Milk fat globule membrane and its role in flavour development in cheese during ripening

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Abstract

Despite the well-recognised role of milk fat in flavour development in cheese, research investigating the importance of the milk fat globule membrane (MFGM) on flavour in cheese is scarce. This study investigated the impact of MFGM composition and structure on the volatile profile of ripened Cheddar cheese samples. Three types of MFGM recombined cheeses were manufactured using MFGM fractions isolated from dairy by-products and were compared to two reference cheeses. After 6 months' maturation the MFGM recombined cheeses had a higher concentration of volatile compounds (short chain fatty acids, alcohols, methyl ketones and sulfide compounds) compared to the reference-cheeses. These results demonstrate that the MFGM composition as well as structural rearrangement at the fat globule interface had a significant effect on the development of volatile compounds in cheese during maturation.

Introduction

Milk fat plays a vital role in determining the texture, flavour, and physico-chemical properties of cheese [1]. The milk fat globule (MFG) consists of a lipid core surrounded by a three-layer membrane termed the MFGM. The MFGM contains a complex mixture of glycoproteins, enzymes, and phospholipids. Phospholipids within the membrane possess a high water-holding capacity and the moisture captured by them can serve as a reservoir where enzymes can act and enhance flavour development [2]. MFGM components can act as substrates for both lactic acid bacteria (LAB) and non-starter lactic acid bacteria (NSLAB) [3] during the later stages of cheese ripening. Furthermore, the MFGM contains redox enzymes such as xanthine oxidase (XO), which are capable of catalysing the oxidation of a broad range of substrates, and therefore may play a role in determining the flavour of cheese.

Buttermilk is a by-product of butter manufacturing, produced during churning; whereas α-serum (αS) and β-serum (βS) are by-products of anhydrous milk fat manufacturing. Buttermilk, αS and βS were once considered to be waste material, but are now recognised as good sources of MFGM. The procedures used to produce buttermilk powder (BMP), αS, and βS affect the protein and lipid moiety of the isolated MFGM fractions, and the content and activity of XO and other enzymes [4]. As such, BMP, αS, and βS were used in this study as a source of MFGM fractions with different protein and lipid composition and XO enzymatic activities. The isolated fractions were then used to investigate the importance of the MFGM structure and composition on development of volatile compounds in model Cheddar cheese samples during six months of ripening.

Experimental

Materials

Raw milk was collected from Jersey cows at late lactation from a local dairy farm (Outram, New Zealand) on the day of milking. Freeze-dried αS and βS and spray dried BMP were obtained from a dairy factory in New Zealand. AMF was obtained from New

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Zealand Milk Products (Auckland, New Zealand). Freeze-dried mesophilic starter culture containing *Lactococcus lactis ssp. lactis* plus *Lactococcus lactis ssp. cremoris* (R704) was obtained from Hansen A/S, Horsholm (Denmark).

MFGM isolation and model cheese production

Freeze-dried αS and βS and BMP were used for MFGM isolation as outlined in Haddadian et al. [4]. Three types of 5% milkfat emulsion were prepared using the three MFGM isolates (2%) as the emulsifier, as previously described [4]. Three separate batches of model Cheddar cheese were manufactured for each treatment using the 5% milk fat emulsions, according to a standard Cheddar cheese making procedure [5]. Two reference cheeses were also manufactured as comparison samples: (1) Native-cheese containing cream and skim milk to evaluate the role of MFGM structure in flavour development; and (2) Tween-cheese containing recombined MFGs emulsified by Tween 80 to evaluate the effect of the MFGM composition in the flavour development process (Table 1). Cheeses were sampled after 1, 90, and 180 d of ripening. Ripened samples taken at each sampling date were frozen at -20°C until the end of the trial so that the complete sample set could be analysed together.

Table 1: Compositional properties of cheese milk samples for model cheese production

Cheese samples	Cheese milk composition (mL)				Emulsifier	PFR*
	Cream#	Emulsion (mL)	Water (mL)	Skim milk (mL)†		
Native	30	-	200	170	-	1.02
Tween	-	230	-	170	Tween80	1.02
α-cheese	-	230	-	170	α-MFGM	1.02
β-cheese	-	230	-	170	β-MFGM	1.02
BMP-cheese	-	230	-	170	BMP-MFGM	1.02

* Protein to fat ratio
# Pasteurised cream; 36% fat and 2.2% protein

Determination of volatile compounds by SPME-GC/MS

The analysis of volatile compounds in miniature model cheeses was carried out using solid phase micro extraction (SPME) with a fibre coated with a film of DVB/CAR/PDMS (Supelco, Bellefonte, PA, USA) and analysed by gas chromatography–mass spectrometry (GC-MS). Volatile compounds were separated on a polyethylene glycol capillary column (Zebron ZB-Wax 60m x 0.32mm x 0.50 µm, Phenomenex, Torrance, CA, USA). A complete randomised design, blocked by replicate, was used for the volatile analysis. Vacuum-packed frozen cheese samples were ground with liquid nitrogen and Na_2SO_4 (1.5 g/g) to give fine particles. A subsample (5 g) of each powdered sample was mixed with 2 µL of an aqueous solution of 12.5 mg L^{-1} fenchol in a 20 mL sealed GC vial. Vials were placed on autosampler tray (PAL3 RSI 85, Agilent Technologies) for analysis.

