International Journal of Food Microbiology 224 (2016) 7–15

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Effect of varying the salt and fat content in Cheddar cheese on aspects of the performance of a commercial starter culture preparation during ripening

Palina Yanachkina [a], Catherine McCarthy [b], Tim Guinee [b], Martin Wilkinson [a,*]

[a] Department of Life Sciences, University of Limerick, Castletroy, Limerick, Ireland
[b] Teagasc, Moorepark Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland

ARTICLE INFO

Article history:
Received 18 June 2015
Received in revised form 15 January 2016
Accepted 7 February 2016
Available online 10 February 2016

Keywords:
Lactic acid bacteria
Permeabilisation
Autolysis
Enzymes
Flow cytometry

ABSTRACT

Production of healthier reduced-fat and reduced-salt cheeses requires careful selection of starter bacteria, as any substantial alterations to cheese composition may prompt changes in the overall performance of starters during cheese ripening. Therefore, it is important to assess the effect of compositional alterations on the individual strain response during cheese ripening for each optimised cheese matrix. In the current study, the effect of varying fat and salt levels in Cheddar cheese on the performance of a commercial *Lactococcus lactis* culture preparation, containing one *L. lactis* subsp. *lactis* strain and one *L. lactis* subsp. *cremoris* strain was investigated. Compositional variations in fat or salt levels did not affect overall starter viability, yet reduction of fat by 50% significantly delayed non-starter lactic acid bacteria (NSLAB) populations at the initial ripening period. In comparison to starter viability, starter autolysis, as measured by release of intracellular lactate dehydrogenase (LDH) or post-proline dipeptidyl aminopeptidase (Pep X) into cheese juices, decreased significantly with lower salt addition levels in full-fat Cheddar. Conversely, reducing fat content of cheese resulted in a significantly higher release of intracellular Pep X, and to a lesser extent intracellular LDH, into juices over ripening. Flow cytometry (FCM) indicated that the permeabilised and dead cell sub-populations were generally lower in juices from cheeses with reduced salt content, however no significant differences were observed between different salt and fat treatments. Interestingly, fat reductions by 30 and 50% in cheeses with reduced or half added salt contents appeared to balance out the effect of salt, and enhanced cell permeabilisation, cell death, and also cell autolysis in these variants. Overall, this study has highlighted that alterations in both salt and fat levels in cheese influence certain aspects of starter performance during ripening, including autolysis, permeabilisation, and intracellular enzyme release. However, it may be possible to reduce the fat and salt content of Cheddar cheese by 30 or 50%, respectively, without largely altering permeabilised and dead cell sub-populations and, in turn, the amount of released intracellular Pep X activity, such that these performance parameters are similar to those observed for control full-fat, full-salt Cheddar cheese.

1. Introduction

Development of flavour during cheese ripening is significantly influenced by the type of starter lactic acid bacteria (LAB) used for cheese manufacturing and subsequently the various enzyme activities originating from them (Fox et al., 1993; Fox and Wallace, 1997; Smit et al., 2005). LAB serve as principal flavour development mediators during cheese ripening, as they provide an array of extracellular and intracellular enzymes that act on various substrates within the cheese matrix to generate important volatile flavour compounds (Lopez et al., 2006; McSweeney, 2004; McSweeney and Sousa, 2000; Wilkinson et al., 1995).

LAB in the form of *Lactococcus lactis* subsp. *cremoris* or *L. lactis* subsp. *lactis* strains are the main starter types added during cheese manufacturing. They possess an extracellular cell envelope proteinase (CEP I or CEP III) and a range of intracellular proteolytic/peptidolytic enzymes, which, during ripening can hydrolyse chymosin-derived casein fragments to generate lower molecular mass peptides and free amino acids (FAA), which directly or indirectly contribute to development of the characteristic flavour and aroma of mature Cheddar cheese (Børsting et al., 2015; Fox et al., 1993; Kunji et al., 1996; McSweeney, 2004; O'Reilly et al., 2002).

