

Effects of Cheese Age on Viscosity of Sauces Made from Process Cheese Loaves and
Comparison of Methods Used to Measure Sauce Viscosity

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Abstract

Natural cheese is the primary ingredient used in the manufacture of process cheese and therefore has a large influence on its performance properties. The objective of this study was to determine the effect of natural cheese age on the viscosity of sauces made from process cheese loaves as well as to compare methods used to measure cheese sauce viscosity. Three replicates of process cheese loaves were made with average cheese blend ages of 10, 30, 90, 150, and 210 days and converted into sauces by diluting with water (3:1 cheese to water ratio) and heating to 70°C while mixing. Viscosity readings (mPa.s) were taken using three different methods (Brookfield, RVA, and MVAG) at the initial time point as well as after 2 hours and 4 hours of hold time at 70°C. Significant differences ($P < 0.05$) in sauce viscosity were found between the cheese blend age treatments for all time points. Increases in sauce viscosity was observed for all treatments during the hold time, however significant differences ($P < 0.05$) were found in the degree of the viscosity change over the hold time. These differences may be correlated with fat:casein and casein:phosphate ratios within the process cheese loaves used to make the cheese sauces. All methods of sauce viscosity testing showed similar trends for all time points, however significant differences ($P < 0.05$) were found in the viscosity values. This is likely attributed to different sensitivity levels of the machines and the apparatuses used to measure viscosity. Overall, the age of the cheese used in the make of process cheese loaves does have an impact on viscosity when converted into cheese sauces and a method of sauce viscosity testing may be found to fit specific cheese sauce applications.

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Chapter 1

Introduction and Objectives

1.1 Introduction

Process cheese is stable, versatile, and highly functioning product that enables the use of cheese into applications that otherwise may not be able to use it. It was first made in Switzerland in 1911 as a way to extend the shelf life of cheese for exporting purposes (Zehren and Nusbaum, 2000). The process cheese industry was started in the U.S. shortly after James Kraft was first granted a patent for process cheese manufacturing in 1916 (Hickey, 2011). Process cheese is made by blending natural cheese(s) with emulsifying salts and other ingredients then heating while mixing until a homogenous material is produced (Kapoor and Metzger, 2008).

Since process cheese is mainly used for its stability and functionality (melting, slicing, shredding, etc.), it is important to understand the factors which affect process cheese stability and functionality. This includes composition parameters like intact casein, moisture, fat, and calcium as well as type and amount of the emulsifying salt. From a process cheese functionality standpoint, intact casein along with type and amount of the emulsifying salt are the most important factors to consider (Berger, et al., 1989). There have been numerous studies which have shown that higher levels of intact casein, or less aged natural cheese, in process cheese produces a firmer texture with less meltability compared to lower levels (Brickley et al., 2007; Olson et al., 1954; Piska, 2004 et al.; Purna et al., 2006; Sommer and Templeton, 1932; Wang et al., 2011 Zahariasen, 1954). Natural cheese age is often referenced as a way to predict intact

casein as increased age is correlated with increased proteolysis (Fox et al., 2000; Guinee, 2011; Kapoor and Metzger, 2008). Therefore, natural cheese age is often referenced as a quick and easy method to determine intact casein content in natural cheese.

Although there has been extensive research done on natural cheese age and its effect on process cheese texture and functionality, there has been minimal research on the effect of cheese age in process cheese when used in performance based applications such as sauces.

1.2 Objectives

The primary goal of this study was to understand the effect cheese age has on the viscosity of process cheese sauces made from a process cheese loaf when held at a high time temperature over time. The effects of cheese age on process cheese itself are well documented, however it is less known the effects cheese age will have when converted into a sauce. This is important to understand as it may help manufacturers provide consist and stable sauces for foodservice operators.

Another goal of this study was to evaluate different methods for testing cheese sauce viscosity. While there are many methods for measuring viscosity, of which many are complex and time consuming, the goal of this study was to evaluate quick and relatively easy methods of viscosity testing that would be more common methods used by manufacturers.

Chapter 2

Review of Literature

2.1 Process cheese and standards of identity

Process cheese is derived from the blending and heating of natural cheeses with emulsifying salts followed by packaging and cooling to produce a homogenous and stable end product (Hickey, 2011). Emulsifying salts such as disodium phosphate (DSP) or trisodium citrate (TSC) disrupt casein crosslinks within natural cheese which allows for dispersion of the newly formed casein complexes and increases the ability to bind with fat via hydrophobic interactions (Kapoor and Metzger, 2008). This process enables the extension of natural cheese shelf as well as improving the quality and stability of natural cheese (Zehren and Nusbaum, 2000). The first recognized process cheese product was developed by Walter Gerber and Fritz Stettler in Switzerland, 1911 (Meyer, 1973). Around this same time the first process cheese product and manufacturing process was being developed by James Kraft. He was granted a patent in 1916 which involved the heating and stirring of Cheddar cheese until a temperature of 170°F was reached and held for 15 minutes. After this pasteurization step was completed, the free flowing and homogenous process cheese was put into sanitary jars or cans which were sealed and cooled (Zehren and Nusbaum, 2000). From this point on, there have been many developments within process cheese manufacturing, formulation, and packaging. This includes the use of batch and continuous cook process, addition of new emulsifying salt types, usage of other dairy and non-dairy ingredients, different varieties of cheese used in the blend, as well as different forms (slice, block, loaf, spread, sauce) (Hickey, 2011).

These developments prompted the need for standards and regulations to be put in place for the manufacturing and labeling of process cheese products.

The FDA (2014) defines the identity of process cheese under three main standards; pasteurized process cheese (aka “American cheese”) (21CFR133.169), pasteurized process cheese food (21CFR133.173), and pasteurized process cheese spread (21CFR133.175). Table 1 represents a summary of the different process cheese standard of identities.

Table 1. Summary of standards and regulations for different types of pasteurized process cheese

	Pasteurized Process Cheese Types			
	"American" Cheese	Cheese Food	Cheese Spread	Cheese Product
Min. Cheese	~75%	51%	51%	None
Max. Moisture	40%	44%	60%	None
Min. Fat	50% fat dry basis	23%	20%	None
Max. Add Fat	5%	None	None	None
Allowable Alternate Dairy Ingredients	Cream, Milkfat	Cream, Milk, Skim Milk, Buttermilk, Whey, Whey Protein Concentrate	Cream, Milk, Skim Milk, Buttermilk, Whey, Whey Protein Concentrate	Milk Protein Concentrate, Caseins, Caseinates, Vegetable Oils
Other Allowable Ingredients			Gum, Sugar, Starch	

Within each of these standards there are different compositional and ingredient usage definitions and parameters. The proceeding definition summaries are based on Cheddar cheese being the cheese used for process cheese manufacture as this is the most common type of cheese manufactured in the United States. From a compositional standpoint,

pasteurized process cheese must meet the following requirements: not more than 40 percent moisture (1% greater than the maximum moisture content for Cheddar cheese), minimum fat content on a dry basis of 50 percent (equal to minimum fat content for Cheddar cheese), and pH not less than 5.3. The allowable ingredients and amounts are as follows; cream and/or milk fat (5 percent maximum), emulsifying salts (3 percent solids maximum, salt, acidulants, artificial color, and sorbic acid/sorbate (preservative) (0.2% maximum). The standard of identity for pasteurized process cheese food states that the product must contain a minimum of 51 percent cheese, have a maximum moisture content of 44 percent, a minimum fat content of 23 percent on a wet basis, and pH not below 5.0. The allowable ingredients and amounts within the previously mentioned pasteurized process cheese definition are included in pasteurized process cheese food, but additionally may also contain: milk, skim milk, buttermilk, whey, whey protein concentrate, and milk fat (no minimum or maximum defined for any). The standard of identity for pasteurized process cheese spread is essentially the same as pasteurized process cheese food but allows a moisture maximum of 60 percent, a minimum fat on a wet basis of 20 percent, and a pH not below 4.0. It also allows the usage of certain non-dairy ingredients which include; sugars, starches, and gums. There is another common naming convention for process cheese known as pasteurized process cheese product. There are no specific defined compositional requirements or standards for this type of product, and it generally contains one or more of the following ingredients; caseins, caseinates, milk protein concentrate, and vegetable oils.

2.2 Natural cheese in process cheese manufacturing

The selection of the natural cheese to be used in the manufacturing of process cheese is a critical step in determining the functionality and flavor of the end product. While there are multiple types of natural cheese (Colby, Swiss, Mozzarella, etc.) that can be used in process cheese manufacturing, the vast majority of process cheese made in the United States is derived from Cheddar cheese (Zehren and Nusbaum, 2000). There are several key factors and compositions of the natural cheese that will have a direct impact on process cheese functionality and flavor including; moisture, fat, salt, calcium, pH, and age (Biswas et al., 2008; Kapoor et al., 2007). Of these, the most crucial to control is age because of its relationship to proteolysis of casein which has a direct impact on texture and flavor development in cheese.

Casein is the primary protein found in natural cheese and thus provides the structural network of process cheese via protein-protein and protein-fat interactions (Garimella Purna et al., 2006). As cheese ages, there is more opportunity for casein to be hydrolyzed which creates smaller and more soluble peptide structures. This hydrolysis also reduces the number of hydrophobic protein-protein and protein-fat interactions thus creating a change in cheese structure and texture (Fox et al., 2000). This makes it vitally important to understand, as well as quantify the proteolysis of natural cheese to be used in process cheese manufacture especially as it relates to age.

Casein consists of 4 individual types of molecules known as; α S1-casein, α S2-casein, β -casein, and κ -casein (CN) (Dalgleish and Corredig, 2012). Proteolysis of these casein molecules in natural cheese is caused by proteolytic enzymes that can be

indigenous to the milk, like plasmin, or from the rennet, starter cultures added during cheese making, and non-starter organisms that either survive pasteurization or are inadvertently introduced during manufacturing. As it relates to texture, the primary casein molecules of concern are α S1-, α S2-, β -CN. κ -CN exists mainly as para- κ -CN in cheese due to the initial coagulation (rennet) of milk in cheese making which cleaves κ -CN at the Phe₁₀₅-Met₁₀₆ bond, producing para- κ -CN and glycomacropeptides, and is fairly resistant to hydrolysis within cheese due to the salt content and pH of cheese (Fox et al., 2000). Initial proteolysis in natural cheese is primarily caused by the proteinases; chymosin (from rennet) and plasmin (McSweeney, 2004). Chymosin cleaves the Phe₂₃-Phe₂₄ peptide bond of the α S1-CN molecule which creates two peptides, α S1-CN (f1-23) and α S1-CN (f24-199) (O'Mahoney et al., 2005). These peptides are further hydrolyzed (mainly by peptidases from starter cultures) creating an initial softening of the cheese (Fox et al., 2000). Plasmin can act on all casein molecules, but is mainly active on α S2- and β -CN and is main cause of hydrolysis of β -CN (Fox et al., 2000). Within 6 months of age, all the α S1-CN is hydrolyzed by chymosin at the Phe₂₃-Phe₂₄ peptide bond and 50% of the β -CN has been hydrolyzed by plasmin (Fox et al. 2000). While these proteinases are the primary causes of initial proteolysis of cheese, starter and non-starter cultures also contribute to proteolysis in cheese. Certain lactic acid bacteria (LAB), the primary type of bacteria used in Cheddar starter cultures, do possess proteinases which can hydrolyze α S1-CN, α S2-CN, or β -CN depending on the type of LAB (Fox et al., 2000). However, the proteolysis of casein from LAB is considered to be relatively weak and less impactful than chymosin and plasmin. While direct proteolysis of casein in

cheese by LAB is minor, they also contain peptidases which can hydrolyze the numerous peptides and polypeptides produced by the hydrolysis of α S1-, α S2- and β -CN from chymosin and plasmin (McSweeney, 2004).