Results and discussion

Effect of MFGM on development of volatile compounds during cheese ripening

A total of 28 significantly different compounds were detected among the model cheese samples for the three time points during the six-month ripening period. The differences between samples over ripening were visualised using PCA on normalised peak areas (Figure 1).

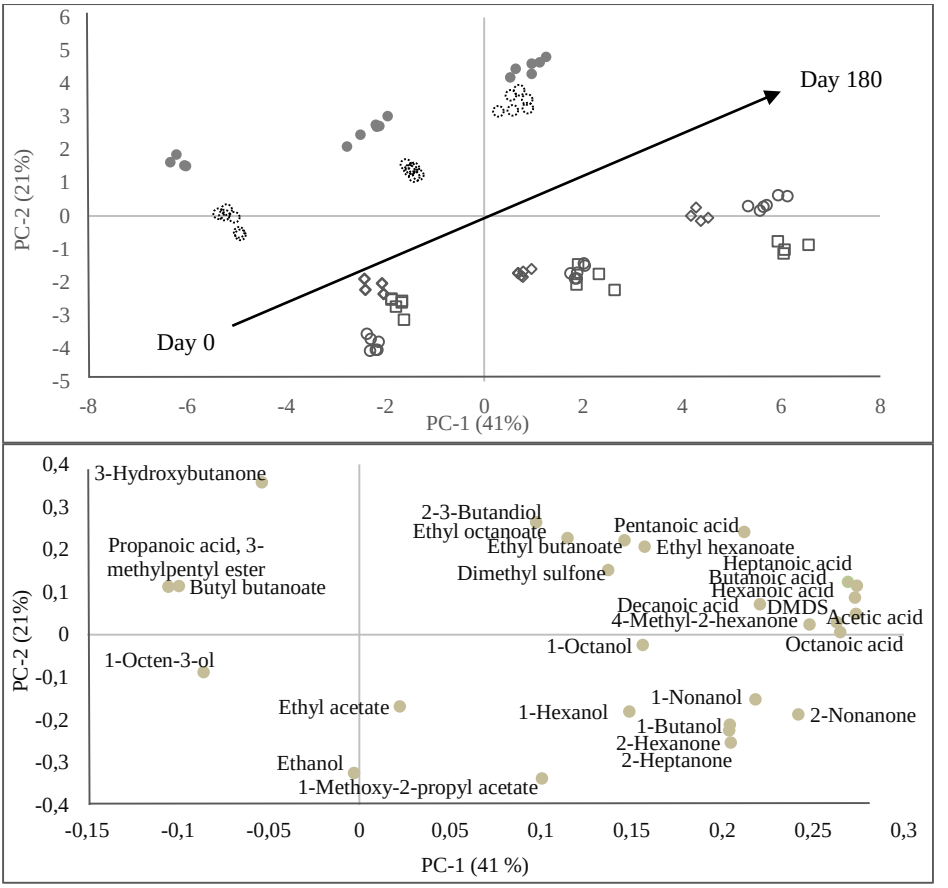

Figure 1: Principal component analysis of significantly different volatile compounds detected in model cheese samples at three time points during six months ripening. Scores plot (top) of cheese samples; α (○), β (□), BMP (◊), Native (●), and Tween (○); Loadings plot (bottom) of significantly different volatile compounds identified by GC-MS. Points represent 3 batches x 2 analytical replicates per treatment per time point.

The first two principal components accounted for 62% of the total variability (PC1 41%, and PC2 21%) and clearly separated the model cheese samples into three groups consisting of Native-cheese, Tween-cheese, and MFGM-recombined cheeses of α, β, and BMP (Figure 1, top). Ripening time proceeded from left to right on PC1. The number and concentration of most compounds increased as ripening proceeded, except for a few compounds, such as ethanol, and octen-3-ol, which decreased significantly over the ripening in all model cheese samples. The volatile profile of the MFGM-recombined cheeses developed the most, while the Tween cheese, followed by the Native-cheese showed the least volatile development during the ripening period.

At six months of ripening, MFGM-recombined cheeses containing αS, βS, and BMP had a higher concentration of short chain fatty acids (SCFAs), alcohols, methyl ketones and sulfide compounds (Table 2). In MFGM recombined cheeses, the higher XO activity in β-serum (7.2 ± 0.8 mmol/L uric acid/min) and α-serum (7.6 ± 0.5 mmol/L uric acid/min) compared to BMP (no detected activity) was correlated to higher concentrations of SCFAs. Native and Tween cheeses had higher concentrations of 2,3-butanediol and 3-hydroxybutanone compared to the recombined cheeses of αS, βS, and

BMP. Tween cheese was uniquely associated with higher levels of three esters; ethyl butanoate, butyl butanoate, and ethyl hexanoate, but overall had the lowest concentration of volatiles, thus showing the importance of MFGM to the flavour development in cheese.

