5-2007

The Effect of Exopolysaccharide-producing *Streptococcus thermophilus* MR1C on Functionality in High Moisture Cheddar-type Cheese

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THE EFFECT OF EXOPOLYSACCHARIDE-PRODUCING *STREPTOCOCCUS THERMOPHILUS* MR1C ON FUNCTIONALITY IN HIGH MOISTURE CHEDDAR-TYPE CHEESE

by

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Nutrition and Food Sciences

Approved:

UTAH STATE UNIVERSITY

Logan, Utah

2007

ABSTRACT

The Effect of Exopolysaccharide-producing *Streptococcus thermophilus* MR1C on Functionality in High Moisture Cheddar-type Cheese

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Utah State University, 2007

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Differences in texture at any particular stage of ripening depend upon differences in the basic structure and the extent to which the basic structure is modified by physical parameters. Thus, very young cheeses of the same variety differ in texture because of variations in pH and in salt, moisture, and fat content. How well a cheese melts and shreds depend on its texture and physical parameters. *Streptococcus thermophilus* MR1C produces an exopolysaccharide (EPS) that is tightly associated with the bacterial cell wall. Addition of *S. thermophilus* MR1C to the cheese make will increase the moisture of the cheese 2-3% and thus affect the texture, melt, and shreddability of that cheese.

To determine the effect of *S. thermophilus* MR1C on the texture, melt, and shreddability of cheese, two stirred-curd cheeses with equivalent physical parameters using EPS-producing *S. thermophilus* MR1C or non-EPS-producing *S. thermophilus* DM10 adjunct cultures were produced. Because MR1C cheese would increase moisture,

the curd size, wash water temperature, and pH at salting had to be altered in order to make the physical parameters the same for both cheeses.

The MR1C cheese was harder and had a higher fracture stress than the DM10 cheese. The MR1C cheese was also more adhesive, but only for one of the two trials. Even with adjustments in the method of manufacture, the MR1C cheese still had a slightly higher SM and pH, which may be partly responsible for the differences between the two cheeses. There were no differences between the MR1C cheese and the DM10 cheese in shreddability as determined by fines, stickiness, and gumminess. Cheese produced without a streptoccus adjunct culture was more cohesive and had fewer fines than the MR1C or DM10 cheese.

(82 pages)

To Jenni

ACKNOWLEDGMENTS

I would like to thank Dr. Donald J. McMahon for providing me with such a valuable experience. I am appreciative of my graduate committee members, Dr. Jeffery Broadbent and Dr. Daren Cornforth, for their valuable contribution. I would also like to thank Robert Fife, Steve White, Bonney Oomman, Andres Pastorino, and Brent Peterson for their assistance and friendship. Finally, I would especially like to thank my wife, Jenni, for her continued patience and support.

The research presented in this thesis was funded by the Western Dairy Center.

Tyler J. Singleton

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LIST OF ABBREVIATIONS

SM = Salt in the Moisture

EPS = exopolysaccharide

MR1C = EPS-producing *S. thermophilus* adjunct culture.

DM10 = Non-EPS-producing *S. thermophilus* adjunct culture.

N = Newton

LITERATURE REVIEW

Texture of Cheese

Texture of cheese is important because (1) after appearance and flavor it is the property by which the consumer first identifies and judges the specific variety (Lawrence et al., 1987), and (2) it impacts how cheese can be presented to the customer (slices, shreds, crumbles, etc). Differences in texture at any particular stage of ripening depend upon differences in the basic structure and the extent to which the basic structure is modified by physical parameters. Thus, very young cheeses of the same variety differ in texture because of variations in pH and in salt, moisture, and fat content. As cheese matures the initial basic structure increasingly breaks down and the texture changes correspondingly (Lawrence et al., 1983).

The structure of a cheese is the spatial arrangement of its components and the texture is, in a sense, the outward manifestation of the inner structure. The basic structure of a cheese is essentially determined by the point at which the curd and the whey are separated, since this determines the mineral content of the curd, and from the residual sugar (lactose and/or galactose) content, the lowest pH that the cheese can attain. The differences between various cheese types are essentially differences in basic structure (Lawrence et al., 1983).

Texture Measurement

The ultimate goal of rheological research on cheese is correlation of measured textural or mechanical properties with sensory characteristics (Holsinger et al., 1995). The rheological characterization of cheese is important as a means of determining body and

texture for quality and identity as well as a means of studying its structure as a function of composition, processing techniques, and storage conditions. Like most solid foods, cheese is viscoelastic in nature, meaning that it exhibits both solid (elastic) and fluid (viscous) behavior (Holsinger et al., 1995; Konstance and Holsinger, 1992).

Historically, firmness and elasticity were evaluated by means of the cheese grader's thumb pressed on the surface of the cheese (Konstance and Holsinger, 1992). Civille and Szczesniak defined parameters for sensory evaluation of cheese (cited in Lee et al., 1978). These parameters were:

- Hardness - the force required to penetrate the sample with the molar teeth,
- Brittleness - breakability of the sample at the first bite,
- Chewiness-number of chews required to swallow a certain amount of sample,
- Springiness - bouncing property of sample through several consecutive bites,
- Adhesiveness - stickiness of sample in the mouth throughout mastication,
- Lumpiness- heterogeneous mouth-feeling of sample throughout mastication.

When a grader assesses the qualities of cheese, one of the main properties he looks at is texture. For Cheddar, he is looking for a body and texture that is closed, firm, and malleable, indicating an appropriate degree of protein structure breakdown (Frances et al., 1993). In developing methods for non-sensory assessment of cheese, one hopes to define these parameters with objective tests that mimic the measurements made subjectively by the grader.

The General Foods Texturometer appeared in the 1960's (Konstance and Holsinger, 1992). This device cyclically compressed a bite-sized sample to 25% of its original height, thereby imitating jaw movement. Strain gauges and a strip-chart recorder produced a force-time curve from which a Texture Profile Analysis (TPA) could be derived. An Instron Universal Testing Machine was then adapted for TPA studies.

There are some problems using TPA for texture assessment. There can be variations in sample dimensions and sample temperature, plus variations in number and orientation of grain junctions in the sample that can greatly affect the results. Thus, multiple replicates are needed to account for sample differences. The slow deformation rates normally employed are also not typical of chewing, plus, the conditions of the test are further removed from chewing by the absence of saliva (O'Hare, 1990). Thus, interpretation of instrument data can be difficult but it does allow comparisons between different cheeses.

A study by Lee et al. (1978) revealed that firmness (or hardness) is the most important characteristic of cheese with respect to consumer preference and sensory perception. Hardness can practically represent brittleness and is closely related to lumpiness and chewiness. Sensory evaluation of hardness by the trained panel correlated with the Instron TPA.

Cheese Make Process

Cheesemaking is best considered simply as the removal of moisture from a rennet coagulum. This is achieved in several ways, but the most important is by decreasing the pH of the curd (Lawrence et al., 1987). In milk, the casein micelles contain relatively large quantities of water. About half of the water is normally removed during the cheesemaking

process, together with varying quantities of calcium and phosphate. The quantity of micellar calcium phosphate lost is determined by the acidity developed before the whey is drained from the curd. The extent to which the sub-microscopic structure of the casein micelles will be retained in the cheese in its original undisrupted form is largely determined by the loss of calcium phosphate (Lawrence et al., 1983).

The manufacture of all cheese varieties involves multiple factors that influence the subsequent change in texture during ripening, including: the pH at which the whey is drained from the curd, since this determines the proportions of chymosin and plasmin in the cheese; the salt-in-the moisture that controls, together with the ripening temperature, the activity of residual rennet and plasmin in the cheese; the pH of the cheese after salting, which, according to Lawrence et al. (1987), is the single most important factor that influences texture, although as shown by McMahon et al. (2005) above pH 5.0 this is because of its correlation with calcium content of the cheese.

Cheesemaking is essentially a procedure for concentrating the casein and the fat of the milk. Electron microscopy has established that cheese consists of a continuous protein matrix, throughout which a discontinuous, discrete fat phase can be observed (Lawrence et al., 1987). Only the casein, therefore, is involved in the formation of the basic structure of a cheese. The basic steps are acidification, coagulation, dehydration (cutting, cooking, stirring, pressing, salting), shaping (moulding, pressing), and salting. The amount of water retained in the product is regulated by the extent and combination of the five steps listed above plus the milk's composition.

In general terms, cheese may be considered as a composite material. The properties of the casein matrix are modified by the presence of fat particles, brine, small holes and

cracks, and the boundaries between curd granules. The rheological properties of the fat are added to those of the casein matrix so that the cheese as a whole is viscoelastic. Generally, the casein network extends in all directions, forming a cage, the rigidity of which depends upon the degree of openness, the amount of water bound to the casein, and the presence of fat and free water (Prentice et al., 1993).

Cook Temperature

Cooking temperature is an easily adjusted manufacturing process variable that interacts with the conditions (i.e., pH, duration of cooking, type of coagulant, and culture strain) in the cheese vat during cooking. Cheese is cooked, i.e., heated to temperatures higher than those used for gelation, mainly as a means of increasing the syneresis of the curd particles. The higher the cooking temperature, the greater the syneresis of the curd particles and the lower the cheese moisture (Lucey et al., 2003).

Proteolysis

The texture of cheese at any specific stage of ripening is determined primarily by its pH and ratio of intact casein to moisture (Lawrence et al., 1987). There is a good correlation between the firmness of a cheese and the quantity of intact α_{s1}-casein present. This is not surprising because the breakdown products of the caseins are largely water-soluble and cannot contribute to the protein matrix (Lawrence et al., 1987).

As each peptide bond is cleaved, two new ionic groups are generated and each of these will compete for the available water in the system. Thus, the water previously available for solvation of the protein chains will become tied up with the new ionic groups.

Relatively low moisture cheese, such as Cheddar, tends, therefore, to become increasingly harder with age and more resistant to slight deformation (Lawrence et al., 1987).

There are two distinct phases in texture development that take place during ripening. The first phase occurs in the first 7 to 14 d when the rubbery texture of young cheese curd is rapidly converted into a smoother, more homogeneous product (Lawrence and Giles, 1993). It has been suggested that proteolysis of the network of caseins that make up the microstructure of cheese causes this change (Fox and McSweeney, 1996), but changes in protein hydration are also taking place (McMahon et al., 1999).

The second phase involves a more gradual change in texture, as the rest of the α_{s1}-casein, along with part of the other caseins, are broken down. This phase takes months as opposed to days for phase one (Lawrence et al., 1987). An examination of commercial Cheddar cheese in the United States showed that 85% of the α_{s1}-casein had been hydrolyzed. However, 95% of the β-casein was still intact after 10 weeks of ripening (Lawrence et al., 1987).

The lower the ratio of moisture to casein, the firmer will be the casein matrix of the cheese and the harder the cheese. Small changes in the moisture to casein ratio result in relatively large changes in available moisture, since much of the moisture is bound to the caseins and their degradation products. Even small decreases in water activity greatly decrease the rate of proteolytic activity in cheese (Lawrence et al., 1987).

Proteolytic activity in cheese is mainly determined by the levels of residual rennet and native milk proteinases present, salt-in-the-moisture ratio, temperature of ripening, type of coagulant used, and changes in pH during ripening (Fox et al., 1993).

The principal pathway of proteolytic degradation during cheese ripening appears to involve a relatively limited breakdown of the caseins by rennet or plasmin. The polypeptides so formed are then further degraded to small peptides and amino acids by the proteinase/peptidase systems of the starter and nonstarter bacteria present (Lawrence et al., 1983). The inability of almost all starter strains to degrade α_{s1}-casein makes it unlikely that starter proteinases contribute significantly to changes in cheese texture, at least in the early stages of ripening (Fox and McSweeney, 1996). The individual proteinases act together synergistically. For instance, the breakdown products of β-casein released by plasmin are further degraded by the starter proteinase/peptidase systems (Lawrence et al., 1987).

Cheese Composition

Fat, pH, moisture, salt-in-the moisture, and calcium and phosphorus are among the most important physical parameters that influence the texture of cheese. Each of these parameters also has an effect on the proteolysis of cheese as well as on each other.

Fat

For cheese, fat contributes to the taste, texture, functionality, and appearance. As the fat content of cheese decreases from 32-34% fat typically in cheddar cheese, the TPA hardness, cohesiveness, and springiness increases (Riddell-Lawrence and Hicks, 1989).

Also for Mozzarella cheese, when fat is removed several undesirable characteristics develop during cooking, including poor melt, a tough and rubbery texture, translucent color, and rapid skin formation (Paulson et al., 1998). These changes occur because fat globules normally act as a filler between the protein fibers that are formed during hot stretching of the cheese curd, thus reducing the interactions among proteins within the

protein matrix. In full fat cheese the protein matrix is relatively open with spaces occupied by the fat globules dispersed through the protein network producing a lace-like appearance (Bryant et al., 1995). Lower fat cheese has a more compact protein matrix with less open spaces because there are fewer fat globules to block fusion of the protein strands during cheesemaking.

Reduced fat cheeses also tend to be harder, more elastic, and more adhesive than their full fat counterparts (Metzger and Mistry, 1994). Increasing moisture content is generally recommended as a means to improve texture of reduced fat cheeses. According to Bryant et al. (1995), the most adhesive cheeses were those containing an open and loose protein matrix as in the higher fat cheeses. As the protein matrix becomes increasingly more compact the cheese loses adhesiveness. Compact appearance of the protein network increased and the number of milk fat globules dispersed within the network decreased with reduction in fat content of cheese. This is a probable explanation for the hard texture observed with lower fat cheeses

A high-fat or a high-moisture content weakens the α_s-casein framework of the cheese structure since the protein molecules must of necessity be further apart. The cheese will thus be smoother (Bryant et al., 1995). Similarly a low-fat and a low-moisture in a cheese results in a tight, strong α_s-casein framework and the textures of parmesan and romano cheeses are therefore tough relative to most other cheeses.

Homogenization of milk also influences cheese hardness. Cheese made from homogenized milk is smoother and has a finer texture than normal, but is also firmer and more elastic because the fat globules contribute to the overall rheological properties (Prentice et al., 1993). Hardness of mozzarella cheese increased with homogenization

pressure and decreased with fat percentage and moisture in nonfat substance (Rudan et al., 1999).

pH

The final cheese pH affects to a greater or lesser extent the basic structure of cheese (Lawrence et al., 1983). Proper control of acid production is a key to the manufacture of good quality cheese. It affects coagulant activity, curd strength, syneresis, and growth of non-starter microorganisms. Syneresis controls the moisture content, which regulates bacterial growth and enzyme activity, which, in turn, influence the rate and pattern of ripening. If the cheese is too acid, the cheese is crumbly; if too basic, the cheese is pasty and sticky (Holsinger et al., 1995).

The extent of acid production in the vat largely determines the mineral content of a cheese since mineral losses after the draining stage are small under normal circumstances. In low pH (i.e., pH<5.0) cheese varieties, the conformation of the caseins changes markedly as the pH approaches their iso-electric point. Similarly, in the camembert-type cheeses, the ammonia produced by the surface mold rapidly increases the pH, resulting in a soft, smooth texture, despite the relatively low degree of proteolysis (Lawrence et al., 1983).

As the pH of cheese curd decreases there is a concomitant loss of colloidal calcium phosphate bound to the casein. (Lawrence et al., 1987). As the pH of the cheese curd drops below pH 5.0, the protein assumes an increasingly more compact conformation and the cheese becomes shorter in texture. The ability of proteins to interact with water and the water-holding capacity of the protein matrix decreases below pH 5.0, which then results in

increased syneresis and decreased moisture content of cheese (Pastorino et al., 2003a). After about 14 d of ripening, cheese can have a texture ranging from springy through to plastic to noncohesive, depending primarily on the pH of the curd and, to a lesser extent, its calcium content (Lawrence et al., 1987).

