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Effect of elevated temperature on the microstructure of full fat Cheddar cheese during ripening

Kevany Soodam[a,b,1], Lydia Ong[a,b,d], Ian B. Powell[c,d], Sandra E. Kentish[a,d], Sally L. Gras[a,b,d,*]

[a] Department of Chemical and Biomolecular Engineering, The University of Melbourne, Parkville, Victoria 3010, Australia
[b] The Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Parkville, Victoria 3010, Australia
[c] Dairy Innovation Australia Limited,180 Princes Highway, Werribee, Victoria 3030, Australia
[d] The ARC Dairy Innovation Hub, Department of Chemical and Biomolecular Engineering, The University of Melbourne, Parkville, Victoria 3010, Australia

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ABSTRACT

Elevated temperatures have been widely studied as a route to accelerate cheese ripening and decrease energy and storage requirements but the impact of temperature on the underlying microstructure of the cheese during prolonged periods of ripening is poorly understood. In this study, Cheddar cheese was matured at four different ripening temperatures (8 °C, 15 °C, 20 °C or a combination of 8 °C and 15 °C) and the impact on cheese microstructure assessed using confocal laser scanning microscopy, cryo scanning electron microscopy and quantitative image analysis of 3D images. An increase in ripening temperature was shown to alter the microstructure of the cheese protein network after only a few weeks of ripening. Incubation at 20 °C significantly reduced branching within the protein network, leading to thicker protein strands and larger pores after 33 days. These structural changes coincided with increased proteolysis, consistent with solubilisation of the protein network; they also led to a softer, less chewy and less cohesive cheese. While the concentration of biogenic amines tryptamine and tyramine were observed to increase with ripening temperature, the concentrations were generally low, confirming that biogenic amines do not represent a health concern under the conditions examined. This study illustrates how 3D image analysis can be used to observe and quantify the effect of process changes on cheese structure, assisting our understanding of the link between structure and function in Cheddar cheese.

1. Introduction

Ripening is a slow and lengthy process for rennet coagulated cheeses, typically lasting from three weeks to two or more years (Fox, 2002). During this period microbiological and biochemical changes occur, leading to changes in cheese texture and the development of flavour (McSweeney, 2004). Accelerated ripening of Cheddar can be accomplished by increasing the ripening temperature, the use of additional cultures (derived from starters or non-starter lactic acid bacteria (NSLAB) in live or attenuated form), or the use of enzymes (in many cases products of genetic modification, where legislatively approved) or cultures with high enzyme activities. Such acceleration can reduce the energy required for temperature control during maturation and decrease storage requirements leading to increased profitability. Among these options the application of a higher ripening temperature is the easiest to implement.

Our understanding of the impact of higher temperatures on the microstructure of Cheddar cheese is far from complete. The microbial and biochemical changes induced by high temperatures have been well documented for Cheddar (Aston, Giles, Durward, & Dulley, 1985; Cromie, Giles, & Dulley, 1987; Folkertsma, Fox, & McSweeney, 1996) and the link between accelerated flavour development and higher ripening temperatures established (Aston et al., 1985; Folkertsma et al., 1996; Hannon et al., 2005). Few studies, however, have probed the link between elevated ripening temperature and Cheddar microstructure. The link between this microstructure and changes in cheese properties has also not been studied.

Industrial Cheddar cheese is conventionally ripened at a constant temperature in the range of 6 °C–8 °C. Short periods of 70 h at 25 °C were shown to have little impact on the structure observed in Cheddar cheese by confocal laser scanning microscopy (CLSM) (O'Reilly et al., 2003). The structure observed was characteristic of Cheddar soon after manufacture, which is typically described as containing a continuous protein matrix with irregular shaped fat globules embedded within the protein network; curd junctions may also be present in the structure (Auty, Twomey, Guinee, & Mulvihill, 2001).

* Corresponding author at: The Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Parkville, Victoria 3010, Australia.
E-mail address: sgras@unimelb.edu.au (S.L. Gras).
[1] Present address: Moorepark Food Research Centre, Teagasc Moorepark, Fermoy, Co. Cork, Ireland.

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Proteolytic activity is known to be higher in Cheddar at elevated ripening temperatures (Aston et al., 1985; Folkertsma et al., 1996), leading to a softer cheese when ripened at 16 °C (Folkertsma et al., 1996). Lipolysis can also be accelerated (Folkertsma et al., 1996). Other textural changes reported include a more brittle and less springy Cheddar cheese when the temperature was increased to 20 °C (Fedrick & Dulley, 1984). These changes all indicate a breakdown in the protein network and potential rearrangement in the underlying microstructure of the cheese that could be visualised using microscopic techniques.

Another potential consequence of ripening at elevated temperatures, not linked directly to the microstructure, is that changes in the bacterial population and proteolysis may affect the concentration of amino acids and biogenic amines, such as putrescine, cadaverine and tyramine. Both amino acids and biogenic amines were observed to significantly increase with temperature and storage in Dutch type cheeses (Komprda et al., 2007; Pachlová, Buňka, Flasarová, Válková, & Buňková, 2012), potentially introducing a health hazard for consumers. The final concentration of amines in Cheddar cheeses ripened at high temperatures at the end of the accelerated ripening period would therefore be valuable data to assess the relative merits of incubation at high temperatures.

The aims of the current study were to investigate the impact of elevated temperature on the microstructure, texture and proteolysis of ripened Cheddar cheese, applying quantitative image analysis to establish structure-function relationships during ripening. Four treatments were applied: ripening at 8 °C, 15 °C, 20 °C, or a two-stage ripening process involving ripening at 15 °C for 33 days followed by ripening at 8 °C for the subsequent ripening period. The presence of biogenic amines was also assessed. This study provides new insights into the microstructural changes that occur within Cheddar during accelerated ripening and the link between changes in cheese structure and functionality.

