



## Physico-chemical and biochemical properties of low fat Cheddar cheese made from micron to nano sized milk fat emulsions

Bal Kumari Sharma Khanal<sup>a</sup>, Chrysanthia Budiman<sup>a</sup>, Mark P. Hodson<sup>b,c</sup>, Manuel R.R. Plan<sup>b</sup>, Sangeeta Prakash<sup>a</sup>, Bhesh Bhandari<sup>a</sup>, Nidhi Bansal<sup>a,\*</sup>

<sup>a</sup> The University of Queensland, School of Agriculture and Food Sciences, Brisbane, QLD, 4072, Australia

<sup>b</sup> The University of Queensland, Metabolomics Australia Queensland Node, Australian Institute for Bioengineering and Nanotechnology, Brisbane, QLD, 4072, Australia

<sup>c</sup> The University of Queensland, School of Pharmacy, Brisbane, QLD, 4072, Australia

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### ABSTRACT

Milk fat emulsions from sodium caseinate (NaCas) and anhydrous milk fat (AMF) were prepared in the size range from 1 to 0.24  $\mu\text{m}$ . These emulsions were used as source of fat to prepare low fat Cheddar cheese (LFC) and their properties such as composition (at day 7), proteolysis, texture profile analysis (TPA), color and microstructure (by confocal laser scanning electron microscopy) were studied during ripening for 180 days. Emulsion size affected the textural, microstructure, compositional, proteolysis and color properties of LFCs, but did not make them comparable to control FFC (control full fat cheese) and textural properties did not change significantly during ripening. This was possibly due to the relatively small size of emulsions added and their inertness that did not lead to higher moisture retention during cheese making and did not coalesce during ripening.

### 1. Introduction

Milk fat is an important component for the development of flavour, texture and other physico-chemical characteristics in cheese (Michalski et al., 2007; O'Connor & O'Brien, 2011). Several studies on low fat cheese (LFCs) have shown reduction of fat levels up to 50% or more results in a poor texture and flavour in the final product (Konuklar et al., 2004; Wijesundera et al., 2000). Although the use of fat replacers to manufacture LFCs is a popular method, several studies have adopted other strategies too. A typical example of this is homogenization and micro-fluidisation. These processes reduce the milk fat particle size (Karaman et al., 2012; Madadlou et al., 2007) and decrease curd firmness and syneresis (Lemay et al., 1994). Homogenization of milk can not only increase the yield of cheese but also reduce the amount of fat loss in to whey (Kelly et al., 2008; Zamora et al., 2007). Furthermore, homogenization has been shown to increase the moisture content in cheese (Karaman and Akalın, 2013; Madadlou et al., 2007), reduce coagulation time and improve acid production rate, curd tension, curd fusion and elasticity of curd (Jana and Upadhyay, 1992; O'Mahony et al., 2005).

Usually, homogenization of whole cheese milk is not suggested because of its deleterious effects on flavour and texture of cheese (Deegan et al., 2014). To preserve optimal flavour and texture,

homogenization of cheese milk by selective method such as homogenization of cream and its incorporation into the skim milk (Deegan et al., 2013; Karaman and Akalın, 2013) is utilized. Earlier studies on manufacture of cheese using selective or complete homogenization of cheese milk reported high moisture content, improved flavour, texture, body, microstructure, sensory qualities and functional properties in baby Gouda, Cheddar, Roquefort, Blue, Edam, Nyamunas, Kariesh, low fat Iranian white and pickled cheeses (Emmons et al., 1980; Jana and Upadhyay, 1992; Madadlou et al., 2007). Another study had reported less free oil in reduced-fat Mozzarella cheese by selective homogenization (Poduval and Mistry, 1999).

Although several studies describe the effect of milk homogenization on cheese quality, much fewer studies have reported the effect of fat globule size. More than 95% of fat is present as spherical milk fat globules (MFG) in milk (Michalski et al., 2007) with average size of 0.1–15  $\mu\text{m}$ , and its composition and size may vary among the cows and within the milk from same cow (Panchal et al., 2017). These MFGs help to disrupt the dense protein network in cheese and act as inert fillers (Michalski et al., 2002). During the preliminary stages of renneting in cheese processing, MFGs and casein micelles affect the rate of gelation, curd firmness and the elasticity of the curd, which in turn effects the texture and flavour characteristics of cheese (Logan et al., 2017; Lucey et al., 2003; Michalski et al., 2007). MFGs' size and the interactions of

\* Corresponding author.

