Influence of Enterococci and Thermophilic Starter Bacteria on Cheddar Cheese Flavour

(Improving Cheddar Cheese Flavour using Enterococci and Thermophilic Starter Bacteria)

Armis No. 4426

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Summary and Conclusions

Cheddar cheese, made traditionally, involved the use of mixed starter cultures, some of which had a high flavouring capacity, and the bacterial flora of the cheese included bacteria of milk origin which survived pasteurisation as well as contaminants from the environment and cheesemaker during the manufacturing process - especially the manual turning of the curd. The unrefrigerated milk used at that time contained high numbers of lactic acid producing souring bacteria such as enterococci and thermophilic (heat-resistant) lactic acid species - a significant proportion of which could survive pasteurisation.

The more mature flavour often attributed to the cheese made at that time, as well as that associated with artisinal/farmhouse Cheddars, which are generally produced under less stringent hygienic conditions, provide strong evidence of the important role of these non-starter bacteria in enhancing the flavour development of Cheddar cheese.

By contrast modern commercial Cheddar cheesemaking has become a highly automated and regulated process with great emphasis on hygiene and a critical dependence on a small number of phage-resistant starter strains, most of which, unfortunately, do not have a significant flavouring capacity during ripening and result in a product often criticised as too bland or lacking in flavour.

Hence this project set out to identify suitable enterococci and thermophilic starter strains which could be added to the cheese during manufacture (as starter adjuncts) with the specific aims of enhancing flavour during ripening as well as facilitating flavour diversity - a trait sought by many commercial Cheddar companies.

The main conclusions were as follows:

- When selected strains of thermophilic lactic acid bacteria, considered to have good flavouring potential in cheese ripening, were added (as starter adjuncts) with the normal starter cultures in controlled Cheddar cheese manufacture it was found that their addition did affect flavour in the ripening cheese, but not beneficially in all cases.
- One strain (Lb. helveticus DPC 4571) however has a strong positive effect on flavour and lost viability quickly during the ripening period as the cells lysed, releasing flavour enhancing enzymes.
- When strain DPC 4571 was used as a starter adjunct in industrial trials, the resulting cheeses were adjudged by commercial graders to have better flavour and body than the control cheeses.
- In a follow-up trial, strain DPC 4571 was compared with four (considered best) selected commercially available starter adjuncts. Sensory analysis of these cheeses indicated that the use of adjuncts did improve flavour and that DPC 4571 was at least as effective as the other (commercial) adjuncts tested. Industrial trials are continuing.

In summary, this project confirmed the potential of thermophilic lactic acid strains to affect flavour when used as starter adjuncts in Cheddar cheese manufacture. Their use can also lead to the development of novel flavours. Many adjunct cultures proposed to-date to enhance Cheddar flavour are composed of strains of lactococcal starter, selected for their flavouring capacity. However, application of such strains in industry would lead to increased probability of phage attack on the primary starter. On the other hand, thermophilic lactic acid strains are phage unrelated to conventional starter and thus would not lead to the introduction of starter specific phage into the cheese plant.

A thermophilic strain from the Moorepark collection (DPC 4571) was shown to have major commercial potential as a flavour enhancer.

Research and Results:

Isolation of Strains

Strains of *Lb. helveticus* and *St. thermophilus* present in the DPRC collection were selected for use as starter adjuncts, these strains had been isolated from artisanal and commercial products over a period of many years. Commercial thermophilic cultures were also obtained from starter companies. Enterococci were isolated from Irish farmhouse cheeses made from raw milk and from a range of artisinal dairy products manufactured in the South of Europe.

Methods of Enumeration

In order to follow the evolution of the thermophilic cultures, when added to Cheddar cheese, it was necessary to develop plating methods which could be used to selectively enumerate the adjuncts in the presence of natural Cheddar flora such as *lactococcal* starter and mesophilic *lactobacilli*. *Lactococci* and *St. thermophilus* grew on M17 agar containing lactose (M17L) as the carbon source. Incubation at 450C for 3 days inhibited growth of *lactococci*. *Lb.helveticus* did not grow on M17L, however, *enterococci* could grow under these conditions. Kanamycin aesculin azide agar (KAA) incubated at 370C for 18 hours was found to be a useful medium for selective enumeration of *enterococci* in a Cheddar cheese environment. *Lactococci* and non-starter lactic acid bacteria were generally found not to grow on this medium and those strains which did grow did not develop a typical colony morphology and thus could be distinguished from *enterococci*. *Lb. helveticus* and *St. thermophilus* did not grow on this medium.

Selective enumeration of *Lb. helveticus* was achieved on deMan, Rogosa and Sharpe (MRS) medium adjusted to pH 5.4 and incubated anaerobically at 420C. *Lactobacillus* selective medium (LBS) incubated at 300C for 5 days was used to enumerate non-starter lactic acid bacteria. Selective conditions to specifically enumerate starter *lactococci* in cheese containing *enterococci* or *St. thermophilus* adjuncts were not determined.

Sensory Evaluation

Application of starter adjuncts during Cheddar cheese manufacture often leads to real but subtle improvement to Cheddar flavour, thus, sensory analysis panellists are required who can discriminate subtle flavour changes. Training of panellists was undertaken and 16 were identified who could objectively discriminate between Cheddar cheeses with similar flavour profiles. Thus, conditions to selectively enumerate thermophilic cultures in a Cheddar cheese environment were identified.

Sensory evaluation of cheese.