Milling pH in conjunction with curd moisture content and residual lactose content determines the pH of dry salted cheese, and differences in cheese pH can affect cheese texture. Proteolysis during refrigerated storage is influenced by pH and proteolysis affects texture and functional properties of cheese. In general, curds with a low pH tend to be crumbly, whereas high pH curds tend to be more elastic (Lucey and Fox, 1993).

Moisture

The more moisture present at any pH, the softer in texture is the cheese (Masi and Addeo, 1986). The rheological properties of cheese with a similar pH and calcium content, and at a similar degree of α_{s1}-casein degradation, are regulated by their moisture contents (Lawrence et al., 1987).

The curd protein matrix contracts (expels moisture) as temperature is raised and pH is lowered and conversely swells (absorb moisture) at low temperature and high pH. Thus increasing temperature or reducing pH during cheese making generally increases curd syneresis (Reinbold et al., 1992).

Cutting the gel when the coagulum is too firm retards syneresis and results in high moisture cheese (Riddell-Lawrence and Hicks, 1988). During healing, a membranous film forms around curd particles as syneresis from the curd surface occurs. As this layer forms and becomes thicker, the rate of syneresis decreases. Thus, moisture content increases

substantially between cheeses manufactured with healing times of 0 and 15 min, and slightly more as heal time is increased to 30 min (Riddell-Lawrence and Hicks, 1988).

Wodicki et al. (1984) studied the effect of water content on the hardness of edam cheese. They made three conclusions about the relationship between moisture and hardness: (1) changes in edam cheese during maturation bring about development of a modulus of elasticity, which is inversely related to the water content of the cheese; (2) there is a close relationship between the hardness of fully matured cheese and the decrease in moisture content during maturation; and (3) It is possible to control the hardness of edam cheese by adjusting the moisture content of freshly-made cheese.

Salt

Salting of curd is a traditional and integral part of the manufacture of most, if not all cheese varieties. Sodium chloride effects cheese texture through its inhibition of microbial growth, control of activity of proteolytic enzymes, and effects on water binding properties of proteins (Cervantes et al., 1983). Calcium content has an overriding influence on the proteins in cheese, and Paulson et al. (1998) observed little effect of salt on microstructure and functionality of cheese other than that occurring with the initial 0.5% salt added.

Sodium chloride affects many constituents of cheese that influence texture. Such factors as structure of casein, moisture content, hydration of the protein networks, and interactions of calcium-paracaseinate-phosphate complex in cheese all are influenced by salt concentration (Cervantes et al., 1983). Also, activity of proteolytic enzymes in cheese is salt concentration dependent, decreasing with an increase in salt concentration. Less proteolysis would be expected in cheese samples with higher salt concentration, and,

conversely, more proteolysis with concomitant softening would occur with less salt concentration (Cervantes et al., 1983).

The addition of salt can affect the texture of the curd, presumably because the presence of relatively high concentrations of sodium ion, at the relatively low pH values found in cheese, interferes with the effectiveness of the calcium ions to neutralize negative charges on the casein (Lawrence et al., 1983). High salt levels tend to result in curdy textures, probably due to insufficient proteolysis. A pasty body, often associated with off-flavors, is common in cheeses with low salt and high moisture levels (Fox, 1987). Salt effects in the normal salting range of 1.5-2.0% are more likely a function of influence on cultures and subsequent breakdown of cheese during aging.

Salt in the Moisture (SM)

Mistry and Kasperson (1998) found that in reduced fat cheddar cheeses, the increased moisture content lowered SM and, consequently, altered the ripening characteristics of cheese. In reduced fat cheeses (fat in dry matter ~ 28%), SM can be increased to levels found in full fat cheese without adversely affecting flavor, but only with a simultaneous increase in TPA hardness. There is a nearly linear relationship between the rate of degradation of casein and the SM level with casein degradation decreasing as SM level increases (Lawrence et al., 1983).

Calcium and Phosphate

During cheesemaking, the pH at draining determines the retention of minerals, mainly calcium and phosphorous, in the cheese curd (Lawrence et al., 1983). The content of calcium then affects the extent and degree of protein aggregation determining the basic

structure and texture of cheese (Lawrence et al., 1983). Calcium and phosphate promote cheese rigidity, as they are responsible for cross-linkage formation within the casein network. Cheeses with a high mineral content will have a more completely cross-linked structure and be more rigid (Masi and Addeo, 1986), thus increasing their TPA hardness.

There is a continuous spectrum of basic structures in the different cheese varieties. At one end of the spectrum are the cheeses with a relatively high mineral content and hard texture, such as Swiss. At the other end are the very acidic cheeses, such as feta, cheshire, and the mould ripened cheeses, with a relatively low mineral content and softer texture in which the casein units have been disrupted (Lawrence et al., 1983). The mineral content of a cheese thus serves as an indication of the extent to which the network structure of the casein is bound together. When the mineral losses from the curd are low, the identity of the casein in the cheese will be almost unchanged from that of the original milk.

Calcium content also affects cheese functionality. According to Paulson et al. (1998), increased calcium content resulted in decreased melting of nonfat Mozzarella cheese. Also, for any given pH value, there is a tendency for Cheddar cheese to become firmer as the calcium content of cheese increases (Lawrence et al., 1993).

Pastorino et al. (2003b) found that as the calcium content of cheese increased by injecting calcium into cheese, serum was released from within the cheese matrix and the moisture content of cheese decreased. This, in turn, decreased cheese cohesiveness. Calcium injection promoted protein-protein interactions, possibly through calcium bridging and charge neutralization, serum was expelled from within the protein matrix, and the cheese became firmer.

Factors that determine changes in texture are basically the same in all cheese varieties. This is not surprising since the components of cheese – rennet, native milk enzymes, caseins, moisture, lactic acid, sodium chloride, fat, and calcium are the same for all cheese varieties. Only the proportion of these components differs (Lawrence et al., 1987).

Exopolysaccharide-Producing Cultures

Many strains of lactic acid bacteria produce extracellular polysaccharides, which may be tightly associated with the bacterial cell wall as capsules or liberated into the growth medium as a loose slime (Cerning, 1995). The term exopolysaccharide (EPS) has been used to refer to either type of external polysaccharide.

Exopolysaccharide-producing bacteria can act as viscosifying, stabilizing, or water-binding agents in various foods and can thus act as alternatives to commercial stabilizers in various foods (Cerning, 1995). However, interest in EPS-producing starters for cheesemaking has generally been restricted by the finding that EPS usually accumulates in cheese whey and increases its viscocity unless the EPS is tightly bound to the bacterial cell as a capsule (Broadbent et al., 2001).

Perry et al. (1997, 1998) investigated the influence of an EPS^+ starter pair on the moisture and melt properties of low fat (6%) Mozzarella cheese. The bacteria used in those studies, *Streptococcus thermophilus* MR1C and *Lactobacillus delbrueckii* subsp. *bulgaricus* MR1R each produce EPS (Perry et al., 1997). The cheese manufactured with MR1C and MR1R contained significantly more moisture and better melt properties than cheese made with a commercial starter pair (*S. thermophilus* TAO61 and *L. helveticus*

LH100). Low et al. (1998) established that this effect was due exclusively to *S. thermophilus* MR1C. Cheese made with *S. thermophilus* DM10, an EPS$^-$ mutant of *S. thermophilus* MR1C, contained an average of 3.2% less water than cheeses made with MR1C (Low et al., 1998). Peterson et al. (2000) found that whey from cheese produced using *S. thermophilus* MR1C was no more viscous than whey from cheese produced without the EPS culture showing that the EPS remained bound to the bacteria in the cheese curd.

Because EPS cultures hold more moisture in the curd, if the effect of the EPS culture is to be evaluated in isolation, then any changes caused by using the EPS culture need to be compensated for during cheesemaking.

Shredding, Slicing

Very little research has been published on the shreddability or sliceability of cheese. Shredded and sliced cheese is a value-added product that is becoming increasingly popular with consumers. Cheese shreds and slices need to be uniformly and precisely cut to meet customer expectations. Often during handling, distribution and storage, shreds crumble, stick, or mat (Hongxu and Guansekaran, 2003). In order to prevent caking or stickiness, powdered cellulose is added to the shreds as an anti-caking agent. Generally, as the moisture in cheese increases, the cheese becomes softer and thus more difficult to shred or slice.

HYPOTHESES AND OBJECTIVES

The hypotheses of this study were:

1. The strong water binding properties of the *S. thermophilus* MR1C capsular (cell-bound) EPS are such that when used in cheese there will be less water available within the protein matrix and for a cheese with fixed total moisture there should be a resultant increase in hardness.
2. Including a capsular EPS-producing culture in cheeses with high moisture (40-45%) will improve shreddability of the cheese with less clumping of shreds during storage.

The objectives of this study were:

1. Manufacture Cheddar-style cheeses with and without EPS cultures to a defined moisture content and compare their texture attributes, shreddability, and clumping of shreds during storage.

PRELIMINARY RESEARCH

Because using the same method to make cheese using an EPS^+ or EPS^- cultures would result in 2% to 3% higher moisture in the EPS^+ cheese, make procedures needed to be developed to produce EPS^+ and EPS^- cheeses of the same composition. There are numerous ways to change the cheese make that will affect the moisture of the cheese. For example, if the curd is cut when the gel is still very soft, the moisture will be lower than if the gel is left for a longer time before cutting. This is probably a reflection of the extent of bonding between and within protein particles, which increases with time (Lucey et al., 2003). The smaller the curd particles the coagulum is cut into, the greater the syneresis and the lower the moisture. Also, the higher the cook temperature, the greater the syneresis and the lower the moisture.

It was not until the seventh attempt that we were able to produce cheeses made with MR1C or DM10 that had very similar physical parameters at around 42% moisture. We first attempted to make high-moisture cheddar by "cheddaring" the curd as opposed to using a stirred-curd method. We found that regardless of the changes to the make, we could only achieve the 42% moisture target with the MR1C cheese while the DM10 cheese remained below 40% moisture.

Using a stirred-curd make it was possible to achieve the higher moisture using the DM10 culture. Draining a portion of the whey and then diluting and cooling with wash water causes some of the calcium to go from the curd to the whey. Less calcium in the curd allows the protein matrix to open and hold more moisture. *S. thermophilus* is a thermophilic bacteria and as such it grows best in warmer temperatures. Therefore, we did

not allow the wash water to cool the curd below 32°C. We were able to change the final moisture by adjusting the temperature of the wash water. If we added cooler water, less water was required to drop the curd temperature to 32°C, less dilution of lactose, less calcium left the curd, and the moisture decreased.

We first used a 2.5-cm curd knife with wire seperator to cut the DM10 cheese and a 0.5-cm knife to cut the MR1C cheese. We also cooked the MR1C cheese 3°C warmer than the DM10 cheese. This resulted in the DM10 cheese with 3% higher moisture. We reduced the knife size for the DM10 cheese to 1.5 cm, but the moisture was still 1.5% higher. We then used the same cook temperature so both cheeses were cooked at the same temperature, which resulted in MR1C with 1.5% higher moisture. While we were adjusting cook temperature and size of the curd to get both cheeses close to equal moisture, we were adjusting the temperature and quantity of the wash water to get both cheeses at the desired 42% moisture. For the final make, we left both cook temperatures at equal temperatures, used the 0.5-cm knife for the MR1C cheese and the 1.5-cm knife for the DM10 cheese, then added cooler wash water to the MR1C cheese to reduce the moisture of the MR1C cheese to equal that of the DM10 cheese.

During the different makes, the MR1C cheese consistently had a high final pH. We salted the MR1C at a lower pH than the DM10 cheese in order to make the final pH the same for both cheeses.

MATERIALS AND METHODS

Cultures

Direct set cultures MA011, suitable for American-style cheese manufacture, containing *L. lactis ssp.lactis* and *L. lactis* ssp. *cremoris*, were obtained from DSM Food Specialties (Millville, UT). Adjunct cultures, EPS^+ *S. thermophilus* MR1C and EPS^- *S. thermophilus* DM10 were grown separately in Sure Set XL® internal pH-controlled medium (DSM Food Specialties). The adjunct streptococcal cultures were grown at 42°C to a pH of 4.4 one day prior to cheese making and kept at 6°C until used. The milk in each vat was inoculated with lactococcal starter and appropriate adjunct.

Cheese Manufacture

Two stainless steel vats were filled with 114 kg of pasteurized, non-homogenized whole milk. The milk in each vat was heated to 36°C and inoculated with 5 units of MA011 and 1.7 L of MR1C or DM10. Rennet was added after 45 min of ripening and the curd was cut 20 min after rennet addition. The curd was allowed to heal for 15 min and then gently agitated for 15 min. Curd was then cooked at 36°C and held at that temperature until a pH of 6.0 was reached and then 40 kg of whey was drained. Water at 12°C or 19°C was added until a curd temperature of 32°C was reached. After 15 min the remainder of the whey was drained, and then the curd stirred until its salting pH was reached (see Table 1). The curd was then salted and placed into a cheese cloth-lined mould and pressed for 20 h. The resulting 10 kg blocks were vacuum-sealed in plastic and cooled to 5°C.

Table 1. Differences in make procedures used to make cheese with MR1C or DM10 adjunct cultures that had similar physical parameters.

Adjunct Culture	Curd Size at Cutting	Wash Water Temperature	Salting pH
	(cm)	°C	
MR1C	0.5	12	5.50
DM10	1.5	19	5.55

Four vats of MR1C and 4 vats of DM10 cheese produced during the summer of 2000 for trial 1 were designated as Trial 1. Five vats of each during the summer of 2001 were designated as Trial 2. During summer 2002, three vats of cheese were made according to DM10 procedure, but without using any of the streptococcal adjunct culture.

Trial 1 cheeses were sampled at d 7, 21, and 42. At d 7 the cheeses were tested for fat, moisture, pH, and salt. At d 7, 21, and 42, TPA (hardness, adhesiveness, cohesiveness, fracture stress) and fines tests were completed. Cheese was shredded and vacuum packaged at d 7, 21, and 42 to determine matting during storage. The shreds were tested for melt at d 42 and 84.

Trial 2 cheeses were sampled at d 3, 7, 14, 21 and 42. At d 7 the cheeses were tested for fat, moisture, pH, and salt. At d 3, 14, 21 and 42, TPA (hardness, adhesiveness, cohesiveness, fracture stress) tests were completed. Fines, gumminess, and stickiness were tested at d 3, 7, 21, and 42.

Trial 3 cheeses were sampled at d 7, 14, 21 and 42. At d 7, the cheeses were tested for fat, moisture, and pH. At d 7, 14, and 21, fines, and gumminess tests were completed. TPA (hardness, adhesiveness, cohesiveness, fracture stress) tests were completed at d 7, 14, 21, and 42.

Cheese Composition

Cheese pH was measured using the gold electrode/quinhydrone method (Marshal, 1992). Moisture was analyzed using a vacuum oven, and moisture was determined as weight loss (AOAC, 1990). Fat content was determined using a modified Babcock method (Richardson, 1985). Total NaCl content was measured using a chloride analyzer (model 926; corning scientific, medfield, MA) (Paulson et al., 1998).

