abstract

Background and Aim High concentrations of acrylamide found in common fried and baked foods attained considerable public concern since it has been classified as a probable human carcinogen. Many analytical methods have been published for the determination of acrylamide in foods. Although these methods perform well for quality control purposes in a food analysis laboratory, they are laborious, costly, and cannot be adopted for process control purposes by the food industry. This study describes a computer vision-based image analysis algorithm using color segmentation for the prediction of acrylamide level in thermally processed foods. *Materials and Methods* Laboratory made experimental potato crisps and cookies were prepared by frying and baking, respectively. The digital images of potato crisps and cookies were used to extract a meaningful browning parameter. *Results and Conclusion* The digital images were analyzed using a semiautomatic segmentation algorithm to calculate the browning ratio of potato crisps and cookies. The calculated browning ration were successfully correlated with acrylamide level of cookies and potato crisps. So, it was possible to predict the levels of acrylamide in test samples by means of their browning ratio values. The image analysis technique described here can be used as an online process control tool for frying and baking industry.

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Introduction

Thermal processing gives rise to several reactions which have direct impact on sensorial and nutritional properties of foods. However, it also forms acrylamide as a result of Maillard reaction between asparagine and reactive carbonyls at elevated temperatures (Mottram *et al.*, 2002; Stadler *et al.*, 2002; Tareke *et al.*, 2002).

Surface color is an important food attribute that can be used to predict the acrylamide level because both brown colored products and acrylamide are formed during the Maillard reaction at high temperatures. Researchers have reported a highly significant correlation between browning and acrylamide formation in heated foods. CIE $L^*a^*b^*$ color parameters have been widely used to correlate surface color and acrylamide in different foods such as French fries (Amrein *et al.*, 2006; Gökmen & Şenyuva, 2006; Pedreschi *et al.*, 2006), potato crisps (Pedreschi *et al.*, 2005), gingerbread (Amrein *et al.*, 2004), wheat bread crust (Surdyk *et al.*, 2004), rye crisp bread (Mustafa *et al.*, 2005), roasted coffee (Şenyuva & Gökmen, 2005) and/or roasted almonds (Lukac *et al.*, 2007). As previously shown for potato crisps and cookies, image analysis can be a viable alternative for the prediction of acrylamide (Gökmen *et al.*, 2006; Gökmen *et al.*, 2007; Gökmen *et al.*, 2008).

As a generic visual feature, the surface browning of thermally processed foods can be monitored by means of computer vision-based image analysis. This study describes a color segmentation algorithm for the calculation of a browning ratio to be correlated with acrylamide level. The potential application of the algorithm is examined for potato crisps and cookies to predict acrylamide concentration.

Experimental procedure

Chemicals and consumables

Acrylamide (99+%) was purchased from Sigma (Diesenhofen, Germany). Hexacyanoferrate and zinc sulfate were analytical grade and purchased from Merck (Darmstadt, Germany). Ultra pure water was used throughout the experiments (MilliQ system, Millipore, Bedford, MA, USA). Oasis MCX solid phase extraction cartridges (30 mg, 1 mL) and Atlantis T3 column (150 × 4.6 mm, 3 μm) were supplied by Waters (Milford, MA, USA).

A stock solution of acrylamide (1 mg/mL) was prepared by dissolving in water. Working standards of acrylamide were prepared daily by diluting the stock solution to concentrations of 1, 2, 5, 10, 20 and 50 μg/L with water. 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.

Preparation of cookies

Flour and shortening were supplied by local producers, and other ingredients were purchased from local supermarkets. Cookies were prepared according to a recipe described in American Association of Cereal Chemists (AACC) Method 10–54 (AACC, 2000) with some modifications. Recipes were formulated with 80 g of wheat flour, 1 g of salt, 17.6 g of deionized water, 0.8 g sodium bicarbonate, 0.4 g ammonium bicarbonate and different proportion of sugars. The ingredients were thoroughly mixed and the dough was rolled out to disks with the diameter of 5.5 cm and the thickness of 2 mm. The disks were baked at 200 and 220 °C for 10, 15, 20 and 25 min in a convection oven.

Preparation of potato crisps

Potatoes were cut into thin slices (1.4 ± 0.1 mm) using an electric slicer. The frying of potato slices was performed by completely immersing the slices in sunflower oil (5 L) contained in an electrical fryer set at 170 °C for different times. Browning development and acrylamide formation were monitored in crisps in a time-dependent manner to build a correlation between them.

Analysis of acrylamide

The samples were prepared for analysis following the procedure described by us elsewhere was used with some modifications (Şenyuva & Gökmen, 2006). Finely ground fried potato sample (1 g) was extracted with 2 × 10 mL of 10 mM formic acid by mixing in a vortex mixer for 2 min. The co-extracted colloids were precipitated by means of Carrez I and Carrez II reagents. The fat was separated by means of cold centrifugation (0 °C) at 9200 ×*g* for 10 min. Because the aqueous extract of fried potato contains positively charged co-extractives, which interfere during the mass spectrometric detection of acrylamide, the extract was cleaned up by means of a cation-exchanger-based SPE cartridge. A final clear supernatant was eluted through a preconditioned Oasis MCX cartridge at a rate of one drop per second. The first 7–8 drops of the eluate were discarded while the remaining drops were collected and filtered through 0.45 μm nylon filter before LC–MS analysis.