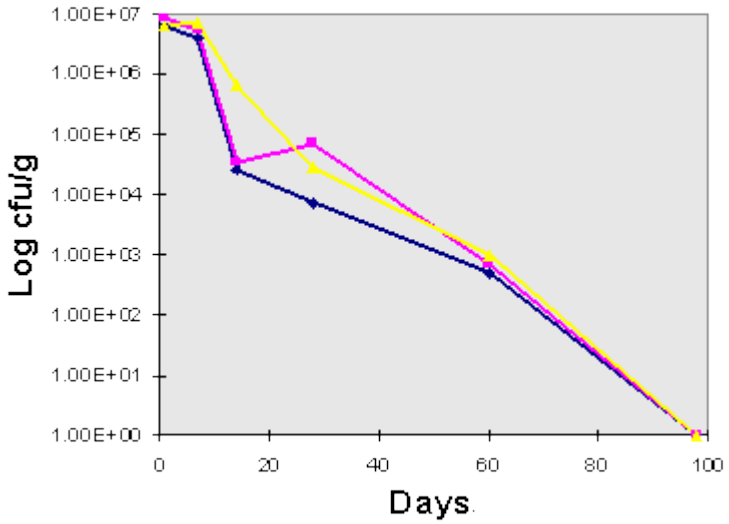
Controlling and monitoring organisms during ripening

The source of non-starter lactic acid bacteria or their influence on Cheddar flavour has not been fully elucidated. Therefore, when conducting trials to assay the effect of specific adjuncts on flavour development it was considered necessary to control the proliferation of non-starter lactic acid bacteria during ripening. An aseptic cheese making facility was used for Cheddar manufacture in this project. Using this system non-starter lactic acid bacteria counts could be controlled at less than 1cfu/g for up to 100 days, increasing to 10^4-10^7 cfu/g at 200 days of ripening. Cheddar cheese was manufactured using conventional Cheddar starters in combination with strains of *St. thermophilus, Lb. helveticus* or *Enterococcus* as starter adjuncts. Adjuncts were added such that their levels at day one in the cheese were 10^6-10^8cfu/g. Seven strains of *St. thermophilus*, 6 strains of *Lb. helveticus* and 4 strains of *Enterococcus* were assayed for their contribution to flavour development. Cheese composition was assayed at 2 weeks post manufacture and was found to be within the accepted range for Cheddar. This was important as composition has a major effect on flavour development during ripening. Proteolysis is considered one of the major biochemical events to occur during ageing of Cheddar cheese. Addition of thermophilic adjuncts lead to higher levels of proteolysis in experimental relative to control cheese.

Lb. helveticus and enterococci had the most significant impact on proteolysis with levels of free amino acids being increased up to 5 fold at 5 months of ripening. The addition of adjuncts usually resulted in flavour variations as determined by sensory analysis. Lb. helveticus and enterococci had the most impact on flavour development which correlates with the increased proteolysis observed with these strains.

Enterococci were found to remain viable in the cheese. No evidence, however, of growth during ripening was observed. Numbers of *St. thermophilus* decrease by 1-2 log units during ripening. *Lb. helveticus* were the least viable. Viability was strain dependent most strains decreased by 2-3 log units during ripening, *Lb. helveticus* DPC 4571 decreased from $2.1x10^{8}$cfu/g at day 1 to <10cfu/g at day 56 (Fig. 1). This rapid loss of viability, coupled with significant increases in the levels of proteolysis associated with this strain implied that DPC 4571 was autolytic. To demonstrate this, cheese was manufactured on 2 occasions, with a non-autolytic commercial starter and with DPC 4571 as an adjunct culture.

The viable count of DPC 4571 in both experiments was in the region of 10^{8}cfu/g at day 1, but viability was lost rapidly over the first 3 week of ripening. Autolysis of DPC 4571 was demonstrated by showing increases in the release of 3 intracellular enzymes, identified in cell extracts of DPC 4571, but not detected in cell extracts of the starter culture, in the cheese juice.



Loss of viability of *Lb. helveticus* 4571 during ripening of Cheddar cheese.

Individual trials using DPRC cultures

Since *Lb. helveticus* DPC 4571 was found to have a positive effect on flavour of cheese manufactured at pilot scale, two industrial trials using this strain were undertaken. Objective sensory analysis of these cheeses demonstrated significant differences between the control and experimental cheeses. The cheese made with DPC 4571 as starter adjunct was less "bitter" and more "sweet", "mature" and "acceptable" than the control cheese. Data from commercial grading indicated that DPC 4571 containing cheese had better flavour and body than the control cheese.

These data indicate that strain DPC 4571 has potential industrial applications as a flavour enhancer. An advantage of this strain as an industrial starter adjunct is that it loses viability in the cheese and would therefore not be easily "recovered" by competitors.

Cheese grading in sensory laboratory

Pilot scale trials using commercial cultures

Commercial adjunct cultures were obtained from 3 of the main suppliers of Cheddar cultures to the Irish industry. The companies selected the adjunct cultures which they considered to be the most beneficial. Four cultures were selected for further study. Two of these cultures contained both mesophilic and thermophilic bacteria, 1 contained only *Lb. helveticus* and one contained only mesophilic lactococci. Cheddar cheese was manufactured with these 4 cultures and *Lb. helveticus* DPC 4571.*The trial was carried out in triplicate.*

Sensory analysis of these cheeses indicated that addition of adjuncts resulted in improved flavour scores and that DPC 4571 was at least equivalent to commercially available adjuncts.