Since proteolysis of natural cheese has a large impact on the texture and functionality of process cheese it is important to be able to quantify the amount of proteolysis which has occurred. While techniques such as electrophoresis and chromatography are able to detect specific peptides and provide detailed information in regards to proteolysis, they are time consuming and costly. It is more beneficial to process cheese manufactures to use faster and less costly methods for measuring proteolysis in cheese. One such method used for determining proteolysis in cheese is by extracting the cheese with water and using the Kjeldahl method to measure the nitrogen content of the water extract and subtracting from the total nitrogen to give the amount of insoluble protein (Kuchroo and Fox, 1982). This value represents the amount of protein that is insoluble as during proteolysis, small and medium sized peptides become water soluble (Bansal et. al., 2010). Thus the insoluble protein can be considered as casein that has not be hydrolyzed to a large extent. One shortcoming of this method is that casein becomes more soluble as it reaches its isoelectric point at pH 4.6. Thus if pH is not controlled during the testing, a higher amount of protein may become water soluble regardless of the amount of proteolysis that has occurred (Bansal et. al., 2010). Another method that can be used for determining proteolysis in cheese is by measuring the total protein via the Kjeldahl method and subtracting the non-casein protein amount from the total protein amount. The non-casein protein of cheese is obtained by using the AOAC

method for Noncasein Nitrogen Content of Milk (AOAC 998.05). In this method cheese is blended in a 4.6 pH buffer solution (acetic acid and sodium acetate) and then filtered. The filtrate is analyzed for nitrogen content via the Kjeldahl method and multiplied by a factor of 6.38 to obtain non-casein protein. The insoluble protein at pH 4.6 amount can be related to intact casein. Intact casein is generally defined as the amount of casein that is still functional within cheese because little to no hydrolysis has occurred on the casein so it is still in its native form. During the typical manufacturing process of process cheese, the network of casein molecules (paracasein) from the natural cheese is dispersed and becomes charged and hydrated which increases the water binding ability and fat emulsification of the casein (Fox et. al, 2000; Guinee, 2011). Therefore, increased amounts of intact casein allow for increased ability of water binding and fat emulsification within process cheese which tends towards a stable and homogenous product. As proteolysis increases in cheese and the casein becomes more hydrolyzed, the protein structures created from the hydrolysis (peptides) become more water soluble and the hydrophilic and lipophilic structures of the casein become altered (Guinee, 2011). This leads to a reduction in the emulsifying ability of the caseins within process cheese and therefore there is an inverse relationship between cheese age and emulsifying capacity (Fox et al., 2000). While more aged cheese may have decreased emulsification capacity, it does offer other flavor and texture benefits that younger cheese cannot. Several studies have shown the effects of cheese age on process cheese texture and functionality. Similar trends are seen throughout the studies which have found process cheese made with younger cheeses to have a firm body, good slicing, and less melt, while

those made with more aged cheeses exhibited a softer body, more melt, and developed a grainy texture (Brickley et al., 2007; Olson et al., 1954; Piska, 2004; Garimella Purna et al., 2006; Sommer and Templeton, 1932; Wang et al., 2011; Zahariasen, 1954). Cheese manufactures often use a combination of young and more aged cheeses in order to deliver a certain texture, optimal performance, and preferred flavor profile.

Age and proteolysis also play a large role in the flavor development and profile of cheeses. There are many flavor compounds formed as cheese ages which are a result of proteolytic, lipolytic, and glycolytic reactions. Proteolysis in natural cheese leads to the formation of peptides (shorter protein fragments) and free amino acids, which when catabolized form compounds that contribute directly to cheese taste and aroma (Singh et al., 2011). It is important to understand these reactions and the compounds made from them as they will have a direct impact to the flavor of the cheese (good or bad) and thus to the flavor of the process cheese using it. Below is a list of some of the compounds formed by the catabolization of certain amino acids in Cheddar cheese and their corresponding aromatic notes.

Catabolic products	Precursor	Aroma note
2-Methyl propanoic acid	Valine	rancid butter, sweaty, sweet, apple-like
2-Methyl-1-propanol	Valine	penetrating, alcohol, wine-like
2-Methyl propanal	Valine	malt
3-Methyl butanoic acid	Leucine	cheesy, sweaty, old socks, rancid, faecal, rotten fruit
3-Methyl-1-butanol	Leucine	fruity, alcohol, solvent-like, grainy
3-Methyl butanal	Leucine	dark chocolate, malt
2-Methyl butanoic acid	Isoleucine	fruity, waxy, sweaty-fatty acid
2-Methyl-1-butanol	Isoleucine	—
2-Methyl butanal	Isoleucine	dark chocolate, malt
3-(Methylthio) propanal	Methionine	cooked/boiled potato
3-(Methylthio) propanol	Methionine	cooked/boiled potato
Methanethiol	Methionine/cysteine	cabbage, boiled cabbage, sulfurous
Methyl sulfide	S-containing	cabbage, sulfurous
Dimethyldisulfide	S-containing	onion
Dimethyltrisulfide	S-containing	garlic
Dimethyltetrasulfide	S-containing	cabbage
Acetophenone	Phenylalanine	almond, musty, glue
Benzaldehyde	Phenylalanine	almond, bitter almond
Phenyl acetaldehyde	Phenylalanine	rosy, violet-like
Phenylethyl alcohol	Phenylalanine	unclean, rose, violet-like, honey
Phenyl acetic acid	Phenylalanine	flowery, rosy, plastic
Phenol	Tyrosine	medicinal
p-OH-phenyl aldehyde	Tyrosine	—
p-OH-phenyl lactate	Tyrosine	—
p-OH-phenyl acetate	Tyrosine	—
p-Cresol	Tyrosine	unclean, medicinal
Indole	Tryptophan	unclean, mothball
Skatole	Tryptophan	unclean, mothball
Benzaldehyde	Tryptophan	almond

Figure 1. Amino acid catabolites formed by lactic acid bacteria in Cheddar Cheese. (Singh et al., 2003)

While the age of the cheese being used in the making of process cheese is an important factor to consider, so is the make process of the natural cheese. It has become common practice in cheese manufacturing to start with ultrafiltered (UF) or vacuum condensed milk. The purpose of condensing milk prior to cheese making is to increase efficiency and cheese yield (Foster et al., 1990). However, it has been found that cheese made from condensed milk does differ from traditional make in both composition and textural attributes. Cheese from condensed milk has been found to have; increased lactose, ash, calcium, total protein, whey protein, fat, and hardness as well as decreased moisture, proteolysis, meltability, and flavor compared to traditional make cheese (Foster et al., 1990; Acharya and Mistry, 2004). These results are a product of the condensing process (UF or vacuum), as milk solids are more easily trapped within the milk matrix

and less likely to be removed during the “wheying off” process. It stands to reason that the observed difference between condensed milk cheese and traditional make cheese would also have an impact on process cheese characteristics when used for manufacturing. Similar to the observed differences between condensed milk and traditional make cheese, Acharya and Mistry (2005) found process cheese products made with condensed milk cheese had higher degrees of hardness and decreased meltability compared to control. Interestingly, it was also found that the process cheese made from condensed milk scored higher in acceptability during sensory evaluation. The samples were found to be less pasty, mealy, and bitter. This is likely a function of the decreased proteolysis observed in condensed milk cheese.

2.3 Compositional, ingredient, and processing effects on process cheese functionality

Process cheese is a complex and intricate system whose functional attributes such as melt, sliceability, sauce viscosity, and emulsion stability as well as texture, are heavily influenced by key compositions and processing conditions and their interactions. Some of the key compositions include casein, fat, and emulsifying salts. While these factors play a large role in process cheese functionality and texture, so do processing parameters like cook time and shear. The effects of these key compositional and processing conditions are described below.

2.3.1 Casein

Casein is the key component related to process cheese texture and functionality. As previously discussed, the structure of process cheese is linked to the relative (intact) casein content which corresponds to long thread-like structures (Berger et al., 1989).

These intact casein structures allow for increased protein-protein and protein-fat interactions which increases firmness (Dimitreli and Thomareis, 2003; Garimella Purna et al., 2006; Shimp, 1985). Inversely, shorter chained casein fragments have weaker protein-protein interactions in the process cheese which can lead to a crumbly texture (Guinee, 2011; Shimp, 1985). The hydrophilic and hydrophobic parts of casein have a greater capacity to bind water and fat, respectively, within a process cheese system when the casein has undergone minimal proteolysis which allows for increased emulsion stability and thus a more stable product (Berger et al., 1989; Guinee, 2011). This relationship also explains the different functionality attributes in process cheeses as higher amounts of intact casein enhances sliceability due to increased firmness from a higher degree of emulsification, while lower intact casein enhances flow (otherwise known as meltability), due to a release of fat from a less stable emulsion (Guinee, 2011; Vakaleris et al., 1962; Wang et al., 2011). These results are similar to the results found in the previously mentioned studies examining cheese age and effects on process cheese texture.

2.3.2 Fat

Fat plays a key role in process cheese texture and functionality due to its relationship with casein. The protein to fat ratio in process cheese is important for determining the limits of texture modification (Shimp, 1985). During the manufacture of process cheese free fat is emulsified into smaller droplets by shear which creates a larger surface area and allows for greater absorption capacity with casein leading to a more stable emulsion (Zehren and Nusbaum, 2000). However, if the fat globules become too

small from overshearing, the surface area of the globules becomes too large for the protein matrix to surround it (Shimp, 1985). This can lead to oiling off of the fat as well as “overcreaming” which creates a thick texture and high viscosity caused by an increase in strength to the protein matrix by the inclusion of more fat particles (Garimella Purna, 2006). However, the presence of fat within process cheese can also inhibit increased protein-protein interactions and cross-linking that may occur in reduced fat process cheeses due higher concentrations of protein, calcium, and phosphates (Guinee and O’Callaghan, 2013). In this regard, fat acts as a “buffer” to the proteins which can enhance melt and decrease firmness. A similar result was observed in a study by Subramanian et al. (2006) in which they found a reduction in fat content had a larger impact on process cheese rheology properties compared to an increase in moisture. Fat content also plays a role in the meltability of process cheese in that higher amounts at high temperatures provide increased heat conductivity within the system and allows protein to flow with the melted fat both which enhances meltability (Guinee, 2011).

2.3.3 Emulsifying salts

Emulsifying salts facilitate the emulsifying ability of casein from natural cheese within process cheese. Casein micelles in natural cheese are bound together by colloidal calcium phosphates bridges making the casein water insoluble and inhibiting its emulsifying capacity (Fox et al., 2000) (Guinee, 2011) (Berger et al., 1989) (Shimp, 1985). Emulsifying salts are able to sequester the calcium (insoluble) from the colloidal calcium phosphate compounds which are bridging the casein micelles and replace it with sodium while also creating a shift in pH. These actions, facilitated by the cooking

process, allow for the dispersion and hydration of casein which increases interactions between proteins as well as emulsification between fat and water (Kapoor and Metzger, 2008) (Lucey et al., 2011). The degree of casein emulsification in process cheese, and in turn the texture and functionality is determined by the insoluble calcium sequestering ability and amount of the emulsifying salts being used. Orthophosphates, such as monosodium phosphate (MSP), disodium phosphate (DSP), and trisodium phosphate (TSP) and citrates, such as monosodium citrate (MSC), disodium citrate (DSC), and trisodium citrate (TSC) are relatively weak calcium binders and produce weaker emulsions within a process cheese which leads to a softer texture and increased meltability (Berger et al., 1989; Kapoor and Metzger, 2008; Shimp, 1985). On the other hand, diphosphates such as disodium dihydrogen diphosphate and tetrasodium diphosphate and polyphosphates, such as pentasodium triphosphate and hexasodium tetraphosphate are strong calcium binders which produce strong emulsions, leading to firm texture and decrease meltability (Berger et al., 1989; Kapoor and Metzger, 2008; Shimp, 1985). The amount of the emulsifying salts being used is also a key factor in regards to process cheese texture and functionality. There needs to be a proper ratio of emulsifying salt solids to casein in order to prevent improper emulsification. If there is too little emulsifying salt, there will be limited insoluble calcium sequestering and casein dispersion, causing oiling off (Lucey et al., 2011; Shimp, 1985). Similarly, too much emulsifying salts will sequester more calcium and enhance emulsification leading to a firm texture as well as oiling off caused by protein-protein interactions overtaking the

protein-fat interactions and pushing the fat out of the matrix (Berger et al., 1989; Lucey et al., 2011; Shimp, 1985).

