EFFECTS OF CHELATING AGENTS ON TEXTURE OF LOWFAT
CHEDDAR CHEESE

A Thesis

presented to the

Faculty of California Polytechnic State University,

San Luis Obispo

In Partial Fulfillment

of the Requirements for the Degree

Master of Science in Agriculture,

with Specialization in Dairy Products Technology

By

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June 2013

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“There are only two mistakes one can make along the road to truth; not going all the way, and not starting”.

- **Buddha**

ABSTRACT

EFFECTS OF CHELATING AGENTS ON TEXTURE OF LOW FAT CHEDDAR CHEESE

Mariela Fernanda Poveda

Effects of two types of chelating agents on proteolysis and texture properties of low fat Cheddar cheese (LFC) were analyzed and compared to full fat Cheddar (FFC) control during ripening for 120 days at 8°C. We hypothesized that chelating agents would bind calcium ions from cheese matrix to give a softer curd due to a decrease of protein-protein interactions and simultaneously increasing moisture content. Cheese milk containing (0.59% fat) was divided into three lots (A, B & C). Sodium citrate (3Na) and disodium EDTA (EDTA) were added to A & B at the rate of (0.02% and 0.2% respectively. C served as control (LFC). Cheesemilk (88°F) was preacidified to pH 6.2 prior to setting using 34 ml chymosin/454 kg and starter culture addition. After cutting, curd was cooked to 96°F for 30 min and held for 10 min. After cooking, the curd was washed, salted, hooped and pressed. FFC was made on subsequent days from same batch of milk by the stirred curd method for Cheddar cheese, cheesemaking was replicated 5 times. Significant difference in moisture content ($P<0.05$) was observed between FFC and LFC. Calcium content on the EDTA and 3Na was significantly reduced ($P<0.05$) compared to FFC. No significant difference ($P>0.05$) in hardness was observed between FFC and LFC at day 7 and 30. After day 30, significant differences ($P<0.05$) among FFC and all LFC were observed. Increase of gumminess in LFC and 3Na compared to FFC was statistically significant ($P<0.05$) during the first 60 days during ripening. Springiness,

cohesiveness and chewiness did not report any differences between LF variants and FFC counterpart. No significant differences in the breakdown of β -casein and α s1₁-casein irrespective of cheese over time. However breakdown of α s1-casein was faster than any other cheese protein, especially in the LF cheddar variants. Significant differences ($P < 0.05$) at all ripening sampling times between cheese samples.

Keywords: low fat cheddar cheese, chelating agents, texture, proteolysis

ACKNOWLEDGMENTS

It is with immense gratitude that I acknowledge the support and help of my advisor Dr. Nana Farkye whose patience and kindness, as well as his academic experience, has been invaluable to me. I cannot find words to express my gratitude for his moral support and wisdom throughout my journey.

It gives me great pleasure in acknowledging the support and help of my committee members: Dr. Andrew Schaffner and Dr. Amy Lammert, who gave me their advice, guidance, and time, and who shared their knowledge for the completion of this thesis.

I praise the enormous amount of help and teaching the technical details of work to Matt Arnold and Andrea Laubscher. Thank you Matt for been always a wonderful person and for helping me out in the use of correct grammar and consistent notation in my writings.

I am indebted to Dr. Bruce Golden and Dr. Philip Tong for their support and I would like to express my gratitude to Dr. Brian Hampson for his encouragement and practical advice.

I will always be grateful to all my DPTC friends for they all helped me stay sane through difficult moments, for all the happy hour and farmers market shared, I will treasure those memories forever. Special thanks to my friend Xiomara Elias-Argote who has been a true friend ever since we met, for all her support and care helped me overcome setbacks and stay focused on my graduate study. I am also grateful to José M. López, Julie Lucca, Juan José Galindo, Luis Gonzalez, Juan Carlos de la Parra, Salomé Ramirez and Anthony Cano for being my second family in the U.S.

Thank you to Keyla Maria, Leonardo Rafael, Aron Pompero, Lira Capitu, Honey Maria and Moro Aroma for all their unconditional love.

This thesis is dedicated to my father Nelson Ernesto Poveda, my mother Mariana Arteaga and my brother Nelson Alejandro Poveda for been my source of love, concern, support and strength all my life.

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I. INTRODUCTION

Obesity is a major public health problem in the United States (Baskin et al., 2005). The prevalence of overweight and obesity in the population is of medical concern because it increases the risk for several diseases, particularly cardiovascular diseases (CVDs) and diabetes mellitus (Panel, 1998). With consumers becoming more conscious about their health conditions, the desire for food products that deliver nutritional and functional benefits has increased (Childs and Drake, 2009). For the past 20 years, in developed countries, trends within consumers have shown an avoidance of high calorie foods (Mistry, 2001). Several efforts to provide in good health options to the consumers have been led by the government (Childs and Drake, 2009). In the United States, for example, the recommended caloric intake from fat is not more than 30% of total calories (McMahon, 2010; Jacobs, 1993) . Cheese is a nutritionally valued dairy product of significant economic interest; nearly a third of all global milk production is consumed as cheese (Kethireddipalli et al., 2010). Even though cheese is considered a nutrient-dense food that is a good source of calcium, phosphorus, and protein, the consumer also perceived this product as high in fat and sodium (Johnson, 2009). During the past 15 years, the demand for reduced and lowfat cheese has increased dramatically (Drake & Swanson, 1995). In the dairy industry, the interests of low calorie foods have given the opportunity to new markets. This incentive has been followed by the dairy industry represented such as Dairy Management Inc. (DMI) that launched several research projects. One of those major efforts is going towards the improvements of LF cheeses. A research conducted through DMI reveals that 16 percent of adults around 20 to 54 years old are cheese restrictors. Fifty percent of the cheese restrictors also say that they might

be willing to buy LF Cheddar cheese if the product with less fat does not compromise on flavor, texture or meltability (Inc, March, 2009). LF cheeses are usually characterized as having poor body, flavor, and functional properties because of high moisture and low salt. Successfully producing lower fat cheese requires mimicking the role of fat in texture, performance, flavor and color of cheese, as well as compensating for the lower salt-in-moisture concentration when moisture content is increased (McMahon, 2010). Procedures developed to improve the texture and flavor attributes of LF cheeses have been modified to simulate the properties of a FFC (Drake et al., 1996). Changes during the manufacture of LF Cheddar cheeses include variation of processing techniques, starter culture selection, and use of additives (Johnson et al., 2009). Significant advances in understanding the biochemical and physicochemical characteristics of LF variants in the past decade have led to novel technological developments (Banks, 2004). Different methods and technology have been developed, for the past 20 years, creating more than 50 patents (Mistry, 2001). Studies on the influence of fat on flavor and flavor development in Cheddar cheese had been reported (Drake et al., 2010). In recent years with the aim to improve quality of LF Cheddar cheeses, researches have been examined unconventional the addition of chelating agents to reduce the calcium colloidal phosphate (CCP) (Pastorino et al., 2003) or increasing total solids using microfiltration (McGregor and White, 1990a, b). Addition of emulsifying salts (Hoffmann et al., 2012), thickening agents (Dabour et al., 2006) and fat mimetics have been implemented (Banks, 2004; Mistry, 2001; Drake 1996) during processing to replace the fat removed, increase the moisture retention and decrease acid accumulation.

II. LITERATURE REVIEW

Cheese

Cheese is the generic name for a group of fermented milk-base food products (Fox et al., 2000) and is one of the classical examples of food preservation that uses lactic acid fermentation, and reduction of water activity through removal of water and addition of NaCl (Fox et al., 2004a). Cheese is a very versatile product with many diverse flavors and texture that can be used as a snack or as part of a dish or prepackaged convenience food (Farkye, 2004). Although traditional cheeses have a rather high fat content, they are rich in protein and in most, cases of calcium and phosphorous and have anticarcinogenic properties (Fox and McSweeney, 1998). Cheese can be classified according to texture, fat content, ripening method or country of origin. The US Code of Federal Regulations classification of cheese is based on moisture and fat content. Content of fat is usually expressed as fat-in-dry matter (FDM) (Farkye, 2004). FDM is the ratio between the % fat and % total solids multiplied by 100. This value is important because it remains constant and excludes variations of water evaporation, especially for ripened cheeses such as cheddar (Fox et al., 2004b). Table 1 shows a classification of cheeses based on moisture content.

Table 1. Classification of cheese based on moisture content (Farkye, 2004).

Consistency	Maximum moisture (%)	Minimum FDM (%)
Hard grating	34	32
Hard	39	50
Semisoft	50 (> 39)	50
Semisoft, part skim	50	45 (< 50)
Soft	Not specified	50

Cheese manufacture essentially involves gelation of the casein via isoelectric point (acid) or enzymatic (rennet) coagulation. Cheeses produced by acid coagulation possess a short shelf life and are usually consumed fresh while the rennet cheeses are almost always matured before consumption (Fox et al., 2004a).

The production of rennet coagulated cheeses can be described in two steps:

- A. Conversion of milk into curds
- B. Ripening of curds

The conversion of milk into curds (A) or coagulation process due to enzymatic reactions can be divided into two phases.

- 1) First phase is represented by the hydrolysis of κ -casein by rennet that cleaves at the Phe105-Met106 bond. As result a molecule of para- κ -casein (hydrophobic) and macropeptides (hydrophilic) are produced as the reaction below. Fat does not participate in this stage.

- 2) Second phase starts when at least 85% of the total κ -casein has been hydrolyzed and micelles begin to aggregate into a gel. Table 2 resumes the grade of participation of factors at first and second stage.

Table 2. Principal factors affecting the rennet-coagulation time of milk (Fox and McSweeney, 1998).

Factor	First phase	Second phase
Temperature	+	++
pH	+++	-
Ca	-	+++
Pre-heating	++	++++
Rennet concentration	++++	-
Protein concentration	+	++++

During ripening of curds (B) three principal events take place:

- 1) Glycolysis: 98% of lactose in milk is separated as lactic acid from the curd and 2% remaining is metabolized by nonstarter lactic acid bacteria (NSLAB).
- 2) Degradation of lipids to fatty acids is called lipolysis that directly contributes to cheese flavor development.
- 3) Proteolysis or break down of proteins is the most complex event during ripening. This process gives the texture and flavor profile to a cheese (Fox and McSweeney, 1998).

Cheddar cheese

Cheddar cheese is a rennet-coagulated cheese with a firm texture. Cheddar cheese can be classified as a First or Second grade according to the composition of the cheese.

Figure 1 suggests ranges of salt in moisture (S/M), moisture in non-fat substance (MNFS), FDM and pH for First and Second Grade.

Figure 1. Cheddar First and Second grade composition ranges (Fox et al., 2004b)

Standards of identity for low and full fat Cheddar cheese

Full fat Cheddar cheese is defined under standards of identity as containing not more than 39% moisture, unless labeled properly as excess of moisture cheese, and not less than 50% FDM (Kosikowski and Mistry, 1997).

According to the U.S Code of Federal Regulations (2005), lowfat cheeses have strict legal definitions. Lowfat refers to cheese containing no more than 6% fat based on definition 21CFR101 – Section 101.62(b)(2)(i). Cheese can be labeled as lowfat food if the fat content has been reduced by 50% or more, such that it contains less than 3g fat per serving (28g) (Drake and Swanson, 1995).

Consumption of Cheddar cheese

U.S. per capita consumption of natural cheese increased by 0.36 pounds over the 2010 amount, reaching a level of 33.50 pounds, the second highest amount on the U.S. record (2012). Even though in 2011 the consumption of American-type cheese consumption dropped by 0.14 pounds to 13.18 pounds per person, Cheddar cheese still remains the second most consumed cheese in the U.S. after mozzarella.

According to the International Dairy Foods Association (IDFA), the sales of natural cheese represented a value of over \$11 billion dollars. The largest volume sales were Cheddar (36.7%), Mozzarella (20.9%) and Colby Jack (9.3%). While mozzarella is the most consumed cheese, sales in billions dollars of Cheddar remain the highest.

Calcium content on a portion of Full fat cheddar

According to the USDA National Nutrient Database for Standard Reference, a portion of a full fat cheddar cheese is 28.35 gr (1 oz) that should contain about 204 mg of Calcium.

Adequate calcium intake recommendations were set at levels associated with desirable retention of body calcium since high bone density is known to be less susceptible to fractures. Recommended intake for adults is 1000 to 1200 mg/day (Bryant et al., 1999). Dairy foods have indisputably been the highest contributor of dietary calcium; thus, it is no surprise that this group remained the top-ranked source of calcium (Cook and Friday, 2003).

Problems in the manufacture of LF cheddar cheese.

The level of fat influences several aspects of cheese, including composition, biochemistry, microstructure, yield, rheological, textural proprieties, and cooking properties. It is well accepted that Cheddar cheese made from skimmilk does not develop a characteristic flavor and texture. Cheese with a FDM less than 50% did not develop a Cheddar profile (Fox et al., 2004b).

Texture of cheddar cheese

The texture of Cheddar cheese is of importance to consumers yet, it is difficult to explain and describe due to its complexity. There are many factors that play a big role developing texture of Cheddar cheese. Table 2 above describes the most important factors during the first phase of rennet-coagulation.

Bovine milk contains 30-35 g protein/L. About 80% of the protein in milk is caseins represented by 4 gene products: α_{s1} , α_{s2} , β , and κ -caseins (Fox, 1992) that in combination with CCP constitute the casein micelles (Lucey et al., 1997)

Natural rennet-curd cheese is essentially a calcium-phosphate-paracasein matrix. In cheese, a dynamic equilibrium exist between concentrations of Ca^{2+} and inorganic phosphate in the paracasein matrix and the cheese serum influenced by pH and other factors such as concentrations of Na^{+} (Fox et al., 2000).

Various studies affirm that the characteristics of cheese structure depends upon pH changes more than any other factor, although the general tendency for the cheese is to become less firm as the calcium content decreases during cheese making, as pH decreases

towards the casein isoelectric point of paracasein, the protein assumes an increasingly more compact conformation and the cheese becomes shorter in texture and fractures at a smaller deformation (Fox et al., 2004b).

Salt plays a major role in cheese texture. Enzyme activity, solubility of protein break down, hydration of the protein network, and interactions of calcium with the paracaseinate complex in cheese are influenced by salt concentration (Fox et al., 2004b).

Proteolysis is the most complex, and perhaps most important event during ripening because it is responsible for textural changes during maturation. Small peptides are responsible for the flavor qualities of the cheese depend on this phenomenon (Fox, 1992).

Impact of fat removal on cheese texture

During the past few decades, consumption of low fat products has grown steadily because of consumer awareness about health associated with obesity (Dabour et al., 2006). Although lowfat cheese may allow for those who want to eat cheese but would like to reduce fat and calorie intake, the removal of fat causes changes in cheese flavor and texture that may be unacceptable to consumers (Childs and Drake, 2009), (Banks, 2004).

The gelation of milk is characterized by the aggregation of the rennet-altered casein micelles into interconnected clusters and forming a network in which fat globules are interspersed as loose inclusions. Continued aggregation of the para-casein, and expulsion of whey leads a gradual fusion of the protein gel network around fat globules (Fox and McSweeney, 2006).

Cheese texture is a sensory characteristic that can be measured using sensorial analysis (Fox et al., 2000) mechanically measured using a Texture Analyzer.

Colloidal Calcium phosphate role in cheese texture

Ca²⁺ ions play a major role in the stability of milk proteins and their functional properties during milk processing but the role of ionic calcium in causing coagulation of milk proteins during heat treatment is still unclear. (Ramasubramanian et al., 2013). The amount and the state of the retained calcium influences the physical characteristics of cheese during ripening (Johnson and Lucey, 2006). Calcium content of Cheddar cheese has an important effect on both texture and long term keeping quality. Some of the factors that influence the calcium content of cheese are:

1. Quantity of the rennet used. The proportion of rennet added should be the minimum necessary to give a firm coagulum. During the manufacture of full fat Cheddar cheese using the milled curd the relation of rennet to kg of milk is 100 ml/1000 kg. In addition to rennet, a small concentration of CaCl₂ is added (0.06% w/w of milk). Addition of Ca reduces the rennet coagulation time of milk that is due to the neutralization of negatively charged residues on casein, which increases the aggregation of renneted micelles. Addition of low concentrations of Ca also increases gel firmness (Lucey and Fox, 1993), For the manufacture of lowfat Cheddar the concentration of rennet is considerably reduced to 34 ml/1000 kg of milk due to the increase of protein content and the reduction of fat globules. The efficacy of the enzyme is inversely proportioned to protein content. For the

purposes of this study, chelating agents have been added in order to reduce calcium colloidal phosphate (CCP) as a novel method to soften the curd strength.

Set time during manufacture after adding rennet. Whey drainage pH is considered the most important factor because it determines the mineral content in the curd as well as the residual concentration of rennet and plasmin in cheese. It also defines the final pH and moisture to casein ratio (Fox et al., 2004b). Decreasing the pH of the whey at draining increases the level of nonmicellar calcium and lowers the Ca^{2+} content of cheese (Metzger et al., 2000).

All of the factors mentioned have an impact in the Calcium retention in the cheese matrix. Changes in the physical properties of cheese during ripening occur in two stages:

1. Changes in the insoluble calcium content led by pH and changes that takes place within a short period after cheesemaking.
2. The second stage is governed by the extent of proteolysis of intact casein that occurs throughout cheese ripening (Johnson and Lucey, 2006).

