Comparisons of Microbial Counts During the Ripening of Monterey Jack Cheese

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Abstract

Three different vats of Monterey Jack cheese, control (Vat A), control plus *Lactobacillus helveticus* adjunct (Vat B), and control plus propionic acid bacteria adjunct (Vat C), were each studied to determine their bacteriological makeup during ripening. Starter cultures and nonstarter lactic acid bacteria (NSLAB) were counted for all three vats. Adjunct cultures added to Vat B (*Lactobacillus helveticus*) and Vat C (propionic acid bacteria) were also enumerated to determine their behavior during cheese ripening. The growth curve for the starter cultures of Vat A displayed an initial count of 10^8 CFU/g that dropped below the detectable limit (1×10^4 CFU/g) during Month 5. The starter bacteria in Vats B and C decreased below the detectable limit earlier than the bacteria in Vat A. The counts of starter bacteria in Vat B were less than the detectable limit after one day while in Vat C they dipped below the detectable limit after one month. The NSLAB counts in Vat A started at 10^3 CFU/g and then began to increase until finally reaching counts in the 10^7 CFU/g range. The NSLAB counts of Vats B and C began at 10^7 CFU/g and maintained this number through Month 6. The counts for the *Lactobacillus helveticus* adjunct added to Vat B started at 1.97E+08 CFU/g. The bacterial numbers maintained counts mostly in the high 10^7 CFU/g range. The propionic acid bacteria adjunct added to Vat C started at 1.16E+08 CFU/g and stayed mostly in the low 10^8 CFU/g range through Month 6. Overall, the trends of the bacterial counts are similar. All three vats display a decrease in starters and an increase in NSLAB. However, Vats B and C differ from the control in the duration of detectable starter cultures and the initial counts of the NSLAB. There is no definitive reason why the starter counts differ but cross contamination and competition are some possibilities. The differences of the NSLAB counts may be due to a synergistic effect of the adjunct bacteria. The adjunct cultures of Vats B and C maintained fairly constant counts throughout the six months.

Introduction

The process of modern cheese making begins with the preparation of the milk. Preparation includes heating, pasteurizing, and the addition of starter cultures of lactic bacteria (Table 1). Then rennet, a substance needed for enzyme induced coagulation, is added to the milk. Coagulation of the milk leads to the separation of whey, a watery liquid, from the curd, a substance consisting of mainly casein. This process is further encouraged by cutting the curd using a 1/4 to 3/8-inch knife to break up the coagulated mass and also increase the surface area of the curd. The next step in the cheese making process is cooking and agitating the curds for a specific amount of time. Cooking the curd is an important step, which serves to contract the curd particles, drive out the free whey, increase the lactic acid production, suppress spoilage microorganisms, and influence the final cheese moisture. After the curd is cooked, the whey is drained off using a metal strainer or sieve. The remaining curd is salted to improve flavor, texture, and appearance and is now ready to be put into hoops and pressed overnight at forty-psi. Pressing the cheese gives it shape and texture, and also extrudes free whey.

After the cheese has been made it must undergo a ripening stage before it is ready for consumption. During the ripening stage the cheese acquires its unique texture, aroma, and taste, due to biochemical changes such as proteolysis, lipolysis, and lactose/lactate metabolism. These changes are mainly attributed to microflora present in the cheese. The purpose of this experiment was to monitor different types of microflora during the ripening process of Monterey Jack cheese. Bacterial counts of three different vats of Jack cheese, Vat A, Vat B and Vat C were monitored in this experiment. Starter bacteria, Nonstarter lactic acid bacteria (NSLAB), *Lactobacillus helveticus*, and propionic acid bacteria were enumerated for the three different vats

of cheese over a six-month period. By calculating the CFU/g of each type of bacteria the bacterial makeup of the cheese can be determined month by month.

During the cheese making process, the initial number of starter bacteria (10^7 CFU/ml) added to the milk increases to greater than 10^9 CFU/ml at the time of curd pressing (Lynch et al., 1997). Starters for Monterey Jack cheese consist of a mix of lactococci. The primary function of these bacteria during cheese manufacture is the production of lactic acid at an appropriate rate, but they also make an important contribution to proteolysis during ripening through the action of lactococcal caseino- and peptidolytic enzymes (Lynch et al., 1997).