2.3.4 Processing conditions

The processing conditions during the manufacture of process cheese can play a pivotal role in the functional and textural attributes of the end product. This includes time and shear during cooking. A study by Glenn et al. (2003) found that process cheese samples exposed to longer cook times and shear had lower melt values. Lee et al. (2003) observed an increase in viscosity of process cheese during manufacture when exposed to longer cook times. Garimella Purna et al. (2006) found an increase in viscosity of process cheese during manufacture and in the end product's firmness when mixed at higher speeds during cooking. All these observations were attributed to the processing conditions causing a decrease in the fat globule size as well an increase in protein surface which enhanced both protein-protein interactions as well as the water binding ability of the proteins.

2.4 Viscosity of process cheese and methods of measurement

2.4.1 Compositional effects on viscosity of process cheese

Viscosity is generally defined as the tendency of a fluid to resist flow (Bourne, 1982). In cheese, viscosity can help quantify melt as "meltability is related to both ease and extent of flow" (Gunasekaran and Ak, 2003). Viscosity is determined by the ratio of stress to strain rate where stress is related to ease of flow and strain rate is related to extent of flow (Gunasekaran and Ak, 2003). Process cheese is considered a viscoelastic material and can have the traits of both a solid and liquid depending on the external

forces being applied to it (Gunasekaran and Ak, 2003). When exposed to higher temperatures process cheese behaves similar to a fluid or liquid material which enables the measurement of viscosity. It is important to understand the viscosity of process cheese at high temperatures as it can provide clues to the functionality and texture of the end product (Dimitreli and Thomareis, 2006). There are several factors that can affect the viscosity of process cheese including; cheese age and intact casein, fat, and emulsifying salt type and amount.

Studies have shown the correlation of increasing cheese age or decreasing intact casein of the natural cheese used in process cheese to decreasing viscosity (Garimella Purna et al., 2006) (Dimitreli and Thomareis, 2004) (Dimitreli and Thomareis, 2006) (Kapoor et al., 2007). As mentioned before, the decrease in intact casein reduces the amount of protein-protein interactions as well as the emulsifying capacity of casein. This causes a decrease in the strength of the protein matrix in process cheese and thus a decrease in viscosity is generally observed. These results are in line with previously mentioned studies which correlated increasing cheese age as well as decreasing intact casein with softer body and increased melt.

Fat content has also been shown to affect viscosity in process cheese. Guinee and O'Callaghan (2013) and Subramanian et al. (2006) both found increases in viscosity and decreases in flowability as fat content was reduced. This was found in spite of an increase in moisture in the reduced fat samples. The conclusions reached in the Guinee and O'Callaghan (2013) study suggested a reduction in fat increases the amount of soluble protein in the moisture phase which allows for increased interactions between

phosphate, calcium, and protein which enhanced gelation. Similarly, Subramanian et al. (2006) proposed the emulsified fat within the protein matrix limits the amount of protein-protein interactions and thus a cheese with a lower fat content will have increased protein-protein interactions. Their results showed that while a decrease in moisture content has a similar effect to decreased fat content in the viscosity and texture of process cheese, the decrease in fat had a significantly higher impact than the moisture decrease. These results are further supported by Rogers et al. (2009). While the study was on natural cheese, they found decreased amounts of fat produced firmer textural attributes of the cheese. They hypothesized that fat acts as a filler within the protein matrix of the cheese and creates some weak spots within the protein matrix when deformed. It also can be theorized, as was done by Glenn et al. (2003) that fat droplet size would also have an impact on the protein network in process cheese (and thus viscosity) as smaller droplet size would provide less fill within protein matrix and enable more protein-protein interactions.

The type and amount of emulsifying salts has also been shown to have a significant effect on process cheese viscosity. Ennis et al. (2000) found differences in time to maximum viscosity (peak viscosity within the cook time of the process cheese) in a rennet casein model system between samples using dipotassium phosphate (DPP) and disodium phosphate (DSP). They observed a shorter time to the maximum viscosity in the samples using DPP as the emulsifying salt. This suggests that the DPP is able to disperse the casein in the system more quickly than DSP. The quicker casein dispersion would lessen the time of the protein matrix formation within the process cheese.

Shirashoji et al. (2010) found process cheese at high temperatures (80°C) had different rheological behaviors when using DSP and sodium hexametaphosphate (SHMP) as the samples using SHMP were thicker and less able to melt compared to the DSP samples. Similarly, to the Ennis et al. (2000) study, the rheology differences were attributed to casein dispersion as SHMP is a more effective calcium chelator and thus is able to disperse casein more effectively than DSP. Sutheerawattananonda and Bastian (1998) observed viscosity differences between process cheeses with DSP and trisodium citrate (TSC) in which the process cheese with TSC had a lower transition temperature to flow and viscosity compared to the DSP process cheese. Garimella Purna et al. (2006) associated low TSC content with low viscosity results due to lack of casein dispersion which limited the protein-protein interactions.

2.4.2 Methods of viscometry in process cheese

There are various rheometry and viscometry methods available which can provide in-depth textural profiles as well as certain viscosity and flow values of materials, including cheese. Dynamic mechanical analysis (DMA) and differential scanning calorimetry (DSC) can be used to understand the viscoelastic behavior as well as measure phase and glass transitions of materials at a certain temperature profile. While these methods can provide valuable insight and data into the viscosity and behavior of a material over a certain temperature range, they are expensive and not as valuable when determining viscosity at a constant temperature or when attempting to quickly measure viscosity of a material. There are other less costly and more empirical methods which can measure the flow of a material which can be related to viscosity. As it relates to

cheese, two such methods include the Schreiber melt and tube test. These methods are valuable when trying to quickly and inexpensively determine the flow or melt characteristics of a cheese. However, they can be limiting in their usefulness for cheeses or cheese sauces with high moisture contents. While there have been numerous studies done on the rheology of process cheese, there are not as many that specifically test viscosity of process cheese at a constant temperatures and even fewer at viscosity of process cheese sauces. The following is a summary of several methods from studies which directly measured viscosity at certain temperatures of process cheese.

2.4.2.1 Rotational viscometry

Rotational viscometry is a method in which one portion of an apparatus is rotated, while another portion remains stationary, to determine apparent viscosity. Certain conditions (temperature and rpms) are applied, while the forces applied to the measuring device (typically a paddle or probe of various configurations) by the material being evaluated or measured. In this method the shear rate is constant and the viscosity value is determined by the strain or torque of the measuring device needed to maintain the shear rate. It is important to understand the geometry and surface area of the measuring device when using this method as different devices will require different strain rates to overcome the resistance to maintain constant shear which will in turn affect the end viscosity result. Lee et al. (1978) was able to determine apparent viscosity of several different cheeses under increasing temperatures, including two process cheeses by applying a constant rotating shear rate using a Brookfield RVT viscometer with a T-F bar spindle. Ennis et al. (1998) determined the viscosity index over time of rennet caseins with varying

amounts of emulsifying salts at a constant temperature by applying a constant rotating shear rate of a paddle apparatus which consisted of two stainless steel plates with two perforations attached to a spindle. The apparatus was then placed inside a water jacketed cup with the material inside the cup. Lee et al. (2003) did a similar study in which a Bohlin CVO rheometer with concentric cylinder apparatus placed in a cup was used to study viscosity creaming profiles of process cheese with and without fat. There have also been several studies which have used a Rapid Visco Analyser (RVA) to measure multiple viscosity profiles of different process cheeses both during and after the manufacturing process under varying conditions (time and temperature) (Kapoor and Metzger, 2005; Metzger et al., 2002; Prow and Metzger, 2005; Garimella Purna et al., 2006). The RVA measures viscosity by applying a constant shear rate with a curved stirring paddle designed to fit inside a canister which filled with the cheese.

2.4.2.2 Dynamic stress/shear rheometry

Dynamic stress or shear rheometry is used to measure mechanical properties of a material when subjected to an oscillating strain or stress (Gunasekaran and Ak, 2003). The most common method used for this type of measurement is small amplitude oscillatory shear (SAOS). In SAOS testing, a material is subjected to an alternating (sinusoidal) strain at a constant amplitude and frequency and the resulting stress is measured (Gunasekaran and Ak, 2003). In SAOS testing, the energy stored and dissipated within a material can be measured via stress output resulting from the alternating strain being applied (Gunasekaran and Ak, 2003). Thus a cross-over point can be determined from when the material goes from being gel-like or elastic to being

more fluid-like or viscous when the value of energy dissipated is closer to a liquid (Newtonian) than a solid (Hookean) which enables viscosity to be determined (Gunasekaran and Ak, 2003). Sutteerawattananonda and Bastian (1998) used SAOS testing to measure the effect emulsifying salt type (DSP and TSC) and moisture content had on the meltability of process cheese. They found that process cheese which used TSC had a lower temperature when the cross-over occurred from a gel-like (elastic) structure to a more fluid-like (viscous) material and had a higher rate of change in viscosity compared to process cheese with DSP.

2.4.2.3 Squeeze flow rheometry

Squeeze flow rheometry is a uniaxial compression test that enables elongational viscosity to be determined. In this method elongational viscosity is determined by compressing a material at a known strain rate between two lubricated plates (Gunasekaran and Ak, 2003). A study by Campanella et al. (1987) compared the elongational viscosities of two different branded process cheese samples over a range of temperatures of 36°C-62°C using uniaxial compression testing on a Universal Testing Machine. They observed a decrease in viscosity with an increase in temperature. While they were unable to find a good model of fit for predicting elongational viscosity with meltability for both samples, the differences they found were small enough that a model of fit may be found if further testing was done with an increased temperature above 62°C.

Chapter 3

Effects of Cheese Age on Viscosity of Sauces Made from Process Cheese Loaves and Comparison of Methods Used to Measure Sauce Viscosity

3.1 Introduction

The ability of process cheese to melt and remain stable and well emulsified (no oiling off or fat separation) at high temperatures compared to natural cheeses which have a tendency to oil off fat at high temperatures, makes it an ideal base for making sauces. The USDA in 2011 estimated that 2.1 billion pounds of process cheese was produced in the U.S (USDA, 2011). Of that, 200 million was used in the foodservice area (Land O'Lakes, 2011). While slices and shreds are common uses for process cheese in the foodservice area, it is estimated that 60 percent of foodservice operators also convert process cheese into sauces (Land O'Lakes, 2013). Operators need the heated sauces to remain consistent in viscosity and stable (no oiling off/fat separation) over long hold times in order to meet customer expectations and reduce labor costs. As previous studies mentioned before have shown, there are many factors that can alter the texture and functionality of process cheese, including cheese age. However, the effects of cheese age on process cheese sauces are relatively unknown. Since higher age of cheese is associated with increased proteolysis and thus lower degree of emulsification (Guinee, 2011), it would stand to reason that there would be textural differences observed in sauces made from process cheese with varying age cheese blends. The increased water binding ability and fat emulsification of process cheese made with younger cheese due to higher amounts of intact casein (Guinee, 2011) would suggest that sauces made from process

cheese with younger cheese blends would have a higher viscosity than sauces made from older cheese blend process cheeses.

While it is well known that composition and age factors of natural cheese have an effect on process cheese performance and texture, processing factors such as time and temperature, also have an effect. Glenn et al. (2003), Lee et al. (2003), and Garimella Purna et al. (2006) all reported an increase in viscosity and firmness as well as a decrease in melt of process cheese when exposed to longer cook times and temperatures. This was concluded to be the result of decreased fat globule size and increased protein surface area which enhanced the water binding ability of the proteins as well protein-protein interactions. The results from these studies suggest that a similar phenomenon may be observed in sauces made from process cheese when held over long periods of time at a high temperature.

The goal of this study is better understand the effect of both natural cheese characteristics, specifically age, and processing conditions on the viscosity of sauces made from process cheese. There have been many studies showing the effect of both factors on process cheese itself, but very few studies that show the effect of both factors especially when being converted into an application based product which is typically done by process cheese users.

This study will also examine multiple methods for testing sauce viscosity as there are several methods available that enable a quick reading for process cheese manufacturer and researchers. This will hopefully enable manufacturers and researchers to select a

method of viscosity measurement that best fits the application and product being measured or studied.

3.1 Materials and methods

3.1.1 Process cheese manufacture

Five process cheese loaf treatments were made in triplicate with cheese blends having average ages of: 10, 30, 90, 150, and 210 days (10d, 30d, 90d, 150d, 210d). Land O'Lakes 18.14 kg. Cheddar cheese blocks (Kiel, WI) were used to create these cheese blends. A Hobart (Troy, OH) food processor model FP100 with a shredding blade attached was used to create cheese shreds of each Cheddar cheese block. The five average cheese blend ages for the process cheese loaves was determined by combining a certain percentage of six separate Cheddar cheese shreds aged to: 10, 34, 42, 44, 454, and 456 days as shown in Table 2.