LC–MS analyses were performed by an Agilent 1200 HPLC system (Waldbronn, Germany) consisting of a binary pump, an autosampler and a temperature controlled column oven, coupled to an Agilent 6130 MS detector equipped with multimode interface. Atmospheric pressure chemical ionization was used with the following interface parameters: drying gas (N_2, 20 psig) flow of 5 L/min, nebulizer pressure of 20 psig, drying gas temperature of 350 °C, capillary voltage of 2000 V, and corona current of 5 μA. The analytical separation was performed on a Atlantis T3 column (150 × 4.6 mm, 3 μm) using the isocratic mixture of 10 mM formic acid at a flow rate of 0.3 mL/min at 25 °C. The LC eluent was directed to the MS system from 10 to 16 min using MSD software. Ions monitored were m/z 72 and 55 for the quantification of acrylamide in the samples. The signal response was linear over a concentration range between 1 and 50 μg/L of acrylamide. The limit of detection and the limit of quantitation for acrylamide were 5 and 15 ng/g in fried potatoes, respectively. The coefficient of variation was 10% or lower for three repetitive measurements of acrylamide in fried potatoes (Gökmen *et al*., 2009).

Image acquisition and analysis

Digital images of potato crisps and cookies were taken from an image acquisition system consisting of a color digital camera placed vertically at a distance of 25 cm above the sample. The angle between the axes of the lens and the sources of illumination was approximately 45° Illumination was achieved with 2 daylight fluorescent lamps with color temperature of 6500 K. Images were captured at a resolution of 5.1 megapixel and stored in a personal computer in .jpeg format without compression.

A typical image captured by a digital camera consists of an array of vectors called pixels. Each pixel $x[n,m]$ has red, green and blue color 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 (R), green (G) and blue (B) components of the (m,n)th pixel $x[n,m]$, respectively. In digital images, x_r, x_g and x_b color components are represented in eight bits, i.e., they are allowed to take integer values between 0 and 255 ($= 2^8 - 1$).

The pixels appearing with potato crisps and cookies were classified based on two predefined color reference values representing the initial color of potato or dough and of brown regions appeared after baking or frying. The mean reference values were used for the segmentation. Pixels of an image were classified into two sets (*Set-I* and *Set-II*) based on their Euclidian distances to the representative mean values. Variable *Set-II* held the number of pixels belonging to brown regions. The browning ratio was computed from the segmented image using the following formula by means of the Matlab code called 'BrowningRatio' given in Appendix 1;

$$\text{Browning Ratio} = \text{Set} - \text{IIPixels}/\text{Total Pixels}.$$

According to this code, representative color reference values are held in vector u together with an additional value for background. This extra third value is to separate the background from the image.

For potato crisps, vector u holds the values given below:

$$u = \begin{bmatrix} 0.833 & 0.587 & 1.000 \\ 0.694 & 0.308 & 1.000 \\ 0.153 & 0.054 & 1.000 \end{bmatrix},$$

and for cookies the values are as follows:

$$u = \begin{bmatrix} 0.980 & 0.863 & 0.326 \\ 0.941 & 0.671 & 0.326 \\ 0.853 & 0.408 & 0.333 \end{bmatrix}.$$

The code accepts two inputs including the image to be segmented and representative mean values. Before segmentation, the image saved in *file_name* should be stored in matrix *im_seg* by converting integer values to doubles using the following instructions:

$$im\_seg = \text{imread}('file\_name');$$
$$im\_seg = \text{im2double}(im\_seg);$$

The segmented image is displayed in a new window and computed browning ratio is printed to the command window at program termination using the following instruction:

$$seg\_im = \text{BrowningRatio}(im\_seq, u).$$

Figure 1 Original and segmented images of a potato chip (browning ratio 5.1%, acrylamide level 250 ng/g)

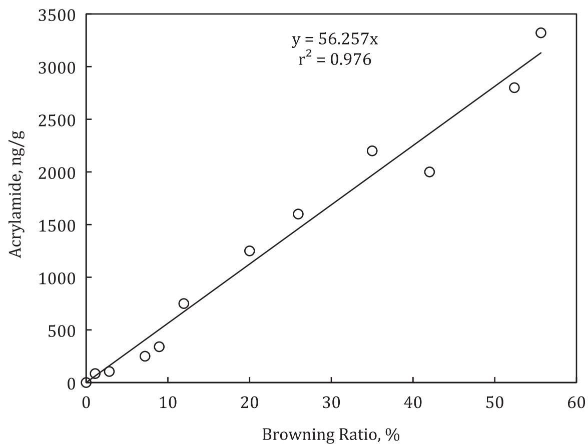
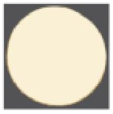
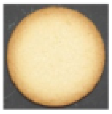
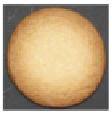
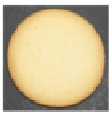
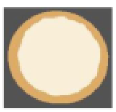
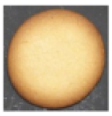
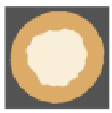
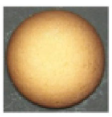
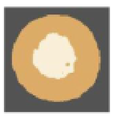


Figure 2 Correlation between browning ratio and acrylamide in potato crisps.