Meltability

Meltability of cheeses was measured using a UW Meltmeter (University of Wisconsin, Madison, WI) as described by Wang et al. (1998). Meltability was expressed as the percentage change in height of cheese after 10 s at a constant force of 0.33 N, when heated to 65°C. The UW Meltmeter tests a cheese plug 7 mm thick and 30 mm in diameter. The plug weighed on average 6.65 g and so for shredded cheese 6.65 g of cheese was pressed into the UW Meltmeter sample chamber using a 30-mm stainless rod.

Texture Profile Analysis

Hardness, adhesiveness, cohesiveness, and fracture stress of the cheeses were measured using a two-bite test to 80% compression (van Vliet, 1991). A force-distance curve (Figure 1) was obtained using an Instron universal testing machine (Model 5500; Instron Corp., Canton, MA) with a crosshead speed of 50 mm/min and a 500-N load cell. Plugs of cheese 1.6-cm in diameter were taken using a stainless steel cork borer and cut to 2-cm length were taken from the cheese immediately after removal from the refrigerator, and tested at approximately 5°C.

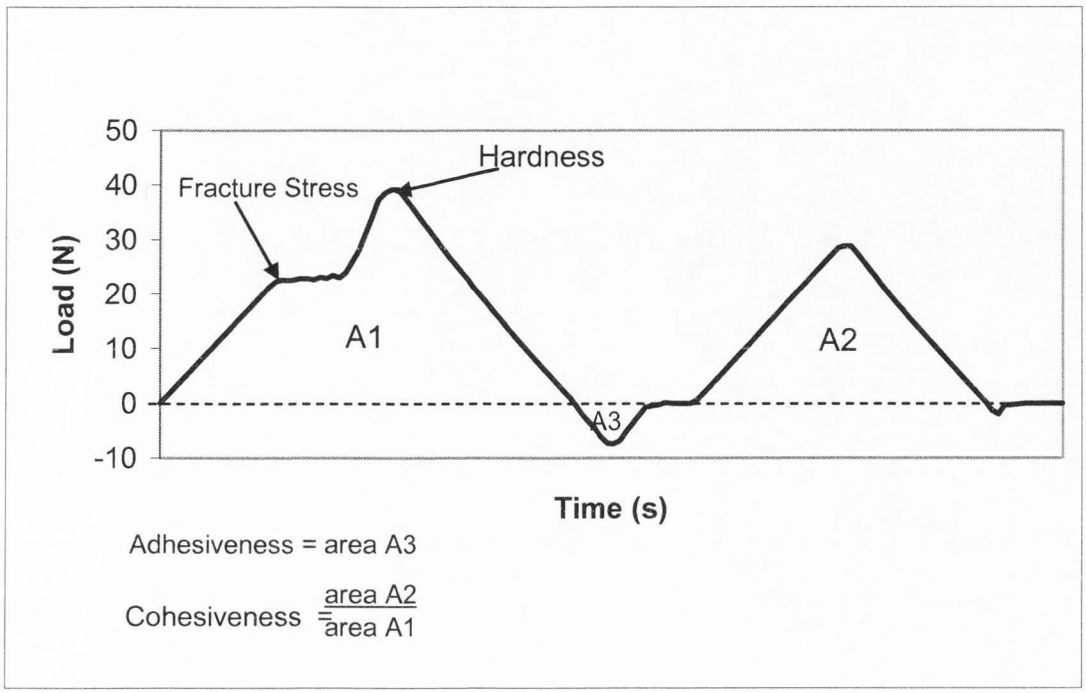

Figure 1. TPA 2-bite compression, force distance curve showing fracture stress, hardness, adhesiveness, and cohesiveness of cheese.

As shown in Figure 1, the point at which the sample first yielded under compression was fracture stress, and the highest force during the first compression was hardness. Adhesiveness was the calculated area of the negative force between the first and second compressions as the crosshead was retracted to its starting position. Cohesiveness was calculated as the area of the second compression divided by the area of the first compression. A less cohesive cheese will be more thoroughly broken down or fractured during the first compression and the area of the second compression will thus be less. For some cheese, mainly aged 21 d or more, there was no apparent fracture points (Figure 2) and so no fracture stress was recorded for those cheeses.

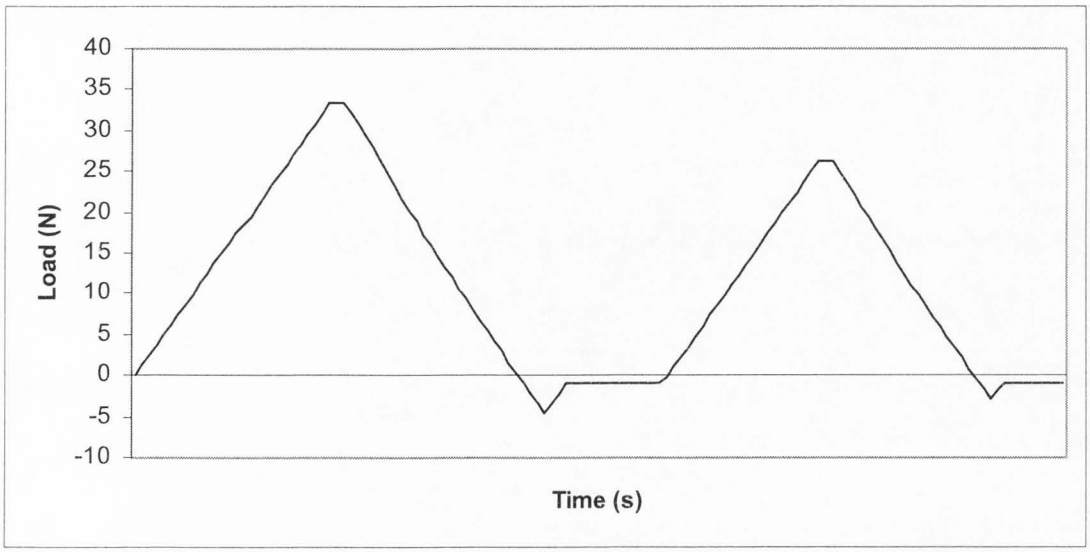

Figure 2. TPA 2-bite compression, force distance curve showing a cheese in which no fracture stress point was apparent.

Fines

Cheese was first sliced into two pieces, 22.5 x 10 x 4.5 cm in size and approximately 225 g in weight. The slices were then shredded in a hand-held electric shredder (Presto Professional SaladShooter, National presto Industries, Inc., Eau Claire, WI). To some shredded cheese, 3.4 g of a cellulose powder anti-caking agent (Solka-floc, International Fiber Corporating, North Tonawanda, NY) was mixed in with the cheese shreds (about 1.5% by weight) to prevent the cheese from sticking. The fines were then spread onto the top of a stack of six U.S.A. Standard Testing Sieves (Fischer Scientific Co.), with sieve openings of 25, 19, 12.5, 9.5, 8.0, and 6.3 mm, from top to bottom, with a pan below the 6.3 mm sieve. The sieves were attached to a shaker (model RX-86, W.S. Tyler, Mentor, OH) and shaken for 5 min. The amount of fines was calculated as the percentage of cheese (by weight) that made it through all the sieves and into the bottom pan.

Stickiness

To determine stickiness, cheese was shredded, but no anticaking agent was added prior to being shaken through the sieves as in the fines test. Stickiness was calculated as the difference in the percentage of cheese that made it through into the pan when anticaking agent was and was not added. If the fines were at 50% of the total cheese shredded when anticaking agent was added and the fines were at 20% when no anticaking agent was added, then the stickiness would be reported as 30%.

Gumminess

Gumminess was calculated as the percentage of cheese (measured by weight) remaining in the shredder following shredding.

Statistical Analysis

A repeated measures design was used to examine the effect of EPS^{+} MR1C over time on TPA, fines, stickiness, and gumminess. For melt, a split-split plot design was used. The whole plot factor was culture (MR1C, DM10), sub-plot factor was shred time (d 7, 21, 42), and sub-subplot factor was time of melt test (d 42, 84). All data were analyzed for statistical signifance using the proc mixed function in Statistical Analysis Software (SAS) version 9.0 (SAS Institute, Inc., Cary, NC). Analysis of variance was used to identify statistically significant differences at the 95% level. Post-hoc means comparisons were made based on p-values (alpha = 0.05) using the Tukey-Kramer adjustment to obtain differences of least means squares. Correlation coefficients were calculated using the proc corr function (see Appendices).

RESULTS AND DISCUSSION

Trial 1

Composition

The MR1C cheese had a slightly higher salt content (Table 2), although this is mainly because of trial 4, which also had a high moisture content as compared to the other cheeses and the differences were not significantly different. The SM percentage was higher for the MR1C cheese for every make. The MR1C cheese probably absorbed more salt initially due to the smaller curd size having more total surface area to contact and absorb salt.

Although the MR1C cheese was salted at a lower pH, the final pH was a little higher. This may be due to the higher SM levels reducing the ability of the starter bacteria to reduce the pH. It may also be a function of wash water temperature and volume.

Table 2. Moisture, fat, fat on the dry basis (FDB), salt, salt-in-the moisture (SM), and pH for trial #1.

Cheese	Rep	Moisture	Fat	FDB	Salt	SM	pH
		----------	----------	---%---	----------	----------	
MR1C	1	41.5	30.8	52.5	1.96	4.72	5.08
	2	41.4	30.0	51.2	2.12	5.12	5.15
	3	41.7	29.8	51.0	2.07	4.96	5.07
	4	43.3	28.5	50.2	2.22	5.13	5.12
	Average	**42.0**	**29.8**	**51.2**	**2.09**	**4.98**	**5.11**
DM10	1	40.5	30.8	51.6	1.89	4.67	5.02
	2	41.6	30.0	51.4	1.92	4.62	5.05
	3	44.4	29.5	53.1	2.02	4.55	5.05
	4	41.7	30.0	51.5	1.95	4.68	5.10
	Average	**42.0**	**30.1**	**51.9**	**1.95**	**4.63**	**5.06**

Component differences were not significant.

Melt

The day the cheese was shredded did not have an effect on melt, nor did the adjunct culture have an effect on melt (Table 3). When the data from both cheeses were pooled together, there was significantly more melt (83.1%) at d 84 than at d 42 (75.6%).

All other factors being the same, there is a nearly linear inverse relationship between the rate of degradation of casein and the SM level with casein degradation decreasing as SM level increases (Lawrence et al., 1983). The hydrolysis of caseins during storage can increase the meltability of cheese by weakening the number and strength of the protein-protein interactions between casein molecules (Lucey et al., 2003). Thus cheese was expected to melt more quickly at d 84 than d 42. Extent of proteolysis during storage of the cheeses was not measured so whether there was a difference between the cheeses is unknown.

Table 3. Mean values for melt expressed as the percentage change in height of cheese after 10 s at 65°C for MR1C and DM10 cheeses tested at d 42 and d 84.

	Melt	
Cheese	d 42	d 84
	----------%----------	
MR1C	76.8[a]	84.5[a]
DM10	70.6[a]	81.7[a]

[a,b]Means with the same letter superscript within the same column were not significantly different.

Texture Profile Analysis

There were not any significant TPA differences between MR1C and DM10 for hardness, adhesiveness, fracture stress, or cohesiveness (Table 4). There was a tendency difference in fracture stress ($P < 0.10$). When pooled over all time periods, the MR1C cheese tended to have a higher fracture stress, meaning that it required more force on the first compression to fracture, although these differences were not significantly different at any individual day tested (Figure 3).

In general, as the cheese aged, adhesiveness increased (Figure 4), cohesiveness decreased (Figure 5), and hardness (Figure 6) and fracture stress remained relatively unchanged. There was a slight correlation between hardness and fracture stress and an inverse correlation between adhesiveness and cohesiveness (Table 5).

Table 4. Means pooled from all time periods for hardness, adhesiveness, cohesiveness, and fracture stress of high moisture Cheddar-type cheese manufactured with MR1C or DM10 adjunct cultures.

Cheese	Hardness (N)	Adhesiveness	Cohesiveness	Fracture Stress (N)
MR1C	35.3[a]	2.96[a]	0.31[a]	30.6[a]
DM10	33.7[a]	2.76[a]	0.30[a]	28.3[a]

[a,b]Means with the same letter superscript within the same column were not significantly different.

Table 5. Correlation between TPA hardness, adhesiveness, cohesiveness, fracture stress and moisture. Table shows R values.

	Adhesiveness	Cohesiveness	Fracture Stress	Moisture
Hardness	-0.06	0.10	0.60	-0.19
Adhesiveness		-0.72	0.13	0.44
Cohesiveness			-0.18	-0.14
Fracture Stress				-0.35

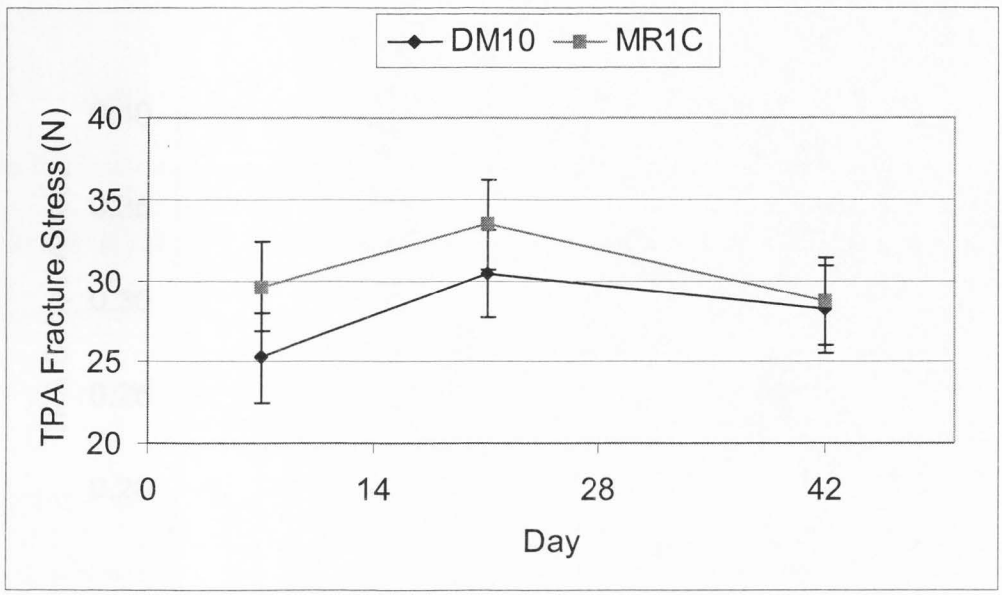

Figure 3. Fracture stress (N) of cheese produced with MR1C or DM10 adjunct cultures over 42 d storage. Error bars show standard error of the mean

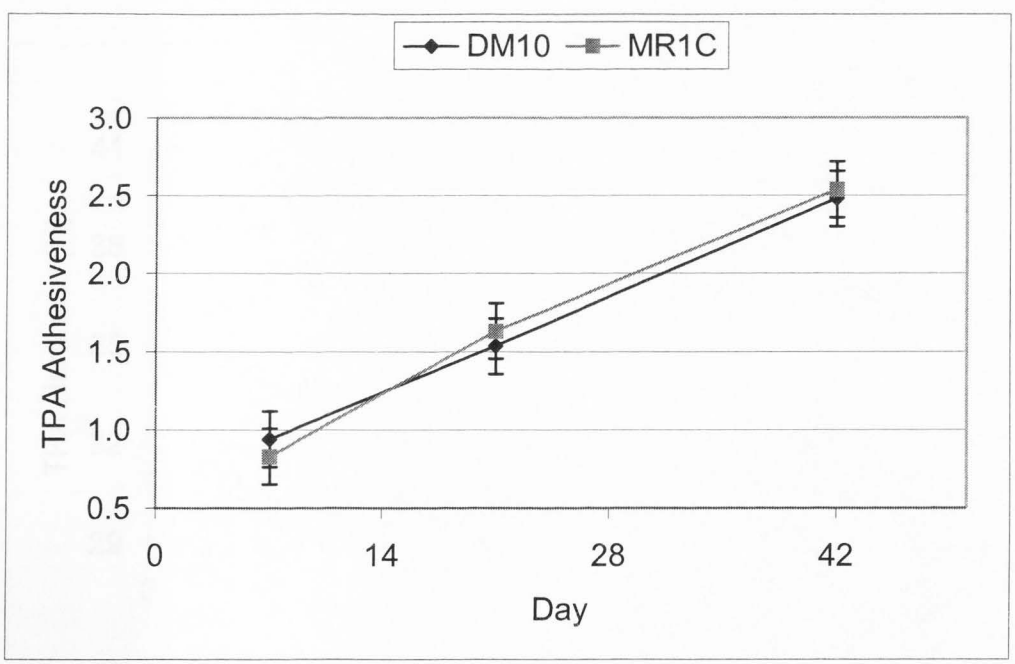

Figure 4. Adhesiveness of cheese produced with MR1C or DM10 adjunct cultures over 42 d storage. Error bars show standard error of the mean.