Removal of colloidal calcium phosphate (CCP) at milk pH has been done successfully in the past using dialysis of skimmilk reducing CCP concentration and demonstrating the importance for micellar integrity (Fox, 1992). In addition, if the pH of milk is lowered by acid addition prior to rennet coagulation, a portion of the micellar calcium is solubilized and this will lower the calcium content of cheese.

Increasing the temperature of acidified milk prior to rennet addition should cause some of the casein to go back into the micelles because of increased hydrophobic attraction among caseins (Metzger et al., 2000).

Chelating agents definition

Chelating agents, in some cases called emulsifier salts, play an important role in food preservation. They are molecules designed to inactivate oxidative degradation that impact quality, shelf-life, and nutritional content of food products, especially of packaged foods. When trace metals are present in foods; they initiate degradation of nutritional compounds such as unsaturated fatty acids, carotenoids, antioxidants, phytosterols, and many vitamins. In consequence, chelating agents are designed to sequester metal ions.

Uses of Chelating agents in the dairy industry

Emulsifying salts such as disodium citrate are widely used in the dairy industry for processed cheese manufacture to control melting, texture and free oil formation (Mizuno and Lucey, 2005). Processed cheese is produced by blending shredded natural cheeses of different types and degrees of maturity with emulsifying agents, and by heating the blend under a partial vacuum with constant agitation until a homogeneous mass is obtained. In addition to natural cheeses, other dairy and non-dairy ingredients may be included in the blend (Carić and Kaláb, 1993).

Sodium phosphates, polyphosphates and citrates are emulsifying salts most commonly used in the manufacture of process cheese either alone or in mixtures. Their role during processing is to sequester calcium in the natural cheese, to solubilize protein and increase its hydration and swelling, to facilitate emulsification of fat, and to adjust and stabilize pH (Carić and Kaláb, 1993).

Chelators such as EDTA can be added to inhibit metal-promoted oxidation, however there is significant consumer and industry demand to eliminate EDTA from product formulations (Goddard et al., 2012).

Chelating agents used for Lowfat Cheddar cheese manufacture

Effect of sodium citrate on structure and function relationships of Cheddar cheese have been already investigated (Pastorino et al., 2003). Using high pressure injection, a buffer (pH 5.27) containing 40% (wt/wt) citric acid and trisodium dehydrated and 6.25 % (wt/wt) anhydrous citric acid from zero (control) to five times successive injections performed 24 hrs apart. Citric acid increased from 0.22 (control) to 1.39% (after five injections). As result, a reduction on the bound phosphate content on cheese decreased from 0.54 mmol/g protein (control) to 0.45 mmol/g protein (after 5 injections) increasing phosphate solubilization and possibly the ionic Ca^{2+} content decreased resulting in expansion of the protein matrix and increasing hardness.

Chelating agents and the impact on Texture of cheese

Calcium chelators such as EDTA, citrate, phosphate are often used to improve the heat stability on dairy products influencing changes in the casein micelles decreasing calcium ions and depletion of CCP (De Kort et al., 2011). In this study, the calcium ion activity was decreased upon the addition of 4 different types of salts in the following order: SHMP (sodium hexametaphosphate), SP (sodium phytate), TSC (trisodium citrate) and Na_2HPO_4 . SHMP caused the most reduction in Ca^{2+} activity. Figure 2 shows efforts of various chelating agents on the decrease of Ca^{2+} ions activity of casein micelles.

Figure 2. Calcium-ion as a function of concentration of salts (De Kort et al., 2011).
 (●) Na₂UMP; (◆) Na₂HPO₄; (■) SHMP (sodium hexametaphosphate); (▲) SP (sodium phytate); (×)
 TSC (trisodium citrate)

III. PRELIMINARY STUDIES ON COAGULATION USING CHELATING AGENTS

Brief outline

If the total Ca^{2+} content of cheese is reduced, then the amount of cross-linking between casein polymers is reduced and the cheese becomes softer (Metzger et al., 2000).

Effects of sodium citrate using high pressure injection was reported (Pastorino et al., 2003) demonstrating an increase on phosphate solubilization, and possible decreased ionic calcium content. The aim of this study is to analyze the effect of the addition of sodium citrate and EDTA to improve the texture of lowfat natural Cheddar.

Disodium citrate and disodium EDTA were selected due to their ability to work well in dairy systems (De Kort et al., 2011).

Determination of disodium citrate concentration and coagulation time

1. Objectives

Determine the adequate concentration of the disodium citrate to act as a Ca^{2+} chelating agent.

2. Procedure

Five liters of pasteurized skimmed milk (0.19% fat) was collected from the California Polytechnic State University Creamery and stored at 4°C over night. Two hundred ml of skimmilk was transferred into 250 ml beakers. Each beaker was previously labeled with different times (5, 10, 15 and 20 min) and concentrations of sodium citrate (0, 0.1, 0.15,

0.2, 0.25 and 0.3 % w/w) and placed into a water bath (ISOTEMP 210, Fisher Scientific) until the milk temperature reached ~31°C. 10% phosphoric acid was added and mixed until the pH was reduced to 6.2. Rennet was added at the rate of 100 ml/475 kg of milk leaving 5 minutes between samples to prevent overlap texture measurements. A timer was set to beep every 5 minutes after rennet addition. The beakers were taken out to measure the curd hardness using TA-XT2 Texture Analyzer using the same setting as yogurt (based on the product similarities). The settings for this measurement are described on Table 4.

Table 3. Texture Analysis Settings. Specific settings selected for yogurt using TA-XT2 Texture Analyzer.

Parameter	Selected Settings
Test Mode	Yogurt
Pre Test Speed	1.2 mm/s
Post Test speed	1.2 mm/s
Distance	80 mm
Compression	50%
Time	5 s
Force	5 g
Probe	40 mm

3. Results and Conclusions

In the case of LF Cheddar cheese manufacture, the set time after rennet addition before curd cutting is reduced to 20 minutes due to a reduction of fat content compared to a FFC set time (30 min) (Kosikowski and Mistry, 1997). In consequence, during the preliminary studies and LF cheese manufacture we maintained this time as a constant.

Figure 3 shows the development of the coagulum hardness of milk containing added sodium citrate after rennet addition. At this point we obtain a curd with a force ~150 g

which makes possible to get a soft curd but still obtain cheese out of it. At 0.025 or 0.3 %wt/wt the curd was too soft and was unacceptable for cheese making.

Figure 3. Effect of Disodium Citrate on rennet gel strength.

Calcium EDTA and Disodium EDTA chemical properties described by manufacture company

In the invention dilute aqueous sodium hypochlorite is stabilized against decomposition during storage by incorporating in it a small proportion of the chelate of disodium ethylenediaminetetraacetic (EDTA). Thus stabilized even at high dilutions the hypochlorite retains nearly its initial strength for many when intended for disinfecting surfaces on considerable organic matter is present the diluted hypochlorite may be formulated to contain also a detergent and an alkaline builder (Burton, 1990).

VERSENE is the provider of the two types of EDTA. They vary on the pH range of action. According to the product specifications VERSENE CA Chelating Agent is more efficient in foods with a pH between 6.5-7.5 (1 wt% solution). VERSENE Disodium EDTA best pH environment falls between 4.3-4.7 (1 wt% solution) and 4.0-6.0 (5 wt% solution).

Selecting the appropriate EDTA for cheese making purposes

1. Objectives

The purpose of this experiment is to define which type of EDTA (calcium or disodium) was most effective for rennet curd formation.

2. Procedure

Five liters of fresh raw whole milk was obtained from the Cal Poly dairy farm and stored at 4°C over night. The cold milk was transferred into a 250 ml and flasks and centrifuged for 25 min at 10,000 rpm to separate the fat from the milk. Skim milk 0.17 % fat was obtained after centrifugation. 200 ml of skim milk were transferred into 250 ml beakers. The beakers were previously labeled with different concentrations of Ca disodium EDTA and disodium EDTA at different concentrations and placed into a water bath (ISOTEMP 210, Fisher Scientific). When milk temperature reached ~31°C, 10% phosphoric acid was added as well as the chelating agents and mixed until the pH was reduced to 6.2. Rennet was added at the rate of 100 ml/475 kg milk. The samples were staggered 5 min apart to prevent overlap texture measurements. After 20 minutes of adding rennet, each beaker was taken out to measure the curd hardness using TA-XT2

Texture Analyzer using the same setting as yogurt (based on the product similarities).

The settings for this measurement are described on Table 5.

Table 4. Texture Analysis Settings. Specific settings selected for yogurt using TA-XT2 Texture Analyzer.

Parameter	Selected Settings
Test Mode	Yogurt
Pre Test Speed	1.2 mm/s
Post Test speed	1.2 mm/s
Distance	80 mm
Compression	50%
Time	5 s
Force	5 g
Probe	40 mm

3. Results and Conclusions

According to the results obtaining by changing the concentration of the two types of EDTA (Table 6), the most appropriate chelating agent for this experiment is the disodium EDTA.

Table 5. Concentration of Ca Disodium EDTA and Disodium EDTA and force.

Ca Disodium EDTA Chelating Agent (%)	Max. Force (g)	Disodium EDTA Chelating Agent (%)	Max. Force (g)
0.03	210	0.03	200
0.1	200	0.1	200
0.2	200	0.2	180
1	200	1	80
2	200	2	55
3	200	3	25
5	200		
7	190		
10	180		

Using Ca Disodium EDTA at 10% w/w did not affect curd firmness.

As result of this experiment, the emulsifying agent selected is disodium EDTA at 0.2% w/w because it gave similar curd characteristics as the disodium citrate after 20 min of rennet addition (Figure 4).

Figure 4. Effect of disodium EDTA and Ca disodium after rennet.

IV. MATERIALS AND METHODS

Ingredients

1. Milk

Pasteurized whole milk from containing 3.97% fat and 3.5% protein was obtained from California Polytechnic State University Creamery. The majority of the milk was skimmed to 0.16% fat and mixed with whole milk to obtain mixed milk for cheese making of 0.59 % fat.

2. Rennet

CHY-MAX[®] by Chr. Hansen's, (Milwaukee, WI) was used for milk clotting. Chy-Max is a 100% pure chymosin produced by fermentation.

3. Starter cultures

A blend of three types of mesophilic commercial cultures of lactic acid bacteria were used; 850, *DSH-hb*, and *LH32*, from Chr. Hansen's, (Milwaukee, WI).

4. 10% Phosphoric acid

Food grade phosphoric acid (Innophop, Cranbury, NJ) solution was used to pre-acidify the mixed milk.

5. Disodium EDTA

Food grade EDTA was obtained from Dow Chemical Company, (Midland, MI)

6. Disodium Citrate

Food grade disodium citrate

Standardization

The milk used for experimental lowfat Cheddar cheeses was HTST pasteurized at 72°C for 16 s, cooled to 4°C, and stored at 4°C overnight until used for cheese manufacture the following day.

Milk for lowfat cheese was standardized to 0.53 % fat by adding 14.3 kg whole milk to 135 kg skim milk. Full fat Cheddar cheese (4.5 kg) manufactured by Cal Poly Creamery from the same lot of whole milk was obtained as full fat cheese was not standardized.

Experimental Design

The project was designed using randomized complete block designed. LF cheese variants and FFC were produced in 5 different occasions. The milk collected for every cheese making was taken from different lots. Cheese milk was divided into three lots (A, B & C). Sodium citrate (3Na) and disodium EDTA (EDTA) were added to vat A & B at the rate of 0.02% and 0.2% respectively. C served as control (LFC). Full fat cheddar (FFC) was manufactured at Cal Poly using the milled curd method and from the cheeses was made from the same batch of milk to avoid variation. Figure 5 summarizes the experimental design for this project.

Figure 5. Flow diagram of experimental design of FFC, 3Na, EDTA and LFC Cheddar cheeses.

Statistical Analysis

A randomized complete block design with one observation per treatment/block combination was used for the purposes of this work. Both, ANOVA and MANOVA models were used to compare treatment means. The specific model parameterization for the MANOVA is shown below. Statistic model nomenclature has been defined in Table 7. A univariate version of the model shown below was used for the ANOVA models.

$$y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij}$$

Table 6. Single factor MANOVA with blocks terminology definition.
Temporal vectors are for ripening measurement times (7, 30, 60, 90 and 120 days)

Single-factor MANOVA terminology	
Parameter	Definition
y_{ij}	Temporal vector response from j^{th} experiment associated with treatment i .
$j = 1, 2, 3, 4, 5$	5 cheese making trials
$i = 1, 2, 3, 4$	Treatments = FFC, LFC, EDTA, 3Na
μ	Overall mean temporal vector
T	Treatment effect vector
B	Experiment (block) effect vector
ε_{ij}	Random error vector

Post-hoc comparisons of individual treatment means following ANOVA analysis were analyzed using Tukey's Method (HSD). Tukey's HSD is more conservative than some other methods such as Fisher's LSD, meaning that we are less likely to declare pairs of means to be statistically significantly different. However, Tukey's HSD is less likely to report erroneous significant differences. As a conservative method, HSD also produces wider confident intervals (CI) for the pairwise differences than Fisher's LSD.

All statistical analysis was performed using JMP (JMP, Version 10. SAS Institute Inc., Cary, NC). All reported intervals are at the 95% level of confidence.

Manufacture of low fat Cheddar cheese

Raw milk from the Dairy California Polytechnic University dairy farm was collected and transported to the Creamery of the same institution for further pasteurization. A sample of the milk was analyzed for fat content by the Babcock method (Standards Methods for the Examination of Dairy Products # 15.083, 2004). Part of the whole pasteurized milk was skimmed and the fat content was determined using the same

protocol. In order to obtain low fat cheese milk, a mass balance using the Pearson Square was performed. Consequently, about 7 parts of skimmed milk was mixed with 1 part of whole milk for each of the three vats LF cheese (A, B & C). The standardized cheese milk contained ~0.57 %. The temperature of the standardized cheese milk for all vats was elevated to 31.1 °C. Cheese milk was acidified to pH 6.5 by circulative 10 % phosphoric acid using a pump. Then sodium citrate (3Na) and disodium EDTA (EDTA) were added to vats A & B at the rates of 0.02% and 0.2% (w/w) respectively, after which additional phosphoric acid was added such that the final pH prior renneting was 6.2. Arnatto was also used to provide color to the cheese (100 ml/454 kg milk). Three types of commercial DVS starter cultures (DSG-hb, LH32, 850) were added 0.0028 %, 0.0028 % & 0.02% (w/w of milk), respectively. The milk was ripened for 30 min with manual agitation. Chymosin rennet (35 ml/1000 kg of milk) was added to the milk, which was held quiescently until a coagulum formed (~ 20 min). The coagulum was cut using 5/8 knives and heated for 5 minutes. Then, the temperature was slowly raised to 35.5 °C in 30 minutes with continuous manual agitation. The whey was drained at pH 5.98. The cheese was immediately hand milled including an extra wash step. The wash water temperature was ~21°C (20 % w/w of milk) and held for 10 minutes. Cheese curd was salted in three installments 5 minutes apart (2.8 % w/w of curd). Curds were placed into a 10 kg Wilson-style stainless steel hoop that was pressed overnight at 276 Kpa. Pressed cheese was packed in polyethylene bags and ripened at 8°C. The flow diagram is shown in Figure 6.

Figure 6. Flow chart for the manufacture of Lowfat Cheddar cheeses.

Manufacture of full fat Cheddar cheese

Commercial FFC cheese (4.5 kg) was acquired from Cal Poly on five different occasions. The FF Cheddar cheese was manufactured using the milled curd method using the same batch of milk employed to manufacture LF cheddar cheeses and has been described below.

Pasteurized cheese milk containing ~4.0 % was tempered at 35.5 °C. Starter bacteria, DVS850, R604 and LH-32 were added at 0.06%, 0.06% and 0.001% w/w of milk, respectively. CaCl₂ and color (Arnnatto) were added at 0.06% and 0.1% w/w of milk, respectively. After 30 minutes of culture addition, chymosin was added (100 ml/1000 kg milk) and stirred for 2 min. Then the agitator and pump were turned off to allow coagulation. The coagulum was cut in 30 min followed by 5 min healing. Stirring begun and steam went on to start cooking the curd for 30 min until temperature reached 39°C. Whey was drained at pH 6.1 and the curd was pushed to both sides to let the curds fuse together. Cheddaring process was done over two hours keeping the vat temperature at 39°C and turning curd cheese piles every 15 min until a pH of 5.4 was reached. Cheese was milled and salt was posterior added at 2.5% w/w of curd in three installments 5 minutes apart. Curds were placed into a 20 kg stainless steel Wilson-style hoop that was pressed overnight at 276 Kpa. Pressed cheese was packed in polyethylene bags and ripened at 8°C. The flow diagram is showed in Figure 7.

Figure 7. Flow chart of manufacture of FFC using the Milled-curd method.

Compositional Analysis

Compositional analysis was performed for the five trials at different time points (Table 8) during 120 days of ripening.

Table 7. Parameters considered over time for all trials.

Parameter	7 days	30 days	60 days	90 days	120 days
pH		X			
Moisture		X			
Total solids		X			
Fat		X			
WSN (Secondary proteolysis)	X	X	X	X	X
Total protein	X				
Total nitrogen	X				
Urea-Page Gel (Primary proteolysis)	X	X	X	X	X
Texture Analysis	X	X	X	X	X
Insoluble Calcium content		X			

pH measurement.

pH was measured in duplicate by weighing 10 g of Cheddar cheese and 10 ml of de-ionized water into a 100 ml blender. Using medium speed, the mix was blended for 30 s. pH electrode was measured using Oakton waterproof spear tip double junction pH electrode (model WD-35634-40, Oakon Instruments, Vernon Hills-IL). Certified buffers solutions of pH 7.00 and 4.00±0.01 (Fischer Scientific, Fair Lawn, NJ) were used to calibrate the electrode. This measurement was carried out in duplicate on the 30th day of ripening.

