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Interaction between starter bacteria and adjunct *Lactobacillus plantarum* INF15D on the degradation of citrate, asparagine and aspartate in a washed-curd cheese

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Abstract

Washed-curd brine-salted cheeses were made with four different starters: citrate degrading (Cit^+) heterofermentative mesophilic DL-starters (CHN-11 and CHN-19), a citrate negative (Cit^-) mesophilic homofermentative O-starter (R-704) and a thermophilic starter TCC-20, all with the addition of an adjunct *Lactobacillus plantarum* INF15D to study the interaction between the starter and adjunct on the degradation of citrate to aspartate (Asp), diacetyl and acetoin in cheese. Citrate degradation by *Lb. plantarum* INF15D in cheese varied according to the type of starter used. In cheese made with Cit^+ DL-starters, the degradation of citrate and Asp by the adjunct *Lb. plantarum* INF15D was less extensive as diacetyl and acetoin were produced from citrate via α-acetolactate by Cit^+ *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* and/or *Leuconostoc mesenteroides* subsp. *cremoris*. In the presence of a Cit^- O-starter, *Lb. plantarum* INF15D degraded citrate mainly to Asp which was further converted to acetoin and diacetyl. However, in the presence of a thermophilic starter, *Lb. plantarum* INF15D degraded citrate mainly to succinic acid. The highest degradation of asparagine (Asn) was found in cheese made with the Cit^- O-starter (R-704) and adjunct *Lb. plantarum* INF15D. Both *Lb. plantarum* INF15D and the starter cultures were capable of deaminating Asn to Asp.

Keywords: Cheese; Starter; *Lactobacillus plantarum*; Citrate; Aspartate; Asparaginase; Asparagine

1. Introduction

Aromatic mesophilic DL-starters are generally used in Dutch type cheeses (Van den Berg, Meijer, Düsterhöft, & Smit, 2004) and in Scandinavian cheese varieties. These starters are often undefined mixed cultures composed of homofermentative *Lactococcus lactis* subsp. *lactis*, *Lc. lactis* subsp. *cremoris*, *Lc. lactis* subsp. *lactis* biovar. *diacetylactis* and the heterofermentative *Leuconostoc mesenteroides* subsp. *cremoris*. The two latter organisms metabolise citrate (Cit^+), giving diacetyl and acetoin, which is important for cheese aroma, and CO_2 production, which is important for eye formation (Hugenholtz, 1993). In Cheddar type cheeses, the degradation of citrate is not considered important for flavour formation. In such cheeses, homofermentative O cultures are used, which do not metabolise citrate (Cit^-) and thus assure the absence of eyes in cheese. Such cultures are mainly composed of *Lc. lactis* subsp. *cremoris* and *Lc. lactis* subsp. *lactis* subspecies. However, the concentration of citrate normally decreases during Cheddar cheese ripening, and this decrease is attributed to the presence of non-starter lactic acid bacteria (NSLAB) (Fox, Guinee, Cogan, & McSweeney, 2000). Thomas (1987) showed that citrate was utilised in Cheddar cheese with various added *Lactobacillus plantarum* strains. Recently, Díaz-Muñiz and Steele (2006), demonstrated that *Lb. casei* ATCC334 was capable of degrading citrate in Cheddar cheese extract.

In Swiss type cheeses, the propionic acid bacteria produce gas for eye formation and thermophilic starter

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cultures are used due to high cooking temperatures of the cheese curd. These cultures are mainly composed of *Streptococcus salivarius* subsp. *thermophilus*, *Lb. delbrueckii* subsp. *lactis* and *Lb. helveticus* species which are all homofermentative and usually considered as Cit^-. However, Torino, Taranto, and de Valdez (2005) showed that *Lb. helveticus* ATCC 15807 was able to metabolise citrate in the presence of lactose with acetate and succinate being the major products. The degradation of citrate in Swiss type cheeses has usually been related to the presence of facultative heterofermentative NSLAB (Fröhlich-Wyder & Bachmann, 2004).