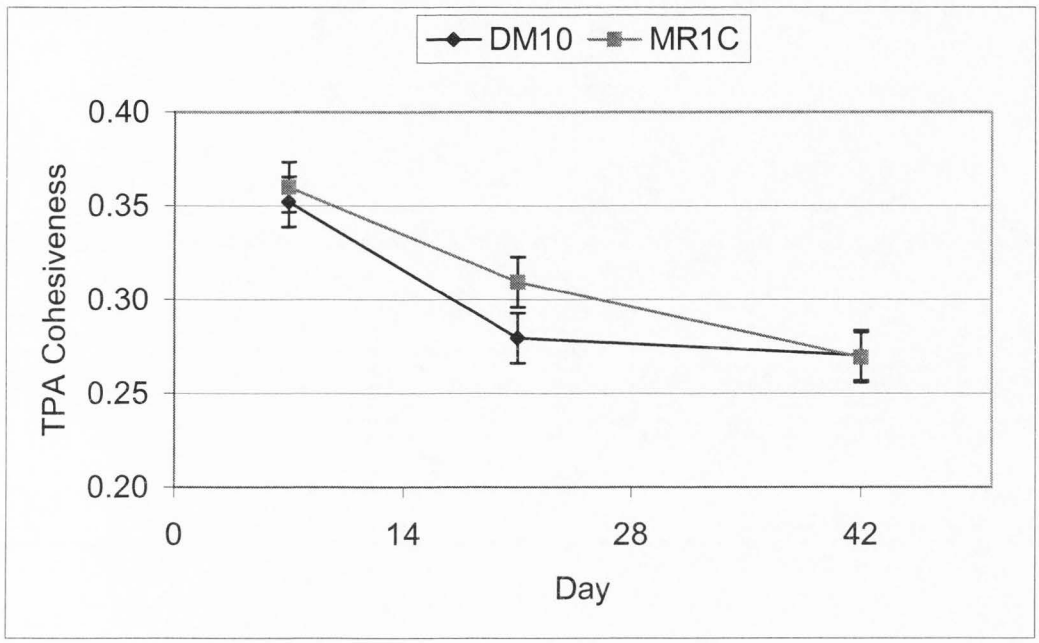

Figure 5. Cohesiveness of cheese produced with MR1C or DM10 adjunct cultures over 42 d storage. Error bars show standard error of the mean.

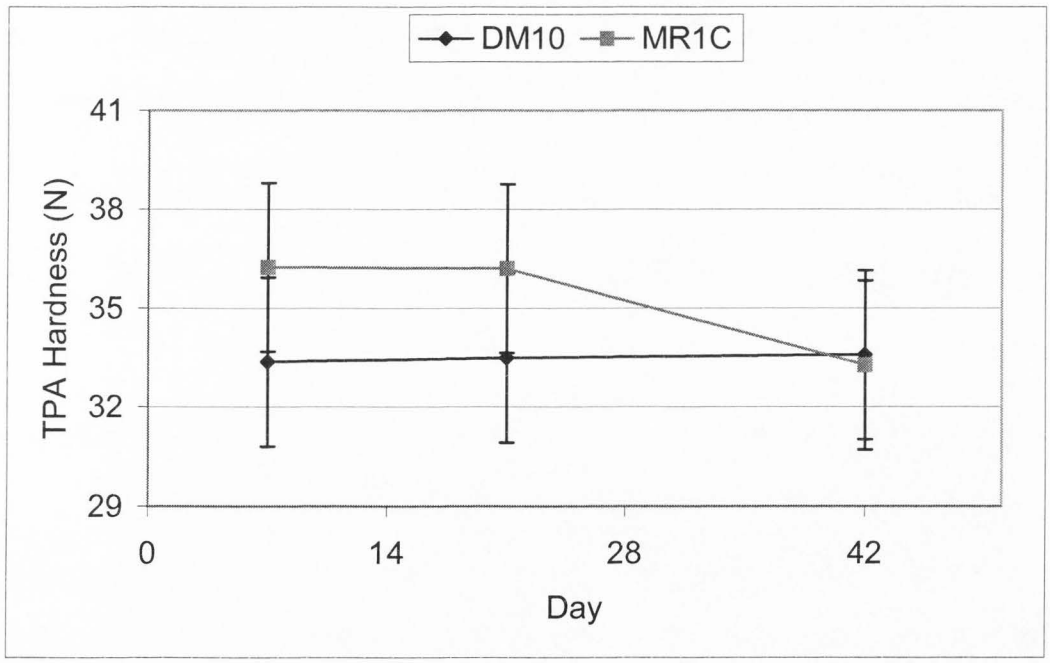

Figure 6. Hardness of cheese produced with MR1C or DM10 adjunct cultures over 42 d storage. Error bars show standard error of the mean.

Fines

There was no significant difference between the MR1C cheese and the DM10 cheese in the amount of fines produced when shredded (37.8% and 42.1%, respectively). At d 7, the MR1C cheese had 22% fewer fines than the DM10 cheese; however at d 21 and 42 the fines were virtually even for both cheeses (Figure 7). Both cheeses had a large reduction in fines between d 7 and 21.

Clumping

When proper anti-caking agent was added to the fines there was no clumping irregardless of the amount of time the fines were in storage.

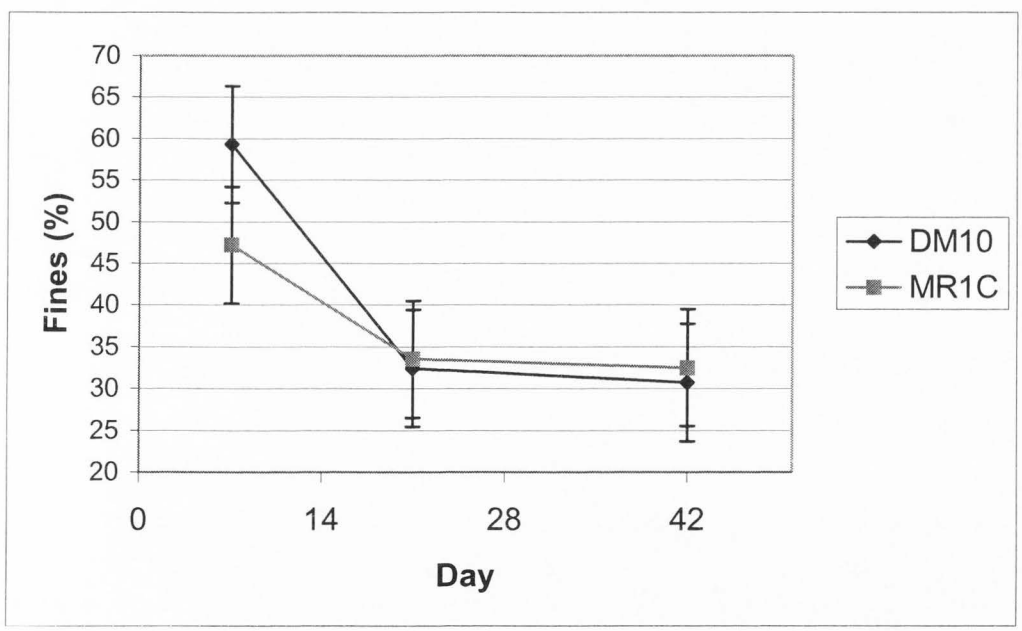

Figure 7. Fines as a percentage of cheese shredded for cheese produced with MR1C or DM10 adjunct cultures cultures over 42 d storage. Error bars show standard error of the mean.

Trial 2

Composition

Below are the average compositions for trial 2 (Table 6). As with trial 1, the MR1C cheese had a slightly higher SM and pH. It was more difficult to make cheese with consistent moistures with trial 2 than it was with trial 1. Rep 2 for both MR1C and DM10 were the highest in moisture of all the makes and rep 4 was the lowest in moisture for both cheeses. As reps for DM10 and MR1C were made on the same day, it is unlikely that both cheeses having the highest and lowest moisture is a coincidence. It is more likely that the differences in moisture are a result of differences in protein and fat content of the milk. No tests were performed on the milk used to make the cheese, however.

Table 6. Moisture, fat, fat on the dry basis, salt, salt-in-the moisture, and pH values for trial #2.

Cheese	Rep	Moisture	Fat	FDB	Salt	SM	pH
		----------	----------	%----------	----------	----------	
MR1C	1	42.8	30.3	52.9	2.06	4.81	5.08
	2	44.9	28.5	51.7	2.27	5.06	5.15
	3	41.9	30.5	52.5	2.03	4.84	5.07
	4	38.9	32.0	52.4	1.82	4.68	5.12
	5	40.5	30.8	51.7	2.00	4.94	5.07
	Average	**41.8**	**30.4**	**52.2**	**2.04**	**4.87**	**5.10**
DM10	1	44.4	28.3	50.8	2.03	4.57	4.86
	2	46.7	27.5	51.6	2.27	4.86	5.04
	3	40.7	31.3	52.7	1.85	4.55	5.06
	4	38.5	32.0	52.0	1.76	4.57	5.12
	5	41.1	30.5	51.8	1.93	4.70	5.05
	Average	**42.3**	**29.9**	**51.9**	**1.97**	**4.65**	**5.03**

Texture Profile Analysis

The MR1C cheese was significantly more adhesive than the DM10 cheese (Table 7). As in trial 1, the MR1C cheese tended to have a higher fracture stress than DM10. Also, in agreement with trial 1, as the cheese aged, adhesiveness increased (Figure 8), cohesiveness decreased (Figure 9), and hardness (Figure 10) and fracture stress (Figure 11) remained relatively unchanged. There was a correlation between fracture stress and hardness and a negative correlation between hardness and moisture as well as fracture stress and moisture (Table 8).

Table 7. Means for hardness, adhesiveness, cohesiveness, and fracture stress of high moisture Cheddar-type cheese manufactured with MR1C or DM10 adjunct cultures.

Adjunct Culture	Hardness	Adhesiveness	Cohesiveness	Fracture Stress
MR1C	40.16[a]	1.69[a]	.33[a]	36.0[a]
DM10	38.01[a]	1.48[b]	.33[a]	32.2[a]

[a,b]Means with the same letter superscript within the same column were not significantly different.

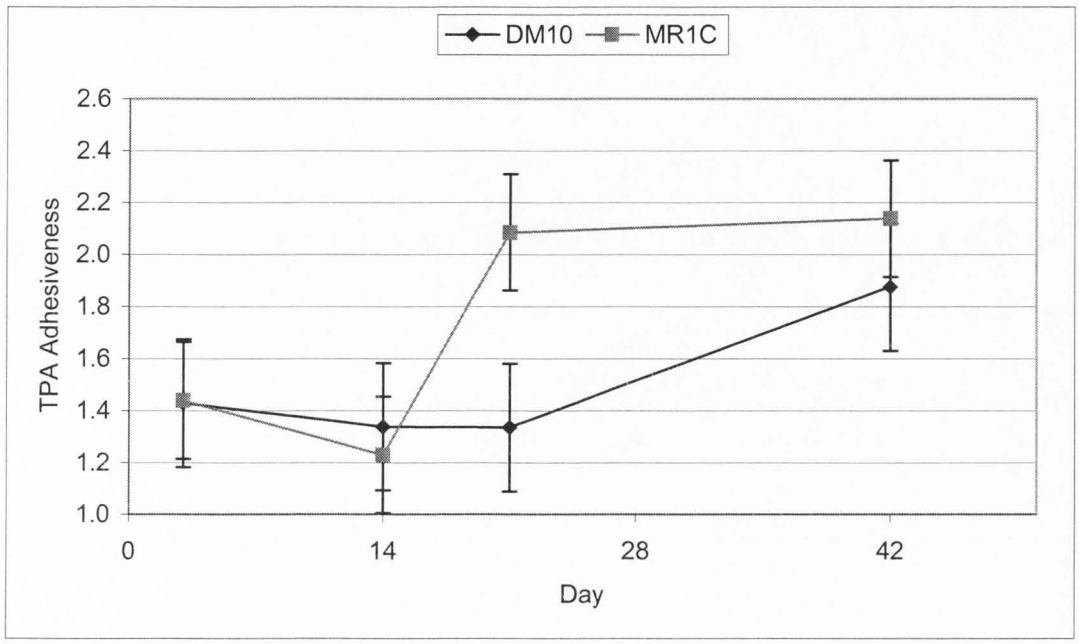

Figure 8. Adhesiveness of cheese produced with MR1C or DM10 adjunct cultures over 42 d storage. Error bars show standard error of the mean.

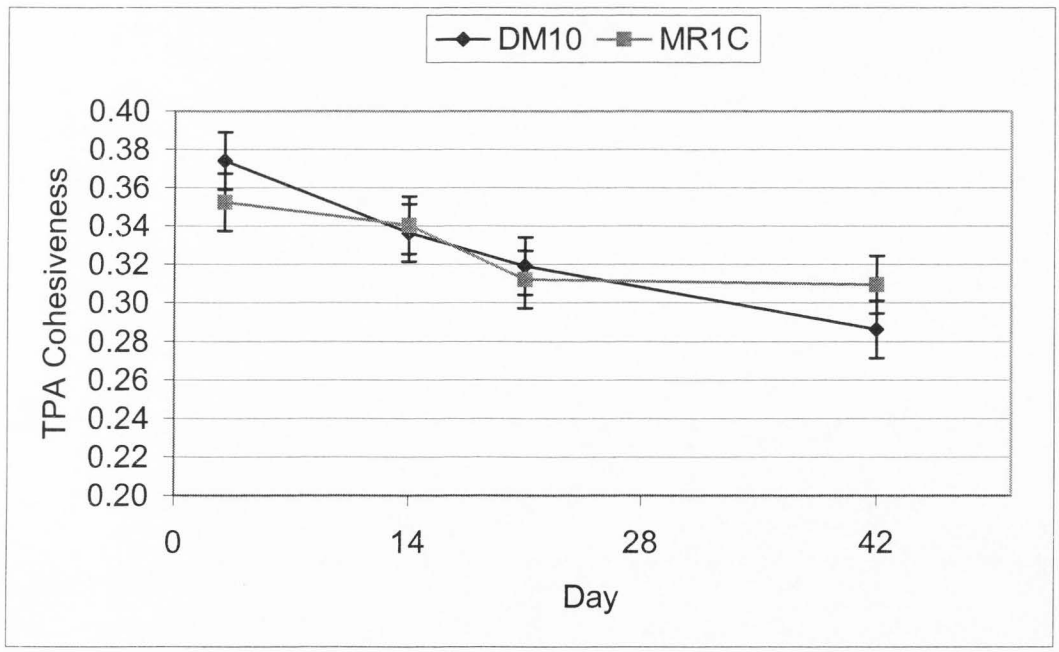

Figure 9. Cohesiveness of cheese produced with MR1C or DM10 adjunct cultures over 42 d storage. Error bars show standard error of the mean.