Moisture and total solids content.

Moisture analyses of the cheese samples were performed using CEM microwave oven model Labwave™ 9000 (CEM Corp., Matthews, NC) AOAC # 926.08 (16th ed., vol. 2, 1995) official method was used. A slight modification in the sample weight was done by using 3 g of hand grated cheese instead of 10 g. The total solids of the sample were calculated and subtracted from 100 to give % moisture. This measurement was carried out in duplicate on the 30th day of ripening.

Salt Analysis using Chloride Method

NaCl content was measured in duplicate by chloride analysis using the Corning 926 Analyzer Salt Analyzer (Corning Medical and Scientific Glass Works). Five grams of cheese were grated and DI water was added to yield a total of 100 g. The water adjustment was determinate by using the next equation:

$$100 - (\text{sample wt. in grams}) * (\% \text{moisture in sample})$$

The cheese sample and the additional deionized (DI) water was added to a Whirlpak bag and homogenized using a Stomacher™ for three min. for three minutes. The content of the bag was filtered through a Whatman™ No. 41 paper. The Chloride Analyzer was adjusted by adding 250 ml of chloride meter standard (200 mg/L Cl, Sherwood) to the combined acid buffer (cat# 131-3751, Nelson James, Inc.). Calibration reading had to be between 97 and 103 for three repeated times. After calibration, 250 ml of filtrate was added to the combined acid buffer to get a reading. The conversion factor used to obtain

the percentage of salt in sample was 0.04 multiplied by the mg/L registered on the Analyzer.

Fat Content of milk

Fat concentration in milk was measured using the Mojonnier ether extraction modified official method # 989.05 - AOAC 16th ed., vol. 2, 1995). This method was the most reliable for milk because the fat content in the samples were expected to be low (4.5 to 0.16%). This measurement was carried out in duplicate the day previous of the cheese manufacture.

Fat Content in LF cheeses

Fat concentration in LF Cheddar cheeses was measured using the Mojonnier ether extraction modified official method # 989.05 (AOAC, 16th ed., vol. 2, 1995), due to an expected low fat content that could be better reported using this procedure. A slight modification during sample preparation was done. One gram of cheese was introduced into a Mojonnier flask and mixed with 8 ml of water at boiling point instead of room temperature. Samples were shaken and cooled prior to adding 3 ml of ammonium hydroxide. The ammonium reagent was mixed with the sample and Mojonnier flasks where placed into a water bath at 70°C for 5 min, rocking by hand every minute. The method was modified due to a poor dilution of the cheese components, mainly caseins. This measurement was carried out in duplicate on the 30th day of ripening and was reported as Fat Dry Matter (FDM).

Fat content in FFC

The fat content was determined by the Babcock Method described in Standard Methods for the Examination of Dairy Products # 15.083 for cheese (Wehr et al., 2004). This measurement was carried out in duplicate on the 30th day of ripening. Fat content in cheese was expressed as FDM.

Total Nitrogen Content

The total N of all the samples was determined by using 1g sample using the Kjeldahl Method # 920.123 (AOAC, 1995) using a the Tecator™ 2020 Digestor (Perstorp Analytical Company, Höganäs, Sweden) and a distillation unit Kjeltac™ 2200 (FOSS Instruments Höganäs, Sweden). Measurements were performed in duplicate for each cheese sample after 30 days of ripening.

Total Protein Analysis.

Total Protein percent was determined by multiplying total N (%) by the conversion factor 6.38.

$$\% \text{ protein} = \% N * 6.38$$

Water Soluble Nitrogen (WSN) Analysis.

Secondary Proteolysis. Water Soluble Nitrogen (WSN) content was determined by weighing 15 g cheese sample and placed into a Whirlpack bag and 30 ml of DI water was added. Samples were homogenized using a Stomacher™ for three minutes. The content

of the bag was filtered through a Whatman™ No. 41 with a glass wool in the bottom. The filtrate was collected into a 50 ml falcon tube, labeled and storage in the freezer until further nitrogen content analysis by the Kjeldahl method. Kjeldahl procedure used for WSN determination is the same described above for the nitrogen analysis of cheese. For this analysis instead of using 1g of cheese as sample, 1 g WSN extract was used. The titrant concentration used for this procedure was 0.01 N HCl (cat# SA54-20, Fisher Scientific) instead of 0.1N HCl due to a low nitrogen content of the samples.

Urea-Polyacrylamide Gel Electrophoresis

Electrophoresis, urea-PAGE, is particularly appropriate for monitoring primary proteolysis. Primary proteolysis was analyzed using urea-PAGE performed on all cheeses in duplicate. The analysis was done at 7, 30, 60, 90 and 120 days of ripening in order to identify the breakdown of caseins.

The preparation described is for two gels. Stacking gel (top part) was done by mixing 5.0 ml of 40% acrylamide solution (cat# 161-0148, Bio-Rad), 0.100 g of N, N' methylene bisacrylamide (cat# M7256-100G, SigmaAldrich) and 14.0 ml of separating gel buffer in a 50 ml falcon tube. To catalyze polymerization, 10 µl of N,N,N',N'-tetramethylethylenediamine or TEMED (cat# 161-800, Bio-Rad) was added with 75.2 µl of 10% (wt./vol.) ammonium persulfate or APS (cat# A7460-500G, Sigma-Aldrich). 7 ml of the stacking gel was pipetted into glass plates. DI water was pipetted on top of the gel to even the surface. 30 min were allowed for polymerization catalysis. The separating gel buffer was prepared by mixing 32.15 g of Tris (hydroxymethyl) methylamine (cat# 42457-5000, ACROS), 192.85 g Urea (BP169-212, Fisher Scientific), 2.86 ml of

concentrated HCl (cat# SA49, Fisher Scientific), and 500 ml DI water using a 50 ml falcon tube. pH was adjusted to 8.9.

The separating gel (bottom part) was prepared by adding 1.0 ml of 40% acrylamide solution, 0.020 gr of N, N' methylene bisacrylamide, and 9.0 ml of stacking gel buffer. The separating gel buffer was prepared by mixing 4.15 g of Tris (hydroxymethyl) methylamine, 150 g of Urea, and 2.2 ml of HCl, dissolving to 500 ml with deionized water and adjusting the pH to 7.6. Polymerization was catalyzed with 5 μ l of TEMED and 60 μ l of 10% (wt./vol.) ammonium persulfate (APS). Water from the surface of the gel was soaked and separating gel was pipetted to the top. 1.5 mm combs were inserted into gel and sat for 15 min to polymerization.

For the electrode buffer, 3.0 g Tris(hydroxymethyl)methylamine or Tris Base and 14.6 g glycine (cat# G8898-1KG, Sigma Aldrich) were dissolved in DI water and made up to 1L and adjusted to a pH 8.4.

Gels were placed into gel holder and inserted into mini gel tank, filled with electrode buffer and the combs were removed. The rest of the tank was filled with ~850 ml of electrode buffer. To pre-equilibrate, gels were run at 120 V for 10 min.

Then, the gels were loaded at equivalent protein level for each sample because were expected to have higher protein content than FFC, 4 μ l of LF cheese samples were loaded while FFC load was ~4.3 μ l. The sample was a mixture containing 10 mg cheese and 1 ml sample buffer. The sample buffer consisted of 0.75 g Tris (hydroxymethyl) methylamine, 49 g Urea, 0.4 ml of concentrated HCl, 0.7 ml β -mercaptoethanol (cat# BP176-100, Fisher Scientific), 0.15 g of Bromophenol Blue (cat# BP115-25, Fisher Scientific) dissolved to 100 ml with deionized water.

The gels were run at 150 V until Bromophenol blue tracking dye was seen at the bottom of the slab, which took between 90 and 120 min.

Gel stain solution was prepared by mixing 250 ml of (0.2% wt./vol) Coomassie Brilliant G250 (cat# BP100-25, Fisher Scientific) and 250 ml of 1 M H_2SO_4 . 1 M H_2SO_4 was prepared by mixing 13.9 ml concentrated H_2SO_4 (cat# A300SI-212, Fisher Scientific) with 236.1 ml of DI water. Coomassie and 1 M H_2SO_4 were combined and held overnight. Next day the mixture was filtered through Whatman™ No. 541 paper. Then 55.5 ml of 10 M KOH prepared by mixing 31.14 g KOH pellets (cat# P250-500, Fisher Scientific) to 24.4 ml of DI water and added to the filtrate. 66.66 g trichloroacetic acid powder (cat# A322-500, Fisher Scientific) was added into the previous solution. Gels were stained overnight. The Gel-Doc (Bio-Rad) and Quantity One® software v.4.6.3 (Bio-Rad) were used to capture gel images.

Texture Analysis for Cheese Samples

Cheese texture is a sensorial characteristic ultimately expressed in sensory descriptors defined by a trained texture panels Table 9. However, to establish and maintain a trained panel could be very costly and time consuming. For this reason, instrumentals methods based on force-compression have been designed to simulate compression of cheese between molars during chewing (Fox et al., 2000).

Test Model used for cheese texture examination was the Texture Profile Analysis (TPA). This method measure specific attributes for solid and semi-solid products such as hardness, cohesiveness, adhesiveness, springiness, gumminess, and chewiness. Figure 9

is a typical cheese TPA graph which includes the areas of compression and lengths in order to calculate the 5 principal texture parameters in cheese (Chen et al., 1979).

Figure 8. Typical TPA cheese behavior.

Table 9 provides the definition of Texture Parameters that are the most used to analyze semisolid products like cheese. TPA have been extensively used in literature (Bourne, 1978, Chen et al., 1979, Bryant et al., 1995, Pons and Fiszman, 1996, Szczesniak, 2002).

Table 8. Definition of Texture Parameters. (Bourne, 1978).

Texture Terminology	
Hardness	The hardness value is the peak force of the first compression of the product. Typically is the deepest compression for most products and is known as “first bite”
Cohesiveness	Cohesiveness is how well the product withstands a second deformation relative to how it behaved under the first deformation. Cohesiveness is the extent to which a material can be deformed before it ruptures. Cohesiveness = Area 2/Area 1
Springiness	Springiness is how well a product physically springs back after it has been deformed during the first compression. Springiness = Length 2/ Length 1
Chewiness	Chewiness only applies for solid products and is the energy required to masticate a product to a state ready for swallowing. Chewiness = Gumminess * Cohesiveness
Gumminess	Gumminess only applies to semi-solid products and is the energy required to disintegrate a semi-solid product to a state ready for swallowing. Gumminess = Hardness/Cohesiveness

TA-XT2[®] Texture Analyzer (Texture Technology Corp., Scarsdale, NY) was used to analyze texture parameters on all cheese samples at 7, 30, 60, 90 and 120 days of ripening. Expert Version 1.22 software (Stable Micro Systems, Scarsdale, NY) was utilized for this purpose.

Eight cheese cubes per samples were prepared by cutting 20 mm cubes; only cheese inside of the block was analyzed. Cheese samples were placed in weight boats, wrapped with foil, labeled and placed in the refrigerator for 3 h. Each weightboat was taken out of the refrigerator, the foil was removed and samples were sitting at room temperature for 30 min. 15 min gap was given between weightboats taking out of the refrigerator. Texture Analyzer Settings are described in Table 10.

Table 9. Texture Analysis Settings. Specific settings selected for TPA with the TA-XT2 Texture Analyzer.

Parameter	Selected Settings
Test Mode	T.P.A
Pre Test Speed	1.2 mm/s
Post Test speed	1.2 mm/s
Distance	10 mm
Compression	50%
Time	5 s
Force	5 g
Probe	40 mm

Calcium Determination

Calcium was determined by an atomic absorption spectroscopy. Ash content of cheese samples were determined by official method # 935.42 (AOAC, 1995). One gram cheese was weighed into a crucible and dried for an hour at 100°C and immediately placed in a Isotemp Programmable Forced-Draft Furnace at 525°C for at least 16 h. Samples were cooled down in a desiccator. Once the samples were cool, the ash was dissolved in 1.0 ml of HNO₃ and a small amount of DI water was added. The solution was transferred to a 250 ml volumetric flask and filled with DI water to the mark on the flask. 10 ml of diluted sample was pipetted into a 100 ml volumetric flask and 10 ml of 1% lanthanum oxide were added and topped with DI water to the mark. 45 ml of the solution was saved into a 50 ml falcon tube labeled and ready to analyze at the Soil Sciences department at California Polytechnic University- San Luis Obispo. The absorption spectrophotometer model SpectrTM AA55B (Varian Analytical Instruments,

Palo Alto, CA). The atomic absorption standard curve and sample were prepared according to the AOAC Official Method # 991.25 (AOAC, 1995).

1% lanthanum oxide was preparing by measuring 11.73g of La_2O_3 in 25ml HNO_3 diluted into 1L of DI water.

To convert the concentration of calcium Ca^{2+} in the cheese (ppm) to mg per portion we used the following equation:

$$\frac{\text{ppm}}{4} = \frac{\% \text{Ca}}{100} \times \frac{28\text{g}}{\text{portion}} \times \frac{1000\text{mg}}{\text{g}} = \frac{\text{mg}}{\text{portion}}$$

V. RESULTS AND DISCUSSION

Milk Composition

Milk was collected in 5 different occasions for LF Cheddar cheeses and FFC manufacture. Milk for LF cheeses were separated in 3 vats A, B, C (3Na, EDTA and LFC, respectively), was standardized (refer to standardization) with a fat content ~0.59% obtained by blending skimmilk standarized and whole milk. There was no evidence (Tukey adjusted $P > 0.05$) that fat content of mix milk for LFC differed between the 5 trials. Whole milk used for FFC was not standardized but the % fat ~4.0 between trials did not showed significant differences in the means according to Tukey LSD ($P > 0.05$). A summary of fat contents of whole milk and standarized milks used for FFC and LF cheeses is shown in Table 11.

Table 10. Means¹ Difference Tukey LSD for cheesemaking milk.

Type of milk		
Trial	%fat mix milk	% fat whole milk
7	0.6 a	4.17a
5	0.59 a	4.10 a
4	0.59 a	4.07 a
6	0.58 a	3.99 a
3	0.58 a	3.91 a

Means within the same column with different superscripts differ significantly ($P < 0.05$)

¹Mean of duplicate determination of five cheesemaking trials.

Since the only source of fat is located in the cream portion of the milk, during the manufacture of LF Cheddar cheese variants milk was standardized using a mixture of skim and full fat milk to a 0.59% fat content.

Cheese Composition

The composition of the cheeses was analyzed to identify changes in the batches.

1. pH

Table 11. Means¹ Difference Tukey LSD pH.

Treatment	Mean	Std Dev	Std error	F	P
3NA	4.62 ^b	0.2448	0.1095	9.1306	0.002
EDTA	4.568 ^b	0.2039	0.0912		
FFC	4.876 ^a	0.2023	0.0905		
LFC	4.662 ^b	0.2145	0.0959		

Means within the same column with different superscripts differ significantly (P<0.05)

¹Mean of duplicate determination of five cheesemaking trials.

3NA = Lowfat Cheddar disodium citrate

EDTA = Lowfat Cheddar disodium EDTA

FFC= Full fat Cheddar control

LFC = Lowfat Cheddar control

Table 12 summarizes the pH values obtained during the five trials, measured at day 7 and shows a significant evidence (P=0.002) among treatments between FFC and all LF cheeses. These results show no evidence that the addition of chelating agents effect the pH of the LF cheeses. Figure 13 shows the least square means plot of the LF Cheddar cheeses and FFC. Differences on the cheese making procedure reducing pH with 10% phosphoric acid and the addition of chelating agents resulted in a variation of the final pH between all LF Cheddar cheeses and FFC.

2. Total Solids (TS)

Table 12. Means¹ Difference Tukey LSD. Total Solids (TS)

Treatment	Mean	Std Dev	Std error	F	P
3NA	46.04 ^{bc}	1.8128	0.8107	186.75	<0.0001
EDTA	44.178 ^c	1.0205	0.4564		
FFC	63.786 ^a	1.4409	0.6444		
LFC	48.05 ^b	0.8911	0.3985		

Means within the same column with different superscripts differ significantly (P<0.05)

¹Mean of duplicate determination of five cheesemaking trials.

3NA = Lowfat Cheddar disodium citrate

EDTA = Lowfat Cheddar disodium EDTA

FFC= Full fat Cheddar control

LFC = Lowfat Cheddar control

% TS among samples show a significant difference (P = 0.0001) between FFC and all LF Cheddar cheeses. The reduction in fat content resulted in increased moisture content in the LF cheeses versions. Of the LF varieties, 3Na and LFC show had a significantly higher (P>0.05) moisture than EDTA.

3. Moisture

An increase of moisture between FFC and the LF Cheddar cheeses are evident showing a significant difference (P>0.05). Increase of moisture in the LF Cheddar cheese samples are due to some procedure modifications having the FFC milling manufacture as control.

- 1) Removal on fat content of the cheesemilk from 4 to 0.6%.
- 2) Reduction of the temperature during the heating from 35.5°C on FFC to 31.1°C of LF Cheddar cheese.

3) Reduction of the cooking temperature from 39.5°C for FFC to 31.1°C for LF Cheddar cheese.

4) Addition of a washing step for LF Cheddar cheeses.