Table 2: Concentrations of significantly different volatile compounds detected in experimental cheese samples after six months ripening.

	AS-cheese	BS-cheese	BMP-cheese	Tween-cheese	Native-cheese
Acetic acid	26.4 ± 1.6 ^{a}	22.1 ± 1.0 ^{b}	12.1 ± 0.6 ^{c}	6.7 ± 0.2 ^{e}	9.8 ± 0.3 ^{d}
Butanoic acid	89.3 ± 5.5 ^{a}	88.3 ± 2.7 ^{a}	66.8 ± 1.8 ^{b}	34.3± 3.0 ^{c}	63.7 ± 2.6 ^{b}
Hexanoic acid	93.8 ± 2.4 ^{a}	99.3 ± 3.4 ^{a}	72.2 ± 2.6 ^{b}	45.0 ± 5.5 ^{d}	61.5± 7.8 ^{c}
Ethanol	322.8 ± 5.0 ^{a}	285.0 ± 2.9 ^{b}	187.1 ± 13.6 ^{c}	248.1 ± 13.4 ^{e}	129.8 ± 8.2^{d}
1-Butanol	7.8 ± 0.4 ^{b}	9.0 ± 0.2 ^{a}	6.8 ± 0.3 ^{c}	5.9 ± 0.5 ^{d}	2.7 ± 0.3 ^{e}
1-Nonanol	1.1 ± 0.05 ^{b}	1.0 ± 0.06 ^{d}	1.1 ± 0.03 ^{b}	0.8 ± 0.03 ^{c}	0.6 ± 0.04 ^{a}
1-Hexanol	4.3 ± 0.1^{c}	4.9 ± 0.3 ^{ab}	4.4 ± 0.2 ^{c}	4.8 ± 0.1^{bc}	2.6 ± 0.4 ^{d}
Dimethyldisulfide	0.7 ± 0.06 ^{a}	0.7 ± 0.02 ^{a}	0.6 ± 0.07 ^{b}	0.04 ± 0.05 ^{c}	0.5 ± 0.04 ^{b}
Dimethyltrisulfide	0.5 ± 0.04 ^{b}	0.6 ± 0.03 ^{a}	0.4 ± 0.03 ^{c}	0.07 ± 0.01 ^{d}	0.1 ± 0.04 ^{c}
2-Hexanone	40.1 ± 0.1 ^{a}	40.7 ± 1.9 ^{a}	40.3 ± 1.4 ^{a}	28.8 ± 0.3 ^{b}	8.6 ± 0.1 ^{c}
2-Heptanone	13.2 ± 0.4 ^{b}	15.0 ± 0.6 ^{a}	13.8 ± 0.4 ^{b}	9.8 ± 0.3 ^{c}	4.0 ± 0.7 ^{d}
2-Nonanone	4.9 ± 0.3 ^{a}	5.4 ± 0.2 ^{a}	5.0 ± 0.2 ^{a}	3.7 ± 0.1 ^{b}	2.4 ± 0.3 ^{c}
4-Methyl-2-hexanone	5.9 ± 0.6^{ab}	6.3 ± 0.4 ^{b}	5.4 ± 0.2 ^{a}	3.5 ± 0.4 ^{c}	2.0 ± 0.2 ^{d}
2-3-Butanediol	0.9 ± 0.1 ^{e}	1.4 ± 0.1 ^{d}	2.0 ± 0.2 ^{c}	5.5 ± 0.07 ^{a}	3.1 ± 0.3 ^{b}
3-Hydroxybutanone	4.6 ± 0.4 ^{d}	4.0 ± 0.2 ^{d}	5.7 ± 0.2 ^{c}	10.1 ± 0.2 ^{a}	8.8 ± 0.3 ^{b}
Ethyl butanoate	1.4 ± 0.05 ^{a}	1.2 ± 0.06 ^{a}	1.0 ± 0.07 ^{a}	1.5 ± 0.08 ^{a}	1.0 ± 0.1 ^{a}
Ethyl hexanoate				0.7 ± 0.2	
Butyl butanoate	3.9 ± 0.2 ^{b}	4.8 ± 0.6 ^{b}	4.3 ± 0.1 ^{b}	11.9 ± 0.6 ^{a}	4.7 ± 0.6 ^{b}

Letters denote significant differences ($p < 0.05$) between samples according to Tukey HSD post-hoc test.

Conclusion

By comparing the volatile profile of the MFGM-recombined cheeses with Tween-cheese, and Native-cheese, new insights were revealed into the role of MFGM and its composition and structure on flavour development in cheese. Rearrangement of the MFGM structure and the higher activity of the MFGM-enzymes such as XO, favoured the production of volatile compounds during ripening. These results demonstrate the potential of using MFGM components from commercial by-products as a functional ingredient to enhance the flavour development of cheese.

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