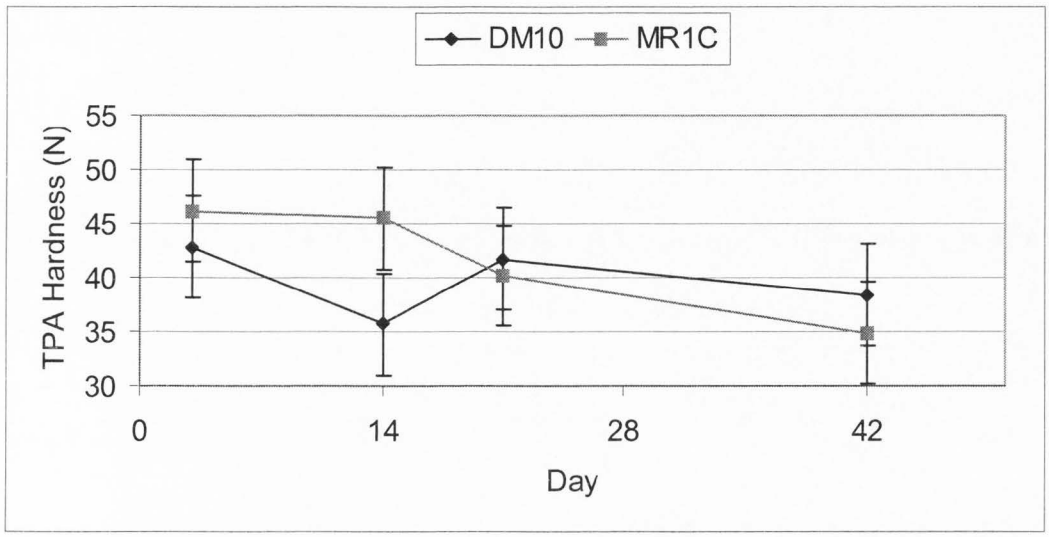

Figure 10. Hardness of cheese (N) produced with MR1C or DM10 adjunct cultures over 42 d storage. Error bars show standard error of the mean.

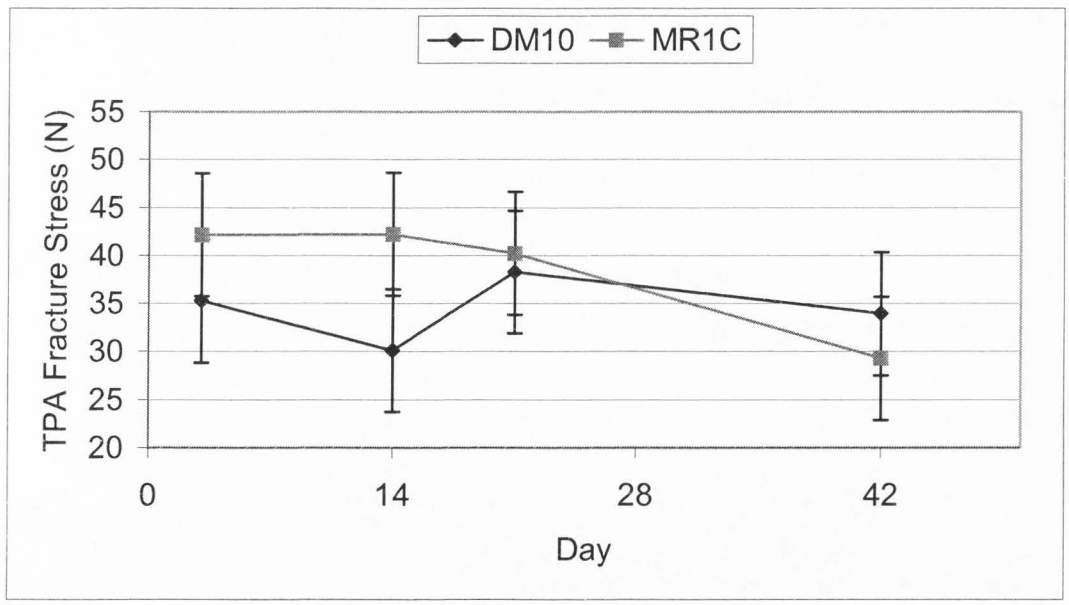

Figure 11. Fracture stress of cheese produced with MR1C or DM10 adjunct cultures over 42 d storage. Error bars show standard error of the mean.

Table 8. Correlation between TPA hardness, adhesiveness, cohesiveness, fracture stress and moisture.

	Adhesiveness	Cohesiveness	Fracture Stress	Moisture
Hardness	0.055	0.317	0.93	-0.8058
Adhesiveness		-0.3247	0.1436	-0.142
Cohesiveness			0.213	-0.68
Fracture Stress				-0.826

Fines, Stickiness, Gumminess

As with experiment 1, there was no significant difference in the amount of fines produced due to culture used. There was also no significant difference between cultures in relation to stickiness and gumminess (Table 9). There was a significant reduction in both fines (Figure 12) and stickiness (Figure 13) between d 7 and d 21. Figure 14 compares fines with and without anticaking agent and stickiness. Gumminess increased between d 21 and d 42 (Figure 15).

Table 9. Means for fines, stickiness, and gumminess of cheese manufactured with MR1C or DM10 adjunct cultures.

Cheese	Fines	Stickiness	Gumminess
	----------%----------		
MR1C	41.11[a]	28.29[a]	3.80[a]
DM10	46.33[a]	31.29[a]	3.65[a]

[a,b]Means with the same letter superscript within the same column were not significantly different.

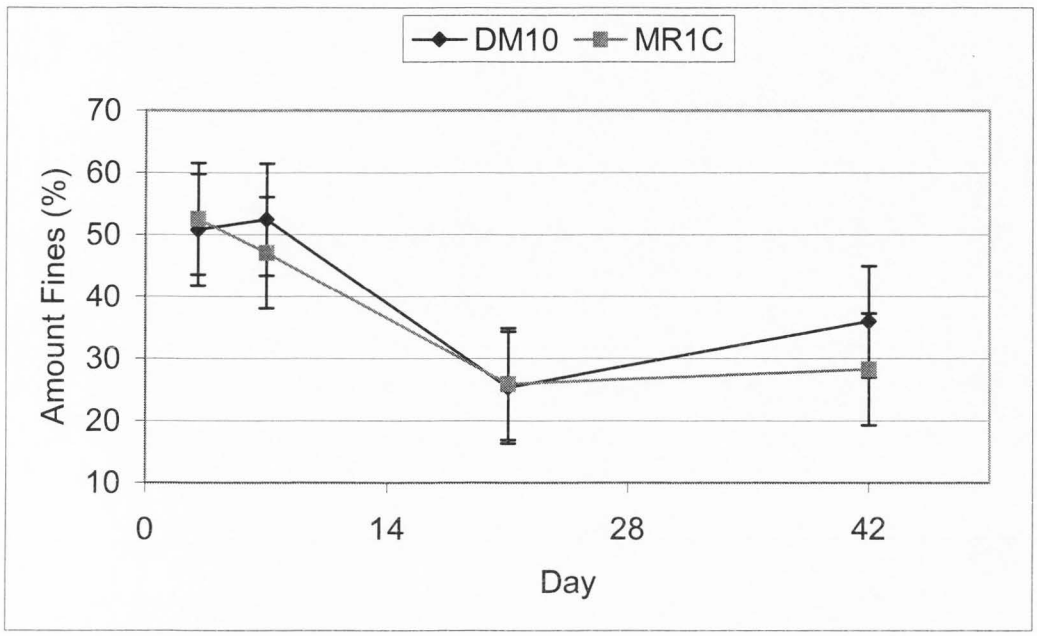

Figure 12. Fines as a percentage of total cheese shredded for cheese produced with MR1C or DM10 adjunct cultures when anticaking agent was added to the shreds. Error bars show standard error of the mean.

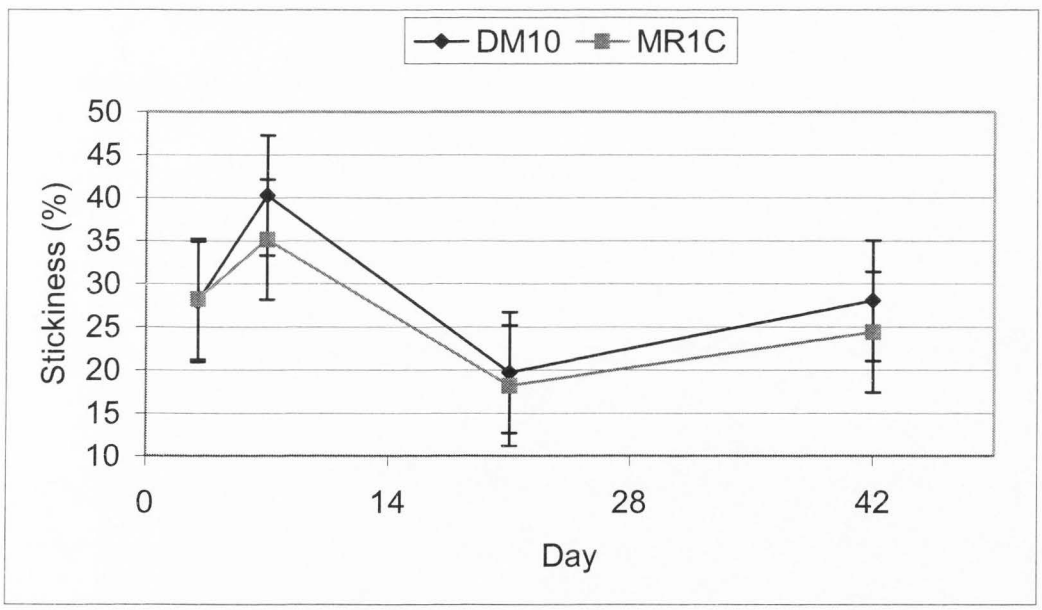

Figure 13. Stickiness calculated as total percent fines when anticaking agent was added to shreds for MR1C or DM10 cheeses less total percent fines when no anticaking agent was added to the shreds. Error bars show standard error of the mean.

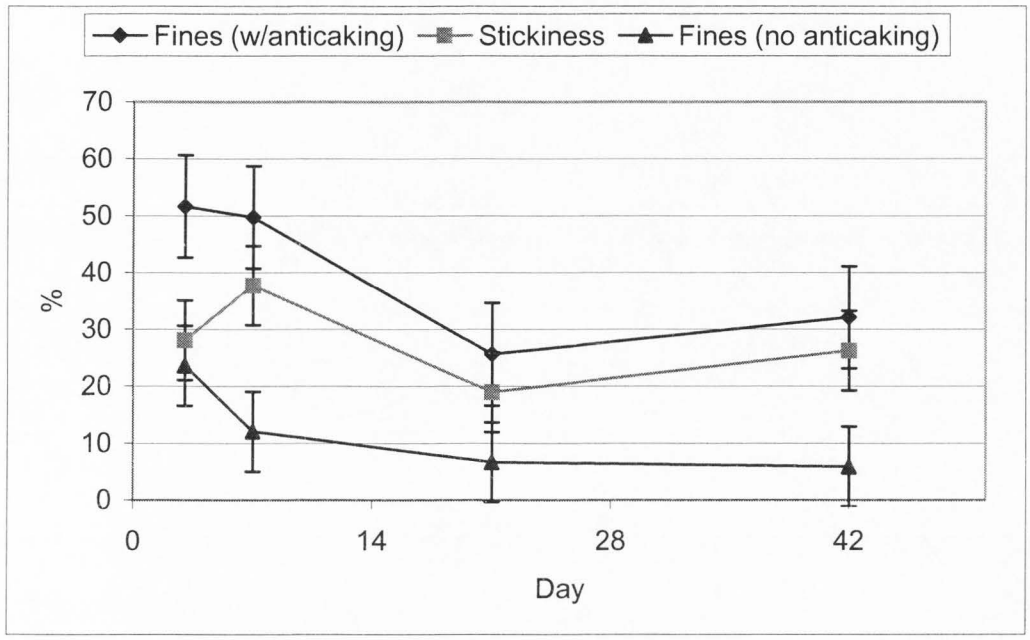

Figure 14. Chart comparing fines with anticaking agent added, fines with no anticaking agent added, and stickiness, which is, calculated as the difference between the 2 fines tests over 42 d storage.

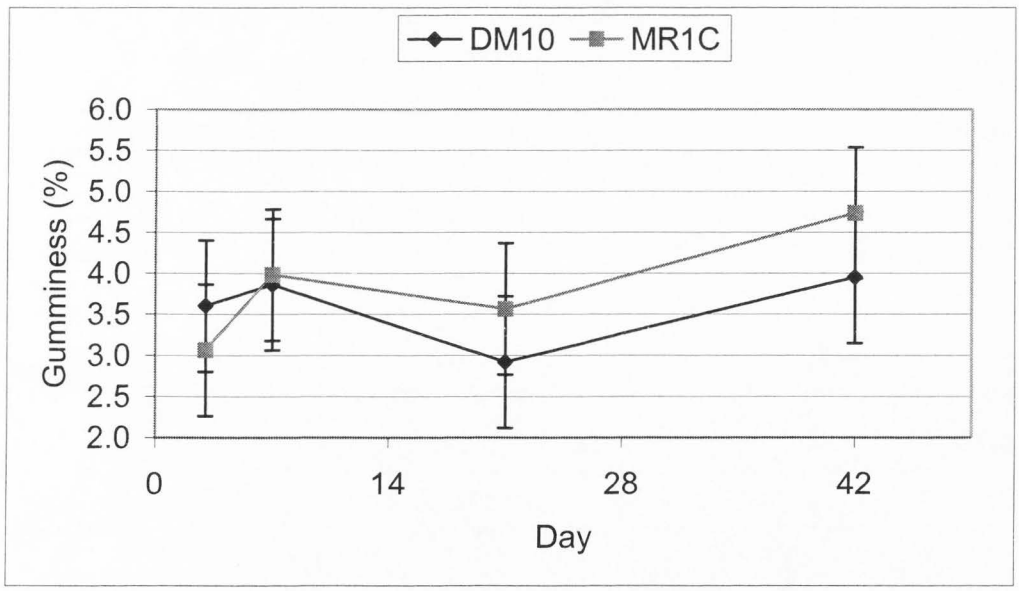

Figure 15. Gumminess as percentage of cheese remaining in shredder following shredding over 42 d storage. Error bars show standard error of the mean.

As with trial 1, the fines were at 50% of the total cheese shreds until the d 21 tests. Lawrence and Giles (1993) reported that there are two distinct phases in texture development that take place during ripening. The first phase occurs in the first 7 to 14 d when the rubbery texture of young cheese curd is rapidly converted into a smoother, more homogeneous product. A smoother, more homogeneous cheese would be less likely to break apart when shredded. The younger cheese (d 3 and d 7) shreds were much rougher than the shreds after the cheese had aged to d 21. The rough shreds were very fragile.