Table 2. Mixture design of Cheddar cheese blend for process cheese manufacture targeting specific average age (10, 30, 90, 150, 210 days).

Average Process Cheese Blend Age (days)	Cheddar Cheese Block Age (days)					
	456	454	44	42	34	10
10						100.00%
30					83.00%	17.00%
90	11.25%		88.75%			
150		26.25%		73.75%		
210	46.00%			54.00%		

The process cheese treatments were formulated to meet the U.S. Food and Drug Administration standard of identity for “Pasteurized Process Cheese” (21CFR133.169) and standardized for moisture (40%), fat (30%), and salt (2%). Certificate of analysis

(COA) data from each of the Cheddar cheese blocks and a Land O’Lakes proprietary Microsoft Excel-based formulation software program was used for compositional standardization. The Cheddar cheese blend amount for all the process cheese treatments was kept constant at 75%. Other ingredients used for the manufacture of the process cheese loaves included; anhydrous milkfat (~0.6-1.6%) (Madison Farms Butter Co. LLC, St. Louis, MO), dried cream (8.6-10.4%) (Kerry Ingredients, Owen, WI), salt (~0.6%) (Cargill, Breaux Bridge, LA), duohydrate disodium phosphate (DSP) (2.5%) (Innophos, Chicago Heights, IL), water (10.8-11.5%) (condensate from the steam accounted for 7.3% of the total water). The emulsifying salt (DSP) amount (2.5%) was kept constant for all process cheese treatments, while the rest of the non-cheese ingredients were used for compositional standardization purposes. The targeted formula and compositions for each of the process cheese treatments are shown in Tables 3 and 4 respectively.

Table 3. Formulation of process cheese loaf treatments

Ingredient	%
Cheddar Cheese	75%
Dry Cream	9.77%
Anhydrous Milkfat	0.98%
Salt	0.60%
Disodium Phosphate (DSP)	2.50%
Water	11.14%

Table 4. Targeted composition values of process cheese treatments

Composition	Target (%)
Moisture	40%
Fat	30.20%
Salt	2%
Total Protein	19.40%
Lactose	2.77%
Fat Dry Basis (FDB)	50.33%
Added Fat	4.98%

The Cheddar cheese blends along with all other ingredients were blended at ambient temperature in a Koss Industrial (Green Bay, WI) steam-injected, twin-screw auger batch cooker for 2 minutes at 110 rpms. After the 2-minute blend time, the steam was turned on and delivered to the cooker at a pressure of 9 psi while the rpms of the augers remained unchanged. The process cheese blends were cooked to a temperature of 74°C and held for 1 minute. The process cheese was then collected from the cooker into 5 lb. (2.2 kg.) loaf cartons (2) and placed into a humidity controlled, air circulated environmental chamber in a single layer which slowly cooled the process cheese samples to 4°C within 20 hours. The process cheese samples were held at 4°C until further testing was completed.

3.1.2 Chemical composition analysis

The process cheese samples were analyzed compositionally for; moisture, fat, salt, total protein, insoluble protein at pH 4.6 (casein), lactose, total calcium, and pH. Moisture content of the process cheese samples was determined using a vacuum oven at 100C for 5 hours (AOAC 926.08). Fat content was determined by the Mojonnier method (AOAC 933.05). Total protein was determined using the Kjeldahl method to obtain the

total Nitrogen content which was then multiplied by a factor of 6.38 (AOAC 2001.14). Insoluble protein at pH 4.6 (referenced as casein throughout the study) was determined by subtracting non-casein protein from total protein. The non-casein protein value was obtained by blending 5 g of the process cheese sample (finely ground) with 42 g of deionized water and 0.05 mL of 10% acetic acid. The solution was allowed to stand for 10 minutes. After the 10-minute hold time, 0.05 mL of 10% sodium acetate was added to the solution and mixed. The precipitate within the solution was allowed to settle and then the solution was filtered through Whatman #1 filter paper. The filtrate was then analyzed for total protein via the Kjeldahl method. This value was considered the non-casein protein content which was then subtracted from the total protein amount to determine the casein content. Lactose was determined enzymatically using a Megazyme (Wicklow, Ireland) Lactose/D-Galactose Rapid Assay Kit (K-LACGAR). Salt was determined using a Corning Chloride Salt Analyzer (Model 926) using (SMEDP, Method 15.053). Total calcium was determined by atomic absorption using the method as described in (AOAC 991.25). pH was determined using a Mettler Toledo (Columbus, OH) FiveEasy pH meter with a glass probe using (SMEDP, Method 15.022). Casein as a percent of total protein was calculated by dividing the insoluble protein % at pH 4.6 (casein) by the total protein results for each of the treatments.

3.1.3 Cheese sauce make

The process cheese loaf samples were cut into 5, 1 lb. cubes and processed through a Hobart (Troy, OH) food processor model FP100 with a shredding blade with ¼ in. pores to create cheese shreds. The shreds were collected in plastic storage bags and

labeled. One pound (453.6 g). of the cheese shreds were then weighed into a stainless steel double boiler along with 0.33 lbs. (151.2 g) of RO (reverse osmosis) water at room temperature for a 3:1 cheese to water ratio. A lid was placed on top of the double boiler which was then set on stovetop over a gas burner set on high and cooked for 3 minutes. After the initial 3-minute cook time, the lid was removed and a 3 bladed stainless steel propeller attached to a Lightnin Labmaster (Rochester, NY) G2Y05R stand mixer set at 400 rpms was placed into the cheese sauce for consistent mixing. The cheese sauce was continually heated and mixed until a temperature of 70°C was achieved. Once the target temperature was reached, the cheese sauce was poured into a 600 mL Pyrex® (Corning Inc., Tewksbury, MA) glass beaker until the sample was needed for viscosity testing. A small portion of the cheese sauce was also sampled into a plastic 2 oz. lidded soufflé cup used for initial moisture testing. A portion of the cheese sauce within the glass beakers was used for initial viscosity testing while the remainder was covered with plastic wrap and placed into a Thermo Scientific (Thermo Fisher Scientific, Marietta, OH) Precision water bath (model 2866) set at 70°C. The cheese sauces were again tested for viscosity after 2 and 4 hours of hold time after initial testing with additional samples taken for moisture testing at each hold time point. Moisture testing was done on the sauces at each time point in order to determine the degree of possible evaporation during the hold times. Moisture loss % was calculated over the entire hold time and at the 2 and 4 hour hold time points for all samples. This was done by subtracting the 2 and 4 hour hold moisture testing result from the initial moisture testing result. Prior to all viscosity tests a kitchen hand mixer with a whisk attachment was placed into the cheese sauce beaker and mixed

on low for 5 seconds. This process was used to prepare cheese sauce samples for all 3 methods of viscosity testing.

3.1.4 Cheese sauce viscosity testing

3.1.4.1 Brookfield DV2T viscometer (BV)

Viscosity readings were obtained from a Brookfield (Brookfield Engineering Laboratories Inc., Middleboro, MA) DV2T Viscometer by depressing an attached RV-5 spindle to the notched set point into the prepared cheese sauce being held in a beaker in a water bath set at 70°C. Similar methods were used by Lee et al. (1978) and Kindstedt et al. (1989) to measure the viscosity of various cheeses at high temperatures using a helical pattern. The spindle was rotated at 50 rpms and viscosity readings measuring the resistance of the rotating spindle in the cheese sauce were recorded in millipascal (mPa.s) every 2 seconds over a 5-minute time sweep using the software installed within the viscometer. The data was then analyzed to determine average viscosity, peak viscosity, low viscosity, and viscosity range during the 5-minute testing time sweep. The process and settings were used for all 3 testing times (initial, 2 hour hold, 4 hour hold).

3.1.4.2 Rapid Visco Analyser (RVA)

Viscosity readings were obtained from a Newport Super 4 RVA (Perten Instruments, Hägersten, Sweden) using similar methods as described by Prow and Metzger (2005). In this method 60 g. of the prepared cheese sauce was weighed into a stainless steel cylinder and a plastic paddle designed for the RVA, was placed into the cylinder which was then attached to an arm on the RVA and depressed into a heated port. Once the cylinder was completely depressed into the heated port, the paddle in the

cylinder was rotated at 50 rpms and the cylinder was continually heated at 70°C while viscosity readings measuring the resistant of the rotating paddle in the cheese sauce were recorded in mPa.s every 2 seconds during a 5-minute time sweep using Thermocline for Windows version 3 (Newport Scientific, Jessup, MA). The data was then analyzed to determine average viscosity, peak viscosity, low viscosity, and viscosity range during the 5-minute testing time sweep. The process and settings were used for all 3 testing times (initial, 2 hour hold, 4 hour hold).

3.1.4.3 Micro Visco-Amylo-Graph® (MVAG)

Viscosity readings were obtained from a MVAG (C.W. Brabender® Instruments, South Hackensack, NJ) by weighing 110 g. of prepared cheese sauce into the MVAG specific stainless steel cup and placing it into a heated port. An attached paddle on the arm of the MVAG was then depressed into the cup. The cup was rotated at 50 rpms while being continually heated at 70°C. Viscosity readings measuring the resistant of the cheese sauce in the rotating cup against the paddle were recorded in mPa.s every 2 seconds over a 5-minute time sweep using a MVAG software program. The data was then analyzed to determine average viscosity, peak viscosity, low viscosity, and viscosity range during the 5-minute testing time sweep. The process and settings were used for all 3 testing times (initial, 2 hour hold, 4 hour hold).

3.1.5 Statistical analysis

Composition and viscosity testing results from triplicate samples within each average cheese blend age treatment were averaged and analyzed using JMP 11 (SAS Institute Inc., Cary, NC) statistical software. ANOVA was used for the overall model, while t-

tests at the 95% confidence interval were used for comparison of results from the different cheese blend age treatments, methods of viscosity testing, and hold times. Due to similar trends observed among treatments for each viscosity test method, average viscosity results from all methods of testing were used for determining blend age effect at each time point. Average viscosity over entire hold time (4 hours) for all treatments was used to determine effect of the differences within each method of viscosity testing. Microsoft Excel scatterplots with best line fit was used to determine r^2 values of fat:casein and casein:DSP ratios vs. viscosity change between hold time points.

3.2 Results

3.2.1 Chemical composition of process cheese loaves

The composition results of the 5 process cheese treatments are shown in Table 5.

Table 5. Composition testing results of process cheese loaves (n=3)

Process Cheese Blend Age	Moisture (%)	Fat (%)	Salt (%)	Total Protein (%)	Insoluble Protein 4.6 pH (Casein) (%)	Casein % of Protein (%)	Lactose (%)	Calcium (mg/100 g)	pH
10 day	40.51% ^{AB}	30.77% ^A	1.92% ^B	19.12% ^A	16.96% ^A	88.70% ^A	3.19% ^A	573 ^B	5.46 ^A
30 day	40.47% ^{AB}	30.72% ^A	1.95% ^B	19.71% ^B	17.23% ^A	87.42% ^B	2.86% ^B	598 ^A	5.47 ^A
90 day	40.97% ^A	29.94% ^B	1.91% ^B	19.27% ^A	16.07% ^B	83.39% ^C	3.31% ^A	582 ^B	5.46 ^A
150 day	40.38% ^B	31.02% ^A	2.00% ^A	19.63% ^B	15.88% ^B	80.90% ^D	2.59% ^C	580 ^B	5.47 ^A
210 day	40.55% ^{AB}	30.64% ^A	2.03% ^A	19.70% ^B	15.24% ^C	77.36% ^E	2.69% ^C	585 ^{AB}	5.50 ^A

Means within columns not sharing similar superscripts are significantly different (P<0.05)

Moisture results ranged from 40.38%-40.97% with no significant differences (P>0.05) among the treatments. Fat results ranged from 29.94% to 31.02% with only a significant difference observed in the 90d (29.94%) from the other treatments. All other treatments were not significantly different (P>0.05). Salt results ranged from 1.91% to 2.03% with significant differences (P<0.05) observed when comparing the 10d, 30d, and 90d to the

remaining treatments which were not significantly different ($P>0.05$) from each other. Total protein values ranged from 19.12% to 19.71% with the 30d having the highest amount and the 10d having the lowest. The 10d and 90d were significantly lower ($P<0.05$) than the remaining treatments which were not significantly different ($P>0.05$) from each other. Casein results ranged from 15.24% to 17.23% with the 30d having the highest amount and the 210d having the lowest amount. The 10d and 30d were significantly higher ($P<0.05$) compared to the remaining blend age treatments as well as the 90d and 150d compared to the 210d. Lactose results ranged from 2.59% to 3.31% between the treatments with 90d having the highest amount and the 150d having the lowest. No significant differences ($P>0.05$) were observed between the 10d and 90d nor between the 150d and 210d. However significant differences ($P<0.05$) were found between the 10d and 90d and the 150d and 210d groupings with the 30d being significantly different ($P<0.05$) when compared to all other treatments. Calcium results ranged from 573 (mg/100 g) to 598 (mg/100 g) with only the 30d being significantly different ($P<0.05$) than the remaining treatments. Although the 30d was statistically significantly different than the remaining treatments, the difference was considered insignificant and within normal variation. pH values ranged from 5.46 to 5.50 with no significant differences ($P>0.05$) observed between the treatments.