Results and discussion

Figure 1 shows a typical color image of a potato crisp with nonhomogenously distributed surface color. The change of surface color during frying is a dynamic process in which certain color transitions occur as the heating proceeds. It is a fact that increasing frying temperature and time also increases the rate of surface browning, as well as the rate of acrylamide formation in potato crisps. The image analysis algorithm mentioned above could easily measure the

Table 1 Original and segmented color images of cookies baked at different conditions

Baking condition (min)	Cookie image: Original	Cookie image: Segmented	Browning ratio (%)
200 °C × 10			2.54
200 °C × 15			6.83
200 °C × 20			37.93
200 °C × 25			71.65
220 °C × 10			6.33
220 °C × 15			30.70
220 °C × 20			60.31
220 °C × 25			76.35

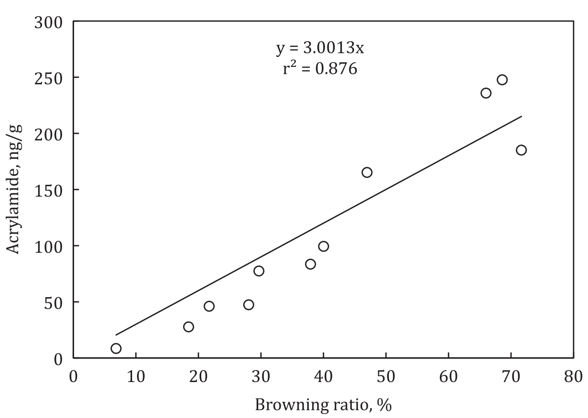


Figure 3 Correlation between browning ratio and acrylamide in cookies.

browning ratio of crisps based on the predefined reference color values. The browning ratio of crisps correlated well ($r^2 > 0.97$) with the level of acrylamide for a given potato variety under different frying conditions (Figure 2).

The original and segmented images of cookies prepared under different baking conditions are given in Table 1. It is clear from cookie images that the browning ratio increased as the thermal load increased during baking. The surface browning in cookies simply developed as a circle that grew from the edge to the center as the baking proceeded. The rate of browning was similar to that of acrylamide formation in cookies during baking. Consequently, browning ratio and acrylamide concentration correlated well for cookies with a correlation coefficient (r^2) higher than 0.87 as shown in Figure 3.

Based on the results of present study, it is concluded that the browning ratio may be considered as a reliable indicator of acrylamide concentration fried potatoes and bakery products. Because its calculation is simply based on the predefined color references, this approach can easily be modified to expand its application for different food formulations, which are to be thermally processed. It should be pointed out that the reference color values reported here are specific for the given potato variety and cookie formulation. These reference values should be revised before this approach is being implemented by the food industry for similar food products. After doing so, the technique can be effectively used as a process control tool to monitor the browning ratio for product safety evaluation purposes.

One potential application of the technique is to sort thermally processed foods in a pass/fail manner. In such a case, it is necessary to define a threshold level for acrylamide to achieve this objective. No maximum permitted concentration has yet been established for acrylamide in foods. The European Commission has recently published a directive aiming to control acrylamide levels among member states (EC, 2007) which could be the starting point for a future regulation. Once a critical level of acrylamide is adopted by the food industry, the segmentation algorithm described may be used as a tool for online safety evaluation and sorting purposes.

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Appendix 1. Matlab code to compute browning ratio of potato crisps and cookies

```
function [seg_im] = BrowningRatio(im_seg,u);
u = im2double(u);
[r c h] = size(im_seg);
% median filter the segmentation image with a [3 x 3] window
filt_im = cat(3,medfilt2(im_seg(:,:,1),'symmetric'),medfilt2
(im_seg
(:,:,2),…
'symmetric'),medfilt2(im_seg(:,:,3),'symmetric'));
figure(1); imshow(filt_im);
% 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 = 1:3
dist(i,:) = sum((im-repmat(u(:,i),[1 r*c])).^2);
end
% find and store the location of minimum distance cluster for each
pixe
[y loc] = min(dist);
seg_im = zeros(r*c,h);
% change pixels values with their representative cluster means for
displaying
% purposes
for i = 1:3
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 x 7] window to
fuse tiny
% unconnected regions
seg_im = cat(3,medfilt2(seg_im(:,:,1),[7
7],'symmetric'),medfilt2(seg_im(:,:,2),…
[7 7],'symmetric'), medfilt2(seg_im(:,:,3),[7 7],'symmetric'));
% display the segmented image in new window
figure(2); imshow(seg_im,[]);
% compute browning ratio
browning_ratio = length(find(loc = = 2))/length(find(loc~ = 3));
% display browning ratio in command prompt
disp(browning_ratio);
```