Most of the fines did not make it to the bottom pan when the anticaking agent was not added (Figure 14). When anticaking agent was added, shreds longer than the sieve opening worked their way through the sieves during shaking. The younger cheese (d 3 and d 7) broke up further as it worked its way down the stack of sieves. When there was not any anticaking agent added, the cheese stuck to the sieves and did not break apart as much during shaking. A company that sells shredded cheese will need to add an anticaking agent or the shreds will cake together during storage. If the cheese is shredded too young, the shaking during transport and handling will cause the shreds to break into many smaller pieces (fines).

Trials 1 and 2 TPA

With both trials 1and 2 the MR1C cheese tended to have a higher fracture stress than the DM10 cheese. In trial 2 the MR1C cheese was significantly more adhesive than the DM10 cheese. The TPA data was combined to encompass both trials and statistical analysis was performed (Table 10).

Table 10. Means for hardness, adhesiveness, cohesiveness, and fracture stress of high moisture Cheddar-type cheese manufactured with MR1C or DM10 adjunct cultures. Means reflect combined results from trials 1 and 2.

Adjunct Culture	Hardness	Adhesiveness	Cohesiveness	Fracture Stress
MR1C	39.3[a]	1.67[a]	.324[a]	35.06[a]
DM10	36.9[b]	1.56[a]	.320[a]	31.30[b]

[a,b]Means with the same letter superscript within the same column were not significantly different.

Table 11. Moisture, fat, fat on the dry basis, salt, salt-in-the moisture, and pH values. Trials 1 and 2 combined.

Cheese	Moisture	Fat	FDB	Salt	SM	pH
	----------%----------					
MR1C	41.9	30.1	51.7	2.06	4.93	5.11
DM10	42.2	30	51.9	1.96	4.64	5.05

With increased number of replicates, the MR1C cheese was shown to be significantly harder and had a significantly higher fracture stress. There were no differences between cultures for adhesiveness or cohesiveness. Table 11 shows the average composition of the cheeses.

Hardness

Although the MR1C cheese was significantly harder than the DM10 cheese, there are too many possible variables to necessarily attribute this difference only to the culture. In order to reduce the moisture in the MR1C cheese as compared to the DM10 cheese, less wash water was used. Less wash water could have the effect of not reducing the calcium and phosphate as much as in the DM10 cheese, which then does not allow the protein

matrix to hold as much water. Cheese with high calcium and phosphate content will have a more completely cross-linked structure and be more rigid (Masi and Addeo, 1986).

The MR1C cheese also had a higher SM and higher pH. The higher the SM, the less the casein is degraded and the harder the cheese (Lawrence et al, 1983). The slightly lower pH of the DM10 cheese probably also indicates a slightly lower calcium phosphate content that would cause the cheese to be softer.

However, these differences in pH and salt are rather minor and would not be expected to cause any large differences. It would appear, therefore, that the use of adjunct culture did produce a harder cheese that was more resistant to fracturing. The MR1C cheese may have been harder because 2 to 3% of the water was bound by the exopolysaccharide and not available in the protein matrix.

Adhesiveness

The MR1C cheese was significantly more adhesive for trial 2, but not when statistics were run on the 2 trials combined. The most adhesive cheeses are those that contain an open and loose protein matrix (Bryant et al., 1995). The more compact the cheese the less adhesive. As cheese ages protein-protein interactions break apart leaving more regions to adhere to something else. This can be seen with both trials as the adhesiveness increased with age of the cheese.

Cohesiveness

There was no difference between cultures for cohesiveness. For both trials there was a decrease in cohesiveness with time. This is expected as the protein-protein interactions are broken down as cheese ages. Lane et al. (1997) reported that among the textural

properties of Cheddar cheese, TPA cohesiveness was most related to primary proteolysis with a trend of decreasing with increasing proteolysis.

Fracture Stress

The MR1C cheese had a significantly higher fracture stress than did the DM10 cheese, meaning that it took more force to cause the MR1C cheese to fracture than the DM10 cheese. Large deformation and fracture properties strongly depend on the size of the largest inhomogeneities or "weak spots" in the cheese matrix (Lucey et al., 2003). The development of cracks and their growth (fracture initiation and propagation) partly depends on the energy required to overcome the adhesive or cohesive stresses in the cheese. Most of the data for TPA fracture stress was from d 3, 7 and 14, with less than 50% measurable fracture stress points at d 21 and less than 10% at d 42. That is, the cheese fractured less as it aged past 14 days. Possibly the polysaccharide capsule produced around MR1C provided reinforcement to the protein matrix making propagation of a major fracture more difficult.

Cracks in Junction Zones

For trial 2 a suitable means of determining the stickiness and gumminess was investigated. An attempt was made to thinly slice the cheese using a small commercial cheese slicer and rate how much cheese stuck to the slicer. This proved to not be an effective way to determine the gumminess or stickiness of cheese. However, an examination of the thinly sliced cheese revealed that the MR1C cheeses had not completely matted together. The extremely thin, nearly unnoticeable cracks in the MR1C cheese were about the length of a curd granule and may have been junction zones between curd pieces.

It appeared that the curd granules did not completely fuse together. A curd granule could be described as a flattened sphere before it is pressed. When curd is initially filled into a hoop, most curd particles are pressed against the adjoining curd particles, with the corners of the particles containing little pockets of air. As the curd is pressed these pockets of air will fill with whey (or remain as a mechanical opening). Through continued pressing and then in the early stages of ripening, the corners of the particles fuse more completely, though the junction zones between curd pieces are still partly visible in aged cheese (Lucey et al., 2003). It appears that the exopolysaccharide prevented the curd particles from completely fusing together. Additional research would be required to determine why this occurred.

The cheeses were sliced again at d 21 and 42 and these same cracks were still present in the MR1C cheese. Even MR1C cheese from trial 1 that had aged over a year had the same size cracks as the younger cheese. In contrast, the DM10 cheese had only a few visible cracks at d 7, but almost no visible cracks by d 21. The aged 1-year-old DM10 cheese did not have any visible cracks.

When plugs of cheese used for TPA analysis were examined, the cracks were not visible. Also, the blocks of cheese that were shred to determine fines did not show signs of these cracks. It was surprising that these cracks did not cause the MR1C cheese to have a lower fracture stress point or to have more fines than the DM10 cheese.

Trial 3

After observing the presence of the cracks during Trial 2, it was decided to make cheeses without an adjunct culture as a comparison. With trial 3 I wanted to determine

what affect adding a streptococcal adjunct culture had on the cheese. The make for trial 3 was the same as for the DM10 cheese in the first two trials.

Composition

The moisture, fat, and FDB were all similar to the MR1C and DM10 cheeses in trials 1 and 2 (Table 12). The pH was a little higher, however. It appears that the adjunct streptococcal cultures caused the pH to drop an additional 0.1 pH after salting.

Texture Profile Analysis

The TPA results showed a dramatic difference between the lower moisture reps 1 and 3 and the higher moisture rep 2 (Table 13). The lower moisture cheeses were significantly harder and more brittle. The higher moisture cheese tended to be more adhesive than the other two cheeses.

There was a strong negative correlation between moisture and hardness, cohesiveness, and fracture stress (Table 14). There was also a strong positive correlation between hardness and fracture stress.

Table 12. Moisture, fat, fat on the dry basis, and pH values for trial #3.

Rep	Moisture	Fat	FDB	pH
	----------%----------			
1	40.1	30.5	50.9	5.22
2	43.3	29	51.1	5.26
3	40.6	31	52.2	5.16
Average	41.3	30.2	51.4	5.21

Table 13. Means for hardness, adhesiveness, cohesiveness, and fracture stress of high moisture Cheddar-type cheese manufactured with MR1C or DM10 adjunct cultures.

Rep	Hardness	Adhesiveness	Cohesiveness	Fracture Stress
1	56.5[a]	1.64[a]	0.422[a]	52.7[a]
2	35.2[c]	1.94[a]	0.360[a]	24.5[b]
3	48.4[b]	1.58[a]	0.394[a]	46.5[a]

[a,,b,c]Means with the same letter superscript within the same column were not significantly different.

Table 14. TPA Correlations for hardness, adhesiveness, cohesiveness, fracture stress, and moisture for trial 3. Table shows R values.

	Adhesiveness	Cohesiveness	Fracture Stress	Moisture
Hardness	-0.174	0.453	0.966	-0.966
Adhesiveness		-0.587	-0.167	-0.371
Cohesiveness			0.345	-0.930
Fracture Stress				-0.996

Both the MR1C and DM10 cheeses from trials 1 and 2 along with the cheese in trial 3 were treated as 21 individual reps and statistical analysis was completed. Reps 1 and 3 from trial 3 were significantly more cohesive than all of the other 19 reps. The increased cohesiveness is probably due to the higher pH of the cheese in trial 3, which is around the optimal pH for cohesion, pH 5.2 (Lawrence et al., 1987).

Figure 16 shows the cohesiveness and adhesiveness over time of the cheese produced for trial 3 and Figure 17 shows the hardness and fracture stress over time. Figure 17 does not show fracture stress at d 42 because none of the trials had a fracture point at d 42. It is interesting that at d 7 and 14, the fracture point occurred at a lower stress than the hardness (maximum stress), while at d 21 the fracture stress was the maximum.

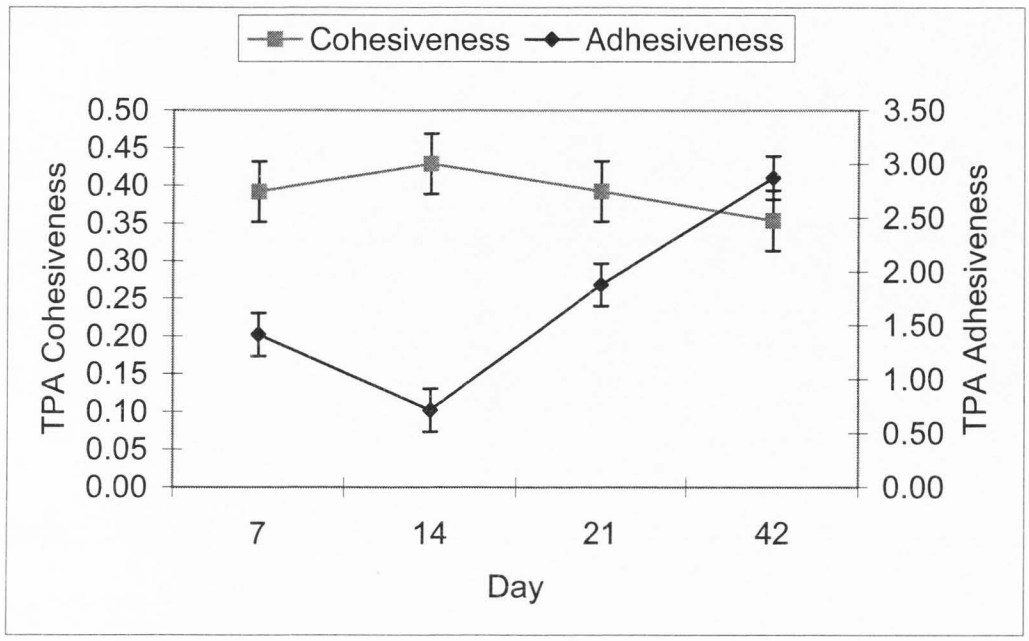

Figure 16. Adhesiveness and cohesiveness of cheese produced with MR1C or DM10 adjunct cultures vs. time for trial 3.

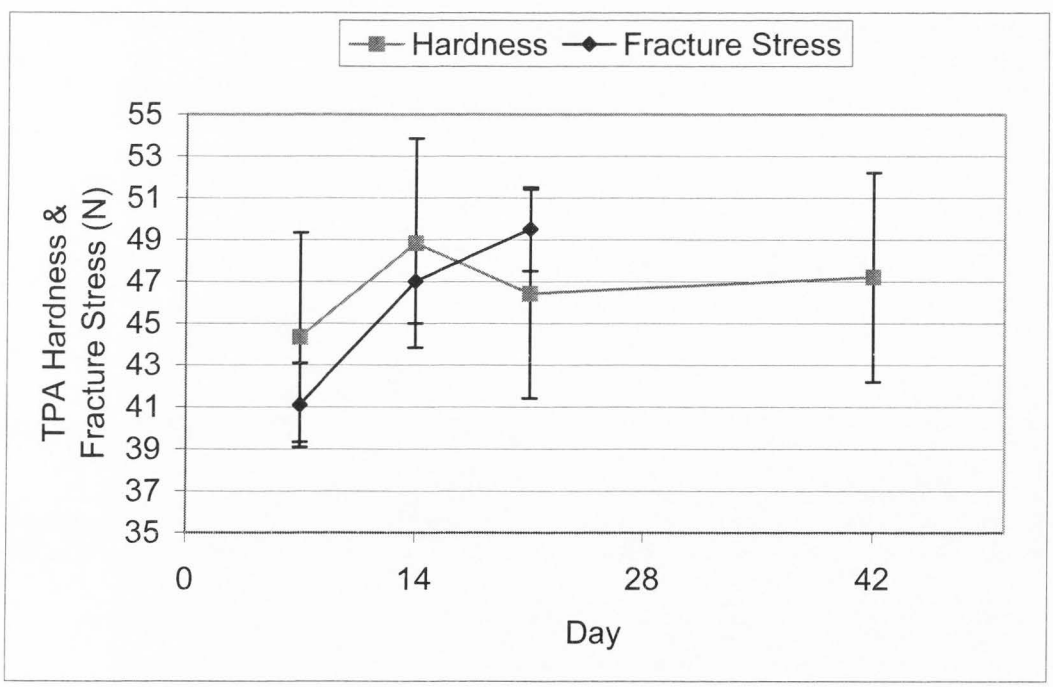

Figure 17. Fracture stress and hardness of cheese produced with MR1C or DM10 adjunct cultures vs. time for trial 3.

Fines, Gumminess

The lower moisture cheeses, reps 1 and 3 had significantly fewer fines than did rep 2 (Table 15). There were no significant differences with gumminess, however.

As with trial 2, there was a dramatic reduction in fines between d 14 and 21. Unlike trial 2, which had a reduction from 50% fines at d 7 to 25% fines at d 21, the reduction was from 25% fines at d 7 to 15% at d 21. Upon visual inspection the shreds did not appear nearly as rough or fragile at d 7 as did the shreds in trials 1 and 2. As with cohesiveness, the higher pH might be responsible for the reduced amount of fines for the cheese in trial 3. Although the MR1C cheese had a higher pH than the DM10 cheese in trials 1 and 2, it is possible that the MR1C cheese did not have fewer fines than the DM10 cheese because the cracks discussed above caused additional fines.

Differences in physical parameters of cheese can have a large impact on the functionality of that cheese. *Streptococcus thermophilus* is used in the industry to help drive quicker acid production. In this research, adding *S. thermophilus* had a negative impact on the shreddability of the cheese. This negative impact may have been because of lower pH (pH 5.05-5.10 compared to pH 5.2) or some other intrinsic factor related to *S. thermophilus*, but it was not because of moisture level. The impact was greatest in younger cheese. In the industry the quicker the cheese can be shredded, the smaller the holding

Table 15. Means for fines and gumminess of cheese manufactured for trial 3.

Rep	Fines	Gumminess
1	15.7[a]	3.22[a]
2	24.7[b]	3.97[a]
3	19.7[a]	3.18[a]

[a,b]Means with the same letter superscript within the same column were not significantly different.

costs of that cheese. Future research is required to discern why the cheese in trial 3 was able to shred at a younger age and produce only half the fines of the streptococcal adjunct cheese. Also, future research is required to know if a difference of 0.10 in pH can influence shredding attributes as shown by the cheeses that were at pH 5.2 rather than pH 5.1 or lower.