The removal of fat from the cheese matrix has resulted in research to find methods to replace the low content of fat for moisture. In order to aid moisture absorption, measures listed above were taken in this regard. It has been proven that higher cooking temperature produced cheese with at lower moisture and decreased proteolysis during 50 days of storage at 4°C (Yun et al., 1993). Studies highlighted the important processing parameters for manufacturing LF cheddar cheeses which includes low temperature cooking and high pH at drain and milling (Mistry, 2001).

A wash step before hopping and pressing the curds was done using water at 21°C (20 % w/w of curd) for 10 minutes to help moisture increase.

In addition, a significant difference ($P>0.05$) between LFC and EDTA was detected.

Table 13. Means¹ Difference Tukey LSD. Moisture, %

Treatment	Mean	Std Dev	Std error	F	P
3NA	53.96 ^{ab}	1.8128	0.8107		
EDTA	55.822 ^a	1.0205	0.4564	186.74	<0.0001
FFC	36.214 ^c	1.4409	0.6444		
LFC	51.95 ^b	0.8911	0.3985		

Means within the same column with different superscripts differ significantly ($P<0.05$)

¹Mean of duplicate determination of five cheesemaking trials.

3NA = Lowfat Cheddar disodium citrate

EDTA = Lowfat Cheddar disodium EDTA

FFC= Full fat Cheddar control

LFC = Lowfat Cheddar control

4. Salt

Significant differences ($P = 0.0154$) between FFC and LF Cheddar cheese variants were detected. No significant differences ($P < 0.05$) among LF sample.

Table 14. Means¹ Difference Tukey LSD. Salt, %

Treatment	Mean	Std Dev	Std error	F	P
3NA	1.964 ^b	0.0089	0.004	5.2308	0.0154
EDTA	1.986 ^{ab}	0.0134	0.006		
FFC	2.012 ^a	0.0415	0.0185		
LFC	1.966 ^b	0.0134	0.006		

Means within the same column with different superscripts differ significantly ($P < 0.05$)

¹Mean of duplicate determination of five cheesemaking trials.

3NA = Lowfat Cheddar disodium citrate

EDTA = Lowfat Cheddar disodium EDTA

FFC = Full fat Cheddar control

LFC = Lowfat Cheddar control

5. Fat

A natural first grade full fat Cheddar cheese should contain % FDM 52-56 (Fox et al., 2004a). Standards of identity define Cheddar cheese as containing not more than 39% moisture and not more than 50% of FDM (Kosikowski and Mistry, 1997). For LF cheddar cheeses, the standard of identity requires a fat content of no more than 6%. As expected, the % fat and % FDM contents between LF cheeses and FFC were significantly different ($P = 0.0001$) (Table 16 & 17).

Table 15. Means¹ Difference Tukey LSD. Fat content, %

Treatment	Mean	Std Dev	Std error	F	P
3NA	5.968 ^b	0.0277	0.0124	12580	<0.0001
EDTA	5.85 ^b	0.142	0.0635		
FFC	32.68 ^a	0.497	0.2223		
LFC	5.908 ^b	0.0709	0.0317		

Means within the same column with different superscripts differ significantly (P<0.05)

¹Mean of duplicate determination of five cheesemaking trials.

3NA = Lowfat Cheddar disodium citrate

EDTA = Lowfat Cheddar disodium EDTA

FFC= Full fat Cheddar control

LFC = Lowfat Cheddar control

Table 16. Fat in Dry Matter, %

Treatment	%Fat	%Moisture	%TS	%FDM	P
3NA	5.97	53.96	46.04	13 ^b	<0.0001
EDTA	5.85	55.82	44.18	13 ^b	
FFC	32.68	36.21	63.79	51.23 ^a	
LFC	6.0	51.95	48.05	12.5 ^b	

Means within the same column with different superscripts differ significantly (P<0.05)

¹Mean of duplicate determination of five cheesemaking trials.

6. Ca^{2+}

Table 18 shows the calcium concentrations in the four cheeses. FFC is significantly different (P <0.05) than the 3Na and EDTA LF cheeses. Therefore, these two LF Cheddar cheeses are expected to have a softer texture because Ca^{+2} concentrations have decreased, and so, protein interaction happens to a lesser degree. On the other hand 3Na and EDTA cheeses have similar calcium concentration with the LFC. Thus, these three samples are expected to have similar textures. These results have a correlation with the preacidification using 10% phosphoric acid step during LF Cheddar cheese making. Examination of preacidification during mozzarella show similar results. At any level of preacidification, the calcium content was reduced. Furthermore, preacidification increased calcium, increased protein, and reduced fat recovery (Metzger et al., 2000).

Table 17. Means¹ Difference Tukey LSD. Calcium, mg/L

Treatment	Mean	Std Dev	Std error	F	P
3NA	1.91 ^b	0.4004	0.1791	9.35	<.0018
EDTA	2.01 ^b	0.2897	0.1296		
FFC	2.924 ^a	0.3891	0.174		
LFC	2.494 ^{ab}	0.1203	0.0538		

Means within the same column with different superscripts differ significantly (P<0.05)

¹Mean of duplicate determination of five cheesemaking trials.

3NA = Lowfat Cheddar disodium citrate

EDTA = Lowfat Cheddar disodium EDTA

FFC= Full fat Cheddar control

LFC = Lowfat Cheddar control

7. Total Nitrogen

Total nitrogen content in the FFC cheese was significantly different (P <0.05) than the other LF versions mainly due to the inclusion of fat in cheesemaking, indicating a lower protein concentration (Table 19). Among the LF Cheddar cheeses, the EDTA cheese had significantly lower (P = 0.0001) nitrogen which may be due to more dissociation of caseins and subsequent losses in the whey.

Table 18. Means¹ Difference Tukey LSD. Nitrogen, %

Treatment	Mean	Std Dev	Std error	F	P
3NA	5.852 ^a	0.2972	0.1329	312.86	<.0001
EDTA	5.578 ^b	0.2175	0.0973		
FFC	4.258 ^c	0.307	0.1373		
LFC	5.872 ^a	0.3227	0.1443		

Means within the same column with different superscripts differ significantly (P<0.05)

¹Mean of duplicate determination of five cheesemaking trials.

3NA = Lowfat Cheddar disodium citrate

EDTA = Lowfat Cheddar disodium EDTA

FFC= Full fat Cheddar control

LFC = Lowfat Cheddar control

Table 19. Means¹ Difference Tukey LSD. Total Protein, %

Treatment	Mean	Std Dev	Std error	F	P
3NA	37.340 ^a	1.9001	0.8497		<.0001
EDTA	35.584 ^b	1.3958	0.6242		
FFC	27.182 ^c	1.9471	0.8708		
LFC	37.466 ^a	2.0677	0.9247		

Means within the same column with different superscripts differ significantly (P<0.05)

¹Mean of duplicate determination of five cheesemaking trials.

3NA = Lowfat Cheddar disodium citrate

EDTA = Lowfat Cheddar disodium EDTA

FFC= Full fat Cheddar control

LFC = Lowfat Cheddar control

Cheese Texture

The texture of a cheese is determined primarily by its pH and the ratio of intact casein to moisture (Lawrence et al., 1987).

1. Hardness

If the total calcium content of cheese is reduced, then the amount of cross-linking between casein polymers is reduced and the cheese becomes softer (Metzger et al., 2000). Calcium plays an integral role in cheese texture by cross-linking proteins. As a result, the amount of calcium in cheese has an effect on texture (Metzger et al., 2000). Literature review correlates with the results. Even though calcium was captured by chelating agents as hypothesized, full fat Cheddar cheese remained as the softer after 120 days of ripening.

Table 20. TPA Hardness. Wilks' Lambda Test.

Hardness	F	df1	df2	P
Equality	2.54	15	22.5	0.0223
Parallel	1.35	12	24	0.26
Overall level	12.38	3	12	0.0006

Wilks' Lambda Test (Table 21) and the profiles (Figure 9) show that the mean TPA hardness profiles over time are significantly different ($P = 0.0223$). While there is no evidence that the relative changes in TPA hardness over the ripening are different (i.e., the hardness temporal profiles appear parallel) ($P = 0.26$), they do exhibit significant differences of mean overall TPA hardness ($P = 0.0006$). Figure 9 shows the changes on hardness of the four treatments during ripening. We can clearly see that overall FFC was the softer variant. EDTA and 3Na follow a very similar pattern during ripening and LFC remained harder compared to any other treatment.

Figure 9. TPA Hardness. Least square means of Lowfat and FFC cheeses during 120 days of ripening.

However as Table 22 shows, TPA hardness by day 7 between FFC, EDTA and 3Na does not show significant differences ($P>0.05$). Results of day 7, cheeses show that the texture between the FFC and the 3Na and EDTA were similar while LFC had the highest TPA hardness due to lack of chelating agents in the formulation.

After 30 d ripen significant differences were detected between the TPA hardness of FFC, LFC and 3Na ($P = 0.0025$). There was no significant differences in TPA hardness between FFC and LF cheese made with EDTA ($P>0.05$).

After 60, 90 and 120 day ripened, significant differences ($P = 0.0003, 0.00034, 0.001$ respectively) were recorded between all the LF Cheddar cheeses and FFC. The FFC had lower mean TPA hardness than all the LF cheeses.

Although no significant differences were detected after 30 days of ripening, Fig 9 shows a decrease on the TPA harness for all cheeses over ripening due to the breakdown of proteins.

An increase in hardness can be due to a more compact protein matrix. Although moisture content was increased in LF Cheddar cheeses it did not soften the cheese after 30 days. Similar results were obtained during texture examination of Cheddar cheeses with different fat content (Bryant et al., 1995).

Table 21. TPA Hardness. Tukey LSD Means¹ for all treatments during ripening.

Parameter by day	Treatment	Mean	Std Dev	Std error	F(3,12)	P
7 Hardness (g)	3NA	10554 ^{ab}	1216.6	544.07	7.48	0.0044
	EDTA	10047 ^b	1668.1	746		
	FFC	8657.7 ^b	1069.4	478.27		
	LFC	12748 ^a	2102.3	940.18		
30 Hardness (g)	3NA	9970 ^a	1779.1	795.64	8.644	0.0025
	EDTA	9216.9 ^{ab}	2036.4	910.69		
	FFC	7656.3 ^b	725.27	324.35		
	LFC	10863 ^a	1718.5	768.55		
60 Hardness (g)	3NA	8664.7 ^a	1490.5	666.57	13.7726	0.0003
	EDTA	9121.4 ^a	1248.8	558.5		
	FFC	6562.8 ^b	534.15	238.88		
	LFC	9965.2 ^a	964.96	431.54		
90 Hardness (g)	3NA	8158.9 ^a	898.58	401.86	7.9882	0.0034
	EDTA	7925.5 ^a	1470.9	657.82		
	FFC	5899.1 ^b	659.49	294.93		
	LFC	9003.1 ^a	1561.5	698.35		
120 Hardness (g)	3NA	7317.7 ^a	737.06	329.63	10.8499	0.001
	EDTA	7445.2 ^a	576.2	257.68		
	FFC	5527.7 ^b	544.24	243.39		
	LFC	8111.5 ^a	1185.9	530.34		

Means within the same column with different superscripts differ significantly (P<0.05)

¹Mean of duplicate determination of five cheesemaking trials.

3NA = Lowfat Cheddar disodium citrate

EDTA = Lowfat Cheddar disodium EDTA

FFC= Full fat Cheddar control

LFC = Lowfat Cheddar control

2. Cohesiveness

Wilks' Lambda Test (Table 23) and the profiles (Figure 10) show that the mean TPA cohesiveness profiles over time are not significantly different (P = 0.0642). While there is no evidence that the relative changes in TPA cohesiveness over the ripening are

different (i.e., the cohesiveness temporal profiles appear parallel) ($P = 0.4$), they do not exhibit significant differences of mean overall TPA cohesiveness ($P = 0.0673$).

Since the MANOVA results did not show any statistically significant differences, subsequent univariate ANOVAs are not needed or recommended. However, for consistency the results are displayed below (Table 24).

Table 22. TPA Cohesiveness. Wilks' Lambda Test.

Cohesiveness	F	Df1	df2	P
Equality	1.67	35	36.1	0.0642
Parallel	1.125	12	24	0.4
Overall level	3.1	3	12	0.0673

Figure 10. TPA Cohesiveness. Least square means of LF cheeses and FFC during 120 days ripening.

Table 23. TPA Cohesiveness. Tukey LSD Means¹ for all treatments during ripening.

Parameter by day	Treatment	Mean	Std Dev	Std error	F(3,12)	P
7 Cohesiveness (g)	3NA	0.586 ^a	0.1539	0.0688	2.76	0.0881
	EDTA	0.444 ^a	0.0467	0.0209		
	FFC	0.444 ^a	0.0573	0.0256		
	LFC	0.54 ^a	0.1428	0.0639		
30 Cohesiveness (g)	3NA	0.462 ^a	0.0835	0.0373	1.14	0.3718
	EDTA	0.446 ^a	0.0619	0.0277		
	FFC	0.424 ^a	0.0773	0.0346		
	LFC	0.51 ^a	0.0946	0.0423		
60 Cohesiveness (g)	3NA	0.48 ^a	0.1098	0.0491	2.691	0.0932
	EDTA	0.434 ^a	0.0684	0.0306		
	FFC	0.42 ^a	0.0367	0.0164		
	LFC	0.484 ^a	0.0783	0.035		
90 Cohesiveness (g)	3NA	0.404 ^a	0.055	0.0246	0.8261	0.5045
	EDTA	0.438 ^a	0.0826	0.0369		
	FFC	0.41 ^a	0.0524	0.0235		
	LFC	0.464 ^a	0.1074	0.048		
120 Cohesiveness (g)	3NA	0.406 ^a	0.0456	0.0204	0.6251	0.6124
	EDTA	0.364 ^a	0.0619	0.0277		
	FFC	0.42 ^a	0.08	0.0358		
	LFC	0.432 ^a	0.1305	0.0583		

Means within the same column with different superscripts differ significantly (P<0.05)

¹Mean of duplicate determination of five cheesemaking trials.

3NA = Lowfat Cheddar disodium citrate

EDTA = Lowfat Cheddar disodium EDTA

FFC= Full fat Cheddar control

LFC = Lowfat Cheddar control

3. Springiness

Since the MANOVA results did not show any statistically significant differences, subsequent univariate ANOVAs are not needed or recommended. However, for consistency the results are displayed below. While the ANOVA for 120 day Springiness Table 25 shows significant evidence for a difference in mean springiness across the cheeses, these results should be interpreted with caution, as the initial MANOVA did not

provide any evidence for difference across all times. This apparent difference could easily be a “false positive” or Type I error.

Table 24. TPA Springiness. Wilks’ Lambda Test.

Springiness	F	df1	df2	P
Equality	1.06	15	22.5	0.44
Parallel	1.15	12	24	0.36
Overall level	1.74	3	12	0.22

Figure 11. TPA Springiness. Least square means of LF cheeses and FFC during 120 days ripening.

There was no significant difference in TPA springiness ($P > 0.05$) irrespective of the type of cheese over ripening time until 90 days (Table 25). After 120 d ripened the TPA springiness of the control FFC was significantly different than control LF cheese. No specific pattern was observed in (Fig 11).

Table 25. TPA Springiness. Tukey LSD Means¹ for all treatments during ripening.

Parameter by day	Treatment	Mean	Std Dev	Std error	F(3,12)	P
7 Springiness (g)	3NA	0.248 ^a	0.1137	0.0508	0.8945	0.4721
	EDTA	0.212 ^a	0.0259	0.0116		
	FFC	0.218 ^a	0.0576	0.0258		
	LFC	0.18 ^a	0.02	0.0089		
30 Springiness (g)	3NA	0.23 ^a	0.1079	0.0483	2.3098	0.1282
	EDTA	0.316 ^a	0.1016	0.0455		
	FFC	0.314 ^a	0.0991	0.0443		
	LFC	0.206 ^a	0.0568	0.0254		
60 Springiness (g)	3NA	0.284 ^a	0.1036	0.0463	0.637	0.6054
	EDTA	0.328 ^a	0.1701	0.0761		
	FFC	0.264 ^a	0.0635	0.0284		
	LFC	0.274 ^a	0.1069	0.0478		
90 Springiness (g)	3NA	0.308 ^a	0.0779	0.0348	0.6153	0.6182
	EDTA	0.3 ^a	0.1102	0.0493		
	FFC	0.294 ^a	0.0706	0.0316		
	LFC	0.248 ^a	0.0589	0.0263		
120 Springiness (g)	3NA	0.31 ^{ab}	0.1168	0.0522	4.064	0.0331
	EDTA	0.3 ^{ab}	0.0381	0.017		
	FFC	0.434 ^a	0.0856	0.0383		
	LFC	0.282 ^b	0.0581	0.026		

Means within the same column with different superscripts differ significantly (P<0.05)

¹Mean of duplicate determination of five cheesemaking trials.

3NA = Lowfat Cheddar disodium citrate

EDTA = Lowfat Cheddar disodium EDTA

FFC= Full fat Cheddar control

LFC = Lowfat Cheddar control

4. Gumminess

Table 26. TPA Gumminess. Wilks' Lambda Test.

