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Monitoring the effect of transglutaminase in semi-hard cheese during ripening by hyperspectral imaging

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ABSTRACT

Semi-hard cheese samples made from pasteurized 3.5% and 5% fat cow's milk were partly enzyme treated with microbial transglutaminase (mTG). Cheese colour and yield was greatly affected by mTG at both fat levels. The cross-linking effect of mTG also led to improved protein content and hardness in cheese. As Texture Profile Analysis (TPA) could only distinguish according to enzyme treatment we studied hyperspectral imaging (HSI) to differentiate also according to fat level and ripening status. HSI was used on a weekly basis during 4–10 weeks period of ripening time. In case of uneven surface, using the spatial information and making proper normalization on the spectra of ROI pixels, the hyperspectral method can provide even better signal-to-noise ratio then the conventional spectrophotometric method. Statistical analysis with Partial Least Squares (PLS) regression and Monte Carlo Cross Validation (MCCV) led to determination of significant wavelengths both for the detection of enzyme treatment (1387 nm) and for fat content (1190, 1234 nm) independent of the actual age of examined semi-hard cheese. Hyperspectral measurement method allowed remote inspection of the product through transparent vacuum foil. The spectrum normalization allowed the elimination of effects originating from inhomogeneous illumination caused by cheese holes.

1. Introduction

The use of non-destructive methods for monitoring of semi-hard cheese during ripening is a topic of great interest nowadays, where attention is mainly focused on the monitoring of eye growth (Grenier et al., 2016).

Ripening can be followed at the first level front face using fluorescent spectroscopy alone (Karoui et al., 2007a) or coupled with chemometric tools (Karoui et al., 2007b).

The effect of ripening has obvious signs like the development of eye growth. Cheese eyes growth is due to CO_2 gas formation, which is released by the activity of propionic acid bacteria (McSweeney, 2004). It was studied by single imaging approaches like X-ray computed tomography (Huc et al., 2014a) and confocal laser scanning microscopy (Soodam et al., 2014). However researchers have tended to use advanced methods by coupling imaging techniques with different image processing, measurement and statistical analysis methods. Therefore cheese ripening has already been observed by MRI combined with CO_2 release measurement (Huc et al., 2014b) or MRI combined with a novel imaging algorithm (Musse et al., 2014). Amulti-scale investigation combining light microscopy, confocal laser scanning microscopy, and scanning electron microscopy has also been used to better understand the process of cheese eyes development. (Huc et al., 2014c).

Among the imaging methods, hyperspectral imaging (HSI) seems to be a very promising tool in the food industry (Sun, 2010; ElMasry et al., 2012). The "hyper" in hyperspectral means "over" as in "too many" and refers to the large number of measured wavelength bands. Hyperspectral images are spectrally over determined, which means that they provide ample spectral information to identify and distinguish spectrally unique materials. Hyperspectral imagery provides the potential for extraction of more accurate and detailed information than possible with any other type of remotely sensed data (Shipert, 2003). Several wavelength selection (assignation) techniques of chemometrics are used to prepare application areas of HSI in food industry (Liu et al., 2014). The application possibilities of HSI for quality and safety analysis and assessment have been already summarized for meat and meat products, fish, fruit, vegetables and grains (Wu and Sun, 2013). The hyperspectral imaging can be used for monitoring disease conditions, ripeness, tenderness, grading or contamination. (Mahalik and Nambiar,

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2010).

The application possibilities for dairy products are also growing (Dufour, 2011; Gowen et al., 2010). According to the literature, HSI could be a useful tool to determine cheese quality characteristics, such as meltability and cheese defects, like calcium lactate crystals or mechanical openings (Sun, 2008). HSI allows the surface changes of cheese samples to be followed. However we did not follow the cheese-eyes development, but the indirect effect of enzyme-treatment (firmer and therefore dry surface).

To our best knowledge the effect of microbial transglutaminase (E.C. 2.3.2.13, official name: glutamine γ-glutamiltransferase, shortly: mTG)on cheese was only monitored with texture profile analysis (TPA). The use of TPA seems to be obvious as the enzyme treatment of cheese causes enhanced hardness by the cross-linking of Glu and Lys. Nevertheless due to mTG, the modified protein texture of cheese can hold back more whey and therefore cause better cheese yield. (Kieliszek and Misiewicz, 2014). However the hardness of semi-hard cheese, was only analyzed at selected ripening states: after 1 week (Cozzolino et al., 2003), 4 weeks (Aaltonen et al., 2014; Di Pierro et al., 2010), 24 weeks (Aaltonen et al., 2014). Therefore our main objective was to follow the effect of mTG enzyme treatment on semi-hard cheese with both TPA and hyperspectral imaging (HSI). Furthermore the effect of milk fat and ripening time was also followed by HSI. The measurement was done on 4 different semi-hard cheese products during the 4–10 weeks period of their ripening. Measurement was done through transparent vacuum pouch in order to see if HSI could have the benefit of observing the sample through vacuum packaging. This could rise up the possibility of use as it on manufacturing line as a hyperspectral sorter. Our final aim was to investigate if hyperspectral imaging could be an alternative to determine the mTG enzyme treatment, fat content and ripening state of the final product.