Nonstarter lactic acid bacteria are described as adventitious bacterial flora capable of growth under the selective conditions of ripening cheeses (Fitzsimons et al., 1999). These bacteria are not added to the milk during cheese making. Instead, they arise from post pasteurization contamination by airborne flora or contamination of cheese making equipment. Since NSLAB have been shown to contribute to flavor development in some varieties of cheese they are considered a desirable contaminant (El Soda et al., 2000).

Propionic acid bacteria and *Lactobacillus helveticus* are both adjunct cultures that are added to supplement the microflora (starter bacteria and NSLAB) of the cheese milk. Adjunct cultures are defined as selected strains of cheese related microorganisms added to the cheese milk to improve development of cheese sensory quality or accelerate cheese ripening (El Soda et al., 2000). In this experiment, adjunct cultures were added to Vats B and C. A *Lactobacillus helveticus* adjunct culture commonly used in Gouda cheese ripening was added to the Vat B cheese while an adjunct propionic acid bacterial culture frequently used in Swiss cheese was added to Vat C.

Table 1. Monterey Jack Cheesemaking Procedures

Step	**Time** (min)	**Temp** (F)	**pH**	**Comments**
Add starter	00	88	6.7	Add DVS 850 @ 360 mls starter/500 gallons milk.
Add Rennett	30	88	6.6	Use 90 ml per 1000# of milk. dilutecalf rennett in 1/40 water.
Cut Curd	60	88	6.58	Use 1/4 ”-3/8” knives. Cut curd and don’t touch for 5 mins.
Start Stir	75	88		No heat.
Start Cook	90	88		Start heating slowly.
End Cook	120	102-104	6.25	Stir 5 min and let curd settle.
Whey Off	130	102-104		Remove _ of the original milk volume.
Add Water	135	88		Add 1/3 of the original milk Volume. Water temp must be Adjusted to obtain the proper Final temp. Water temp 120 C.
Drain	160	86		Remove all the whey. Stir for 25 min.
Salt	175	86		Add 2% of curd weight. Add in three even additions.
Hoop	200	78		Puts 25# of cheese into 20# hoops.
Press				Press overnight at 40 psi.
Package			5.2	Vacuum pack in air tight bags.
Age				6 months at 4° C.

Materials and Methods

At the start of each sampling day, the vacuum packed blocks of cheese are removed from the ripening room. Using a sterilized knife, a small block of cheese from each vat is cut and placed in a labeled whirlpak bag. After the samples are collected the remaining portion of the cheese is vacuum packaged and returned to the ripening room.

The small blocks of cheese are then taken to the lab where the bacteriological work can be done. First, the outer layer of the sample block of cheese is scraped off to reduce contamination. Once the cheese surface is removed, 10g of cheese is taken from the middle portion and placed in a stomacher bag. Next, 90 mls of a 2% Trisodium citrate solution (TSC) were added to the stomacher bag. The TSC solubilizes the casein molecules in the cheese to release the bacteria. The stomacher bag containing the cheese and TSC is then placed in a stomacher for three minutes. Using the stomached samples, serial dilutions are made for each vat sample.

One milliliter of the serial dilutions was pipetted into the labeled petri plates. After the sample was added to the plate, 30 mls of cooled media was poured on top and allowed to solidify. Four types of media were used to enumerate the different bacterial types. Rogosa agar selects for the NSLAB, LM17 was used to detect the starter bacteria, Lactobacilli MRS selects for *Lactobacillus helveticus* adjunct culture, and Sodium Lactate Agar selects for the propionic acid bacteria adjunct culture. Rogosa and LM17 were used for all three vats whereas MRS was used for Vat B and SLA was used for Vat C. After the agar solidified, the plates were placed into the incubator. The LM17 and Rogosa plates were incubated aerobically at 32ºC. The LM17 plates were incubated for three days and the Rogosa plates were incubated for five days. The other two sets of plates were incubated anaerobically for three days at different temperatures.

The Sodium Lactate Agar plates were incubated at 32ºC while the MRS plates were incubated at 34ºC. At the end of the incubation period, the plates were counted to determine the CFU/g for each bacterial type in each trial. Using extra calculations, overlapping growth was taken into consideration for non-selective LM17 media. To get the numbers for the starter bacteria, the equation, LM17- MRS (or SLA) - Rogosa = starters, was used. The Rogosa and SLA counts were graphed directly because they are specialized media. The MRS counts were also graphed directly. This media supposedly selects for the thermophilic *Lactobacillus helveticus* adjunct culture but there may be some question as to whether mesophilic *Lactobacillus* will also grow on this media. However, this media was the best one found so the counts were used. After calculating the counts of each trial, the CFU/g of the three replicate trials was averaged plotted against time.