3.2.2 Cheese sauce viscosity

3.2.2.1 Cheese age effect

Table 6 shows the average sauce viscosity results (combined average of all 3 test methods) of each blend age treatment at all 3 testing time points.

Table 6. Average cheese sauce viscosity of all test methods at initial, 2 hour, and 4 hour time points (n=3)

Process Cheese Blend Age	Average Cheese Sauce Viscosity (mPa.s) of All Test Methods		
	Initial	2 hour hold	4 hour hold
10 day	1018 ^{CD}	1236 ^{AB}	1372 ^A
30 day	717 ^{EF}	886 ^{DE}	955 ^D
90 day	517 ^{FG}	821 ^{DE}	1021 ^{BCD}
150 day	504 ^G	929 ^{DE}	1222 ^{ABC}
210 day	478 ^G	738 ^E	1003 ^D

Means not sharing similar superscripts are significantly different (P<0.05)

The 10d had the highest viscosity and was observed to be significantly different (P<0.05) than the remaining treatments. Also, the 30d was significantly different (P<0.05) compared to the 150d and 210d, while the 90d, 150d, and 210d were not significantly different (P>0.05) from each other. A constant decrease in viscosity at the initial time point was observed with an increase in cheese blend age of the process cheese samples as shown in Table 6 and Figure 2.

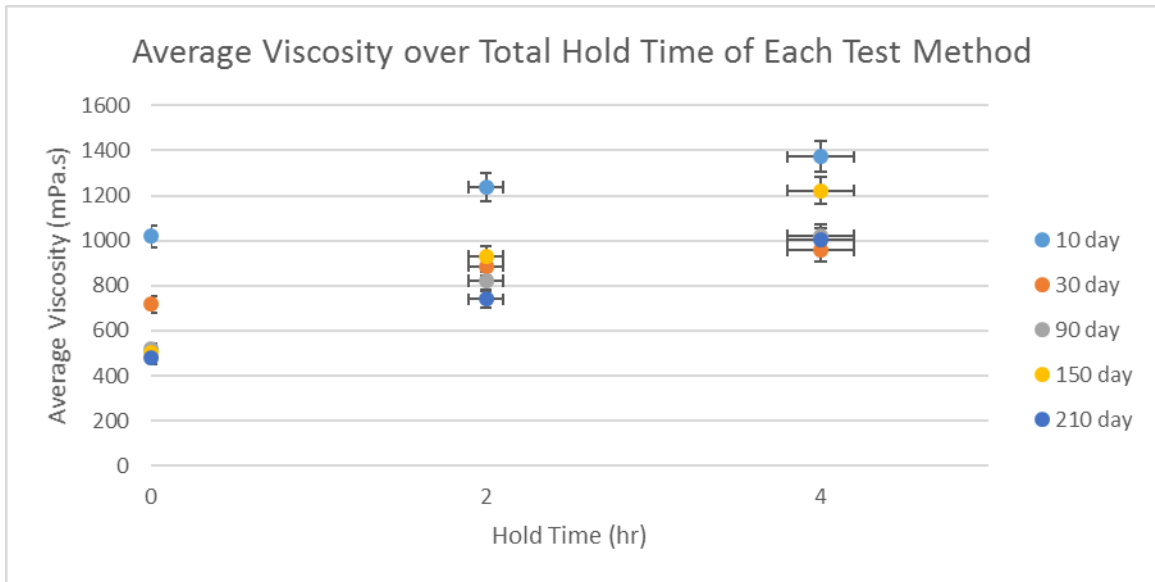


Figure 2. Average viscosity of all test methods at initial, 2 hour, and 4 hour time points

The average viscosity results at the 2 hour hold showed the 10d having the highest viscosity and the 210d having the lowest. The 10d was significantly different ($P < 0.05$) than the remaining blend age treatments. All other treatments were not significantly different ($P > 0.05$) from each other (Table 6). A constant decrease in viscosity was observed with increased blend age between all samples except for the 150d which was higher than the 30d and 90d (Figure 2). Also, there was an observed increase in viscosity for all treatments at the 2 hour hold time point compared to the initial time point.

The average viscosity results at the 4 hour hold showed the 10d having the highest viscosity and the 30d having the lowest. The 10d was significantly different ($P < 0.05$) than all remaining treatments except the 150d. The 150d was significantly higher ($P < 0.05$) in viscosity than the 30d and 210d and although the difference was not significant ($P > 0.05$) the 150d was also higher than the 90d. The 30d, 90d, and 210d were not significantly different ($P > 0.05$) from each other (Table 6). A decrease in viscosity

with increase blend age was only observed between the 10d and 30d treatments at the 4 hour hold time. The 90d and 150d treatments increased in viscosity compared to the 30d. However, another viscosity decrease was observed between the 150d and 210d treatments (Figure 2). As observed between the 2 hour and initial hold points, there is an increase in viscosity for all treatments at the 4 hour hold compared to the 2 hour hold.

3.2.2.2 Methods of viscosity testing

Table 7 and Figures 3, 4, and 5 shows the cheese sauce viscosity results of each test method (Brookfield, RVA, and MVAG) respectively at the initial (0), 2 hour, and 4 hour hold time points.

Table 7. Cheese sauce viscosity results (mPa.s) for each test method at each hold time point

Cheese Blend Age	Brookfield			RVA			MVAG		
	Hold Time (hr)			Hold Time (hr)			Hold Time (hr)		
	0	2	4	0	2	4	0	2	4
10	886 ^{EF GHIJKL}	1275 ^{ABC}	1525 ^A	1159 ^{BCDEF}	1411 ^{AB}	1527 ^A	1008 ^{CDEFGHI}	1022 ^{CDEFGHI}	1066 ^{BCDEFG}
30	731 ^{GHIJKLMNO}	930 ^{CDEFGHIJ}	1049 ^{CDEFGH}	811 ^{FGHIJKLMNO}	1027 ^{CDEFGHI}	1070 ^{BCDEFG}	609 ^{JKLMNO}	703 ^{HIJKLMNO}	747 ^{GHIJKLMNO}
90	536 ^{LMNO}	903 ^{DEFGHIJK}	1203 ^{ABCDE}	566 ^{KLMNO}	945 ^{CDEFGHIJ}	1111 ^{BCDEF}	451 ^{NO}	617 ^{JKLMNO}	750 ^{GHIJKLMNO}
150	459 ^O	1068 ^{BCDEFG}	1528 ^A	608 ^{JKLMNO}	1048 ^{CDEFGH}	1283 ^{ABC}	447 ^{NO}	673 ^{JKLMNO}	855 ^{EF GHIJKLM}
210	477 ^{NO}	824 ^{FGHIJKLMN}	1254 ^{ABCD}	534 ^{LMNO}	874 ^{EF GHIJKL}	1069 ^{BCDEFG}	422 ^O	518 ^{MNO}	686 ^{JKLMNO}

Means not sharing similar superscripts are significantly different (P<0.05)

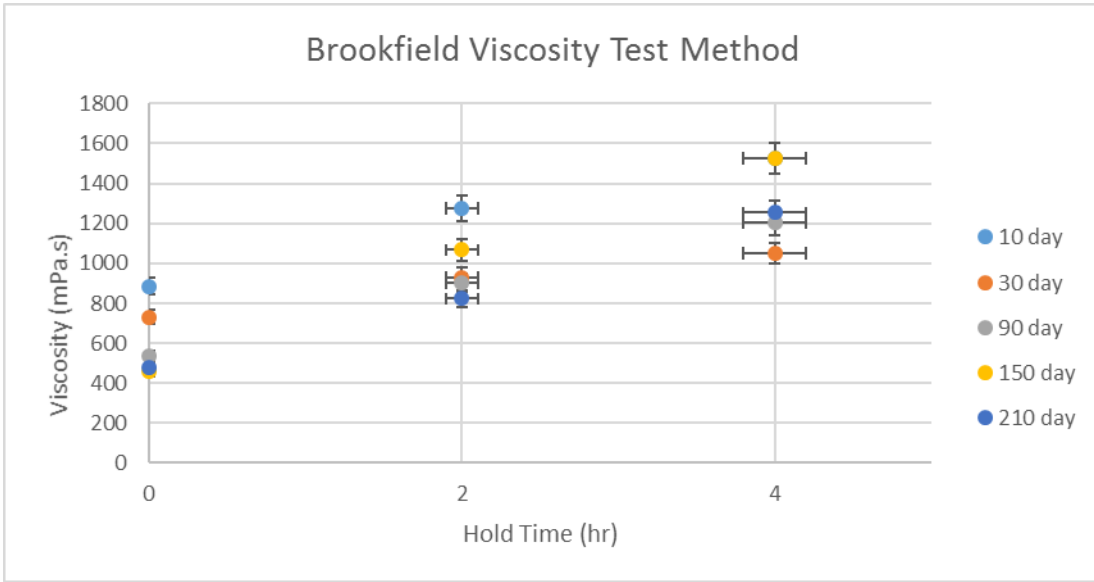


Figure 3. Brookfield test method viscosity results of each treatment at initial (0), 2 hour, and 4 hour hold time points

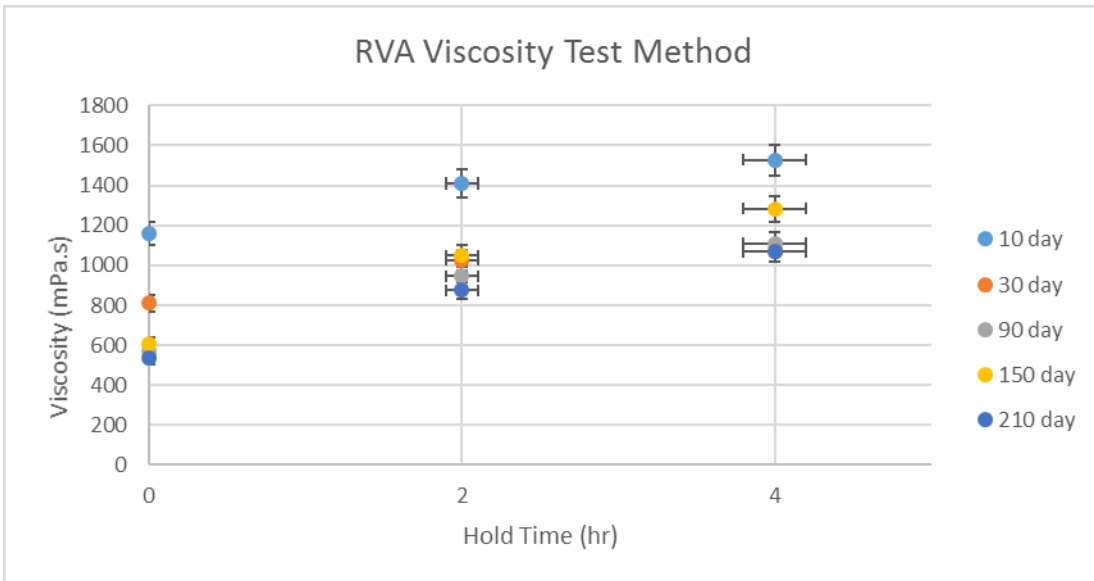


Figure 4. RVA test method viscosity results of each treatment at initial (0), 2 hour, and 4 hour hold time points