Gumminess	F	df1	df2	P
Equality	3.43	15	22.5	0.0042
Parallel	2.85	12	24	0.14
Overall level	11.13	3	12	0.0009

Wilks' Lambda Test (Table 26) and the profiles (Figure 12) show that the mean TPA gumminess profiles over time are significantly different ($P = 0.0042$). While there is no evidence that the relative changes in TPA gumminess over the ripening are different (i.e., the hardness temporal profiles appear parallel) ($P = 0.14$), they do exhibit significant differences of mean overall TPA hardness ($P = 0.0009$). Figure 12 shows the changes on hardness of the four treatments during ripening. We can clearly see that overall FFC was the gummiest variant. EDTA and 3Na follow a very similar pattern during ripening and LFC remained harder compared to any other treatment.

Figure 12. TPA Gumminess. Least square means of LFC and FFC during ripening.

Table 27. TPA Gumminess. Tukey LSD Means¹ for all treatments during ripening.

Parameter by day	Treatment	Mean	Std Dev	Std error	F(3,12)	P
7 Gumminess (g)	3NA	6254.8 ^{ab}	1441	644.45	8.884	0.0022
	EDTA	4376.1 ^{bc}	902.12	403.44		
	FFC	3804.5 ^c	421.13	188.33		
	LFC	7075 ^a	2629.5	1176		
30 Gumminess (g)	3NA	4726.9 ^{ab}	1540.2	688.81	6.7708	0.0063
	EDTA	4018.2 ^{ab}	1560.4	697.84		
	FFC	3217 ^b	531.86	237.86		
	LFC	5549.4 ^a	1393.6	623.22		
60 Gumminess (g)	3NA	4648.1 ^a	1014.3	453.6	14.6422	0.0003
	EDTA	3859 ^a	1158.5	518.09		
	FFC	2744.7 ^b	293.73	131.36		
	LFC	4869.6 ^a	1191.5	532.87		
90 Gumminess (g)	3NA	3279.2 ^a	572.11	255.86	4.5797	0.0233
	EDTA	3436.4 ^a	781.57	349.53		
	FFC	2435.3 ^a	519.58	232.36		
	LFC	4278.3 ^a	1400.1	626.14		
120 Gumminess (g)	3NA	2963.1 ^a	477.26	213.44	1.5792	0.2458
	EDTA	2738.7 ^a	471.08	210.67		
	FFC	2366.8 ^a	660.15	295.23		
	LFC	3506.6 ^a	1250.8	559.39		

Means within the same column with different superscripts differ significantly (P<0.05)

¹Mean of duplicate determination of five cheesemaking trials.

3NA = Lowfat Cheddar disodium citrate

EDTA = Lowfat Cheddar disodium EDTA

FFC= Full fat Cheddar control

LFC = Lowfat Cheddar control

TPA Gumminess in FFC was significantly lower (P<0.05) than those of LFC and 3Na during the first 60 days of ripening. After 90 and 120 days ripen the mean TPA treatments were not significantly different (P>0.05). Increase in TPA Gumminess in LF Cheddar cheeses can be attributed to the firm and elastic protein (Table 27).

5. Chewiness

Wilks' Lambda Test (Table 28) and the profiles (Figure 13) show that the mean TPA chewiness profiles over time are not significantly different ($P = 0.636$). While there is no evidence that the relative changes in TPA chewiness over the ripening are different (i.e., the chewiness temporal profiles appear parallel) ($P = 0.31$), they do exhibit significant differences of mean overall TPA chewiness ($P = 0.0055$).

Table 28. TPA Chewiness. Wilks' Lambda Test.

Chewiness	F	df1	df2	P
Equality	2.02	15	22	0.636
Parallel	1.25	12	24	0.3098
Overall level	7.05	3	12	0.0055

Figure 13. TPA Chewiness. Least square means of LFC and FFC during ripening.

There was no significant difference in TPA chewiness ($P>0.05$) between all cheeses over ripening time as shown in Table 29.

Table 29. TPA Chewiness. Tukey LSD Means¹ for all treatments during ripening.

Parameter by day	Treatment	Mean	Std Dev	Std error	F (3,12)	P
7 Chewiness (g)	3NA	1356 ^a	372.93	166.78	3.158	0.0643
	EDTA	969.35 ^a	113.96	50.964		
	FFC	825.38 ^a	180.58	80.759		
	LFC	1242.4 ^a	368.14	164.64		
30 Chewiness (g)	3NA	983.55 ^a	232.07	103.78	0.7425	0.547
	EDTA	1200.3 ^a	387.75	173.41		
	FFC	971.03 ^a	213.91	95.664		
	LFC	1095.6 ^a	152.83	68.349		
60 Chewiness (g)	3NA	1178.3 ^a	179.55	80.299	10.3542	0.0012
	EDTA	1124 ^a	335.82	150.18		
	FFC	710.59 ^a	114.31	51.121		
	LFC	1242.2 ^a	300.17	134.24		
90 Chewiness (g)	3NA	991.49 ^a	145.03	64.859	3.1925	0.0626
	EDTA	970.19 ^a	213.53	95.495		
	FFC	694.63 ^a	103.98	46.499		
	LFC	1011.4 ^a	220.02	98.397		
120 Chewiness (g)	3NA	896.4 ^a	354.56	158.57	0.4928	0.6939
	EDTA	857.57 ^a	197.38	88.271		
	FFC	1040.7 ^a	360.58	161.26		
	LFC	939.24 ^a	193.8	86.669		

Means within the same column with different superscripts differ significantly ($P<0.05$)

¹Mean of duplicate determination of five cheesemaking trials.

3NA = Lowfat Cheddar disodium citrate

EDTA = Lowfat Cheddar disodium EDTA

FFC= Full fat Cheddar control

LFC = Lowfat Cheddar control

Cheese Proteolysis

1. Primary Proteolysis

The firmness and elasticity of cheese is more related to casein breakdown than to fat (Ardö, 1997). The flavor of LF Cheddar cheeses, especially the hard varieties, is usually atypical of their full fat counterparts (Childs and Drake, 2009). In LF Cheddar cheese the lack of and an imbalance of flavor has been associated with lowered levels of fatty acids such as butanoic and hexanoic acids and methyl ketones (Mistry, 2001). In this thesis project, amino acid determination was not performed. Primary and secondary proteolysis was examined during the ripening of the cheeses as proteolysis of α_{s1} -casein to α_{s1} -I casein is thought to lead to initial softening of cheese.

Urea-PAGE (Fig 14, 15, and 16) shows how α_{s1} -casein is hydrolyzed by chymosin during ripening of the LF cheddar cheeses and FFC. Although β -casein is very resistant to rennet hydrolysis, in cheese, β -casein in solution is hydrolyzed slowly by plasmin, producing γ^1 , γ^2 , γ^3 -caseins. Chymosin, to a lesser extent, and plasmin are mainly responsible for primary proteolysis (Fox and McSweeney, 1998).

Fig 14, 15 and 16 during 120 d ripen shows a decreasing concentration in concentration of β -CN. Although densitometry analysis was not taking account in this project, figures showed a slow decrement of this casein over time. Proper breakdown of the casein matrix, β -casein in particular, has been shown to make the cheese softer (Andersen et al., 2010).

On the SDS-PAGE qualitatively differences were observed in the proteolysis of α_{s1} -casein which is visible after 30 days of ripening and becomes more obvious at 60, 90 and

120 days. The α_{s1} -casein in the EDTA sample, especially, seems to undergo more proteolysis compared to the other treatments. Individual images in the polyacrylamide gels show a rate of degradation of α_{s1} -casein, in LF Cheddar variants, faster than β -casein. Although there is no difference in proteolysis rate by statistical analysis, we can clearly see a decrease in the casein band through all the treatments, therefore we can accredit these findings to methodology limitations.

Figure 14. Polyacrylamide gel electrophoresis of LFC and FFC after 7 and 30 days of ripening. (Trial 3). Standard (STD), full fat Cheddar cheese control (FFC), lowfat Cheddar cheese control (LFC), disodium citrate lowfat Cheddar cheese (3Na) and lowfat Cheddar cheese disodium EDTA.

Figure 15. Polyacrylamide gel electrophoresis of LFC and FFC cheeses after 60 and 90 days of ripening.
(Trial 3). Full fat Cheddar cheese control (FFC), lowfat Cheddar cheese control (LFC), disodium citrate lowfat Cheddar cheese (3Na) and lowfat Cheddar cheese disodium EDTA, standard (STD).

Figure 16. Polyacrylamide gel electrophoresis of LFC and FFC after 120 days of ripening. (Trial 3). Full fat cheddar cheese control (FFC), lowfat Cheddar cheese control (LFC), standard (STD), disodium citrate lowfat Cheddar cheese (3Na) and lowfat Cheddar cheese disodium EDTA.

2. Secondary Proteolysis. Water Soluble Nitrogen (WSN)

Secondary proteolysis of the cheeses was quantitatively examined by determination of water soluble nitrogen (WSN).

Table 30. Water Soluble Nitrogen (WSN) content. Wilks' Lambda Test.

WSN	F	df1	df2	P
Equality	3.15	35	36	0.0004
Parallel	1.17	12	24	0.36
Overall level	16.5	3	12	

Wilks' Lambda Test (Table 30) and the profiles (Figure 17) show that the mean WSN profiles over time are significantly different ($P = 0.0004$). While there is no evidence that the relative changes in WSN over the ripening are different (i.e., the WSN temporal

profiles appear parallel) ($P = 0.36$), they do exhibit significant differences of mean overall WSN ($P < 0.0001$). Fig 17 shows % WSN in cheeses increase overtime for all the variants.

Figure 17. Water Soluble Nitrogen (WSN) content. Least square means of LFC and FFC during ripening.

Significant differences ($P = 0.0125$) were detected at day 7 (Table 31) between the FFC and all the LF cheddar variants being FFC the sample with lesser value. By day 30, no significant difference ($P > 0.05$) was detected between FFC and 3Na. LF cheeses after day 60 no significant differences between cheese were detected with the exception of day 90 where EDTA and FFC show a significant difference ($P < 0.05$). Results correlate with the literature, secondary proteolysis is due to the action of chymosin and starter

proteinases (Farkye, 1995). Therefore, this method may be a better indicator of residual rennet or whether proteases have been used (Rank et al., 1985).

Table 31. Water Soluble Nitrogen (WSN) content. Tukey LSD Means¹ for all treatments during ripening.

Parameter by day	Treatment	Mean	Std Dev	Std error	F(3,12)	P
7 % WSN	3NA	6.858 ^a	0.4716	0.2109	5.577	0.0125
	EDTA	6.766 ^a	0.5108	0.2284		
	FFC	5.654 ^b	0.679	0.3036		
	LFC	6.91 ^a	0.5236	0.2342		
30 % WSN	3NA	10.824 ^{ab}	2.2051	0.9861	6.3	0.0082
	EDTA	11.568 ^a	1.1843	0.5296		
	FFC	9.264 ^b	1.2873	0.5757		
	LFC	12.472 ^a	0.5771	0.2581		
60 % WSN	3NA	14.878 ^a	2.8747	1.2856	2.006	0.1669
	EDTA	16.624 ^a	4.7387	2.1192		
	FFC	14.256 ^a	2.8012	1.2527		
	LFC	16.544 ^a	3.4921	1.5617		
90 % WSN	3NA	17.226 ^{ab}	1.7588	0.7865	4.7512	0.0208
	EDTA	19.55 ^a	3.2607	1.4582		
	FFC	17.132 ^b	0.9166	0.4099		
	LFC	18.902 ^{ab}	2.7136	1.2136		
120 % WSN	3NA	20.574 ^a	2.023	0.9047	2.3679	0.122
	EDTA	22.168 ^a	0.5622	0.2514		
	FFC	20.316 ^a	2.1948	0.9815		
	LFC	21.044 ^a	2.0167	0.9019		

Means within the same column with different superscripts differ significantly (P<0.05)

¹Mean of duplicate determination of five cheesemaking trials.

3NA = Lowfat Cheddar disodium citrate

EDTA = Lowfat Cheddar disodium EDTA

FFC= Full fat Cheddar control

LFC = Lowfat Cheddar control

VI. CONCLUSIONS

- 1) Significant reduction of Ca^{2+} content in EDTA and 3Na Cheddar cheeses.
- 2) Addition of chelating agents, especially EDTA, showed to be the most effective in soften the cheese before 60 days.
- 3) Reduction of Ca^{2+} content was quantified. At 0.02% sodium citrate and 0.2 % disodium EDTA addition, Ca^{2+} decreased in a rate of 34.7 and 31.3% respectively compared to FFC. Reduction of 14.7 % on the content of Ca^{2+} for the LF cheese control was registered in comparison to FFC.

VII. DIRECTIONS FOR FUTURE RESEARCH

Further research of the complexity of the texture of LF Cheddar cheeses is a potential research due to a customer need of having a natural cheese low in fat.

Possible research directions are as follows:

1. Investigate the effects of addition of higher concentrations of chelating agents in the cheese making process.
2. Explore the possibility to combine LF Cheddar cheese technologies, such as ultrafiltration, addition of yeast or application of fat mimetics.
3. Flavor profile of the LF cheeses could be examined using Gas Chromatography Mass.
4. A trained panel to develop a descriptive sensory evaluation will lead to a better understanding of the texture of LF cheeses.

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APPENDIX A

Table 32. Dietary Reference Intake Values for Calcium by Life-Stage Group for U.S. and Canada^a

Life-Stage Group	(mg/day)
0 to 6 months	210
6 to 12 months	270
1 through 3 years	500
4 through 8 years	800
9 through 13 years	1300
14 through 18 years	1300
19 through 30 years	1000
31 through 50 years	1000
51 through 70 years	1200
>70 years	1200
Pregnancy	
≤18 years	1300
19 through 50 years	1000
Lactation	
≤18 years	1300
19 through 50 years	1000

^aFood and Nutrition Board, Institute of Medicine, National Academy Press, Washington, DC, 1997.

APPENDIX B

Cheddar Cheese Composition

1. Statistical Data for pH

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	0.27437500	9.1306	0.0020*
trial	4	4	0.63368000	15.8156	<.0001*

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Sq Mean	Std Error	Mean
3NA	4.6200000	0.04475861	4.62000
EDTA	4.5680000	0.04475861	4.56800
FFC	4.8760000	0.04475861	4.87600
LFC	4.6620000	0.04475861	4.66200

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ $Q=2.9688$

LSMean[i]	LSMean[j]			
	3NA	EDTA	FFC	LFC
Mean[i]-Mean[j]				
Std Err Dif				
Lower CL Dif				
Upper CL Dif				
3NA				
	0	0.052	-0.256	-0.042
	0	0.0633	0.0633	0.0633
	0	-0.1359	-0.4439	-0.2299
	0	0.23992	-0.0681	0.14592
EDTA	-0.052	0	-0.308	-0.094
	0.0633	0	0.0633	0.0633
	-0.2399	0	-0.4959	-0.2819
	0.13592	0	-0.1201	0.09392
FFC	0.256	0.308	0	0.214
	0.0633	0.0633	0	0.0633
	0.06808	0.12008	0	0.02608
	0.44392	0.49592	0	0.40192
LFC	0.042	0.094	-0.214	0
	0.0633	0.0633	0.0633	0
	-0.1459	-0.0939	-0.4019	0
	0.22992	0.28192	-0.0261	0

Level		Least Sq Mean
FFC	A	4.8760000
LFC	B	4.6620000
3NA	B	4.6200000
EDTA	B	4.5680000

Levels not connected by same letter are significantly different.

3. Statistical Data for Total Solids (TS) content

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	1211.8943	186.7461	<.0001*
trial	4	4	2.8333	0.3274	0.8542

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
3NA	46.040000	0.65775021	46.0400
EDTA	44.178000	0.65775021	44.1780
FFC	63.786000	0.65775021	63.7860
LFC	48.050000	0.65775021	48.0500

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ Q= 2.9688

	LSMean[j]			
	3NA	EDTA	FFC	LFC
Mean[i]-Mean[j]				
Std Err Dif				
Lower CL Dif				
Upper CL Dif				
3NA				
	0	1.862	-17.746	-2.01
	0	0.9302	0.9302	0.9302
	0	-0.8996	-20.508	-4.7716
	0	4.62358	-14.984	0.75158
EDTA				
	-1.862	0	-19.608	-3.872
	0.9302	0	0.9302	0.9302
	-4.6236	0	-22.37	-6.6336
	0.89958	0	-16.846	-1.1104
FFC				
	17.746	19.608	0	15.736
	0.9302	0.9302	0	0.9302
	14.9844	16.8464	0	12.9744
	20.5076	22.3696	0	18.4976
LFC				
	2.01	3.872	-15.736	0
	0.9302	0.9302	0.9302	0
	-0.7516	1.11042	-18.498	0
	4.77158	6.63358	-12.974	0

Level	Least Sq Mean
FFC A	63.786000
LFC B	48.050000
3NA B C	46.040000
EDTA C	44.178000

Levels not connected by same letter are significantly different.

