Changes in viability and physiology of starter culture Lactococcus lactis subsp. cremoris AM2 during Cheddar cheese manufacture as monitored by flow cytometry and cell sorting.

Imelda Doolan*†[1] and Martin Wilkinson[2]

[1]University of Limerick (UL) – Life Sciences Department, Schrodinger Builidng, Castletroy, Limerick, Ireland

[2]University of Limerick (UL) – Life Sciences Department, Schrodinger Builidng, Castletroy, Limerick, Ireland

Abstract

Cheddar cheese is manufactured using starter cultures which are inoculated into milk and subsequently grow to high levels (_˜ 10 9 cfu/ml), generate lactic acid and towards the end of the process become non-viable. These properties of various starter cultures impact on the final cheese quality and are therefore monitored by selective agar plate counting or by measurement of released intracellular marker enzymes. However, the application of flow cytometry with cell sorting has emerged as a potentially useful technique to further differentiate sub-populations of starter cultures into live, dead or permeabilised cells which may develop over the manufacturing process. The initial stage of acidification in the cheese vat typically takes 4 – 6 hours and the cheese is then ripened at a low temperature for up to 1 year. The contribution of starter during ripening principally arises from permeabilisation or autolysis releasing intracellular enzymes principally peptidases which generate key flavour compounds. In this study we applied FCM and cell sorting using a MoFlo (Beckman Coulter) to follow the evolution of various sub populations of the highly autolytic culture AM2 in a simulated manufacturing process covering the acidification phase of manufacture. Syto9/PI and DiBAC4(3) were used to assess changes in viability or membrane potential on samples taken at various intervals over the process. In tandem, various sub-populations identified by FCM were sorted and plated onto L-M17 agar to assess their recovery and viability. Over the manufacturing process which featured a temperature elevation step from 32 to 38 C cells stained with Syto 9/PI became progressively PI positive and cells stained with DiBAC4(3) showed an increase in green fluorescence. By the end of the process (6h) the population consisted predominantly of permeabilised/dead cells and correlated well with release of an intracellular peptidase Pep X. Sorting of the predominant populations onto L-M17 agar showed a progressive loss in viability as temperature was elevated and thereafter with the appearance of micro-colonies indicating a loss in ability to recover and grow which reflected the changes noted in cytometric profiles. This study has further demonstrated the major influence of process temperature elevation on the commencement of the permeabilisation process during the initial phase of cheese manufacture for this strain. We believe that FCM

*Speaker

†Corresponding author: imelda.doolan@ul.ie

can provide additional physiological information beyond the scope of traditional methodology to identify strain related responses to process variables which may influence product quality and ultimately lead to improvements in strain selection and process optimisation.

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