In dairy products, diacetyl and acetoin may be produced from citrate metabolism performed by Cit^+ lactic acid bacteria (LAB) or by aspartate (Asp) metabolism by mesophilic *Lactobacillus* sp. (Kieronczyk, Skeie, Langsrud, Le Bars, & Yvon, 2004). The Cit^+ *Leuconostoc* ssp. and *Lc. lactis* subsp. *lactis* biovar. *diacetylactis* degrade citrate to acetate and oxaloacetate, where the latter is decarboxylated to pyruvate. The unstable α-acetolactate, which is derived from pyruvate, is quickly decarboxylated to acetoin or diacetyl. Production of diacetyl requires O_2, and acetoin is therefore most likely to be formed in cheese. Diacetyl may also be dehydrogenated to acetoin which may be further dehydrogenated to 2,3-butanediol, by bacteria possessing butanediol dehydrogenase for instance *Leuconostoc* ssp. (Hugenholtz, 1993; McSweeney & Sousa, 2000). Kieronczyk, Skeie, Olsen, and Langsrud (2001) showed that the NSLAB *Lb. plantarum* INF15D isolated from good quality Norvegia cheese (Narvhus, Hulbækdal, & Abrahamsen, 1993) could degrade Asp in vitro under cheese-like conditions. As previously reported, this strain possesses glutamate dehydrogenase (GDH) activity and produces α-ketoglutarate (α-KG) (Tanous, Kieronczyk, Helinck, Chambellon, & Yvon, 2002), which is mainly used in transamination of Asp leading to formation of oxaloacetate (Kieronczyk et al., 2004). It is likely that oxaloacetate is then metabolised via the same pathway as described above for citrate, resulting in the production of such metabolites as acetoin and diacetyl. Indeed, Kieronczyk et al. (2004) also showed that *Lb. plantarum* INF15D degraded Asp to diacetyl and acetoin in a cheese paste.

Many LAB have GDH activity, which make them capable of deaminating glutamate (Glu) to α-KG which is used for amino acid transamination. Tanous et al. (2002) and Williams, Withers, Brechany, and Banks (2006) found that GDH activity was common in several *Lactobacillus* sp., and among the NSLAB flora normally present in cheese, *Lb. plantarum* strains usually exhibited the highest activity. Fernandez de Palencia, De la Plaza, Amarita, Requena, and Pelaez (2006) tested 156 wild LAB isolates and found GDH activity in all species tested, but with species and strain variability. Of particular interest was the high incidence of GDH activity in the *Leuconostoc* strains. Thage et al. (2005) found GDH activity in a commercial DL-starter, CHN-11 from Chr. Hansen. Helinck, Le Bars, Moreau, and Yvon (2004) showed that GDH activity was common in *Str. thermophilus*.

Lb. plantarum INF15D has previously shown some interesting properties as an adjunct to cheese (Skeie, Kieronczyk, Næss, & Østlie, 2007; Skeie, Lindberg, & Narvhus, 2001): In cheese made with CHN-11 starter, *Lb. plantarum* INF15D degraded serine (Ser) to acetate and citrate to Asp, which was degraded further to acetoin during early ripening. The question then arises: How does *Lb. plantarum* INF15D work as an adjunct in combination with other types of starter cultures commonly used in cheese?

In light of results obtained in cheese made with starter CHN-11 (Skeie et al., 2007) the objective of this work was to examine the influence of *Lb. plantarum* INF15D in combination with different commercial starter cultures on the degradation of citrate and Asp in a washed-curd cheese.

2. Materials and methods

2.1. Experimental design

Washed-curd brine-salted cheeses, were made in four replicate blocks (i.e., cheesemaking days) with one experimental factor, the starter type: CHN-11 and CHN-19, both DL-starters; R-704, an O-starter and a thermophilic starter TCC-20 (selected for its good growth at 32 °C), all freeze-dried direct vat set (DVS) cultures from Chr. Hansen (Hørsholm, Denmark). The characteristics of the starters are given in Table 1 (information provided by Chr. Hansen). In total, 16 vats of cheese were manufactured.

All cheeses were produced with an adjunct strain, *Lb. plantarum* INF15D, isolated from good quality Norvegia cheese. *Lb. plantarum* INF15D, previously described as *Lb. paracasei* ssp. *paracasei* INF15D (Kieronczyk et al., 2001, 2004; Skeie et al., 2001; Tanous et al., 2002), has been reclassified as *Lb. plantarum* by 16s rDNA sequence analysis. In order to achieve high cell numbers, the adjunct starter was inoculated (10%) in 1 L MRS broth and grown at 30 °C for 20 h, before centrifugation for 10 min at $9617 \times g$ at 4 °C in a Sorvall RC 5B Plus centrifuge (Du Pont, Wilmington, US). The pellet was resuspended in cheese milk and added to the cheese vat together with the starter, giving average adjunct inoculums of log 6.9 cfu mL^{-1}. A high adjunct inoculum was aimed at, in order to suppress the growth of other NSLAB strains in the cheese.

The milk was obtained from the university herd. Cheese was made according to Skeie et al. (2001), with modifications as described by Skeie et al. (2007).

Significant differences ($P < 0.05$) between replicate block, treatment factor (starter) and age were found by using the ProcMixed procedure with repeated measurements using SAS/Stat 8.2 package (SAS Institute Inc., Cary, USA). A toeplitz covariance structure was used when analysing the data. Principal component analysis of free amino acids (FAA) was made by using The Unscrambler