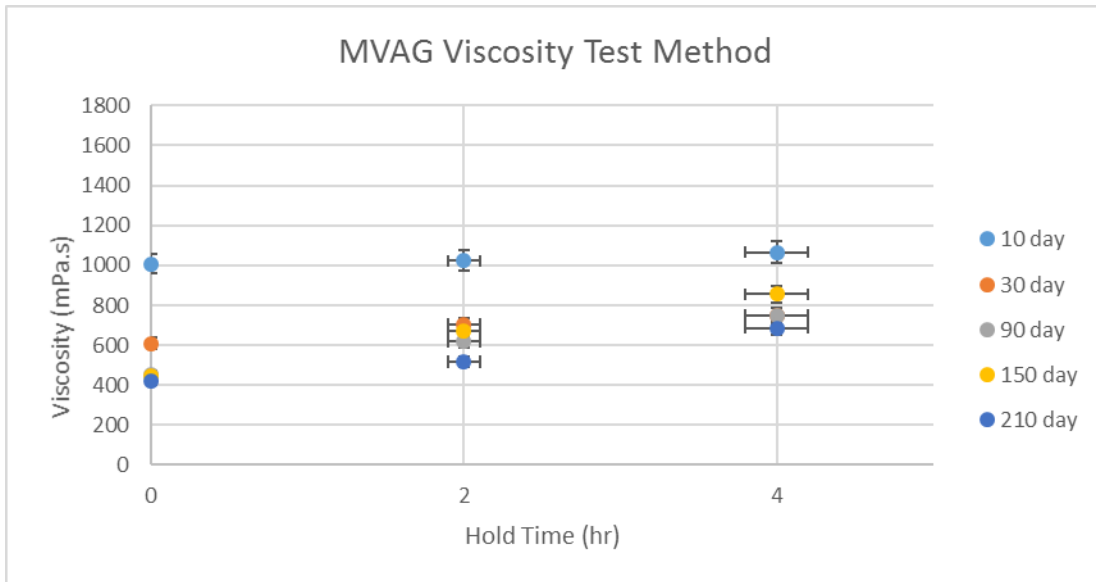


Figure 5. MVAG test method viscosity results of each treatment at initial (0), 2 hour, and 4 hour hold time points

While significant differences are observed among all three methods at each of the hold time points, each method had similar viscosity trends for both cheese blend age and hold time factors. A lower viscosity is observed as cheese blend age increases at the initial time point for all methods except for the RVA where a slight viscosity increase is observed between the 150d and 90d treatments.

At the 2 hour time point, all methods show a decrease in viscosity with increasing cheese age except for the 150d as this treatment was higher than the 30d and 90d except for the MVAG method where the 150d was only higher than the 90d and slightly lower than the 30d. An increase in viscosity of all treatments at the 2 hour hold time point compared to the initial time point is also observed for all methods.

At the 4 hour time point, all methods show a decrease in viscosity between the 10d and 30d treatments. However, after the 30d there is an increase in viscosity for the 90d and 150d with both being higher than the 30d and even the 10d as seen in the

Brookfield method. After the 150d, another decrease is observed in the 210d treatment for all methods. The 210d viscosity is lower than the 10d and 30d for all methods except the Brookfield, where the 210d is higher than the 30d and 90d. Again, an overall increase in viscosity for all treatments is observed between the 2 hour and 4 hour time points for all methods.

When comparing the averaged viscosity results of each method (BV, RVA, and MVAG) over the total 4 hour hold time, significant differences ($P < 0.05$) are observed between the MVAG and both the BV and RVA. However, no significant differences ($P > 0.05$) were observed between the BV and MVAG (Table 8).

Table 8. Average viscosity of cheese sauce treatments over 4 hour hold for all test methods (BV, RV, MVAG) (n=3)

Process Cheese Blend Age	Average Viscosity (mPa.s) over 4 hour hold by Method		
	Brookfield	RVA	MVAG
10 day	1229 ^A	1366 ^A	1032 ^B
30 day	903 ^{BCD}	969 ^{BCD}	686 ^{EFG}
90 day	881 ^{BCD}	874 ^{BCD}	608 ^G
150 day	1018 ^{BC}	980 ^{BCD}	653 ^{FG}
210 day	852 ^{CDE}	826 ^{DEF}	540 ^G

Means not sharing similar superscripts are significantly different ($P < 0.05$)

In all methods, the 10d had the highest viscosity and the 210d had the lowest. The 150d was higher, although not significantly ($P > 0.05$), than the 30d and 90d for both the BV and RVA. The 150d was only higher than the 90 day with the MVAG method and was not a significant difference ($P > 0.05$). While there were significant differences

for average viscosity observed between the methods of testing, the viscosity trends over the total holding time were essentially the same between the treatments (Figure 6).



Figure 6. Average viscosity over total hold time (initial-4 hour) of each test method

When comparing the average viscosity range (peak-low viscosity during 5-min time sweep) of each method (BV, RVA, and MVAG) over the total 4 hour hold time, significant differences ($P < 0.05$) are observed between the RVA and both the BV and MVAG for all treatments (Table 9 and Figure 7).

Table 9. Average viscosity range (peak-low) of cheese sauce treatments over 4 hour hold for each test method (BV, RV, MVAG) (n=3).

Process Cheese Blend Age	Average Viscosity Range (mPa.s) over 4 hour hold by Method		
	Brookfield	RVA	MVAG
10 day	319 ^C	735 ^A	193 ^{DE}
30 day	205 ^D	428 ^B	105 ^{DEF}
90 day	154 ^{DEF}	333 ^{BC}	76 ^F
150 day	164 ^{DEF}	345 ^{BC}	113 ^{DEF}
210 day	144 ^{DEF}	318 ^C	84 ^{EF}

Means not sharing similar superscripts are significantly different (P<0.05)



Figure 7. Average viscosity range (peak-low) over total hold time (initial-4 hour) of each test method

The BV and MVAG had only one significant difference (P>0.05) between them which was observed in the 10d treatment.

3.3 Discussion

3.3.1 Chemical composition of process cheese loaves

Minimal composition differences were observed between all treatments except for casein. While some of the non-casein compositions differences were found to be statistically significant, the differences were considered to be within typical variation for process American cheese. All treatments were formulated the same and balanced for moisture, fat, and salt based on composition data of the various natural cheese samples used for targeting specific blend ages. Therefore, the minimal differences observed in the non-casein compositions and the larger difference observed in casein due to average blend age differences was as expected. It is well known that intact protein, particularly casein, in natural cheese becomes hydrolyzed as the cheese is ripened or aged due to enzymatic proteolysis (Fox, 1987) (Fox et al., 2000). While the higher casein result in the 30d compared to the 10d was not expected based on what is known about age and protein degradation in cheese, this result is likely due to a higher overall total protein result in the 30d than the 10d. When the casein percentage of total protein was calculated a constant and significant decrease ($P < 0.05$) was observed with an increase in cheese blend age (Figure 8) and is in line with expected results.

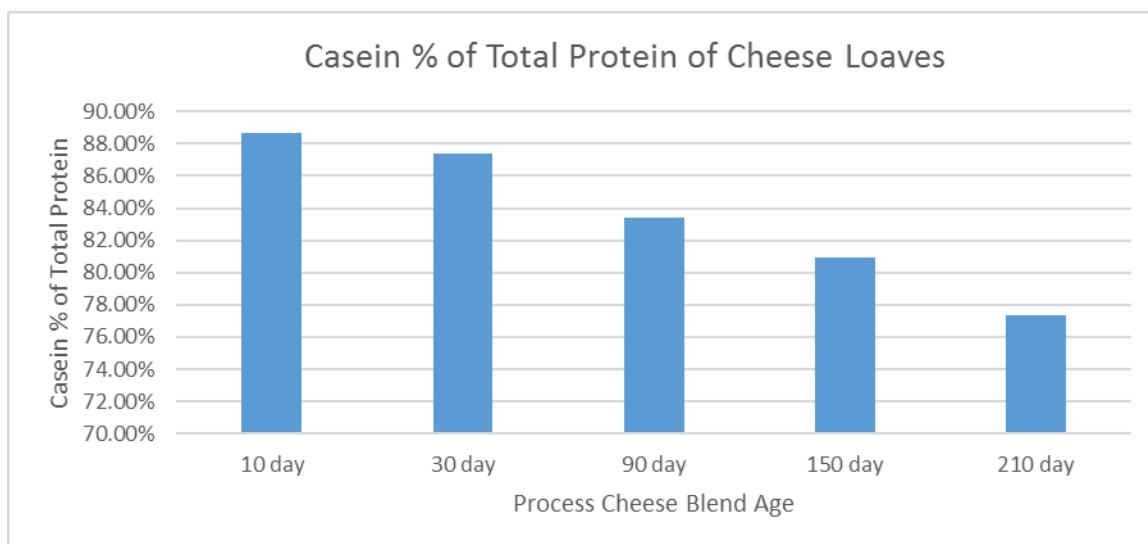


Figure 8. Casein % of total protein of process cheese loaves (n=3)

It is also possible that the test method for casein used in this study caused the higher casein result in the 30d sample compared to the 10d sample. There may be a higher number of large protein structures due to increased proteolysis in the 30d sample which are still insoluble at pH 4.6 compared to the 10d sample which would have more intact protein and less protein structures from decreased proteolysis. However, the increased total protein percentage in the 30d sample seems like the most likely cause of the higher casein result compared to the 10d sample.

3.3.2 Cheese sauce viscosity

3.3.2.1 Cheese age effect

A constant decrease in average viscosity (average between all viscosity test methods) at the initial time point was observed with an increase in cheese blend age of the process cheese samples (Table 6 and Figure 2). This trend is likely associated with the same trend seen in the casein percentage of total protein results. Emulsifying salts increase casein dispersion in process cheese when heated and mixed which allow for casein-casein

interactions to occur (Lee et al., 2003) (Mizuno and Lucey, 2005). This dispersion of casein in the presence of shear also enables increased interactions with other particles such as water and fat due to exposure of hydrophilic and hydrophobic regions which in turn causes an increase in viscosity (Ennis and Mulvihill, 1999). Therefore, a higher amount of casein in a process cheese should lead to a higher amount of casein dispersion and network formations which in turn would lead to an increase in viscosity. A decrease in casein within process cheese having similar total protein content may lead to increased amounts of peptides formed during proteolysis, which may interact and bind with water and fat causing an increase in viscosity. However, these formations may also fill the gaps within the casein network and reduce the connection strength of the casein matrix thus decreasing viscosity initially (Rogers et al., 2009). This suggests that the interactions within the casein matrix have a larger impact on viscosity than the interactions of the peptides created during proteolysis with other components such as water and fat at the initial time point. Also, the peptides formed from proteolysis in natural cheese become water-soluble and thus may not be a part of the protein matrix (Venugopal and Muthukumarappan, 2003). This would likely cause a process cheese which used more aged natural cheese to be softer and lower in viscosity when made into a sauce as there would be less structure. Lower viscosity in process cheese with more aged cheese was also supported in a study by Garimella Purna et al. (2006). They found process cheese samples made with more aged cheese produced lower viscosity results, which was attributed to weaker protein-protein and protein-fat interactions caused by higher amounts of hydrolyzed casein, or less intact casein. The initial time point testing of the cheese

sauce may be considered a close representation to the process cheese loaf itself and therefore would likely exhibit similar texture and viscosity profiles to the loaf based on cheese age and proteolysis.

