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Effect of milk centrifugation and incorporation of high heat-treated centrifugate on the microbial composition and levels of volatile organic compounds of Maasdam cheese

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ABSTRACT

Centrifugation is a common milk pretreatment method for removal of *Clostridium* spores which, on germination, can produce high levels of butyric acid and gas, resulting in rancid, gassy cheese. The aim of this study was to determine the effect of centrifugation of milk, as well as incorporation of high heat-treated centrifugate into cheese milk, on the microbial and volatile profile of Maasdam cheese. To facilitate this, 16S rRNA amplicon sequencing in combination with a selective media-based approach were used to study the microbial composition of cheese during maturation, and volatile organic compounds within the cheese matrix were analyzed by HPLC and solid-phase microextraction coupled with gas chromatography–mass spectrometry. Both culture-based and molecular approaches revealed major differences in microbial populations within the cheese matrix before and after warm room ripening. During warm room ripening, an increase in counts of propionic acid bacteria (by $\sim 10^{1.5}$ cfu) and nonstarter lactic acid bacteria (by $\sim 10^{8}$ cfu) and a decrease in the counts of *Lactobacillus helveticus* (by $\sim 10^{2.5}$ cfu) were observed. *Lactococcus* species dominated the curd population throughout ripening, followed by *Lactobacillus, Propionibacterium*, and *Leuconostoc*, and the relative abundance of these accounted for more than 99% of the total genera, as revealed by high-throughput sequencing. Among subdominant microflora, the overall relative abundance of *Clostridium sensu stricto* was lower in cheeses made from centrifuged milk than control cheeses, which coincided with lower levels of butyric acid. Centrifugation as well as incorporation of high heat-treated centrifugate into cheese milk seemed to have little effect on the volatile profile of Maasdam cheese, except for butyric acid levels. Overall, this study suggests that centrifugation of milk before cheesemaking is a suitable method for controlling undesirable butyric acid fermentation without significantly altering the levels of other volatile organic compounds of Maasdam cheese.

Key words: centrifugation, microbial composition, high-throughput sequencing, volatile profile, Maasdam cheese

INTRODUCTION

Centrifugation at ~9,000 × *g* is a milk pretreatment method for removal of *Clostridium* spores. Some species of *Clostridium*, on germination, can produce gas and a high level of butyric acid via butyric acid fermentation, resulting in rancid, gassy cheeses (Su and Ingham, 2000; Le Bourhis et al., 2007). As well as removal of bacterial spores, centrifugation removes indigenous bacterial cells present in milk (Te Giffel and Van Der Horst, 2004). Some of these indigenous milk microorganisms can survive pasteurization and can grow during ripening of cheese (Grappin and Beuvier, 1997; Jordan and Cogan, 1999; Quigley et al., 2013; Sheehan, 2013). Therefore, it may be assumed that the reduction in microbial load in cheese milk by centrifugation may influence the microbial composition of cheese during maturation, as the environment would be less competitive, thus further favoring the growth of the most abundant bacteria. The microbial composition within the cheese matrix is known to play an important role in determining biochemical and ripening characteristics, including flavor development through production of enzymes and metabolites, of different varieties of cheese (Beuvier et al., 1997; Beresford et al., 2001; Montel et al., 2014; Guarrasi et al., 2017).

Although traditional culture-based approaches are effective for quantifying common starter or nonstarter

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bacteria, these approaches are not sensitive to those microorganisms that are difficult to culture, present as subdominant populations, or both (Quigley et al., 2013; O'Sullivan et al., 2015). Moreover, recent studies based on culture-independent approaches have suggested that some bacterial cells in a highly stressed condition are viable but not culturable (Quigley et al., 2013; Ruggirello et al., 2014; Hickey et al., 2018). Alternatively, molecular approaches, including high-throughput sequencing, can provide a detailed insight into the composition of both dominant and subdominant microflora. More recently, 16S rRNA amplicon sequencing has been increasingly used in the study of microbial composition within fermented food products, including cheese (Quigley et al., 2012; O'Sullivan et al., 2015; Alessandria et al., 2016). For the first time, we profiled the microbiota of cheese made from centrifuged milk, as well as cheese made from centrifuged milk containing high heat-treated (**HHT**) centrifugate compared with control cheeses, using high-throughput sequencing.