4. Statistical Data for Moisture content

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	1211.8943	186.7461	<.0001*
trial	4	4	2.8333	0.3274	0.8542

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Sq Mean	Std Error	Mean
3NA	53.960000	0.65775021	53.9600
EDTA	55.822000	0.65775021	55.8220
FFC	36.214000	0.65775021	36.2140
LFC	51.950000	0.65775021	51.9500

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ Q= 2.9688

		LSMean[j]			
Mean[i]-Mean[j]	3NA	EDTA	FFC	LFC	
Std Err Dif					
Lower CL Dif					
Upper CL Dif					
3NA		0	-1.862	<u>17.746</u>	2.01
		0	0.9302	<u>0.9302</u>	0.9302
		0	-4.6236	<u>14.9844</u>	-0.7516
		0	0.89958	<u>20.5076</u>	4.77158
EDTA	1.862	0	<u>19.608</u>	<u>3.872</u>	
	0.9302	0	<u>0.9302</u>	<u>0.9302</u>	
	-0.8996	0	<u>16.8464</u>	<u>1.11042</u>	
	4.62358	0	<u>22.3696</u>	<u>6.63358</u>	
FFC		<u>-17.746</u>	<u>-19.608</u>	0	<u>-15.736</u>
		<u>0.9302</u>	<u>0.9302</u>	0	<u>0.9302</u>
		<u>-20.508</u>	<u>-22.37</u>	0	<u>-18.498</u>
		<u>-14.984</u>	<u>-16.846</u>	0	<u>-12.974</u>
LFC		-2.01	<u>-3.872</u>	<u>15.736</u>	0
		0.9302	<u>0.9302</u>	<u>0.9302</u>	0
		-4.7716	<u>-6.6336</u>	<u>12.9744</u>	0
		0.75158	<u>-1.1104</u>	<u>18.4976</u>	0

Level	Least Sq Mean
EDTA A	55.822000
3NA A B	53.960000
LFC B	51.950000
FFC C	36.214000

Levels not connected by same letter are significantly different.

5. Statistical Data for Salt content

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	0.00748000	5.2308	0.0154*
trial	4	4	0.00292000	1.5315	0.2549

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
3NA	1.9640000	0.00976388	1.96400
EDTA	1.9860000	0.00976388	1.98600
FFC	2.0120000	0.00976388	2.01200
LFC	1.9660000	0.00976388	1.96600

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ $Q=2.9688$

		LSMean[j]			
	Mean[i]-Mean[j]	3NA	EDTA	FFC	LFC
	Std Err Dif				
	Lower CL Dif				
	Upper CL Dif				
LSMean[i]	3NA				
		0	-0.022	-0.048	-0.002
		0.01381	0.01381	0.01381	
		0	-0.063	-0.089	-0.043
		0.01899	-0.007	0.03899	
	EDTA				
		0.022	0	-0.026	0.02
		0.01381	0	0.01381	0.01381
		-0.019	0	-0.067	-0.021
		0.06299	0	0.01499	0.06099
	FFC				
		0.048	0.026	0	0.046
		0.01381	0.01381	0	0.01381
		0.00701	-0.015	0	0.00501
		0.08899	0.06699	0	0.08699
	LFC				
	0.002	-0.02	-0.046	0	
	0.01381	0.01381	0.01381	0	
	-0.039	-0.061	-0.087	0	
	0.04299	0.02099	-0.005	0	

Level	Least Sq Mean
FFC A	2.0120000
EDTA A B	1.9860000
LFC B	1.9660000
3NA B	1.9640000

Levels not connected by same letter are significantly different.

6. Statistical Data for Fat content

Whole Model

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	2687.6759	12580.10	<.0001*
trial	4	4	0.2372	0.8326	0.5298

treatment

Least Squares Means Table

Level	Sq Mean	Std Error	Mean
3NA	5.968000	0.11934404	5.9680
EDTA	5.850000	0.11934404	5.8500
FFC	32.680000	0.11934404	32.6800
LFC	5.908000	0.11934404	5.9080

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha = 0.050$ Q = 2.9688

LSMean[i]	LSMean[j]			
	3NA	EDTA	FFC	LFC
Mean[i]-Mean[j]				
Std Err Dif				
Lower CL Dif				
Upper CL Dif				
3NA		0 0.118 -26.712 0.06	0 0.16878 0.16878 0.16878	0 -0.3831 -27.213 -0.4411
		0 0.16878 0.16878 0.16878	0 -0.3831 -27.213 -0.4411	0 0.61907 -26.211 0.56107
EDTA	-0.118 0 -26.83 -0.058		0 0.16878 0.16878 0.16878	-0.6191 0 -27.331 -0.5591
	0.16878 0 0.16878 0.16878	-0.6191 0 -27.331 -0.5591	0 0.16878 0.16878 0.16878	0.38307 0 -26.329 0.44307
FFC	26.712 26.83 0 26.772	0.16878 0.16878 0 0.16878		26.2109 26.3289 0 26.2709
	27.2131 27.3311 0 27.2731	26.2109 26.3289 0 26.2709	27.2131 27.3311 0 27.2731	
LFC	-0.06 0.058 -26.772 0	0.16878 0.16878 0.16878 0	-0.5611 -0.4431 -27.273 0	
	0.44107 0.55907 -26.271 0	0.44107 0.55907 -26.271 0	0.44107 0.55907 -26.271 0	0

Level		Least Sq Mean
FFC	A	32.680000
3NA	B	5.968000
LFC	B	5.908000
EDTA	B	5.850000

Levels not connected by same letter are significantly different.

7. Statistical Data for C^{2+} . Day7

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	3.2922550	9.3545	0.0018*
trial	4	4	0.2326700	0.4958	0.7393

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
3NA	1.9100000	0.15317582	1.91000
EDTA	2.0100000	0.15317582	2.01000
FFC	2.9240000	0.15317582	2.92400
LFC	2.4940000	0.15317582	2.49400

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ $Q=2.9688$

		LSMean[j]			
	Mean[i]-Mean[j]	3NA	EDTA	FFC	LFC
	Std Err Dif				
	Lower CL Dif				
	Upper CL Dif				
LSMean[i]	3NA				
		0	-0.1	-1.014	-0.584
		0.21662	0.21662	0.21662	0.21662
		0	-0.7431	-1.6571	-1.2271
		0.54311	-0.3709	0.05911	0.05911
	EDTA				
		0.1	0	-0.914	-0.484
		0.21662	0	0.21662	0.21662
		-0.5431	0	-1.5571	-1.1271
		0.74311	0	-0.2709	0.15911
	FFC				
		1.014	0.914	0	0.4311
	0.21662	0.21662	0	0.21662	
	0.37089	0.27089	0	-0.21311	
	1.65711	1.55711	0	1.07311	
LFC					
	0.584	0.484	-0.43	0	
	0.21662	0.21662	0.21662	0	
	-0.0591	-0.1591	-1.0731	0	
	1.22711	1.12711	0.21311	0	

Level	Least Sq Mean
FFC A	2.9240000
LFC A B	2.4940000
EDTA B	2.0100000
3NA B	1.9100000

Levels not connected by same letter are significantly different.

8. Statistical Data for Nitrogen.

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	8.8126800	312.8670	<.0001*
trial	4	4	1.2234500	32.5761	<.0001*

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Sq Mean	Std Error	Mean
3NA	5.8520000	0.04333397	5.85200
EDTA	5.5780000	0.04333397	5.57800
FFC	4.2580000	0.04333397	4.25800
LFC	5.8720000	0.04333397	5.87200

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ $Q=2.9688$

	LSMean[j]			
Mean[i]-Mean[j]	3NA	EDTA	FFC	LFC
Std Err Dif				
Lower CL Dif				
Upper CL Dif				
3NA				
	0	0.274	1.594	-0.02
	0	0.06128	0.06128	0.06128
	0	0.09206	1.41206	-0.2019
	0	0.45594	1.77594	0.16194
EDTA	-0.274			
	0.06128	0	1.32	-0.294
	-0.4559	0	0.06128	0.06128
	-0.0921	0	1.13806	-0.4759
	-0.0921	0	1.50194	-0.1121
FFC	-1.594	-1.32		
	0.06128	0.06128	0	-1.614
	-1.7759	-1.5019	0	0.06128
	-1.4121	-1.1381	0	-1.7959
	-1.4121	-1.1381	0	-1.4321
LFC	0.02	0.294	1.614	
	0.06128	0.06128	0.06128	0
	-0.1619	0.11206	1.43206	0
	0.20194	0.47594	1.79594	0

Least

Level	Sq Mean
LFC A	5.8720000
3NA A	5.8520000
EDTA B	5.5780000
FFC C	4.2580000

Levels not connected by same letter are significantly different.

9. Statistical Data for TPA Hardness. Day 7

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	43270160	7.4787	0.0044*
trial	4	4	16161283	2.0949	0.1445

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
3NA	10554.072	621.06480	10554.1
EDTA	10046.818	621.06480	10046.8
FFC	8657.706	621.06480	8657.7
LFC	12747.598	621.06480	12747.6

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ $Q=2.9688$

	LSMean[j]			
	3NA	EDTA	FFC	LFC
Mean[i]-Mean[j]				
Std Err Dif				
Lower CL Dif				
Upper CL Dif				
3NA				
		0.507.254	1896.37	-2193.5
		0.878.318	0.878.318	0.878.318
		0	-2100.3	-711.19
		0.3114.81	4503.92	414.029
EDTA				
	-507.25		0.1389.11	-2700.8
	0.878.318		0.878.318	0.878.318
	-3114.8	0	-1218.4	-5308.3
	2100.3	0.3996.67		-93.225
FFC				
	-1896.4	-1389.1		0
	0.878.318	0.878.318		0
	-4503.9	-3996.7		0
	711.189	1218.44		0
LFC				
	2193.53	2700.78	4089.89	0
	0.878.318	0.878.318	0.878.318	0
	-414.03	93.2254	1482.34	0
	4801.08	5308.33	6697.45	0

Level		Least Sq Mean
LFC	A	12747.598
3NA	A B	10554.072
EDTA	B	10046.818
FFC	B	8657.706

Levels not connected by same letter are significantly different.

10. Statistical Data for TPA Hardness. Day 30

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	27678896	8.6440	0.0025*
trial	4	4	30357330	7.1103	0.0036*

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Sq Mean	Std Error	Mean
3NA	9970.036	462.03147	9970.0
EDTA	9216.894	462.03147	9216.9
FFC	7656.322	462.03147	7656.3
LFC	10862.774	462.03147	10862.8

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ Q= 2.9688

		LSMean[j]				
	Mean[i]-Mean[j]	3NA	EDTA	FFC	LFC	
	Std Err Dif					
	Lower CL Dif					
	Upper CL Dif					
LSMean[i]	3NA		0.753.142	2313.71	-892.74	
			0.653.411	653.411	653.411	
			0. -1186.7	373.864	-2832.6	
			0. 2692.99	4253.56	1047.11	
	EDTA	-753.14		0.1560.57	-1645.9	
		653.411		0.653.411	653.411	
		-2693		0. -379.28	-3585.7	
		1186.71		0.3500.42	293.97	
	FFC	-2313.7	-1560.6		0. -3206.5	
		653.411	653.411		0. 653.411	
		-4253.6	-3500.4		0. -5146.3	
		-373.86	379.278		0. -1266.6	
	LFC	892.738	1645.88	3206.45		0
		653.411	653.411	653.411		0
		-1047.1	-293.97	1266.6		0
		2832.59	3585.73	5146.3		0

Level	Least Sq Mean
LFC A	10862.774
3NA A	9970.036
EDTA A B	9216.894
FFC B	7656.322

Levels not connected by same letter are significantly different.

11. Statistical Data for TPA Hardness. Day 60

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	31441106	13.7726	0.0003*
trial	4	4	10859192	3.5676	0.0387*

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
3NA	8664.6720	390.11724	8664.67
EDTA	9121.4080	390.11724	9121.41
FFC	6562.7940	390.11724	6562.79
LFC	9965.2140	390.11724	9965.21

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ $Q=2.9688$

		LSMean[j]			
	Mean[j]-Mean[j]	3NA	EDTA	FFC	LFC
	Std Err Dif				
	Lower CL Dif				
	Upper CL Dif				
LSMean[i]	3NA				
		0	-456.74	<u>2101.88</u>	-1300.5
		0	551.709	<u>551.709</u>	551.709
		0	-2094.7	<u>463.962</u>	-2938.5
		0	1181.18	<u>3739.79</u>	337.374
	EDTA	456.736	0	<u>2558.61</u>	-843.81
		551.709	0	<u>551.709</u>	551.709
		-1181.2	0	<u>920.698</u>	-2481.7
		2094.65	0	<u>4196.53</u>	794.11
	FFC	<u>-2101.9</u>	<u>-2558.6</u>	0	<u>-3402.4</u>
		<u>551.709</u>	<u>551.709</u>	0	<u>551.709</u>
		<u>-3739.8</u>	<u>-4196.5</u>	0	<u>-5040.3</u>
		<u>-463.96</u>	<u>-920.7</u>	0	<u>-1764.5</u>
	LFC	1300.54	843.806	<u>3402.42</u>	0
		551.709	551.709	<u>551.709</u>	0
		-337.37	-794.11	<u>1764.5</u>	0
	2938.46	2481.72	<u>5040.34</u>	0	

Least

Level		Sq Mean
LFC	A	9965.2140
EDTA	A	9121.4080
3NA	A	8664.6720
FFC	B	6562.7940

Levels not connected by same letter are significantly different.

12. Statistical Data for TPA Hardness. Day 90

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	25970435	7.9882	0.0034*
trial	4	4	10373485	2.3931	0.1085

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
3NA	8158.9240	465.55221	8158.92
EDTA	7925.4860	465.55221	7925.49
FFC	5899.0940	465.55221	5899.09
LFC	9003.1020	465.55221	9003.10

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ Q= 2.9688

		LSMean[j]			
	Mean[j]-Mean[j]	3NA	EDTA	FFC	LFC
	Std Err Dif				
	Lower CL Dif				
	Upper CL Dif				
LSMean[i]	3NA		0 233.438	2259.83	-844.18
			0 658.39	658.39	658.39
			0 -1721.2	305.199	-2798.8
			0 2188.07	4214.46	1110.45
	EDTA	-233.44	0 2026.39	-1077.6	
		658.39	0 658.39	658.39	
		-2188.1	0 717605	-3032.2	
		1721.19	0 3981.02	877.015	
	FFC	-2259.8	-2026.4	0	-3104
		658.39	658.39	0	658.39
		-4214.5	-3981	0	-5058.6
		-305.2	-71.761	0	-1149.4
	LFC	844.178	1077.62	3104.01	0
		658.39	658.39	658.39	0
		-1110.5	-877.02	1149.38	0
		2798.81	3032.25	5058.64	0

Least Sq Mean

Level		Least Sq Mean
LFC	A	9003.1020
3NA	A	8158.9240
EDTA	A	7925.4860
FFC	B	5899.0940

Levels not connected by same letter are significantly different.

13. Statistical Data for Hardness. Day 120

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	18307984	10.8499	0.0010*
trial	4	4	3561544	1.5830	0.2417

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
3NA	7317.7380	335.39875	7317.74
EDTA	7445.1520	335.39875	7445.15
FFC	5527.7460	335.39875	5527.75
LFC	8111.4720	335.39875	8111.47

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ Q= 2.9688

		LSMean[j]			
	Mean[i]-Mean[j]	3NA	EDTA	FFC	LFC
LSMean[i]	Std Err Dif				
	Lower CL Dif				
	Upper CL Dif				
	3NA				
		0	-127.41	1789.99	-793.73
		0	474.325	474.325	474.325
		0	-1535.6	381.813	-2201.9
		0	1280.77	3198.17	614.445
	EDTA	127.414	0	1917.41	-666.32
		474.325	0	474.325	474.325
		-1280.8	0	509.227	-2074.5
		1535.59	0	3325.59	741.859
	FFC		-1790	-1917.4	0
			474.325	474.325	0
			-3198.2	-3325.6	0
			-381.81	-509.23	0
LFC		793.734	666.32	2583.73	
		474.325	474.325	474.325	
		-614.45	-741.86	1175.55	
		2201.91	2074.5	3991.91	

Level	Least Sq Mean
LFC A	8111.4720
EDTA A	7445.1520
3NA A	7317.7380
FFC B	5527.7460

Levels not connected by same letter are significantly different.

14. Statistical Data for TPA Cohesiveness. Day 7

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	0.07609500	2.7601	0.0881
trial	4	4	0.08788000	2.3906	0.1088

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
3NA	0.58600000	0.04287190	0.586000
EDTA	0.44400000	0.04287190	0.444000
FFC	0.44400000	0.04287190	0.444000
LFC	0.54000000	0.04287190	0.540000

LS Means Plot

LSMeans Differences Tukey HSD

α=0.050 Q= 2.9688

		LSMean[j]				
	Mean[i]-Mean[j]	3NA	EDTA	FFC	LFC	
LSMean[i]	Std Err Dif					
	Lower CL Dif					
	Upper CL Dif					
	3NA		0	0.142	0.142	0.046
			0	0.06063	0.06063	0.06063
			0	-0.038	-0.038	-0.134
			0	0.322	0.322	0.226
	EDTA		-0.142	0	0	-0.096
			0.06063	0	0.06063	0.06063
			-0.322	0	-0.18	-0.276
			0.038	0	0.18	0.084
	FFC		-0.142	0	0	-0.096
			0.06063	0.06063	0	0.06063
			-0.322	-0.18	0	-0.276
			0.038	0.18	0	0.084
	LFC		-0.046	0.096	0.096	0
		0.06063	0.06063	0.06063	0	
		-0.226	-0.084	-0.084	0	
		0.134	0.276	0.276	0	

Level	Least Sq Mean
3NA A	0.58600000
LFC A	0.54000000
EDTA A	0.44400000
FFC A	0.44400000

Levels not connected by same letter are significantly different.