2. Materials and methods

2.1. Cheese treatment

Three 20 kg blocks of commercial full fat Cheddar cheese, made with pasteurised milk and a defined-strain *Lactococcus lactis* bulk starter culture, were obtained from the same vat of cheese and the blocks were then cut and packaged into at least 84 ~500 g block samples, which were then individually vacuum packed into commercial cheese barrier bags. The cheese samples were then stored at 8 °C (T8), 15 °C followed by 8 °C (T15-8),15 °C (T15) or 20 °C (T20) for the ripening period (330 days, 21 blocks of cheeses for each treatment). For one treatment (T15-8), the cheese was stored at 15 °C for 33 days and then 8 °C for the remainder of the trial. At selected time points during ripening, three 500 g block samples of each treatment were analysed using the methods described below, except for the amine analysis which used six 500 g block samples.

2.2. Compositional analysis of cheese

Cheeses were analysed in triplicate for fat, protein (%N x 6.38), salt and moisture using the gravimetric method AS2300.1.3 (Australian Standard, 2008), the Kjeldahl method AS2300.1.2.1 (Australian Standard, 1991), a potentiometric method (AOAC, 2012) and the method described in AS2300.1.1 (Australian Standard, 1988) respectively. The pH of the fresh and mature cheese was determined by blending 10 g of cheese with 10 g of water purified to a resistivity of 18.2 MΩ (Millipore MilliQ, Billerica, MA, USA) and the pH of the resultant slurry was then measured using an electrode pH meter (Orion 720A, Orion Pacific, Frankston, Australia).

2.3. Microscopic techniques

Cheese samples were analysed during ripening at days 1, 12, 33, 98, 194 by confocal laser scanning microscopy (CLSM, Leica TCS SP2; Leica Microsystems, Heidelberg, Germany), using a method described previously (Soodam, Ong, Powell, Kentish, & Gras, 2015). Stock solutions of Nile Red and Fast Green FCF were prepared at a concentration of 1 mg/mL in dimethyl sulfoxide and water respectively. These solutions were then diluted 10 fold just prior to staining. A total of 6 images and 3 three-dimensional pictures were collected for each cheese treatment at each time point during ripening. Reconstruction and analysis of the three-dimensional images was carried out using Imaris image processing software (Bitplane, South Windsor, CT, USA) as described previously (Ong, Dagastine, Kentish, & Gras, 2012) and yielded parameters as described by Soodam et al. (2015).

Cheese samples were also analysed with cryo scanning electron microscopy (Cryo-SEM, Quanta; Fei, Hillsborro, OR, USA) using a method described in a previous study (Ong, Dagastine, Kentish, & Gras, 2011). A total of 6 images were taken at a magnification of 2000×, 4000× or 8000× from one representative cheese sample for each treatment at each time-point (days 1, 12, 33, 98, 194) during ripening.

2.4. Proteolysis determination

The proteolytic pattern of the cheese during ripening was determined using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The cheese stock solution was prepared using the method of Ong, Henriksson, and Shah (2006), except that 25 mg samples of cheeses were used. The stock solutions produced were then diluted ~4-fold with Trisaminomethane (Tris, 10 mmol/L)–Ethylenediaminetetraacetic acid (EDTA 1 mmol/L) pH 8.0 buffer. Standards of α-casein (CN), β-CN and κ-CN (Sigma-Aldrich, Castle Hill, Australia) were also prepared at a concentration of 0.25 mg/mL. An aliquot of 6.5 μL was mixed with 2.5 μL of Bolt LDS Sample Buffer (4×) and 1 μL Bolt Reducing Agent (10×) (Invitrogen, Mt Waverley, Australia). The solution was heated at 70 °C for 10 min and then loaded into a 12% acrylamide Bis-Tris Plus gel (Invitrogen). A set of molecular standards, SeeBlue Plus2 pre-stained (Invitrogen), was also loaded in each gel. Bolt 2-(*N*-morpholino) ethanesulphonic acid SDS Running Buffer (1×) (Invitrogen) was used as buffer and the system was run for 75 min at 125 V. The protein bands were then stained and destained as described by Ong et al. (2006) except 7.5% v/v acetic acid was used and the destaining solution did not contain methanol. The gel was finally scanned using a Fujifilm Intelligent Dark Box II with LAS-3000 Lite V2.2 software and LAS-3000 Image Multi Gauge (Fujifilm, Brookvale, Australia) to quantify the intensity of α- and β-CN bands during ripening. The data presented are representative of three gels.

2.5. Determination of amine content

2.5.1. Extraction of samples

Hydrochloric acid (HCl, 1 mL, 0.1 M) was added to 0.1 g of grated cheese. The samples were then cryo-milled (Precellys 24, Bertin Technologies, Île-de-France, France) at −10 °C (program 2, 3 min total cycle; per cycle: 30 s spinning, 45 s waiting), followed by centrifugation for 10 min at 10,000 rpm (~9000*g*). The fat layer was removed and the supernatant was then collected (10 μL) for derivatization, avoiding the casein fraction at the bottom of the tube.

2.5.2. Derivatization of samples

The derivatization was performed using a method described by Boughton et al. (2011). Borate buffer (200 mM boric acid (Univar, Ajax Finechem, Seven Hills, Australia), 35.7 μM ^{13}C, ^{15}N L-Valine, 10 mM Tris (2-carboxyethyl)-phosphine (Sigma Aldrich), 1 mM ascorbic acid (Sigma Aldrich) was prepared and adjusted to pH 8.8 using 2 M sodium hydroxide. The supernatant (10 μL) was diluted 10 times using 0.1%