It is interesting to note that all treatments were higher in average viscosity at the 2 hour hold time compared to the initial time point. Also, the same trend of decreased viscosity with increased blend age as observed at the initial time point testing was not observed at the 2 hour hold time as the 150d blend age sample was higher in viscosity than 30d and 90d blend age treatments (Table 6 and Figure 2). The increased viscosity results for all treatments is likely due to the solubility changes of casein structures and the increased protein-protein interactions over the long heating time. Kawasaki (2008) found large viscosity increases in process cheese over longer cooking times which was attributed to an increase in insoluble casein structures observed over longer cooking times. At the beginning of the cook time most of the casein structures were soluble and dispersed throughout the system. Kawasaki hypothesized that insoluble casein structures were continually forming and creating networks or aggregations as the cook times were increased which was causing the increased viscosity. This is further supported by an increase in grainy appearance of the sauces which was observed over the hold time in this study likely associated with an increase in insoluble casein structures. A study by Lee et al. (2003) lends further support to this hypothesis. They found process cheese viscosity increased from initial cook time until a peak viscosity was reached, after which a significant drop in viscosity was observed. Based on microstructure observations of the process cheese throughout the cook process, they postulated that the initial viscosity

increase was due to increased protein-protein interactions of the dispersed caseins which eventually reached a maximum (peak viscosity). After further cooking and shear, the protein network began to break down and resulted in “large, compacted protein structures”. This was a result of the protein-protein interactions “overriding” the protein-fat and protein-water interactions which caused a drop in viscosity and gave the process cheese a grainy texture. It is possible that a similar phenomenon occurred during the hold time of the cheese sauces in this study as the sauces were held at temperatures close to typical cook temperatures of process cheese. However as there was a lack of shear during the hold time, the protein-protein interactions within the cheese sauce continued to occur but never reached the advanced state of “overriding” the protein-fat and protein-water interactions. While an increase in viscosity was observed in all treatments from the initial to 2 hour hold time points, there were varying degrees of viscosity increases observed between the treatments. Table 10 and Figure 9 shows the change in viscosity between each testing time point as well as the total change over the total hold time (4 hour-initial).

Table 10. Average viscosity change of all test methods over total hold time (initial-4 hour) (n=3).

Process Cheese Blend Age	Viscosity Change- Initial-2 hour (mPa.s)	Viscosity Change- 2 hour-4 hour (mPa.s)	Viscosity Change- Initial-4 hour (mPa.s)
10 day	218	137	355
30 day	169	69	238
90 day	304	200	504
150 day	425	293	717
210 day	261	264	525

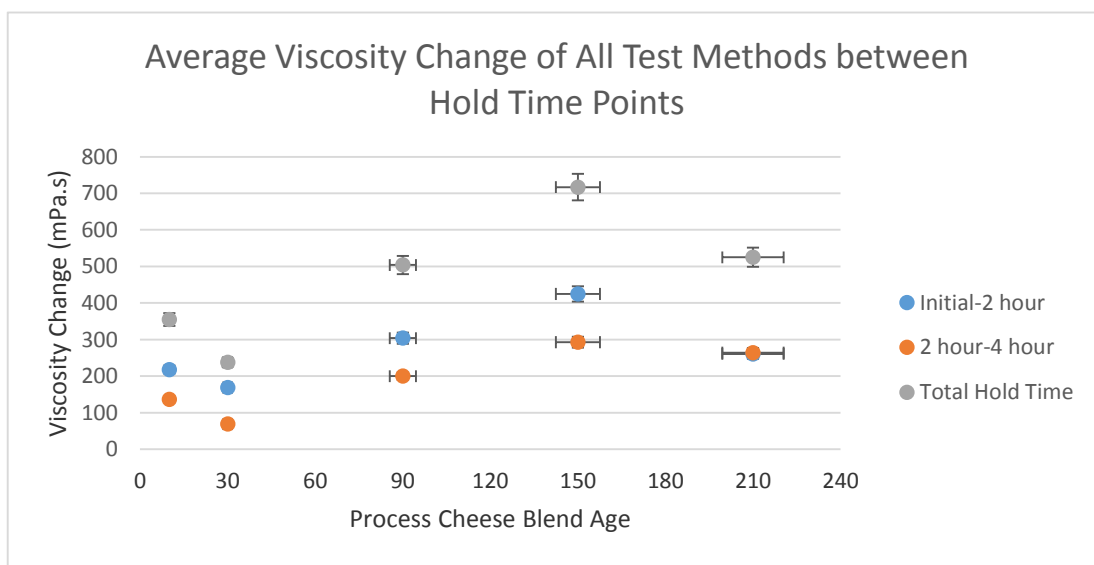


Figure 9. Average viscosity change of all test methods between each hold time point

At the 2 hour time point the 10d and 30d had the lowest viscosity change from the initial time point at 218 and 169 mPa.s increase respectively. The 150d had the largest change with an increase of 425 mPa.s. The increase in viscosity for the 90d and 210d were in between these values at 304 and 261 mPa.s respectively. The differences in degree of viscosity increase could be explained by a couple of factors. While one possible factor appears to be the protein-protein interactions mentioned earlier as the initial viscosity results show that casein was likely the main factor in determining viscosity. This factor does not explain the results observed at the 2 hour hold time as the lower casein treatments (90d, 150d, 210d) had larger viscosity increases than the higher casein treatments (10d and 30d). In the case of the 150d to the 30d blend age treatment, it had an overall higher viscosity altogether. This suggests that non-casein structures (peptides, fat, phosphate, etc.) may be providing additional interaction sites over time leading towards more molecular bonds being formed and in turn, higher viscosity. When

looking at the fat:casein ratio of the process cheese loaves and viscosity change over time of the cheese sauces there appears to be a relationship ($r^2=0.99$) from the initial time point to the 2 hour hold time point within the first four treatments (10d, 30d, 90d, 150d). A higher fat:casein ratio trended towards a higher degree of increased viscosity from the initial to 2 hour time points (Figure 10).

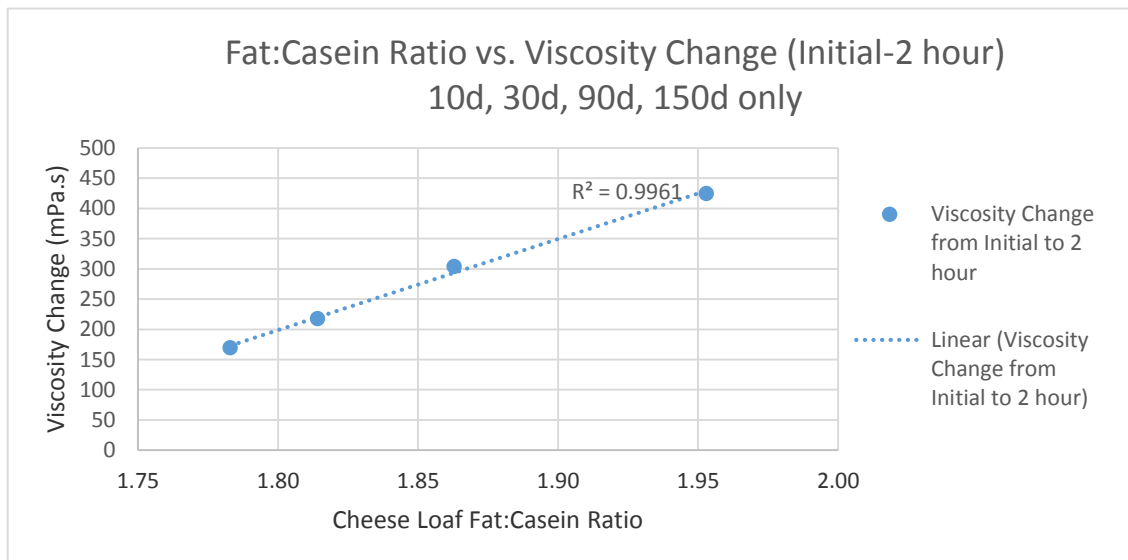


Figure 10. Plot of cheese loaf fat:casein ratio vs. viscosity change from initial to 2 hour hold time points for 10d, 30d, 90d, and 150d cheese sauce treatments

A study by Aguilera and Kinsella (1993) found that the addition of fat globules to protein-containing dairy gels when heated, led to increased gel formation. They postulated that the fat globules acted as a nucleation site for a “copolymerization process” of a gel matrix containing casein micelles and fat globules. This may explain the higher viscosity increases observed in the 90d and 150d as these had higher fat:casein ratios compared to the 10d and 30d treatments (Table 11).

Table 11. Average fat:casein ratio for process cheese treatments (n=3)

Process Cheese Blend Age	Fat:Casein Ratio
10 day	1.81
30 day	1.78
90 day	1.86
150 day	1.95
210 day	2.01

There does though appear to be a limit or optimum ratio of fat:casein as it relates to cheese sauce viscosity changes over time. When the data from the 210d treatment is included in the model, the linear r^2 value drops to 0.37 (Figure 11) as the viscosity increase over time is less than the 150d despite a higher fat:casein ratio.

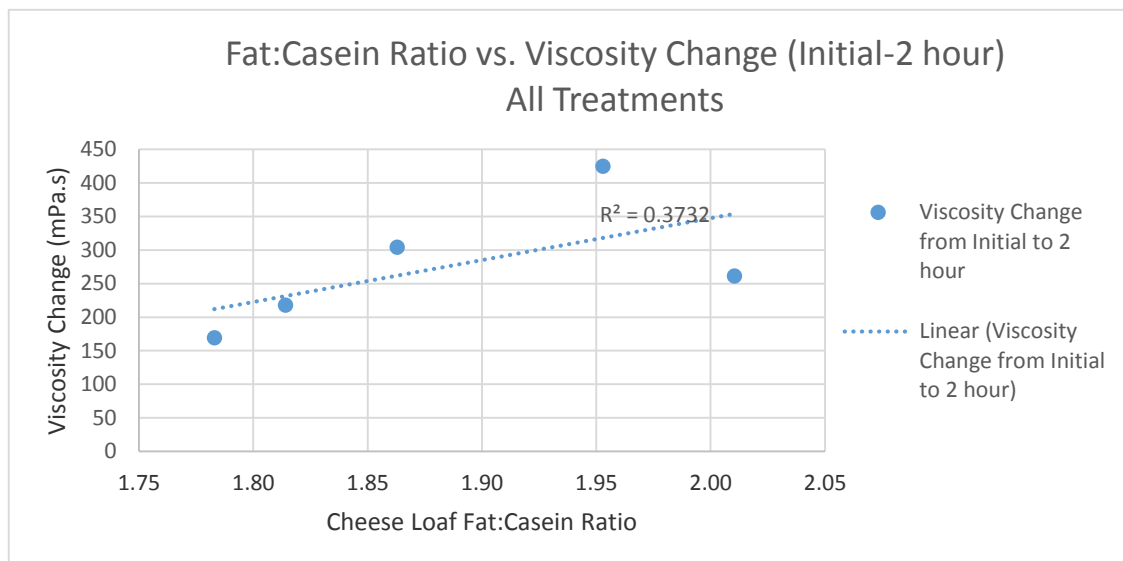


Figure 11. Plot of cheese loaf fat:casein ratio vs. viscosity change from initial to 2 hour hold time points for all cheese sauce treatments

However, it appears that a 2nd order polynomial (or quadratic) relationship is starting to emerge. When analyzing the same data set (all samples) as a 2nd order polynomial relationship, the r^2 value increases to 0.84 (Figure 12).

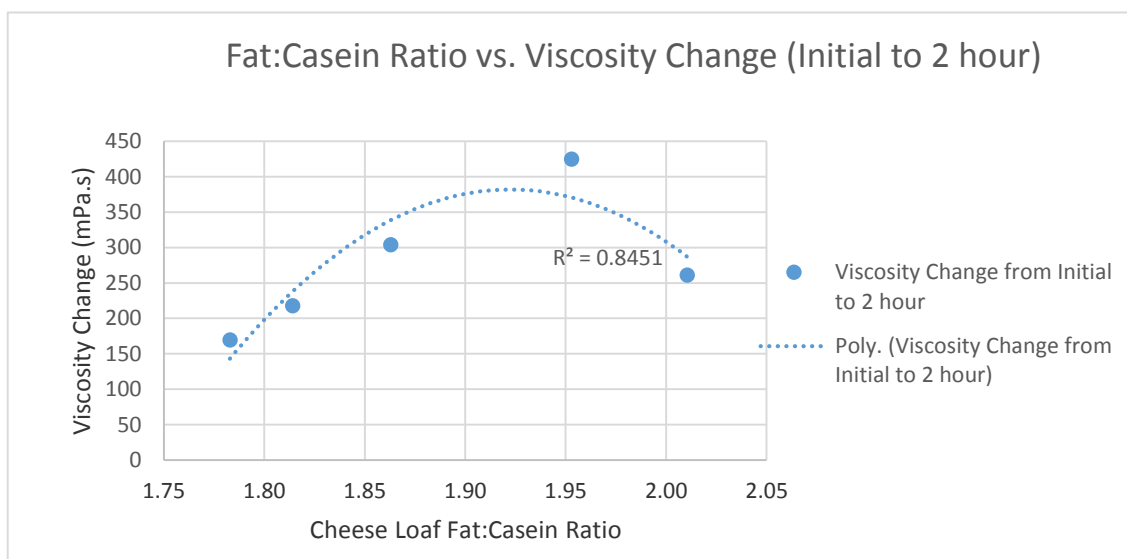


Figure 12. Plot of cheese loaf fat:casein ratio vs. viscosity change from initial to 2 hour hold time points for all cheese sauce treatments (2nd order polynomial)

It is possible that the fat:casein ratio of the 210d was such that the higher amount of fat globules relative to casein, caused a decrease in the amount of protein-protein interactions by filling in the gaps of the casein matrix as suggested earlier by Rogers et al. (2009) thus decreasing the effect of protein-protein interactions on viscosity.