15. Statistical Data for TPA Cohesiveness. Day 30

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	0.01997500	1.1414	0.3718
trial	4	4	0.03292000	1.4109	0.2890

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
3NA	0.46200000	0.03415650	0.462000
EDTA	0.44600000	0.03415650	0.446000
FFC	0.42400000	0.03415650	0.424000
LFC	0.51000000	0.03415650	0.510000

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ $Q=2.9688$

		LSMean[j]			
Mean[i]-Mean[j]	3NA	EDTA	FFC	LFC	
Std Err Dif					
Lower CL Dif					
Upper CL Dif					
3NA					
	0	0.016	0.038	-0.048	
	0	0.0483	0.0483	0.0483	
	0	-0.1274	-0.1054	-0.1914	
	0	0.15941	0.18141	0.09541	
EDTA					
	-0.016	0	0.022	-0.064	
	0.0483	0	0.0483	0.0483	
	-0.1594	0	-0.1214	-0.2074	
	0.12741	0	0.16541	0.07941	
FFC					
	-0.038	-0.022	0	-0.086	
	0.0483	0.0483	0	0.0483	
	-0.1814	-0.1654	0	-0.2294	
	0.10541	0.12141	0	0.05741	
LFC					
	0.048	0.064	0.086	0	
	0.0483	0.0483	0.0483	0	
	-0.0954	-0.0794	-0.0574	0	
	0.19141	0.20741	0.22941	0	

Level	Least Sq Mean
LFC A	0.51000000
3NA A	0.46200000
EDTA A	0.44600000
FFC A	0.42400000

Levels not connected by same letter are significantly different.

16. Statistical Data for TPA Cohesiveness. Day 60

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	0.01565500	2.6910	0.0932
trial	4	4	0.07357000	9.4847	0.0011*

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
3NA	0.48000000	0.01969348	0.480000
EDTA	0.43400000	0.01969348	0.434000
FFC	0.42000000	0.01969348	0.420000
LFC	0.48400000	0.01969348	0.484000

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ $Q=2.9688$

		LSMean[j]			
	Mean[i]-Mean[j]	3NA	EDTA	FFC	LFC
LSMean[i]	Std Err Dif				
	Lower CL Dif				
	Upper CL Dif				
	3NA				
		0	0.046	0.06	-0.004
		0.02785	0.02785	0.02785	
		0	-0.0367	-0.0227	-0.0867
		0.12868	0.14268	0.07868	
	EDTA				
		-0.046	0	0.014	-0.05
		0.02785		0.02785	0.02785
		-0.1287	0	-0.0687	-0.1327
		0.03668	0	0.09668	0.03268
	FFC				
		-0.06	-0.014	0	-0.064
		0.02785	0.02785	0	0.02785
	-0.1427	-0.0967	0	-0.1467	
	0.02268	0.06868	0	0.01868	
LFC					
	0.004	0.05	0.064	0	
	0.02785	0.02785	0.02785	0	
	-0.0787	-0.0327	-0.0187	0	
	0.08668	0.13268	0.14668	0	

Level	Least Sq Mean
LFC A	0.48400000
3NA A	0.48000000
EDTA A	0.43400000
FFC A	0.42000000

Levels not connected by same letter are significantly different.

17. Statistical Data for TPA Cohesiveness. Day 90

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	0.01146000	0.8261	0.5045
trial	4	4	0.04103000	2.2182	0.1282

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
3NA	0.40400000	0.03041107	0.404000
EDTA	0.43800000	0.03041107	0.438000
FFC	0.41000000	0.03041107	0.410000
LFC	0.46400000	0.03041107	0.464000

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ $Q=2.9688$

		LSMean[j]			
	Mean[i]-Mean[j]	3NA	EDTA	FFC	LFC
LSMean[i]	Std Err Dif				
	Lower CL Dif				
	Upper CL Dif				
	3NA				
		0	-0.034	-0.006	-0.06
		0.04301	0.04301	0.04301	
		0	-0.1617	-0.1337	-0.1877
		0	0.09368	0.12168	0.06768
	EDTA				
		0.034	0	0.028	-0.026
		0.04301	0	0.04301	0.04301
		-0.0937	0	-0.0997	-0.1537
		0.16168	0	0.15568	0.10168
	FFC				
		0.006	-0.028	0	-0.054
		0.04301	0.04301	0	0.04301
	-0.1217	-0.1557	0	-0.1817	
	0.13368	0.09968	0	0.07368	
LFC					
	0.06	0.026	0.054	0	
	0.04301	0.04301	0.04301	0	
	-0.0677	-0.1017	-0.0737	0	
	0.18768	0.15368	0.18168	0	

Level	Least Sq Mean
LFC A	0.46400000
EDTA A	0.43800000
FFC A	0.41000000
3NA A	0.40400000

Levels not connected by same letter are significantly different.

18. Statistical Data for TPA Cohesiveness. Day 120

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	0.01317500	0.6251	0.6124
trial	4	4	0.03302000	1.1751	0.3701

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
3NA	0.40600000	0.03748333	0.406000
EDTA	0.36400000	0.03748333	0.364000
FFC	0.42000000	0.03748333	0.420000
LFC	0.43200000	0.03748333	0.432000

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ $Q=2.9688$

	LSMean[j]			
	3NA	EDTA	FFC	LFC
Mean[i]-Mean[j]				
Std Err Dif				
Lower CL Dif				
Upper CL Dif				
3NA				
	0	0.042	-0.014	-0.026
	0.05301	0.05301	0.05301	0.05301
	0	-0.1154	-0.1714	-0.1834
	0.19937	0.14337	0.13137	0.13137
EDTA				
	-0.042	0	-0.056	-0.068
	0.05301	0	0.05301	0.05301
	-0.1994	0	-0.2134	-0.2254
	0.11537	0	0.10137	0.08937
FFC				
	0.014	0.056	0	-0.012
	0.05301	0.05301	0	0.05301
	-0.1434	-0.1014	0	-0.1694
	0.17137	0.21337	0	0.14537
LFC				
	0.026	0.068	0.012	0
	0.05301	0.05301	0.05301	0
	-0.1314	-0.0894	-0.1454	0
	0.18337	0.22537	0.16937	0

Level		Least Sq Mean
LFC	A	0.43200000
FFC	A	0.42000000
3NA	A	0.40600000
EDTA	A	0.36400000

Levels not connected by same letter are significantly different.

19. Statistical Data for TPA Springiness. Day 7

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	0.01165500	0.8945	0.4721
trial	4	4	0.01712000	0.9854	0.4518

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Sq Mean	Std Error	Mean
3NA	0.24800000	0.02947315	0.248000
EDTA	0.21200000	0.02947315	0.212000
FFC	0.21800000	0.02947315	0.218000
LFC	0.18000000	0.02947315	0.180000

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ Q= 2.9688

		LSMean[j]			
	Mean[i]-Mean[j]	3NA	EDTA	FFC	LFC
Std Err Dif					
Lower CL Dif					
Upper CL Dif					
LSMean[i]	3NA				
		0	0.036	0.03	0.068
		0.04168	0.04168	0.04168	
		0	-0.0877	-0.0937	-0.0557
		0	0.15974	0.15374	0.19174
	EDTA				
		-0.036	0	-0.006	0.032
		0.04168	0	0.04168	0.04168
		-0.1597	0	-0.1297	-0.0917
		0.08774	0	0.11774	0.15574
	FFC				
		-0.03	0.006	0	0.038
		0.04168	0.04168	0	0.04168
		-0.1537	-0.1177	0	-0.0857
		0.09374	0.12974	0	0.16174
	LFC				
	-0.068	-0.032	-0.038	0	
	0.04168	0.04168	0.04168	0	
	-0.1917	-0.1557	-0.1617	0	
	0.05574	0.09174	0.08574	0	

Level	Least Sq Mean
3NA A	0.24800000
FFC A	0.21800000
EDTA A	0.21200000
LFC A	0.18000000

Levels not connected by same letter are significantly different.

20. Statistical Data for TPA Springiness. Day 30

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	0.04849500	2.3098	0.1282
trial	4	4	0.05618000	2.0069	0.1576

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Sq Mean	Std Error	Mean
3NA	0.23000000	0.03741212	0.230000
EDTA	0.31600000	0.03741212	0.316000
FFC	0.31400000	0.03741212	0.314000
LFC	0.20600000	0.03741212	0.206000

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ $Q=2.9688$

		LSMean[j]			
	Mean[i]-Mean[j]	3NA	EDTA	FFC	LFC
LSMean[i]	Std Err Dif				
	Lower CL Dif				
	Upper CL Dif				
	3NA				
		0	-0.086	-0.084	0.024
		0.05291	0.05291	0.05291	
		0	-0.2431	-0.2411	-0.1331
		0.07108	0.07308	0.18108	
	EDTA				
		0.086	0	0.002	0.11
		0.05291	0	0.05291	0.05291
		-0.0711	0	-0.1551	-0.0471
		0.24308	0	0.15908	0.26708
	FFC				
		0.084	-0.002	0	0.108
		0.05291	0.05291	0	0.05291
	-0.0731	-0.1591	0	-0.0491	
	0.24108	0.15508	0	0.26508	
LFC					
	-0.024	-0.11	-0.108	0	
	0.05291	0.05291	0.05291	0	
	-0.1811	-0.2671	-0.2651	0	
	0.13308	0.04708	0.04908	0	

Level	Least Sq Mean
EDTA A	0.31600000
FFC A	0.31400000
3NA A	0.23000000
LFC A	0.20600000

Levels not connected by same letter are significantly different.

21. Statistical Data for TPA Springiness. Day 60

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	0.01193500	0.6370	0.6054
trial	4	4	0.14550000	5.8247	0.0077*

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
3NA	0.28400000	0.03534119	0.284000
EDTA	0.32800000	0.03534119	0.328000
FFC	0.26400000	0.03534119	0.264000
LFC	0.27400000	0.03534119	0.274000

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ Q= 2.9688

		LSMean[j]			
	Mean[i]-Mean[j]	3NA	EDTA	FFC	LFC
	Std Err Dif				
	Lower CL Dif				
	Upper CL Dif				
LSMean[i]	3NA	0	-0.044	0.02	0.01
		0.04998	0.04998	0.04998	
		0	-0.1924	-0.1284	-0.1384
		0.10438	0.16838	0.15838	
	EDTA	0.044	0	0.064	0.054
		0.04998	0.04998	0.04998	
		-0.1044	0	-0.0844	-0.0944
		0.19238	0.21238	0.20238	
	FFC	-0.02	-0.064	0	-0.01
		0.04998	0.04998	0	0.04998
		-0.1684	-0.2124	0	-0.1584
		0.12838	0.08438	0	0.13838
LFC	-0.01	-0.054	0.01	0	
	0.04998	0.04998	0.04998	0	
	-0.1584	-0.2024	-0.1384	0	
	0.13838	0.09438	0.15838	0	

Level	Least Sq Mean
EDTA A	0.32800000
3NA A	0.28400000
LFC A	0.27400000
FFC A	0.26400000

Levels not connected by same letter are significantly different.

22. Statistical Data for TPA Springiness. Day 90

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	0.01089500	0.6153	0.6182
trial	4	4	0.03585000	1.5184	0.2584

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Sq Mean	Std Error	Mean
3NA	0.30800000	0.03435841	0.308000
EDTA	0.30000000	0.03435841	0.300000
FFC	0.29400000	0.03435841	0.294000
LFC	0.24800000	0.03435841	0.248000

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ Q= 2.9688

		LSMean[j]			
	Mean[i]-Mean[j]	3NA	EDTA	FFC	LFC
Std Err Dif					
Lower CL Dif					
Upper CL Dif					
LSMean[i]	3NA				
		0	0.008	0.014	0.06
		0.04859	0.04859	0.04859	0.04859
		0	-0.1363	-0.1303	-0.0843
		0	0.15225	0.15825	0.20425
	EDTA				
		-0.008	0	0.006	0.052
		0.04859	0	0.04859	0.04859
		-0.1523	0	-0.1383	-0.0923
		0.13625	0	0.15025	0.19625
	FFC				
		-0.014	-0.006	0	0.046
		0.04859	0.04859	0	0.04859
		-0.1583	-0.1503	0	-0.0983
		0.13025	0.13825	0	0.19025
	LFC				
	-0.06	-0.052	-0.046	0	
	0.04859	0.04859	0.04859	0	
	-0.2043	-0.1963	-0.1903	0	
	0.08425	0.09225	0.09825	0	

Level	Least Sq Mean
3NA	A 0.30800000
EDTA	A 0.30000000
FFC	A 0.29400000
LFC	A 0.24800000

Levels not connected by same letter are significantly different.

23. Statistical Data for TPA Springiness. Day 120

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	0.07205500	4.0640	0.0331*
trial	4	4	0.03228000	1.3655	0.3030

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Sq Mean	Std Error	Mean
3NA	0.31000000	0.03438023	0.310000
EDTA	0.30000000	0.03438023	0.300000
FFC	0.43400000	0.03438023	0.434000
LFC	0.28200000	0.03438023	0.282000

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ Q= 2.9688

		LSMean[j]			
	Mean[i]-Mean[j]	3NA	EDTA	FFC	LFC
	Std Err Dif				
	Lower CL Dif				
	Upper CL Dif				
LSMean[i]	3NA	0	0.01	-0.124	0.028
		0	0.04862	0.04862	0.04862
		0	-0.1343	-0.2683	-0.1163
		0	0.15435	0.02035	0.17235
	EDTA	-0.01	0	-0.134	0.018
		0.04862	0	0.04862	0.04862
		-0.1543	0	-0.2783	-0.1263
		0.13435	0	0.01035	0.16235
	FFC	0.124	0.134	0	0.152
		0.04862	0.04862	0	0.04862
		-0.0203	-0.0103	0	0.00765
		0.26835	0.27835	0	0.29635
	LFC	-0.028	-0.018	-0.152	0
		0.04862	0.04862	0.04862	0
		-0.1723	-0.1623	-0.2963	0
		0.11635	0.12635	-0.0077	0

Level		Least Sq Mean
FFC	A	0.43400000
3NA	A B	0.31000000
EDTA	A B	0.30000000
LFC	B	0.28200000

Levels not connected by same letter are significantly different.

24. Statistical Data for TPA Gumminess. Day 7

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	35642412	8.8839	0.0022*
trial	4	4	23880922	4.4642	0.0193*

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Sq Mean	Std Error	Mean
3NA	6254.7960	517.17446	6254.80
EDTA	4376.0540	517.17446	4376.05
FFC	3804.5120	517.17446	3804.51
LFC	7075.0420	517.17446	7075.04

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ $Q=2.9688$

		LSMean[j]				
	Mean[i]-Mean[j]	3NA	EDTA	FFC	LFC	
	Std Err Dif					
	Lower CL Dif					
	Upper CL Dif					
LSMean[i]	3NA		0 1878.74	2450.28	-820.25	
			0 731.395	731.395	731.395	
			0 -292.63	278.915	-2991.6	
			0 4050.11	4621.65	1351.12	
	EDTA	-1878.7		0 571.542	-2699	
		731.395		0 731.395	731.395	
		-4050.1		0 -1599.8	-4870.4	
		292.627		0 2742.91	-527.62	
	FFC	-2450.3	-571.54		0 -3270.5	
		731.395	731.395		0 731.395	
		-4621.7	-2742.9		0 -5441.9	
		-278.92	1599.83		0 -1099.2	
	LFC	820.246	2698.99	3270.53		0
		731.395	731.395	731.395		0
		-1351.1	527.619	1099.16		0
		2991.61	4870.36	5441.9		0

Level	Least Sq Mean
LFC A	7075.0420
3NA A B	6254.7960
EDTA B C	4376.0540
FFC C	3804.5120

Levels not connected by same letter are significantly different.

25. Statistical Data for TPA Gumminess. Day 60

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	13841544	14.6422	0.0003*
trial	4	4	11726314	9.3035	0.0012*

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
3NA	4648.1180	251.04011	4648.12
EDTA	3858.9700	251.04011	3858.97
FFC	2744.6960	251.04011	2744.70
LFC	4869.6320	251.04011	4869.63

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ Q= 2.9688

		LSMean[j]			
	Mean[i]-Mean[j]	3NA	EDTA	FFC	LFC
	Std Err Dif				
	Lower CL Dif				
	Upper CL Dif				
LSMean[i]	3NA		0.789.148	1903.42	-221.51
			0.355.024	355.024	355.024
			0.264.85	849.424	-1275.5
			0.1843.15	2957.42	832.484
	EDTA	-789.15		0.1114.27	-1010.7
		355.024		0.355.024	355.024
		-1843.1		0.60.2764	-2064.7
		264.85		0.2168.27	43.3356
	FFC	-1903.4	-1114.3		0.2124.9
		355.024	355.024		0.355.024
		-2957.4	-2168.3		0.3178.9
		-849.42	-60.276		0.1070.9
	LFC	221.514	1010.66	2124.94	
		355.024	355.024	355.024	
		-832.48	-43.336	1070.94	
		1275.51	2064.66	3178.93	

Level	Least Sq Mean
LFC A	4869.6320
3NA A	4648.1180
EDTA A	3858.9700
FFC B	2744.6960

Levels not connected by same letter are significantly different.