E-mail address: [n.bansal@uq.edu.au](mailto:n.bansal@uq.edu.au) (N. Bansal).

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MFGs and/or free fat within the casein matrix may have a leading role in cheese meltability (Rowney et al., 2003).

Preparation of full-fat Cheddar cheese (Logan et al., 2017; O'Mahony et al., 2005) and Emmental cheese (Michalski et al., 2007) with small MFGs ( $D[4,3] = 2.76$  and  $3.45 \mu\text{m}$  in Cheddar cheeses; and  $D[4,3] = 3.31 \mu\text{m}$  in Emmental cheese) have been reported to improve their textural, flavour and sensory properties. Additionally, Logan et al. (2014) reported firmer texture of the cheese curd prepared from large MFGs ( $D[4,3] = 3.88$ – $5.78 \mu\text{m}$ ) in combination of small casein micelles ( $D[4,3] = 153$ – $159 \text{nm}$ ). The higher gel strength is achieved when the size of MFGs is compatible with the pore size of protein network. The gel strength is increased in this case because of the role of MFGs as local filler particles and MFGs did not break the protein network (Logan et al., 2014). Michalski et al. (2003) have found higher moisture content, enhanced proteolysis and less firmer texture in Camembert cheese prepared from small MFGs ( $D[4,3] = 3 \mu\text{m}$ ) compared to the large MFGs ( $D[4,3] = 6 \mu\text{m}$ ). However, O'Mahony et al. (2005) reported decrease in firmness (storage modulus) of rennet curd in miniature Cheddar-type cheese by large size MFGs ( $D[4,3] = 4.68 \mu\text{m}$ ) compared to smaller MFGs ( $D[4,3] = 3.45 \mu\text{m}$ ). Similarly, use of bigger size MFGs ( $D[3,2] = 4.6 \mu\text{m}$ , obtained from fractionated milk via microfiltration) in rennet gels exhibited decrease in  $G'$  compared to the smaller ( $D[3,2] = 1.89 \mu\text{m}$ ) and medium sized ( $1.46 \mu\text{m}$ ) MFGs (Michalski et al., 2002). The porous area within a protein gel network is too large to fit the small and medium sized MFGs as filler particles to reinforce the overall gel firmness; while, MFGs larger than the size casein micelles are deleterious to the gel strength (Michalski et al., 2002). Hence, so far conflicting results have been reported in literature on the effect of MFG size on textural properties of cheese.

Also, the studies so far (Deegan et al., 2013; Karaman and Akalin, 2013; Logan et al., 2015; O'Mahony et al., 2005) have reported on the effect of size of MFGs and homogenization on reduced fat and full fat cheeses only. Other studies (Karaman et al., 2012; Nair et al., 2000; Tahereh et al., 2017; Van Hekken et al., 2007) suggested homogenization as one of the modifying techniques to improve the textural and other characteristics of low fat cheeses, but did not study the effect of emulsion size. Incorporation of milk fat emulsions in micron to nano-size range as a texture modifier in low-fat Cheddar cheese has not been reported yet.

Emulsions with different structures, physico-chemical and functional properties can be produced by controlling several factors such as environmental conditions (pH, ionic strength, temperature, etc.), properties of colloidal particles (size, concentrations, surface charge, etc.) and preparation techniques (conditions of mixing, addition of ingredients, etc.) (De Figueiredo Furtado et al., 2016). To manufacture dairy-based emulsions, different constituents of milk such as caseins, whey proteins (WP), anhydrous milk fat (AMF) and lactose are extensively used. Sodium caseinate (NaCas) acts as a more effective encapsulant than whey proteins because of its superior emulsifying properties and resistance to heat denaturation owing to its amphiphilicity, good colloidal stability, stearic and electrostatic repulsions (Karthik and Anandharamkrishnan, 2016; Liang et al., 2017; Vega and Roos, 2006). Therefore, NaCas was selected as a stabilizer to make emulsions with AMF in this study to make low-fat Cheddar cheese.