Results and Discussion

Vat A cheese was the control cheese (Fig 1). Since no adjunct cultures were added, only starter bacteria and nonstarter lactic acid bacteria were considered in the graph. On Day 1 the starter bacteria count was 2.81E+08 CFU/g. After Day 1 the starter counts began to decrease until less than 1 x 10^4 CFU/g were detected in Month 6. The NSLAB count in the Vat A cheese started out at 7.45E+03 CFU/g. By Month 1, the bacterial counts had risen to 6.02E+05 CFU/g and continued to increase through Month 3. The Month 3 and Month 4 counts both were in the 1E+07 CFU/g range. The Month 5 NSLAB count increased to 3.77E+07 CFU/g and remained in this range through the final count in Month 6.

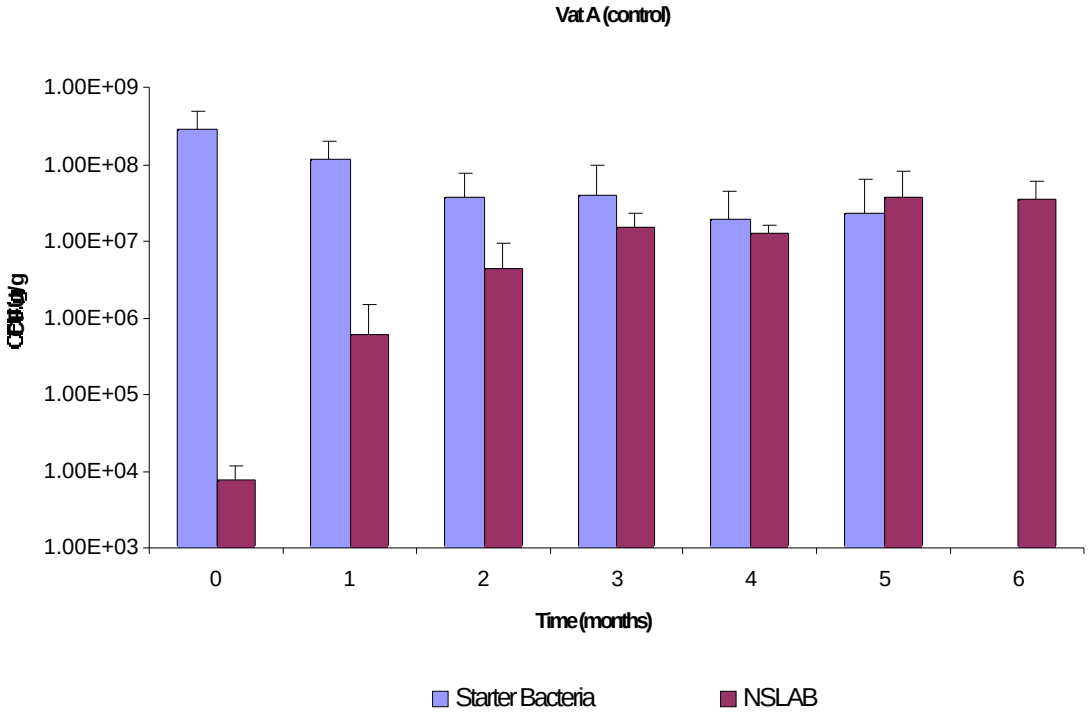

Fig. 1. Bacterial counts in Vat A Cheese. Detection limit was 1E+03 CFU/g.

The starter bacteria in Vat B demonstrated numbers different than those of Vat A (Fig. 2). On Day 1 the starter bacteria were at 5.50+06 CFU/g, two-fold less than the bacterial count detected in the control. After Day 1, the bacterial counts dropped below the detection limit (1E+04 CFU/g). NSLAB counts in Vat B began at 1.01E+07 CFU/g on Day 1. This is higher than the counts seen in the control vat. The counts remain close to the original number until an increase in Month 3 (7.06E+07 CFU/g). After Month 3, the counts level off and no more increases are seen. The counts for the adjunct culture, *Lactobacillus helveticus*, started at 1.98E+08 CFU/g on Day 1. This was the highest count seen for the adjunct culture. After the first day, the counts stayed mainly in the high 10^7 CFU/g range with the exception of a decrease in Month 1.