Gumminess was mainly affected by the moisture level. A scatterplot comparing moistures and gumminess (Figure 18) that includes the 5 MR1C and 5 DM10 reps from trial 2 and the 3 reps from trial 3 shows a strong linear relationship between moisture and gumminess. As moisture increased from 38.5% to 46.7%, gumminess increased from 2.63 percent to 5.84%, or about .39% for every percent increase in moisture.

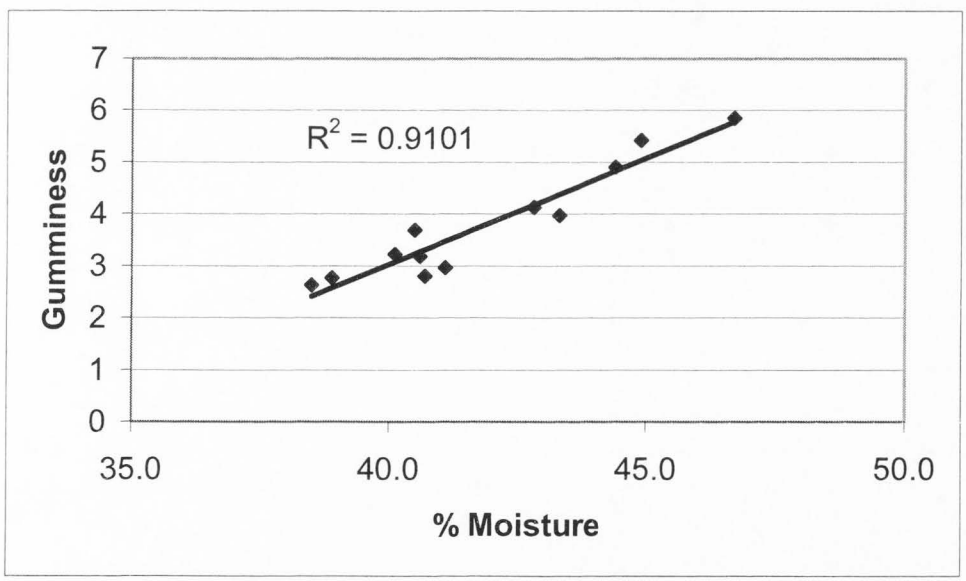

Figure 18. Gumminess scatterplot comparing moistures and gumminess for cheese made during trials 2 and 3.

CONCLUSIONS

The MR1C cheese was harder and had a higher fracture stress than the DM10 cheese. The MR1C cheese had a slightly higher SM and pH, which may be partly responsible for the differences between the two cheeses as well as the presence of the exopolysaccharide. There were no differences between the MR1C cheese and the DM10 cheese in shreddability as determined by fines, stickiness, and gumminess. When proper anti-caking agent was used, there was no clumping of MR1C or DM10 cheeses. Cheese produced without a streptoccus adjunct culture was more cohesive and had fewer fines than the MR1C or DM10 cheese, although this may be a function of a slightly higher pH.

It was observed that cheese produced with the MR1C adjunct culture had very small cracks throughout, probably a result of poor curd fusion. Future research is needed to determine the cause of these cracks.

Future research is also necessary to determine if the streptococcal adjunct was responsible for increased fines or if it was due to the lower pH or another physical parameter of the cheese made with the streptococcal adjunct. Future research could also be useful to determine how different physical parameters affect the amount of fines produced when cheese is shredded.

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APPENDICES

A. Analysis for Differences by Culture and Time – Trial 1 only

ANOVA for Hardness

Effect	DF	DF	F Value	Pr > F
cult	1	88	1.39	0.2414
Time(cult)	4	88	0.44	0.7758

Least Squares Means

Effect	Culture	(Weeks)	Estimate	Error
cult	dm10		33.4686	1.0695
cult	mr1c		35.2321	1.0450
Time(cult)	dm10	1	33.3607	1.9349
Time(cult)	dm10	3	33.4700	1.8099
Time(cult)	dm10	6	33.5750	1.8099
Time(cult)	mr1c	1	36.2356	1.8099
Time(cult)	mr1c	3	36.1963	1.8099
Time(cult)	mr1c	6	33.2644	1.8099

ANOVA for Adhesiveness

Effect	DF	DF	F Value	Pr > F
cult	1	87	0.02	0.8825
Time(cult)	4	87	40.90	<.0001

Least Squares Means

Effect	Culture	(Weeks)	Estimate	Error
cult	dm10		1.6507	0.07453
cult	mr1c		1.6663	0.07362
Time(cult)	dm10	1	0.9379	0.1348
Time(cult)	dm10	3	1.5344	0.1261
Time(cult)	dm10	6	2.4800	0.1261
Time(cult)	mr1c	1	0.8300	0.1261
Time(cult)	mr1c	3	1.6313	0.1303
Time(cult)	mr1c	6	2.5375	0.1261

ANOVA for Cohesiveness

Effect	DF	DF	F Value	Pr > F
cult	1	88	2.44	0.1217
Time(cult)	4	88	21.73	<.0001

Least Squares Means

Effect	Culture	(Weeks)	Estimate	Error
cult	dm10		0.3003	0.005599
cult	mr1c		0.3125	0.005470
Time(cult)	dm10	1	0.3514	0.01013
Time(cult)	dm10	3	0.2788	0.009474
Time(cult)	dm10	6	0.2706	0.009474
Time(cult)	mr1c	1	0.3600	0.009474
Time(cult)	mr1c	3	0.3088	0.009474
Time(cult)	mr1c	6	0.2687	0.009474

ANOVA for Brittleness

Effect	DF	DF	F Value	Pr > F
cult	1	88	2.96	0.0889
Time(cult)	4	88	1.82	0.1324

Least Squares Means

Effect	Culture	(Weeks)	Estimate	Error
cult	dm10		27.9646	1.0976
cult	mr1c		30.6046	1.0724
Time(cult)	dm10	1	25.2300	1.9856
Time(cult)	dm10	3	30.4069	1.8574
Time(cult)	dm10	6	28.2569	1.8574
Time(cult)	mr1c	1	29.6275	1.8574
Time(cult)	mr1c	3	33.4500	1.8574
Time(cult)	mr1c	6	28.7362	1.8574

ANOVA for Fines

Effect	DF	DF	F Value	Pr > F
cult	1	11	0.51	0.4916
Time(cult)	4	11	6.26	0.0071

Least Squares Means

Effect	Culture	(Weeks)	Estimate	Error
cult	dm10		40.8111	3.0714
cult	mr1c		37.8333	2.8435
Time(cult)	dm10	1	59.3000	4.9251
Time(cult)	dm10	3	32.4333	4.9251
Time(cult)	dm10	6	30.7000	6.0320
Time(cult)	mr1c	1	47.1667	4.9251
Time(cult)	mr1c	3	33.8333	4.9251
Time(cult)	mr1c	6	32.5000	4.9251

ANOVA for Melt Test

Split-Split Plot Design

Effect	DF	DF	F Value	Pr > F
culture	1	7.02	1.38	0.2788
shred	2	7.02	0.91	0.4456
culture*shred	2	7.02	2.42	0.1583
test	1	123	32.98	<.0001
culture*test	1	123	1.16	0.2839
shred*test	2	123	0.04	0.9639
culture*shred*test	2	123	1.01	0.3675

Least Squares Means

Effect	Type	Shredded	Tested	Estimate	Error
culture	DM10			70.7195	5.3731
culture	MR1C			79.4388	5.2276
shred		7		79.9536	6.3838
shred		21		68.1292	6.5967
shred		42		77.1546	6.4350
test			42	71.3607	3.8239
test			84	78.7976	3.8502
culture*shred	DM10	7		68.1638	9.0016
culture*shred	DM10	21		59.8896	9.5877
culture*shred	DM10	42		84.1050	9.1443
culture*shred	MR1C	7		91.7434	9.0016
culture*shred	MR1C	21		76.3688	9.0016
culture*shred	MR1C	42		70.2042	9.0016
culture*test	DM10		42	66.3040	5.4173
culture*test	DM10		84	75.1349	5.4897
culture*test	MR1C		42	76.4173	5.3040
culture*test	MR1C		84	82.4603	5.3040
shred*test		7	42	76.0597	6.4777
shred*test		7	84	83.8476	6.4777
shred*test		21	42	64.6539	6.6097
shred*test		21	84	71.6044	6.7825
shred*test		42	42	73.3684	6.5514
shred*test		42	84	80.9408	6.5093
culture*shred*test	DM10	7	42	62.3113	9.1348
culture*shred*test	DM10	7	84	74.0163	9.1348
culture*shred*test	DM10	21	42	56.6016	9.4989
culture*shred*test	DM10	21	84	63.1776	9.9709
culture*shred*test	DM10	42	42	79.9992	9.3403
culture*shred*test	DM10	42	84	88.2108	9.2226
culture*shred*test	MR1C	7	42	89.8080	9.1348
culture*shred*test	MR1C	7	84	93.6789	9.1348
culture*shred*test	MR1C	21	42	72.7063	9.1348
culture*shred*test	MR1C	21	84	80.0313	9.1348
culture*shred*test	MR1C	42	42	66.7375	9.1348
culture*shred*test	MR1C	42	84	73.6709	9.1348

B. Analysis for Differences by Culture and Time –Trial 2 only

ANOVA for Hardness

Effect	DF	DF	F Value	Pr > F
cult	1	108	1.41	0.2378
Time(cult)	6	108	2.42	0.0310

Least Squares Means

Effect	Culture	(Weeks)	Estimate	Error
cult	dm10		39.6735	1.7805
cult	mr1c		42.6926	1.8163
Time(cult)	dm10	0.5	42.8580	2.8681
Time(cult)	dm10	2	35.6980	2.8681
Time(cult)	dm10	3	41.7187	4.5348
Time(cult)	dm10	6	38.4192	3.7026
Time(cult)	mr1c	0.5	46.2000	2.8681
Time(cult)	mr1c	2	49.4808	3.7026
Time(cult)	mr1c	3	40.2025	4.5348
Time(cult)	mr1c	6	34.8869	3.2066

ANOVA for Adhesiveness

Effect	DF	DF	F Value	Pr > F
cult	1	108	6.15	0.0147
Time(cult)	6	108	9.22	<.0001

Least Squares Means

Effect	Culture	(Weeks)	Estimate	Error
cult	dm10		1.4939	0.06234
cult	mr1c		1.7147	0.06359
Time(cult)	dm10	0.5	1.4290	0.1004
Time(cult)	dm10	2	1.3380	0.1004
Time(cult)	dm10	3	1.3338	0.1588
Time(cult)	dm10	6	1.8750	0.1296
Time(cult)	mr1c	0.5	1.4395	0.1004
Time(cult)	mr1c	2	1.1950	0.1296
Time(cult)	mr1c	3	2.0863	0.1588
Time(cult)	mr1c	6	2.1381	0.1123

ANOVA for Cohesiveness

Effect	DF	DF	F Value	Pr > F
cult	1	108	0.11	0.7464
Time(cult)	6	108	8.29	<.0001

Least Squares Means

Effect	Culture	(Weeks)	Estimate	Error
cult	dm10		0.3287	0.005690
cult	mr1c		0.3313	0.005804
Time(cult)	dm10	0.5	0.3735	0.009165
Time(cult)	dm10	2	0.3350	0.009165
Time(cult)	dm10	3	0.3188	0.01449
Time(cult)	dm10	6	0.2875	0.01183
Time(cult)	mr1c	0.5	0.3530	0.009165
Time(cult)	mr1c	2	0.3517	0.01183
Time(cult)	mr1c	3	0.3113	0.01449
Time(cult)	mr1c	6	0.3094	0.01025

ANOVA for Brittleness

Effect	DF	DF	F Value	Pr > F
cult	1	108	2.91	0.0907
Time(cult)	6	108	2.19	0.0491

Least Squares Means

Effect	Culture	(Weeks)	Estimate	Error
cult	dm10		34.3851	1.9652
cult	mr1c		39.1771	2.0046
Time(cult)	dm10	0.5	35.2500	3.1655
Time(cult)	dm10	2	30.0900	3.1655
Time(cult)	dm10	3	38.2638	5.0051
Time(cult)	dm10	6	33.9367	4.0866
Time(cult)	mr1c	0.5	42.1355	3.1655
Time(cult)	mr1c	2	45.0842	4.0866
Time(cult)	mr1c	3	40.2025	5.0051
Time(cult)	mr1c	6	29.2863	3.5391

ANOVA for Fines

Effect	DF	DF	F Value	Pr > F
cult	1	48	1.15	0.2883
Time(cult)	6	48	4.14	0.0020

Least Squares Means

Effect	Culture	(Weeks)	Estimate	Error
cult	dm10		43.0981	3.1412
cult	mr1c		38.4299	3.0053
Time(cult)	dm10	0.5	50.7625	5.4070
Time(cult)	dm10	1	52.4100	4.8362
Time(cult)	dm10	3	25.3000	7.6466
Time(cult)	dm10	6	43.9200	6.8394
Time(cult)	mr1c	0.5	52.5375	5.4070
Time(cult)	mr1c	1	47.0500	4.8362
Time(cult)	mr1c	3	25.8750	7.6466
Time(cult)	mr1c	6	28.2571	5.7803

ANOVA for Stickiness

Effect	DF	DF	F Value	Pr > F
cult	1	48	0.50	0.4832
Time(cult)	6	48	2.65	0.0266

Least Squares Means

Effect	Culture	(Weeks)	Estimate	Error
cult	dm10		28.9763	2.5602
cult	mr1c		26.4727	2.4495
Time(cult)	dm10	0.5	27.9250	4.4070
Time(cult)	dm10	1	40.2600	3.9417
Time(cult)	dm10	3	19.7000	6.2324
Time(cult)	dm10	6	28.0200	5.5744
Time(cult)	mr1c	0.5	28.2000	4.4070
Time(cult)	mr1c	1	35.1300	3.9417
Time(cult)	mr1c	3	18.1750	6.2324
Time(cult)	mr1c	6	24.3857	4.7113

ANOVA for Gumminess

Effect	DF	DF	F Value	Pr > F
cult	1	52	0.44	0.5099
Time(cult)	6	52	1.34	0.2548

Least Squares Means

Effect	Culture	(Weeks)	Estimate	Error
cult	dm10		3.5819	0.2762
cult	mr1c		3.8353	0.2638
Time(cult)	dm10	0.5	3.6010	0.4333
Time(cult)	dm10	1	3.8610	0.4333
Time(cult)	dm10	3	2.9175	0.6851
Time(cult)	dm10	6	3.9480	0.6128
Time(cult)	mr1c	0.5	3.0630	0.4333
Time(cult)	mr1c	1	3.9790	0.4333
Time(cult)	mr1c	3	3.5650	0.6851
Time(cult)	mr1c	6	4.7343	0.5179

C. Analysis for Differences by Culture and Time – Trial 3 only

ANOVA for Hardness

Effect	DF	DF	F Value	Pr > F
trial	2	36	82.77	<.0001
Time(trial)	9	36	3.12	0.0071

Least Squares Means

Effect	Trial	(Weeks)	Estimate	Error
trial	1		56.5225	1.1853
trial	2		35.1594	1.1853
trial	3		48.4056	1.1853
Time(trial)	1	1	59.9475	2.3707
Time(trial)	1	2	56.5425	2.3707
Time(trial)	1	3	55.1100	2.3707
Time(trial)	1	6	54.4900	2.3707
Time(trial)	2	1	28.3475	2.3707
Time(trial)	2	2	35.9775	2.3707
Time(trial)	2	3	34.8625	2.3707
Time(trial)	2	6	41.4500	2.3707
Time(trial)	3	1	44.7125	2.3707
Time(trial)	3	2	53.9825	2.3707
Time(trial)	3	3	49.2675	2.3707