Contact between intracellular peptidases of LAB and various substrates within the cheese matrix, depends on the extent of cell permeabilisation and/or cell autolysis (Doolan and Wilkinson, 2009; Sheehan et al., 2005), which correlates with elevated levels of intracellular enzyme release with a significant impact on proteolysis and flavour development in cheese (Wilkinson et al., 1994b). However,

* Corresponding author.
E-mail address: martin.wilkinson@ul.ie (M. Wilkinson).

http://dx.doi.org/10.1016/j.ijfoodmicro.2016.02.006
0168-1605/

starter enzyme release profiles are strain dependent and can vary as a result of microenvironmental changes in cheese during the maturation period (O'Donovan et al., 1996; Sheehan et al., 2005; Wilkinson et al., 1994b). Some of the important strain dependent aspects of starter performance relevant to cheese ripening include: starter viability, permeabilisation potential, autolytic response and ability to release intracellular enzymes (Chapot-Chartier et al., 1994; O'Donovan et al., 1996; Wilkinson et al., 1994a). These properties appear to be affected by cheese compositional parameters including moisture level, fat and also salt contents, which subsequently impact the final pH of cheese and water activity levels (Beresford et al., 2001; Feirtag and McKay, 1987; Lortal and Chapot-Chartier, 2005; O'Donovan et al., 1996; Steele et al., 2013; Wilkinson et al., 1994b).

Fat and salt are both fundamental components of cheese, which directly and indirectly control starter activity and various enzymes associated with cheese ripening (Fenelon et al., 2000; Hickey et al., 2013; Thomas and Pearce, 1981). Fat content has been shown to influence starter bacteria retention during whey drainage (Laloy et al., 1996), while the action of starter esterases can hydrolyse fat to free fatty acids (Collins et al., 2003; Hickey et al., 2006; Smittle et al., 1972), which can be converted to other volatile and non-volatile flavour compounds (Hickey et al., 2006; McSweeney and Sousa, 2000; Singh et al., 2003; Smit et al., 2005). Salt addition, on the other hand, promotes a higher rate of starter permeabilisation and cell autolysis during early stages of ripening (Wilkinson et al., 1994a, b), which can allow intracellular enzymes of LAB access to substrates within the cheese matrix (Bunthof et al., 2001; Doolan and Wilkinson, 2009; Guinee, 2004; Sheehan et al., 2006, 2005).

Individual studies of cheeses with either reduced-salt or reduced-fat contents have shown that both compositional alterations (e.g. salt and fat) significantly influence moisture and salt in moisture (S/M) levels in such cheese varieties (Banks, 2004; Banks et al., 1989; Fenelon et al., 2000; Rulikowska et al., 2013; Schroeder et al., 1988). A substantial increase in moisture with a significant decrease in salt content results in cheeses having very low S/M levels, which is a primary factor controlling microbial growth, cell permeabilisation, cell autolysis, and subsequently intracellular enzyme release during cheese ripening (Rulikowska et al., 2013; Wilkinson et al., 1994b). As a result, bitterness is the most common flavour defect in aged reduced-fat and reduced-salt Cheddar (Mistry, 2001). The latter parameters influence starter activity through higher cell densities and a prolonged viability during ripening with an accumulation of excessive levels of hydrophobic bitter peptides (Wilkinson et al., 1994a). Furthermore, significantly lower S/M levels result in lower cell autolysis and decreased intracellular enzyme release from starter cells, in particular Pep X, which assists de-bittering by cleaving proline residues from hydrophobic bitter peptides originating from proline rich caseins (Wilkinson and Kilcawley, 2005).

Substantial alterations in fat and salt levels may have a more significant impact on overall starter culture performance and, in turn, the flavour characteristics of such cheese varieties. However, very little is known regarding the extent of composition-related microbiological changes when both the salt and the fat content of cheese are lowered, and how this influences intracellular enzyme release, especially from defined strain starters in cheese.

Therefore this study was carried out in order to assess the impact of reducing salt and fat levels in Cheddar cheese by 30 and 50% on aspects of the performance of a commercial starter culture in relation to its viability, permeabilisation and autolytic response during the early ripening phase.