Maasdam is a washed-curd, brine-salted, large eye-forming, semihard cheese, which is developed by combining the cultures and technologies of Emmental and Gouda cheese. Apart from thermophilic lactobacilli, mesophilic mixed-strain cultures comprising *Lactococcus* and *Leuconostoc* are used as starters (as in Gouda cheese) and propionic acid bacteria (**PAB**) are used as secondary starters (as in Emmental cheese). To date, very little has been published regarding the microbial and volatile profile of Maasdam cheese, a better understanding of which will aid manufacturers to consistently achieve the desirable cheese aroma profile (Johnson and Lucey, 2006).

The objective of our study was to investigate the effect of (1) centrifugation and (2) the incorporation of the HHT centrifugate into cheese milk on microbial composition and levels of volatile organic compounds (**VOC**) of Maasdam cheese during maturation. In our study, centrifugation refers to the separation of bacteria and spores at a centrifugal force of ~9,000 × *g* (in some studies this is also referred as bactofugation; Te Giffel and Van Der Horst, 2004), whereas centrifugal separation refers to separation of milk into cream and skim milk. A parallel study was conducted investigating the effect of milk centrifugation and incorporation of HHT centrifugation on the composition, texture, and ripening characteristics of Maasdam cheese.

MATERIALS AND METHODS

Cheese Manufacture

Cheese milks were prepared as described by Lamichhane et al. (2018) and in Supplemental Figure S1 (https://doi.org/10.3168/jds.2017-14180). In summary, raw milk from a local dairy company (Dairygold Co Operative Society Limited, Cork, Ireland) was divided into 2 portions. One portion of the raw milk was separated into skim milk and cream using a cream separator. Control milk (**CT**) was prepared by adding a portion of cream and skim milk obtained from cream separator to achieve a protein to fat ratio of 1.13: 1. Another portion of the raw milk was centrifuged at 9,000 × *g* (at 50°C with flow rate of 1,000 L/h), resulting in centrifuged whole milk and centrifugate. Centrifuged whole milk was separated into cream and skim milk and high heat treatment (120°C for 26 s) was applied to centrifugate. A second cheese milk type (i.e., centrifuged milk; **CF**) was prepared by adding a portion of cream and skim milk obtained from separation of centrifuged whole milk, and a third cheese milk type was prepared by mixing a portion of cream and skim milk obtained from separation of centrifuged whole milk and HHT centrifugate (**CFHHT**; at a level of 6 to 10%, wt/wt, depending on the protein content of centrifugate). The protein-to-fat ratio of all cheese milks were standardized to 1.13: 1. All cheese milks were pasteurized before Maasdam cheese manufacture. Maasdam cheeses were manufactured as per Lamichhane et al. (2018). Three experimental Maasdam cheese types [i.e., cheese made from control milk (CT cheese), centrifuged milk (CF cheese) and centrifuged milk containing HHT centrifugate (CFHHT cheese)] were each manufactured on 3 different occasions in replicate cheesemaking trials over a 3-mo period as per Lamichhane et al. (2018). Starters and secondary starters (frozen direct vat inoculate, Chr. Hansen Ltd., Cork, Ireland) used for the manufacture of Maasdam cheese were (1) mesophilic mixed-strain (C950, 18 mg/kg of milk), consisting of *Lactococcus lactis* ssp. *cremoris*, *Lactococcus lactis* ssp. *lactis*, and *Leuconostoc*; (2) *Lactobacillus helveticus* (LH-B01, 4.8 mg/kg of milk); and (3) PAB (PS-60, 7.0 mg/kg of milk).

Enumeration of Starter and Nonstarter Lactic Acid Bacteria and PAB

Samples were aseptically removed from cheese wheels using a cheese trier at 1, 11, 41, 65, 97, 140, and 180 d of ripening. The cheese samples (10 g) were placed in a sterile stomacher bag (Grade, Leicestershire, UK), diluted (10-fold) with 2% (wt/vol) trisodium citrate buffer (VWR, Dublin, Ireland), and stomached for 10 min using a stomacher (Iul Instruments, Barcelona, Spain). Serial dilutions of 10-fold diluted cheese samples were made using maximum recovery diluent, containing low levels of peptone (1 g/L) and sodium chloride (8.5 g/L). Total numbers of nonstarter lactic acid bacteria