Time(trial)	3	6	45.6600	2.3707

ANOVA for Adhesiveness

Effect	DF	DF	F Value	Pr > F
trial	2	36	2.70	0.0805
Time(trial)	9	36	16.13	<.0001

Least Squares Means

Effect	Trial	(Weeks)	Estimate	Error
trial	1		1.6381	0.1181
trial	2		1.9413	0.1181
trial	3		1.5794	0.1181
Time(trial)	1	1	1.7100	0.2362
Time(trial)	1	2	0.2950	0.2362
Time(trial)	1	3	1.5075	0.2362
Time(trial)	1	6	3.0400	0.2362
Time(trial)	2	1	1.4850	0.2362
Time(trial)	2	2	1.2200	0.2362
Time(trial)	2	3	2.2750	0.2362
Time(trial)	2	6	2.7850	0.2362
Time(trial)	3	1	1.0450	0.2362
Time(trial)	3	2	0.6250	0.2362
Time(trial)	3	3	1.8500	0.2362
Time(trial)	3	6	2.7975	0.2362

ANOVA for Brittleness

Effect	DF	DF	F Value	Pr > F
trial	2	10	27.15	<.0001
Time(trial)	5	10	1.00	0.4644

Least Squares Means

Effect	Trial	(Weeks)	Estimate	Error
trial	1		53.3333	2.6461
trial	2		24.6667	3.0555
trial	3		47.0278	2.2198
Time(trial)	1	1	52.0000	2.6461
Time(trial)	1	2	56.0000	5.2923
Time(trial)	1	3	52.0000	5.2923
Time(trial)	2	1	24.3333	3.0555
Time(trial)	2	2	25.0000	5.2923
Time(trial)	3	1	42.7500	2.6461

Time(trial)	3	2	51.3333	3.0555
Time(trial)	3	3	47.0000	5.2923

ANOVA for Fines

Effect	DF	DF	F Value	Pr > F
trial	2	9	12.63	0.0024
Time(trial)	6	9	7.43	0.0044

Least Squares Means

Effect	Trial	(Weeks)	Estimate	Error
trial	1		15.7333	1.2696
trial	2		24.7333	1.2696
trial	3		19.6500	1.2696
Time(trial)	1	1	18.2500	2.1989
Time(trial)	1	2	14.1000	2.1989
Time(trial)	1	3	14.8500	2.1989
Time(trial)	2	1	28.5000	2.1989
Time(trial)	2	2	26.5000	2.1989
Time(trial)	2	3	19.2000	2.1989
Time(trial)	3	1	28.7000	2.1989
Time(trial)	3	2	19.3000	2.1989
Time(trial)	3	3	10.9500	2.1989

ANOVA for Gumminess

Effect	DF	DF	F Value	Pr > F
trial	2	9	1.09	0.3758
Time(trial)	6	9	0.87	0.5512

Least Squares Means

Effect	Trial	(Weeks)	Estimate	Error
trial	1		3.2233	0.4234
trial	2		3.9667	0.4234
trial	3		3.1783	0.4234
Time(trial)	1	1	2.9150	0.7333
Time(trial)	1	2	4.1000	0.7333
Time(trial)	1	3	2.6550	0.7333
Time(trial)	2	1	3.1300	0.7333
Time(trial)	2	2	4.7350	0.7333
Time(trial)	2	3	4.0350	0.7333
Time(trial)	3	1	3.5600	0.7333
Time(trial)	3	2	3.2200	0.7333
Time(trial)	3	3	2.7550	0.7333

D. Analysis for Differences by Culture and Time – Trial 1 and Trial 2

ANOVA for Hardness

Effect	DF	DF	F Value	Pr > F
cult	1	200	6.54	0.0113
Time(cult)	8	200	4.63	<.0001

Least Squares Means

Effect	Culture	(Weeks)	Estimate	Error
cult	dm10		36.7575	1.0701
cult	mr1c		40.7047	1.1121
Time(cult)	dm10	0.5	42.8580	2.3985
Time(cult)	dm10	1	33.3607	2.8667
Time(cult)	dm10	2	35.6980	2.3985
Time(cult)	dm10	3	36.2196	2.1895
Time(cult)	dm10	6	35.6511	2.0271
Time(cult)	mr1c	0.5	46.2000	2.3985
Time(cult)	mr1c	1	36.2356	2.6816
Time(cult)	mr1c	2	49.4808	3.0964
Time(cult)	mr1c	3	37.5317	2.1895
Time(cult)	mr1c	6	34.0756	1.8961

ANOVA for Adhesiveness

Effect	DF	DF	F Value	Pr > F
cult	1	199	0.31	0.5789
Time(cult)	8	199	24.79	<.0001

Least Squares Means

Effect	Culture	(Weeks)	Estimate	Error
cult	dm10		1.4786	0.04950
cult	mr1c		1.5184	0.05162
Time(cult)	dm10	0.5	1.4290	0.1110
Time(cult)	dm10	1	0.9379	0.1326
Time(cult)	dm10	2	1.3380	0.1110
Time(cult)	dm10	3	1.4675	0.1013
Time(cult)	dm10	6	2.2207	0.09378
Time(cult)	mr1c	0.5	1.4395	0.1110
Time(cult)	mr1c	1	0.8300	0.1241
Time(cult)	mr1c	2	1.1950	0.1432
Time(cult)	mr1c	3	1.7896	0.1035
Time(cult)	mr1c	6	2.3378	0.08772

ANOVA for Cohesiveness

Effect	DF	DF	F Value	Pr > F
cult	1	200	1.30	0.2549
Time(cult)	8	200	17.70	<.0001

Least Squares Means

Effect	Culture	(Weeks)	Estimate	Error
cult	dm10		0.3260	0.004062
cult	mr1c		0.3327	0.004221
Time(cult)	dm10	0.5	0.3735	0.009104
Time(cult)	dm10	1	0.3514	0.01088
Time(cult)	dm10	2	0.3350	0.009104
Time(cult)	dm10	3	0.2921	0.008311
Time(cult)	dm10	6	0.2779	0.007694
Time(cult)	mr1c	0.5	0.3530	0.009104
Time(cult)	mr1c	1	0.3600	0.01018
Time(cult)	mr1c	2	0.3517	0.01175
Time(cult)	mr1c	3	0.3096	0.008311
Time(cult)	mr1c	6	0.2891	0.007198

ANOVA for Brittleness

Effect	DF	DF	F Value	Pr > F
cult	1	200	10.53	0.0014
Time(cult)	8	200	4.39	<.0001

Least Squares Means

Time Effect	Culture	Standard (Weeks)	Estimate	Error
cult	dm10		30.8574	1.1655
cult	mr1c		36.3118	1.2113
Time(cult)	dm10	0.5	35.2500	2.6123
Time(cult)	dm10	1	25.2300	3.1223
Time(cult)	dm10	2	30.0900	2.6123
Time(cult)	dm10	3	33.0258	2.3847
Time(cult)	dm10	6	30.6911	2.2078
Time(cult)	mr1c	0.5	42.1355	2.6123
Time(cult)	mr1c	1	29.6275	2.9207
Time(cult)	mr1c	2	45.0842	3.3725
Time(cult)	mr1c	3	35.7008	2.3847
Time(cult)	mr1c	6	29.0112	2.0652

E. Analysis for Differences by Culture and Time – Trial 2 and Trial 3

ANOVA for Fines

Effect	DF	DF	F Value	Pr > F
trial	12	36	13.80	<.0001
Time(trial)	25	36	5.84	<.0001

Least Squares Means

Effect	Trial	(Weeks)	Estimate	Error
trial	1		15.7333	3.5039
trial	1d		67.9350	4.2914
trial	1m		50.8500	4.2914
trial	2		24.7333	3.5039
trial	2d		54.2000	4.2914
trial	2m		43.7500	3.5039
trial	3		19.6500	3.5039
trial	3d		41.0000	3.0345
trial	3m		42.5875	3.0345
trial	4d		42.8333	4.0460
trial	4m		31.3833	4.0460
trial	5d		33.7500	3.5039
trial	5m		36.0333	3.5039
Time(trial)	1	1	18.2500	6.0690
Time(trial)	1	2	14.1000	6.0690
Time(trial)	1	3	14.8500	6.0690
Time(trial)	1d	1	62.5500	6.0690
Time(trial)	1d	6	73.3200	6.0690
Time(trial)	1m	1	59.1000	6.0690
Time(trial)	1m	6	42.6000	6.0690
Time(trial)	2	1	28.5000	6.0690
Time(trial)	2	2	26.5000	6.0690
Time(trial)	2	3	19.2000	6.0690
Time(trial)	2d	0.5	54.8000	6.0690
Time(trial)	2d	1	53.6000	6.0690
Time(trial)	2m	0.5	46.3000	6.0690
Time(trial)	2m	1	57.5000	6.0690
Time(trial)	2m	6	27.4500	6.0690
Time(trial)	3	1	28.7000	6.0690
Time(trial)	3	2	19.3000	6.0690
Time(trial)	3	3	10.9500	6.0690
Time(trial)	3d	0.5	65.4500	6.0690
Time(trial)	3d	1	53.0000	6.0690
Time(trial)	3d	3	21.5500	6.0690
Time(trial)	3d	6	24.0000	6.0690
Time(trial)	3m	0.5	72.4000	6.0690
Time(trial)	3m	1	55.7500	6.0690

Time(trial)	3m	3	22.8500	6.0690
Time(trial)	3m	6	19.3500	6.0690
Time(trial)	4d	0.5	49.2500	6.0690
Time(trial)	4d	1	54.2500	6.0690
Time(trial)	4d	6	25.0000	8.5828
Time(trial)	4m	0.5	53.9500	6.0690
Time(trial)	4m	1	21.2000	6.0690
Time(trial)	4m	6	19.0000	8.5828
Time(trial)	5d	0.5	33.5500	6.0690
Time(trial)	5d	1	38.6500	6.0690
Time(trial)	5d	3	29.0500	6.0690
Time(trial)	5m	0.5	37.5000	6.0690
Time(trial)	5m	1	41.7000	6.0690
Time(trial)	5m	3	28.9000	6.0690

ANOVA for Stickiness

Effect	DF	DF	F Value	Pr > F
trial	9	27	5.77	0.0002
Time(trial)	19	27	3.31	0.0023

Least Squares Means

Effect	Trial	(Weeks)	Estimate	Error
trial	1d		42.2600	4.2111
trial	1m		45.3750	4.2111
trial	2d		40.1250	4.2111
trial	2m		34.7000	3.4384
trial	3d		27.2875	2.9777
trial	3m		27.0375	2.9777
trial	4d		29.0500	3.9703
trial	4m		18.3000	3.9703
trial	5d		23.6500	3.4384
trial	5m		19.9167	3.4384
Time(trial)	1d	1	39.3000	5.9555
Time(trial)	1d	6	45.2200	5.9555
Time(trial)	1m	1	52.7500	5.9555
Time(trial)	1m	6	38.0000	5.9555
Time(trial)	2d	0.5	31.9000	5.9555
Time(trial)	2d	1	48.3500	5.9555
Time(trial)	2m	0.5	30.9500	5.9555
Time(trial)	2m	1	48.1000	5.9555
Time(trial)	2m	6	25.0500	5.9555
Time(trial)	3d	0.5	35.7500	5.9555
Time(trial)	3d	1	42.8500	5.9555
Time(trial)	3d	3	15.2500	5.9555
Time(trial)	3d	6	15.3000	5.9555
Time(trial)	3m	0.5	39.3500	5.9555

Time(trial)	3m	1	36.5500	5.9555
Time(trial)	3m	3	17.4500	5.9555
Time(trial)	3m	6	14.8000	5.9555
Time(trial)	4d	0.5	23.6000	5.9555
Time(trial)	4d	1	44.4500	5.9555
Time(trial)	4d	6	19.1000	8.4223
Time(trial)	4m	0.5	29.0000	5.9555
Time(trial)	4m	1	10.9000	5.9555
Time(trial)	4m	6	15.0000	8.4223
Time(trial)	5d	0.5	20.4500	5.9555
Time(trial)	5d	1	26.3500	5.9555
Time(trial)	5d	3	24.1500	5.9555
Time(trial)	5m	0.5	13.5000	5.9555
Time(trial)	5m	1	27.3500	5.9555
Time(trial)	5m	3	18.9000	5.9555

ANOVA for Gumminess

Effect	DF	DF	F Value	Pr > F
trial	12	38	7.67	<.0001
Time(trial)	27	38	1.46	0.1406

Least Squares Means

Effect	Trial	(Weeks)	Estimate	Error
trial	1		3.2233	0.3591
trial	1d		4.8950	0.3591
trial	1m		4.1250	0.3591
trial	2		3.9667	0.3591
trial	2d		5.8350	0.4398
trial	2m		5.4217	0.3591
trial	3		3.1783	0.3591
trial	3d		2.8038	0.3110
trial	3m		3.0825	0.3110
trial	4d		2.5950	0.4147
trial	4m		2.7733	0.4147
trial	5d		2.9550	0.3591
trial	5m		3.6800	0.3591
Time(trial)	1	1	2.9150	0.6220
Time(trial)	1	2	4.1000	0.6220
Time(trial)	1	3	2.6550	0.6220
Time(trial)	1d	0.5	4.1950	0.6220
Time(trial)	1d	1	4.6850	0.6220
Time(trial)	1d	6	5.8050	0.6220
Time(trial)	1m	0.5	3.8700	0.6220
Time(trial)	1m	1	2.9000	0.6220
Time(trial)	1m	6	5.6050	0.6220
Time(trial)	2	1	3.1300	0.6220
Time(trial)	2	2	4.7350	0.6220

Time(trial)	2	3	4.0350	0.6220
Time(trial)	2d	0.5	5.8550	0.6220
Time(trial)	2d	1	5.8150	0.6220
Time(trial)	2m	0.5	3.6350	0.6220
Time(trial)	2m	1	6.7300	0.6220
Time(trial)	2m	6	5.9000	0.6220
Time(trial)	3	1	3.5600	0.6220
Time(trial)	3	2	3.2200	0.6220
Time(trial)	3	3	2.7550	0.6220
Time(trial)	3d	0.5	2.6400	0.6220
Time(trial)	3d	1	2.9450	0.6220
Time(trial)	3d	3	2.7700	0.6220
Time(trial)	3d	6	2.8600	0.6220
Time(trial)	3m	0.5	2.5850	0.6220
Time(trial)	3m	1	3.1200	0.6220
Time(trial)	3m	3	2.9800	0.6220
Time(trial)	3m	6	3.6450	0.6220
Time(trial)	4d	0.5	2.4850	0.6220
Time(trial)	4d	1	2.8900	0.6220
Time(trial)	4d	6	2.4100	0.8797
Time(trial)	4m	0.5	2.4450	0.6220
Time(trial)	4m	1	3.0350	0.6220
Time(trial)	4m	6	2.8400	0.8797
Time(trial)	5d	0.5	2.8300	0.6220
Time(trial)	5d	1	2.9700	0.6220
Time(trial)	5d	3	3.0650	0.6220
Time(trial)	5m	0.5	2.7800	0.6220
Time(trial)	5m	1	4.1100	0.6220
Time(trial)	5m	3	4.1500	0.6220