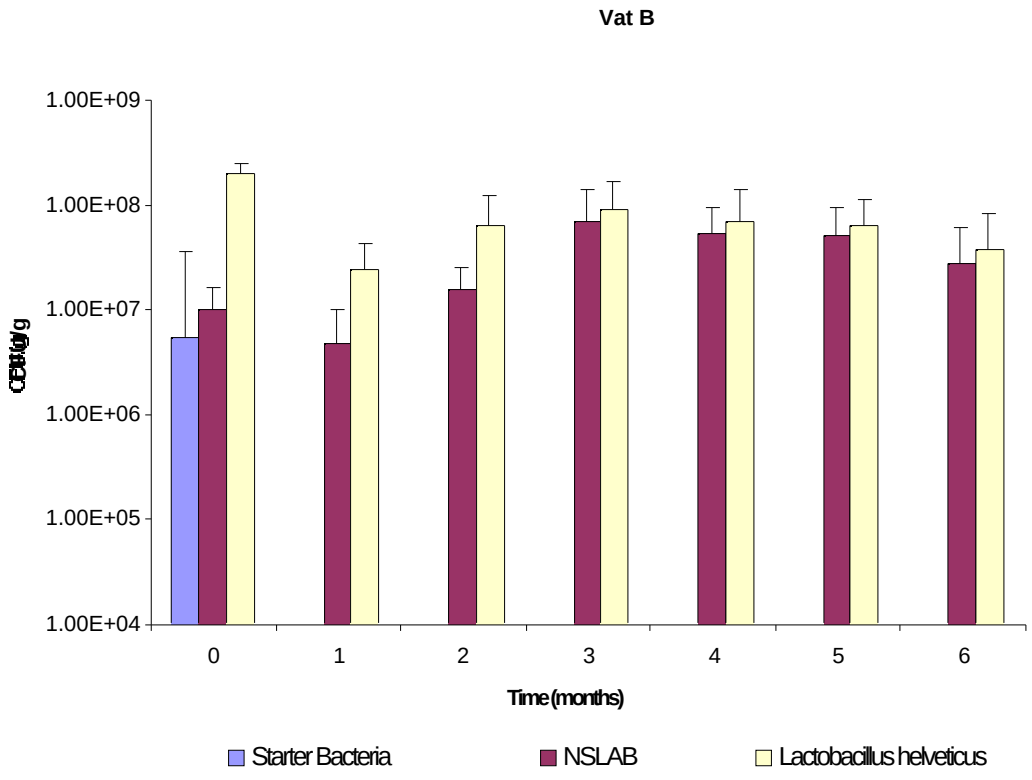

Fig. 2. Bacterial counts of Vat B Cheese. Detection limit was 1E+04 CFU/g.