2. Materials and methods

2.1. Manufacturing and ripening process of semi-hard cheese

Semi-hard cheese samples were made from 10 L 3.5% fat and 5% fat pasteurized non homogenized cows' milk (Cserpes Sajtműhely Ltd., Kapuvár, Hungary). The fat content was adjusted with whipping cream (30% fat) of Fuchstej Ltd. (Valkó, Hungary). The milk was then heated up to 30 °C in a 10 L Armfield FT-20A cheese-vat (Bridge House, Great Britain) and 0.025% (w/v) $CaCl_2$ was added. A cheese curd stirrer was used for proper mixing during the whole process, except during curdling. In case of enzyme treated samples, 0.01% (w/v) mTG (Probind CH, nominal activity: 40–65 EU, BDF Natural Ingredients, Girona, Spain) was used simultaneously with MA 4001 cheese culture (LYO 5DCU, Danisco, Epernon, France). After 15 min incubation 0.05% (v/v) chymosin (Présure Simple Brun, Danisco, Melle, France) was added. Stirring was stopped 5 min after the addition of chymosin. The sol-gel transformation took 1 h, which was necessary to reach a curd texture hard enough to cut with a cheese harp. After cutting the curd, it was heated to 39 °C to help whey separation. Then whey was separated. The curd was pressed for 24 h with a force of 20 kg per kg cheese and then cured in 20% (w/v) NaCl solution for one day. After 1 day desiccation, 1 kg cheese samples were cutted (size: 40 mm × 40 mm × 10 mm, cuboid shape) and were vacuum-packaged in Flexo-vacuum PS650 five-layers vacuum package (oxygen permeability: 100 $cm^3/m^2/24$ h, steam permeability: 6 $g/m^2/24$ h). These samples were ripened at 10 ± 1 °C for 10 weeks. Finally 4 samples were measured by each milk fat level. The cheeses were identified by the fat level of cheese milk, because the fat content of cheese, varies (generally 2–3%) according to manufacture (see in our case on Table 1).

2.2. Surface colour

CIELAB values (lightness, L*; redness, a* and yellowness, b*) of cheese were evaluated on a Minolta CHROMA METER CHR-400 tristimulus colour measuring system (Konica Minolta Sensing Inc., Japan) at 4 weeks of ripening. Ten replicates of the analysis were performed for each formulation.

2.3. Determination of chemical characteristics of semi-hard cheese samples

The pH of cheese samples was measured once, after 4 weeks ripening, with a Testo 206 portable pH meter (Testo AG., Limburg an der Lahn, Germany). Dry matter content was measured both in cheese (after 4 weeks) with KERN MLS-N Moisture Analyser (Kern & Sohn GmbH, Balingen, Germany) using 105 °C as the drying temperature. The fat content of cheese samples was determined by Lindner method (Seidel and Lindner, 1993) after 4 weeks. The protein content of cheese was determined by Kjeldahl method (Kjeldahl, 1883).

2.4. Hyperspectral imaging

During the 4–10 weeks period of ripening at 10 ± 1 °C, hyperspectral images were measured on both sides of each sample between 960 and 1694 nm on a weekly basis. The measurement was done always on the same 2 sides of the samples.

Hyperspectral data were taken by Head Wall push broom system (900–1700 nm range, 256 × 320 InGaAs sensor matrix, 14 bit A/D, px = 5.16 nm/px spectral resolution, 58 × 100 mm–184 × 320 px image size, 100 mm/320 px = 312 μm/px spatial resolution, 45/0 illumination geometry, HeadWall Photonics, Inc, Fitchburg, MA, USA) and Argus calibration and controlling software (Firtha, 2010). The stable setup, proper calibration method and pixel-noise handling provided the same result for a flat surface, as a conventional spectrophotometer.

Both sides of each cheese sample were measured through packaging on each week of ripening. One measurement resulted in

Table 1
Cheese yield, yellowness and chemical composition of semi-hard cheese samples.

Cheese type	Cheese yield (kg cheese/10 L milk)	Yellowness (a*)	Fat in dry matter (%)	Protein of cheese (%, w/w)
control - 3.5% (untreated cheese made from 3.5% milk)	1.33 ± 0.15	13.02 ± 0.30	47.32 ± 0.92	21.57 ± 0.43
enzyme-treated - 3.5% (mTG treated cheese made from 3.5% milk)	1.36 ± 0.16	14.21 ± 0.34	44.60 ± 1.26	23.62 ± 0.71
control - 5% (untreated cheese made from 5% milk)	1.44 ± 0.14	14.07 ± 0.32	53.61 ± 1.04	25.98 ± 1.02
enzyme-treated - 5% (mTG treated cheese made from 5% milk)	1.45 ± 0.17	15.92 ± 0.31	48.58 ± 0.49	27.14 ± 0.75