2. Material and methods

2.1. Cheddar cheese manufacture

Cheddar cheese was manufactured in triplicate in 500 L vats using a frozen DVS preparation of a mixed starter culture R604Y containing one *L. lactis* subsp. *lactis* and one *L. lactis* subsp. *cremoris* strain (Chr. Hansen's Ireland Ltd., Little Island, Co. Cork, Ireland). Starter culture was added to cheese milk at the rate of 0.012% (*w*/*v*), the curd was cooked to 38.5 °C, pitched at pH 6.15, and milled at pH 5.35. Salt was added at the rate of 0.9, 1.22 and 1.8% (*w*/w) to milled curd after which cheeses were pressed overnight at 264.6 kPa. Subsequently all cheeses were vacuum packed and ripened at 8 °C for 60 days. Resultant cheeses were denoted as FFFS, FFRS, FFHS, RFFS, RFRS, RFHS, HFFS, HFRS and HFHS based on target compositions (Fig. 4). Grated cheese samples (10 g) were taken at 1, 14, 28 and 56 days of ripening to assess starter and NSLAB populations. Cheese juice extracted using hydraulic pressure was used to assess the levels of released intracellular enzyme activities—LDH and Pep X. Flow cytometry (FCM) was used to evaluate the physiological status of starter bacteria present in juices during Cheddar cheese ripening.

2.2. Extraction of cheese juice

Cheese juices were expressed from all cheeses on each sampling day using a hydraulic cheese press (Wilkinson et al., 1994a). Grated cheese (300 g) was mixed with general purpose grade sand (600 g; Fisher Scientific, UK) and subjected to a gradual pressure increase to 32 MPa over one hour. The expressed aqueous phase was collected over three hours at room temperature following incubation at 4 °C for 30 min. Subsequently, the solidified fat layer was removed and cheese juice was centrifuged at 10,000 ×*g* for 10 min. The centrifuged juice was used to determine released LDH and Pep X activities. Addition of a filtration step through a 10 μm pore size Partec filter (CellTrics®, Partec, Germany) generated the filtrate used for FCM analysis.

2.3. Determination of water activity (a_w) in cheese juice during ripening

Water activity (a_w) was measured in disposable sample cups each containing 3 mL of a freshly expressed cheese juice at 21 °C with an Aqualab Series 3TE (Decagon Devices, Inc.,WA, USA) dewpoint electronic water activity meter. Data is presented as the mean of three replicate measurements of three replicate trials.

2.4. Starter and NSLAB viability in cheese during ripening

Cheese extracts were prepared by diluting 10 g of cheese 1:10 (*w*/*v*) with sterilised tri-sodium citrate (2% *w*/*v*) in a sterile stomacher bag. The mixture was homogenised for 5 min using a stomacher (Seward Stomacher® 400 Circulator, Seward Ltd., UK). The resultant homogenate was used for microbiological analysis. L-M17 agar was used to enumerate starter LAB counts (Terzaghi and Sandine, 1975). NSLAB populations were enumerated on LBS selective agar (Rogosa et al., 1951). L-M17 and LBS agar plates were incubated at 30 °C for 3 and 5 days, respectively, after which plates were counted and the cfu/g of cheese were calculated. Data is presented as the mean of three replicate measurements of three replicate trials.

2.5. Starter autolysis in cheese

Starter autolysis was monitored by the release of the intracellular marker enzymes LDH and Pep X into cheese juice during ripening (Hickey et al., 2006; Sheehan et al., 2005; Wilkinson et al., 1994a). LDH activity was assayed by modification of the method of Wittenberger and Angelo (Wittenberger and Angelo, 1970) by measuring the decrease in absorbance at 340 nm (UV–vis spectrophotometer, Shimadzu, Japan) resulting from pyruvate-dependent oxidation of NADH. Activity was expressed as units/mL/min of cheese juice, where one unit is defined as the amount of LDH that catalyses the oxidation of 1 μmol NADH per min. Pep X activity was determined in cheese juice samples by a modification of the method of Booth (Booth et al., 1990) based on the ability of Pep X enzyme to hydrolyse Gly-Pro-AMC substrate (Bachem Feinchemikalien, Bubendorf, Switzerland). The release of AMC from the synthetic substrates was determined spectrofluorimetrically using