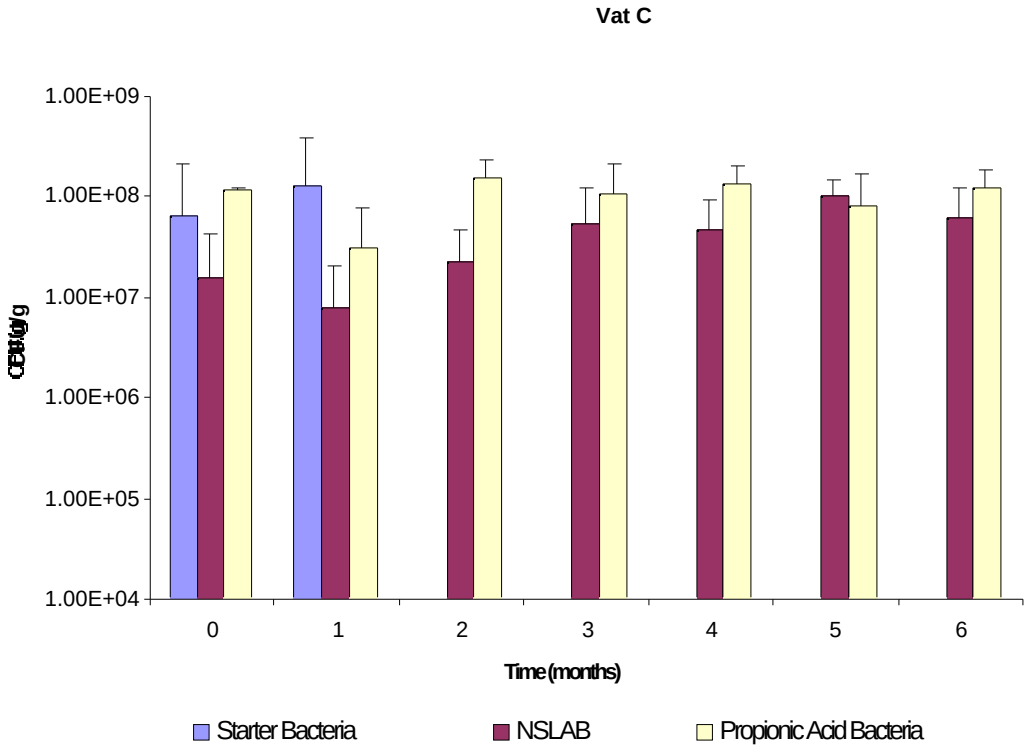

Fig. 3. Bacterial counts of Vat C Cheese. Detection limit was 1E+04 CFU/g.

The Day 1 bacterial counts of the Vat C starter culture (6.37E+07 CFU/g) are lower than the counts seen in the control but higher than the counts in Vat B. After Month 1 the bacterial counts decreased below the detection limit (1E+04 CFU/g). The initial NSLAB count of Vat C was 1.55E+07 CFU/g. This is similar to the count seen in Vat B but higher than the count seen in the control. During Month 1 through Month 6, the NSLAB counts stay mainly in the mid 10^7 CFU/g range. The final count for the NSLAB in Vat C was 6.22E+07 CFU/g. The counts of the propionic acid bacteria adjunct culture in Vat C began at 1.16E+08 CFU/g. After an initial decrease in Month 1 (3.09E+07 CFU/g), the bacterial count climbed to the 1E+08 CFU/g range and remained there through Month 6. The final count in Month 6 was 1.22E+08 CFU/g.

The starter bacteria in Vats B and C behaved differently than the starters in the control vat. Each of the three vats demonstrated decreasing trends with the starter bacterial counts dropping below the detectable limit. This decreasing trend is typical of starter bacteria in most cheeses (Lynch et al., 1997). Bacteriocidal effects of salt, low pH, and the depletion of a fermentable sugar all contribute to the decrease in starter bacterial numbers during the early maturation stages of cheese (Lynch et al., 1997). Although all three vats displayed a decreasing trend, the counts of Vats B and C drop below the detectable limit before Month 2 whereas bacterial counts of Vat A are detectable through Month 5. One possible reason for the difference between the vats is that the adjunct cultures may have the ability to out compete the starters, causing them to die off sooner. Another possibility is that the adjunct cultures may be able to grow on the specialized Rogosa agar, thereby throwing off the calculations for starter bacterial counts.

An increasing trend in the counts of NSLAB is typical in most cheeses (Rehman et al., 1999). The NSLAB grow during ripening and sometimes reach higher counts than the starter organisms (Rehman et al., 1999). The increasing trend is seen in the control vat but not in Vats B or C. The Day 1 count of Vat A begins at 1E+03 CFU/g whereas Vats B and C begin at 1E+07 CFU/g. The higher counts seen in Vats B and C may be due to a synergistic effect of the adjunct bacteria. In the early readings, the counts on Rogosa agar display numbers significantly lower than the counts on the adjunct bacterial agar. The conclusion that can be drawn from this is that the adjunct bacteria did not grow on Rogosa. Instead, the higher counts may be attributed to the adjunct cultures encouraging the growth of the NSLAB. By Month 6, the NSLAB counts for all three vats reached the 1E+07 CFU/g range.

Little information could be found about the typical behavior of adjunct cultures in cheese. In the Vat B cheese, the bacterial counts for *Lactobacillus helveticus* adjunct culture began at 1E+08 CFU/g. After an initial decrease in Month 1, the numbers stayed mainly in the 1E+07 CFU/g range through Month 6. With the exception of a decrease in Month 1, the propionic acid bacteria adjunct culture added to Vat C remained in the 1E+08 CFU/g range for the entire six months.

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