**Table 1**  
Size of fat particles in emulsions (at different pressure) and in cream.

Emulsion samples	Pressure (Bar)	D [4, 3]	D [3, 2]	d (0.1)	d (0.5)	d (0.9)	Sample code for cheese
Cream	–	$4.59 \pm 0.13^a$	$3.57 \pm 0.02^a$	$2.18 \pm 0.03^a$	$4.04 \pm 0.07^a$	$7.75 \pm 0.37^a$	Control FFC for full fat control and Control LFC for low fat control cheese
Large	100	$0.97 \pm 0.04^b$	$0.49 \pm 0.01^b$	$0.21 \pm 0.00^b$	$0.81 \pm 0.03^b$	$1.95 \pm 0.10^b$	Low fat cheese with large emulsion (LFC-large)
Medium	250	$0.5 \pm 0.04^c$	$0.28 \pm 0.01^c$	$0.14 \pm 0.01^{bc}$	$0.39 \pm 0.02^c$	$1.02 \pm 0.05^c$	Low fat cheese with medium emulsion (LFC-medium)
Small	1000	$0.24 \pm 0.00^d$	$0.15 \pm 0.00^d$	$0.08 \pm 0.00^c$	$0.18 \pm 0.00^d$	$0.52 \pm 0.00^c$	Low fat cheese with small emulsion (LFC-small)

Results are expressed as the mean  $\pm$  standard error ( $n = 3$ ). Means in a single column with different superscripts are significantly different ( $P < 0.05$ ).

The aim of this study was to investigate the effect of size of emulsions (from micron to nano-size) prepared from NaCas and AMF on the physicochemical properties of low fat Cheddar cheese during ripening. It was hypothesized that the smaller emulsions will have larger number of particles and more surface area as compared to the native milk fat particles. Hence, smaller size emulsions would act as fillers and break the densely cross-linked protein network in LFCs. Consequently, that would influence the development of physico-chemical, textural and functional properties of low fat Cheddar cheese during ripening.

## 2. Materials and methods

### 2.1. Materials

AMF (composition: 99.9% butter fat, 0.08% moisture and 0.13% free fatty acids) was purchased from Rogers and Company Foods Pty. Ltd. (VIC, Australia). Sodium caseinate (NaCas: 96.6% protein, 0.25% lactose, 0.7% fat, 1.2% sodium) was obtained from Murray Goulburn Co-op (VIC, Australia) and starter culture FD-DVS R-707 (*Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*) was received from Chr. Hansen Pty. Ltd. (VIC, Australia). Rennet (Chymax Plus, FPC, 200 IMCU/mL) was purchased from Cheeselinks (VIC, Australia). Skim milk powder (SMP) (moisture: 3.9 g/100 g, protein: 32.5 g/100 g, fat: 0.8 g/100 g, lactose: 55 g/100 g, minerals: 7.8 g/100 g) and cream (protein: 2.1 g/100 g, fat: 39.4 g/100 g, carbohydrate: 2.9 g/100 g) were bought from a local supermarket. Trichloro acetic acid (A11560) was purchased from Alfa Aesar (NSW, Australia). Urea (U12500), Trizma<sup>®</sup> base (T1503), methyl red (ACS dye) and bromocresol (ACS dye) green were procured from Sigma (NSW, Australia). Glycine, Mini-PROTEAN<sup>®</sup> TBE urea precast gels (15%, 15 wells comb, 15  $\mu\text{L}$ ), bromophenol blue and Coomassie brilliant blue were purchased from Bio-Rad (VIC, Australia). Sodium hydroxide (40%) and copper sulphate catalyst were bought from Therommo-fisher scientific (QLD, Australia). Hydrochloric acid (concentration  $\sim 32\%$  and 0.1 M), sulphuric acid (98%) were purchased from Merck (NSW, Australia). Boric acid and sodium sulphate, were procured from Chem Supply (SA, Australia).

### 2.2. Preparation of emulsion and analysis of particle size distribution

At first, 15% solution of NaCas was prepared by mixing it with water using an overhead stirrer (IKA<sup>®</sup> 20, John Morris Scientific, NSW, Australia) for 2 h and was stored overnight for complete protein hydration. AMF was mixed with NaCas and heated in water bath at  $45^\circ\text{C}$  followed by coarse emulsion preparation using digital ultraturrax (IKA<sup>®</sup> T25, GmbH, Staufen, Baden-Württemberg, Germany) at 8200 rpm for 20 min. The coarse emulsion was further homogenized using a homogenizer (Avestin Emulsiflex C5, ATA Scientific, NSW, Australia) to obtain emulsions in size range from  $\sim 1$  to  $0.25 \mu\text{m}$ . The pressures used to achieve different emulsion size, expected particle size and the sample codes for cheeses are summarised in Table 1. Particle size distribution in emulsions was determined using Malvern Laser Diffraction Particle Size Analyser Mastersizer 2000 (Malvern Instrument Ltd, Worcester, UK). The refractive index of the milk and water used were 1.39 and 1.33, respectively. Triplicate analysis was carried out for each sample.

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