26. Statistical Data for TPA Gumminess. Day 90

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	8553268.3	4.5797	0.0233*
trial	4	4	5202927.4	2.0893	0.1453

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Sq Mean	Std Error	Mean
3NA	3279.2420	352.86147	3279.24
EDTA	3436.4060	352.86147	3436.41
FFC	2435.3200	352.86147	2435.32
LFC	4278.3080	352.86147	4278.31

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ Q= 2.9688

		LSMean[j]				
	Mean[i]-Mean[j]	3NA	EDTA	FFC	LFC	
	Std Err Dif					
	Lower CL Dif					
	Upper CL Dif					
LSMean[i]	3NA					
		0	-157.16	843.922	-999.07	
		0	499.021	499.021	499.021	
		0	-1638.7	-637.57	-2480.6	
		0	1324.33	2325.42	482.43	
	EDTA	157.164		0	1001.09	-841.9
		499.021		0	499.021	499.021
		-1324.3		0	-480.41	-2323.4
		1638.66		0	2482.58	639.59
	FFC	-843.92	-1001.1		0	-1843
		499.021	499.021		0	499.021
		-2325.4	-2482.6		0	-3324.5
		637.575	480.411		0	-361.49
	LFC	999.066	841.902	1842.99		0
		499.021	499.021	499.021		0
		-482.43	-639.59	361.491		0
	2480.56	2323.4	3324.48		0	

Level	Least Sq Mean
LFC A	4278.3080
EDTA A B	3436.4060
3NA A B	3279.2420
FFC B	2435.3200

Levels not connected by same letter are significantly different.

27. Statistical Data for TPA Gumminess. Day 120

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	3410685.7	1.5792	0.2458
trial	4	4	1161170.1	0.4032	0.8028

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Sq Mean	Std Error	Mean
3NA	2963.0940	379.45516	2963.09
EDTA	2738.7020	379.45516	2738.70
FFC	2366.8160	379.45516	2366.82
LFC	3506.6360	379.45516	3506.64

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ Q= 2.9688

		LSMean[j]			
	Mean[i]-Mean[j]	3NA	EDTA	FFC	LFC
	Std Err Dif				
	Lower CL Dif				
	Upper CL Dif				
LSMean[i]	3NA		0.224.392	596.278	-543.54
			0.536.631	536.631	536.631
			0. -1368.8	-996.87	-2136.7
			0.1817.54	2189.43	1049.61
	EDTA	-224.39		0.371.886	-767.93
		536.631		0.536.631	536.631
		-1817.5		0. -1221.3	-2361.1
		1368.76		0.1965.04	825.217
	FFC	-596.28	-371.89		0. -1139.8
		536.631	536.631		0.536.631
		-2189.4	-1965		0. -2733
		996.873	1221.27		0.453.331
	LFC	543.542	767.934	1139.82	
		536.631	536.631	536.631	
		-1049.6	-825.22	-453.33	
		2136.69	2361.09	2732.97	

Level	Least Sq Mean
LFC A	3506.6360
3NA A	2963.0940
EDTA A	2738.7020
FFC A	2366.8160

Levels not connected by same letter are significantly different.

28. Statistical Data for TPA Chewiness. Day 7

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	891415.33	3.1582	0.0643
trial	4	4	151793.76	0.4033	0.8027

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Sq Mean	Std Error	Mean
3NA	1356.0080	137.17388	1356.01
EDTA	969.3520	137.17388	969.35
FFC	825.3800	137.17388	825.38
LFC	1242.3720	137.17388	1242.37

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ Q= 2.9688

		LSMean[j]			
	Mean[i]-Mean[j]	3NA	EDTA	FFC	LFC
	Std Err Dif				
	Lower CL Dif				
	Upper CL Dif				
LSMean[i]	3NA		0.386.656	530.628	113.636
			0.193.993	193.993	193.993
			0. -189.27	-45.3	-462.29
			0.962.584	1106.56	689.564
	EDTA	-386.66		0.143.972	-273.02
		193.993		0.193.993	193.993
		-962.58		0. -431.96	-848.95
		189.272		0. 719.9	302.908
	FFC	-530.63	-143.97		0. -416.99
		193.993	193.993		0.193.993
		-1106.6	-719.9		0. -992.92
		45.2996	431.956		0.158.936
LFC	-113.64	273.02	416.992		0
	193.993	193.993	193.993		0
	-689.56	-302.91	-158.94		0
	462.292	848.948	992.92		0

Level	Least Sq Mean
3NA A	1356.0080
LFC A	1242.3720
EDTA A	969.3520
FFC A	825.3800

Levels not connected by same letter are significantly different.

29. Statistical Data for TPA Chewiness. Day 30

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	173373.78	0.7425	0.5470
trial	4	4	159209.71	0.5113	0.7288

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Sq Mean	Std Error	Mean
3NA	983.5500	124.77033	983.55
EDTA	1200.2680	124.77033	1200.27
FFC	971.0280	124.77033	971.03
LFC	1095.5840	124.77033	1095.58

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ $Q=2.9688$

	LSMean[j]			
	3NA	EDTA	FFC	LFC
Mean[i]-Mean[j]				
Std Err Dif				
Lower CL Dif				
Upper CL Dif				
3NA				
	0	-216.72	12.522	-112.03
	0	176.452	176.452	176.452
	0	-740.57	-511.33	-635.89
	0	307.133	536.373	411.817
EDTA				
	216.718	0	229.24	104.684
	176.452	0	176.452	176.452
	-307.13	0	-294.61	-419.17
	740.569	0	753.091	628.535
FFC				
	-12.522	-229.24	0	-124.56
	176.452	176.452	0	176.452
	-536.37	-753.09	0	-648.41
	511.329	294.611	0	399.295
LFC				
	112.034	-104.68	124.556	0
	176.452	176.452	176.452	0
	-411.82	-628.54	-399.3	0
	635.885	419.167	648.407	0

Level	Least Sq Mean
EDTA A	1200.2680
LFC A	1095.5840
3NA A	983.5500
FFC A	971.0280

Levels not connected by same letter are significantly different.

30. Statistical Data for TPA Chewiness. Day 60

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	866581.24	10.3542	0.0012*
trial	4	4	657962.39	5.8962	0.0073*

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Sq Mean	Std Error	Mean
3NA	1178.2940	74.696490	1178.29
EDTA	1123.9840	74.696490	1123.98
FFC	710.5860	74.696490	710.59
LFC	1242.1960	74.696490	1242.20

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ Q= 2.9688

		LSMean[j]			
	Mean[j]-Mean[j]	3NA	EDTA	FFC	LFC
	Std Err Dif				
	Lower CL Dif				
	Upper CL Dif				
	3NA				
		0	54.31	467.708	-63.902
		0	105.637	105.637	105.637
		0	-259.3	154.093	-377.52
		0	367.925	781.323	249.713
	EDTA	-54.31	0	413.398	-118.21
		105.637	0	105.637	105.637
		-367.92	0	99.7831	-431.83
		259.305	0	727.013	195.403
	FFC	-467.71	-413.4	0	-531.61
		105.637	105.637	0	105.637
		-781.32	-727.01	0	-845.22
		-154.09	-99.783	0	-218
	LFC	63.902	118.212	531.61	0
		105.637	105.637	105.637	0
		-249.71	-195.4	217.995	0
		377.517	431.827	845.225	0

Level	Least Sq Mean
LFC A	1242.1960
3NA A	1178.2940
EDTA A	1123.9840
FFC B	710.5860

Levels not connected by same letter are significantly different.

31. Statistical Data for TPA Chewiness. Day 90

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	333659.03	3.1925	0.0626
trial	4	4	85352.19	0.6125	0.6617

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Sq Mean	Std Error	Mean
3NA	991.4900	83.471612	991.49
EDTA	970.1940	83.471612	970.19
FFC	694.6340	83.471612	694.63
LFC	1011.3760	83.471612	1011.38

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ Q= 2.9688

		LSMean[j]			
Mean[i]-Mean[j]	3NA	EDTA	FFC	LFC	
Std Err Dif					
Lower CL Dif					
Upper CL Dif					
3NA					
	0	21.296	296.856	-19.886	
	0	118.047	118.047	118.047	
	0	-329.16	-53.601	-370.34	
	0	371.753	647.313	330.571	
EDTA					
	-21.296	0	275.56	-41.182	
	118.047	0	118.047	118.047	
	-371.75	0	-74.897	-391.64	
	329.161	0	626.017	309.275	
FFC					
	-296.86	-275.56	0	-316.74	
	118.047	118.047	0	118.047	
	-647.31	-626.02	0	-667.2	
	53.6015	74.8975	0	33.7155	
LFC					
	19.886	41.182	316.742	0	
	118.047	118.047	118.047	0	
	-330.57	-309.28	-33.715	0	
	370.343	391.639	667.199	0	

Level	Least Sq Mean
LFC A	1011.3760
3NA A	991.4900
EDTA A	970.1940
FFC A	694.6340

Levels not connected by same letter are significantly different.

32. Statistical Data for TPA Chewiness. Day 120

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	93293.98	0.4928	0.6939
trial	4	4	571725.38	2.2650	0.1226

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Sq Mean	Std Error	Mean
3NA	896.4020	112.34380	896.40
EDTA	857.5720	112.34380	857.57
FFC	1040.6660	112.34380	1040.67
LFC	939.2380	112.34380	939.24

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ Q= 2.9688

		LSMean[j]			
Mean[i]-Mean[j]		3NA	EDTA	FFC	LFC
Std Err Dif					
Lower CL Dif					
Upper CL Dif					
3NA					
		0	38.83	-144.26	-42.836
		0	158.878	158.878	158.878
		0	-432.85	-615.94	-514.51
		0	510.508	327.414	428.842
EDTA					
	-38.83		0	-183.09	-81.666
	158.878		0	158.878	158.878
	-510.51		0	-654.77	-553.34
	432.848		0	288.584	390.012
FFC					
	144.264	183.094		0	101.428
	158.878	158.878		0	158.878
	-327.41	-288.58		0	-370.25
	615.942	654.772		0	573.106
LFC					
	42.836	81.666	-101.43		0
	158.878	158.878	158.878		0
	-428.84	-390.01	-573.11		0
	514.514	553.344	370.25		0

Level	Least Sq Mean
FFC A	1040.6660
LFC A	939.2380
3NA A	896.4020
EDTA A	857.5720

Levels not connected by same letter are significantly different.

33. Statistical Data for Water Soluble Nitrogen (WSN) content in Cheese. Day 7

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	5.3695000	5.5779	0.0125*
trial	4	4	1.0231700	0.7972	0.5496

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
3NA	6.8580000	0.25332950	6.85800
EDTA	6.7660000	0.25332950	6.76600
FFC	5.6540000	0.25332950	5.65400
LFC	6.9100000	0.25332950	6.91000

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ Q= 2.9688

	LSMean[j]			
Mean[i]-Mean[j]	3NA	EDTA	FFC	LFC
Std Err Dif				
Lower CL Dif				
Upper CL Dif				
3NA				
	0	0.092	1.204	-0.052
	0.35826	0.35826	0.35826	0.35826
	0	-0.9716	0.14039	-1.1156
	0.115561	2.26761	1.01161	
EDTA				
	-0.092	0	1.112	-0.144
	0.35826	0	0.35826	0.35826
	-1.1556	0	0.04839	-1.2076
	0.97161	0	2.17561	0.91961
FFC				
	-1.204	-1.112	0	-1.256
	0.35826	0.35826	0	0.35826
	-2.2676	-2.1756	0	-2.3196
	-0.1404	-0.0484	0	-0.1924
LFC				
	0.052	0.144	1.256	0
	0.35826	0.35826	0.35826	0
	-1.0116	-0.9196	0.19239	0
	1.11561	1.20761	2.31961	0

Level		Least Sq Mean
LFC	A	6.9100000
3NA	A	6.8580000
EDTA	A	6.7660000
FFC	B	5.6540000

Levels not connected by same letter are significantly different.

34. Statistical Data for Water Soluble Nitrogen (WSN) content in Cheese. Day 30

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	27.649920	6.2929	0.0082*
trial	4	4	15.445170	2.6364	0.0865

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
3NA	10.824000	0.54122438	10.8240
EDTA	11.568000	0.54122438	11.5680
FFC	9.264000	0.54122438	9.2640
LFC	12.472000	0.54122438	12.4720

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ $Q=2.9688$

		LSMean[j]				
	Mean[i]-Mean[j]	3NA	EDTA	FFC	LFC	
	Std Err Dif					
	Lower CL Dif					
	Upper CL Dif					
LSMean[i]	3NA		0	-0.744	1.56	-1.648
			0	0.76541	0.76541	0.76541
			0	-3.0163	-0.7123	-3.9203
			0	1.52834	3.83234	0.62434
	EDTA	0.744	0	2.304	-0.904	
		0.76541	0	0.76541	0.76541	
		-1.5283	0	0.03166	-3.1763	
		3.01634	0	4.57634	1.36834	
	FFC	-1.56	-2.304	0	-3.208	
		0.76541	0.76541	0	0.76541	
		-3.8323	-4.5763	0	-5.4803	
		0.71234	-0.0317	0	-0.9357	
	LFC	1.648	0.904	3.208		
		0.76541	0.76541	0.76541		
		-0.6243	-1.3683	0.93566		
		3.92034	3.17634	5.48034		

Level		Least Sq Mean
LFC	A	12.472000
EDTA	A	11.568000
3NA	A B	10.824000
FFC	B	9.264000

Levels not connected by same letter are significantly different.

35. Statistical Data for Water Soluble Nitrogen (WSN) content in Cheese. Day 60

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	21.32466	2.0060	0.1669
trial	4	4	160.52162	11.3253	0.0005*

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
3NA	14.878000	0.84183351	14.8780
EDTA	16.624000	0.84183351	16.6240
FFC	14.256000	0.84183351	14.2560
LFC	16.544000	0.84183351	16.5440

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha = 0.050$ Q = 2.9688

	LSMean[j]			
	3NA	EDTA	FFC	LFC
Mean[i]-Mean[j]				
Std Err Dif				
Lower CL Dif				
Upper CL Dif				
3NA				
	0	-1.746	0.622	-1.666
	0.119053	1.19053	1.19053	1.19053
	0	-5.2805	-2.9125	-5.2005
	0.178846	4.15646	1.86846	1.86846
EDTA	1.746	0	2.368	0.08
	1.19053	0	1.19053	1.19053
	-1.7885	0	-1.1665	-3.4545
	5.28046	0	5.90246	3.61446
FFC	-0.622	-2.368	0	-2.288
	1.19053	1.19053	0	1.19053
	-4.1565	-5.9025	0	-5.8225
	2.91246	1.16646	0	1.24646
LFC	1.666	-0.08	2.288	0
	1.19053	1.19053	1.19053	0
	-1.8685	-3.6145	-1.2465	0
	5.20046	3.45446	5.82246	0

Level	Least Sq Mean
EDTA A	16.624000
LFC A	16.544000
3NA A	14.878000
FFC A	14.256000

Levels not connected by same letter are significantly different.

36. Statistical Data for Water Soluble Nitrogen (WSN) content in Cheese. Day 90

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	22.022895	4.7512	0.0208*
trial	4	4	69.177200	11.1932	0.0005*

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
3NA	17.226000	0.55589088	17.2260
EDTA	19.550000	0.55589088	19.5500
FFC	17.132000	0.55589088	17.1320
LFC	18.902000	0.55589088	18.9020

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ Q= 2.9688

	LSMean[j]			
Mean[i]-Mean[j]	3NA	EDTA	FFC	LFC
Std Err Dif				
Lower CL Dif				
Upper CL Dif				
3NA				
	0	-2.324	0.094	-1.676
	0.78615	0.78615	0.78615	0.78615
	0	-4.6579	-2.2399	-4.0099
	0.00992	2.42792	0.65792	0.65792
EDTA	2.324	0	2.418	0.648
	0.78615	0	0.78615	0.78615
	-0.0099	0	0.08408	-1.6859
	4.65792	0	4.75192	2.98192
FFC	-0.094	-2.418	0	-1.77
	0.78615	0.78615	0	0.78615
	-2.4279	-4.7519	0	-4.1039
	2.23992	-0.0841	0	0.56392
LFC	1.676	-0.648	1.77	0
	0.78615	0.78615	0.78615	0
	-0.6579	-2.9819	-0.5639	0
	4.00992	1.68592	4.10392	0

Level	Least Sq Mean
EDTA A	19.550000
LFC A B	18.902000
3NA A B	17.226000
FFC B	17.132000

Levels not connected by same letter are significantly different.

37. Statistical Data for Water Soluble Nitrogen (WSN) content in Cheese. Day 120

Whole Model

Actual by Predicted Plot

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
treatment	3	3	10.064455	2.3679	0.1220
trial	4	4	36.169320	6.3823	0.0054*

Residual by Predicted Plot

treatment

Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
3NA	20.574000	0.53231131	20.5740
EDTA	22.168000	0.53231131	22.1680
FFC	20.316000	0.53231131	20.3160
LFC	21.044000	0.53231131	21.0440

LS Means Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ Q= 2.9688

	LSMean[j]			
	Mean[i]-Mean[j]	Std Err Dif	Lower CL Dif	Upper CL Dif
3NA				
	0	-1.594	0.258	-0.47
	0	0.7528	0.7528	0.7528
	0	-3.8289	-1.9769	-2.7049
EDTA				
	0	0.64092	2.49292	1.76492
	1.594	0	1.852	1.124
	0.7528	0	0.7528	0.7528
FFC				
	-0.6409	0	-0.3829	-1.1109
	-3.82892	0	4.08692	3.35892
	-0.258	-1.852	0	-0.728
LFC				
	0.7528	0.7528	0	0.7528
	-2.4929	-4.0869	0	-2.9629
	1.97692	0.38292	0	1.50692
LFC				
	0.47	-1.124	0.728	0
	0.7528	0.7528	0.7528	0
	-1.7649	-3.3589	-1.5069	0
	2.70492	1.11092	2.96292	0

Level	Least Sq Mean
EDTA A	22.168000
LFC A	21.044000
3NA A	20.574000
FFC A	20.316000

Levels not connected by same letter are significantly different.