Lactone formation ability of a chosen *Lactococcus lactis* subsp. *lactis* var. *diacetylactis* strain from the Agroscope Strain Collection during fermentation in cream

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Introduction

Lactones are potent flavour compounds contributing to creamy, fruity and coconut-like aroma notes in milk products such as cream, butter and cheese (1).

In milk products, the chemical formation of lactones from hydroxy fatty acid (HFA) triglycerides is widely accepted (2, 3). The ability of yeasts e.g. *Sporobolomyces odorus* to produce lactones is also well known (6). However, to date, their production by lactic acid bacteria (LAB) and the corresponding metabolic pathways are still uncertain (4, 5).

LAB have been reported to produce γ-decalactone and γ-dodecalactone from 10-hydroxypalmitic acid and 10-hydroxystearic acid in malt whiskey (4), but no influence of LAB activity on the formation of lactones was found during Gouda cheese ripening (5).

Objective

Investigation of the microbial formation of lactones by LAB during fermentation in cream with and without supplementation of HFA, supposed precursors of lactones (4).

Experimental

Lc. lactis subsp. *lactis* biovar. *diacetylactis* FAM18027 was selected from the Agroscope Strain Collection out of 65 strains of different LAB species for its ability to develop buttery and fruity flavour notes during fermentation in cream. Two experimental conditions were explored at 30° C for 24 h with and without supplementation of HFA:

- Fermentation: full-fat cream + FAM18027
- Incubation: full-fat cream without strain addition

Fermentation and incubation were carried out in 0.5 L bioreactors without oxygen addition and *pH* regulation, with a low pale mixing at 160 rpm.

The formation of lactones in cream was evaluated by head-space solid phase microextraction–gas chromatography–mass spectrometry (HS-SPME-GC-MS) using a DVB/CAR/PDMS 50/30 µm 2 cm-fibre. Results were normalised using paraldehyde as internal standard (IS).

Table 1 Composition of the hydroxy fatty acid mix added to the cream (10 % *w/w*) before fermentation or incubation

Hydroxy fatty acid (HFA)	mmol	Hydroxy fatty acid (HFA)	mmol
10-Undecenoic acid (C10:1)	0.50	Linoleic acid (C18:2Δ9,12)	0.15
Z-5-Dodecenoic acid (C12:1)	0.28	Ricinoleic acid (12-hydroxy-C18:1Δ9)	0.04
Myristoleic acid (C14:1)	0.05	*Z*-10-Nonadecenoic acid (C19:1)	0.10
Oleic acid (C18:1Δ9)	0.12		

Results

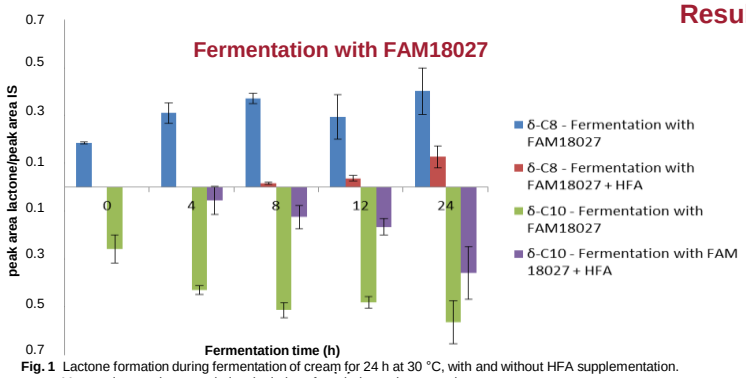

Fig. 1 Lactone formation during fermentation of cream for 24 h at 30 °C, with and without HFA supplementation. Mean values and mean relative deviation of two independent experiments

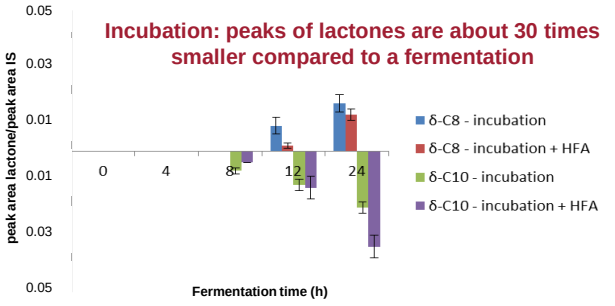

Fig. 2 Lactone formation during incubation of cream for 24 h at 30 °C, with and without HFA supplementation. Mean values and mean relative deviation of two independent experiments

- main volatile lactones found in the samples: *δ*-octalactone (*δ*-C8) and *δ*-decalactone (*δ*-C10)
- GC-MS analyses show increase of the signals of these two main lactones after 4 h of fermentation already (Fig. 1).
- addition of HFA to the cream in presence of FAM18027: less important lactone formation
- especially formation of *δ*-C8 is affected by the HFA addition (slower formation and a three times lower signal after 24 h of fermentation)

- in cream incubated without LAB (Fig. 2), *δ*-C8 and *δ*-C10 signals were about thirty times lower than in samples fermented with LAB over the same period of time
- similar effect on lactone formation in presence of HFA
- increase of *δ*-C10 was detected only after 24 h of incubation with HFA

Conclusion

The results clearly show that the GC-MS signals of *δ*-C8 and *δ*-C10 in cream fermented with LAB were higher than in samples incubated without LAB, over the same period of time. It can hence be concluded that FAM18027 forms lactones during 24 h of fermentation at 30°C. As expected, lactones were also slowly formed during cream incubation *via* a non-enzymatic reaction, where probably the HFA naturally present in the cream undergo trans-esterification to release lactones (5).

The addition of HFA as lactone precursors seemed to not have any effects on the increase of the lactones, except for a formation of *δ*-C10 after 24 h incubation.

In presence of HFA, the LAB might show different metabolic activities than without supplementation, which might influence the formation of different secondary metabolites such as lactones. Further studies will be needed to elucidate these questions.

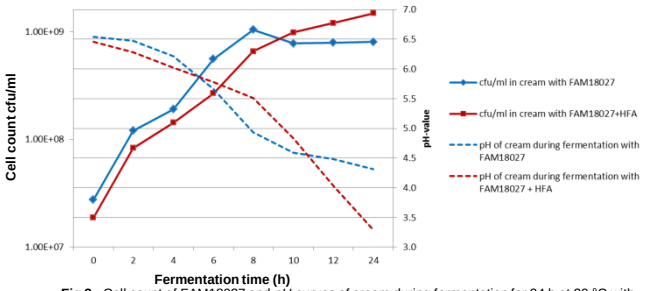

Fig 3. Cell count of FAM18027 and *pH*-curves of cream during fermentation for 24 h at 30 °C with and without HFA supplementation. Mean values and mean relative deviation of two independent experiments

- similar growth of FAM18027 with or without HFA supplementation (Fig. 3)
- with HFA, exponential phase slightly continues until 24 h
- slower acidification in presence of HFA between 6 and 10 h
- after 10 h of fermentation, HFA supplementation leads to a lower *pH*

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