There appears to also be a similar phenomenon, albeit inverse, to fat:casein and viscosity change between the casein:DSP ratio and viscosity change from the initial time point to the 2 hour time point ($r^2=0.89$) as shown in Table 12 and Figure 13.

Table 12. Average casein:DSP ratio of process cheese loaves (n=3)

Process Cheese Treatments	Casein:DSP Ratio
10d	6.79
30d	6.89
90d	6.43
150d	6.35
210d	6.10

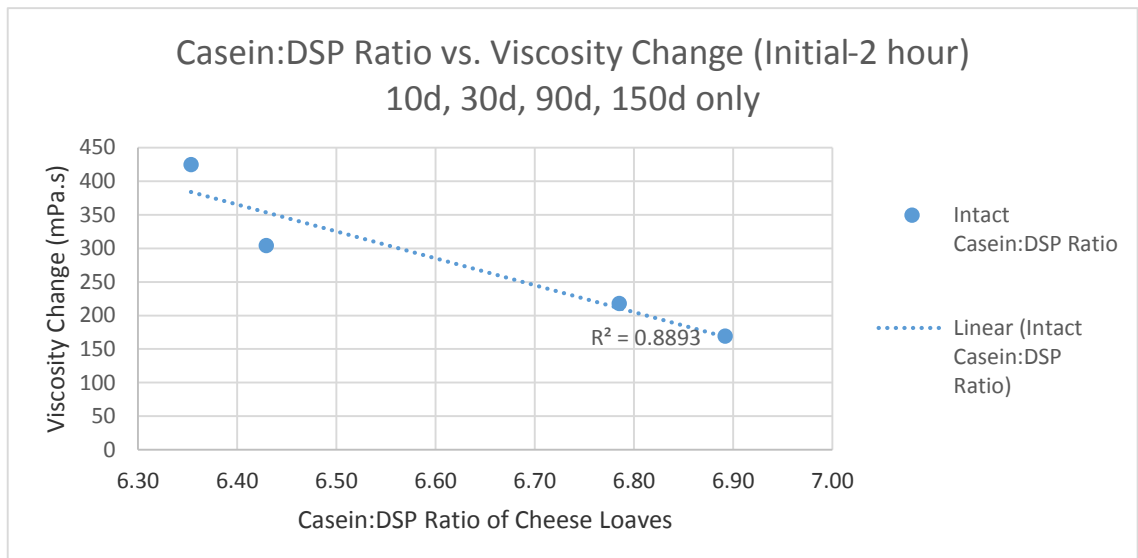


Figure 13. Plot of casein:DSP ratio of cheese loaves vs. viscosity change from initial to 2 hour hold time points for 10d, 30d, 90d, 150d treatments

As the ratio of casein:DSP decreases the viscosity change increases. This potential relationship may be explained by casein-phosphate interactions. Phosphate based emulsifying salts, like the one used in this study, may bond with casein during heating forming casein-phosphate complexes which lead to increases in gel formation and reduced melt (Mizuno and Lucey, 2005) (Kaliappan and Lucey, 2011). A decrease in the casein:DSP ratio suggests that there is more “unreacted” phosphate (phosphate which did not bind with insoluble calcium) available in the system as lower casein content would create lower amounts of insoluble calcium available for the phosphate to react with. Also similar to the fat:casein ratio and viscosity change linear relationship, a decrease in the r^2 value (0.36) is observed when inputting the data from the 210d treatment as the viscosity change over time was less than the 150d treatment despite a lower casein:DSP ratio (Figure 14).

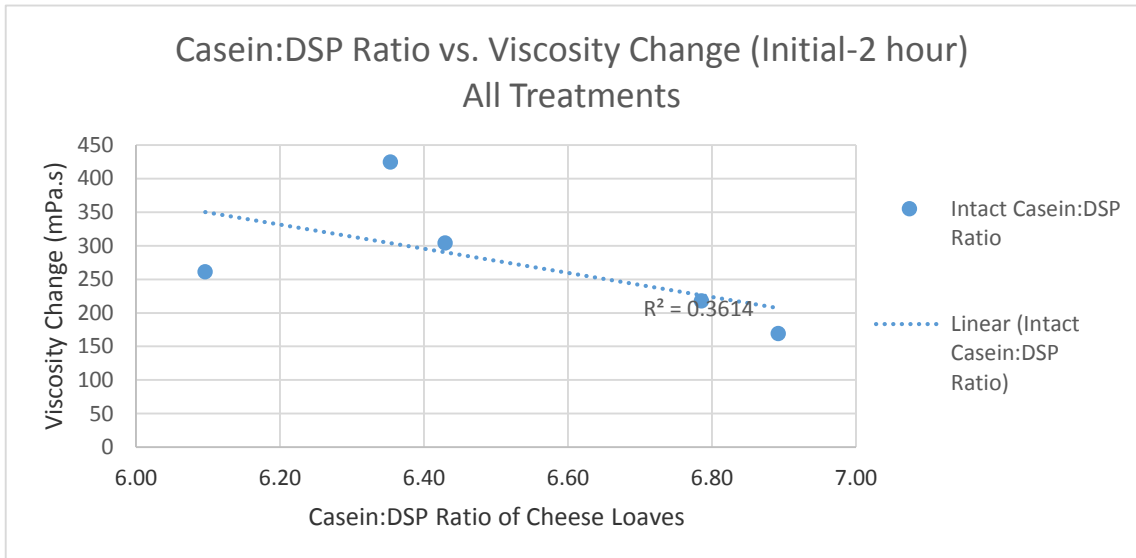


Figure 14. Plot of casein:DSP ratio of cheese loaves vs. viscosity change from initial to 2 hour hold time points for all treatments

As with the fat:casein ratio, there appears to be a limit or optimal level of casein:DSP as it relates to viscosity change over time for cheese sauces as when analyzed as a 2nd order polynomial relationship the r^2 value increase to 0.78 (Figure 15).

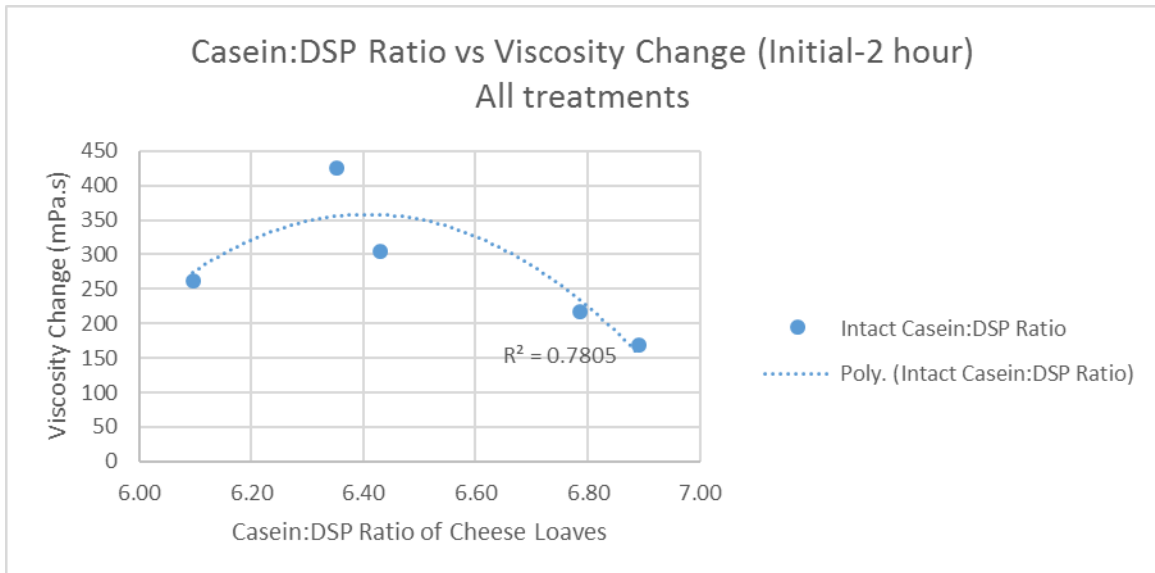


Figure 15. Plot of casein:DSP ratio of cheese loaves vs. viscosity change from initial to 2 hour hold time points for all treatments (2nd order polynomial)

The fat:casein ratio (1.95) as well as the casein:DSP ratio (6.35) in the 150d may be such as to allow for complete and optimal dispersion of the casein within the system which in turn creates a maximum amount of casein-casein and casein-fat interactions while also creating increased casein-phosphate interactions. However, beyond these levels (higher fat:casein and lower casein:DSP) there could be less molecular interactions occurring which creates a decrease in the cheese sauce viscosity change over time. Bunka et al. (2013) presumed that an optimum amount of DSP does exist in a process cheese system which would lead to a stronger gel matrix. It may also be possible that a similar level exists for fat:casein and that the 150d was close to the optimal fat:casein and casein:DSP ratio levels comparative to the rest of the set in this study and thus led to a higher viscosity change over the hold time. These optimal ratios in the process cheese loaves may also explain the similar viscosity increases observed between the 90d and 210d as the fat:casein and casein:DSP ratios for those treatments were on the high and low ends of the fat:casein and casein:DSP ratios of the 150d. This creates a quadratic relationship in terms of viscosity increase for these treatments with the 150d treatment being at the top of the curve and the 90d and 210d on either side of the curve. More data is needed to confirm the significance of the relationships between the fat:casein and casein:DSP ratios and cheese sauce viscosity change, but the data in this study does suggest that there is some correlation between these ratios and cheese sauce viscosity changes over time. Moisture loss did not play a role in the increased viscosity of the treatments as the results from the 2 hour hold time point testing were 0.5% or less difference from the initial time point results (Table 13) which was considered an insignificant difference.

Table 13. Average cheese sauce moisture and moisture loss over hold time (n=3)

Process Cheese Blend Age	Average Sauce Moisture			Average Moisture Loss %		
	Initial	2 hr.	4 hr.	Initial-2 hr.	2-4 hr.	Total (Initial-4 hr.)
10 day	55.08%	55.15%	54.83%	0.07%	-0.32%	-0.25%
30 day	55.81%	55.89%	55.50%	0.09%	-0.39%	-0.30%
90 day	55.81%	55.66%	55.36%	-0.15%	-0.30%	-0.45%
150 day	55.06%	55.05%	54.76%	-0.01%	-0.29%	-0.30%
210 day	55.09%	54.59%	54.44%	-0.50%	-0.15%	-0.65%

As observed at the 2 hour hold time point compared to the initial time point, there was a viscosity increase for all treatments at the 4 hour hold time point compared to the 2 hour hold time point. As noted earlier, the continued heating likely led to further casein-casein and casein-phosphate interactions and were the main causes for the observed viscosity increase over the hold time. There was a minimal increase in viscosity change between the 2 hour and 4 hour hold time points for the 10d and 30d at 137 and 69 mPa.s respectively. The other treatments (90d, 150d, 210d) had viscosity increases of; 200, 293, and 264 mPa.s respectively (Table 10). These viscosity changes are noticeably less than observed from the initial time point to the 2 hour hold. However, the same relationships between the fat:casein and casein:DSP ratios to viscosity change from the 2 hour to 4 hour hold time points are observed as was from the initial to 2 hour hold. An increase in fat:casein and decrease in casein:DSP ratios led to a higher viscosity change for the first four treatments (10d, 30d, 90d, 150d) (Figures 16 and 17) with r^2 values of 0.98 and 0.90 respectively.

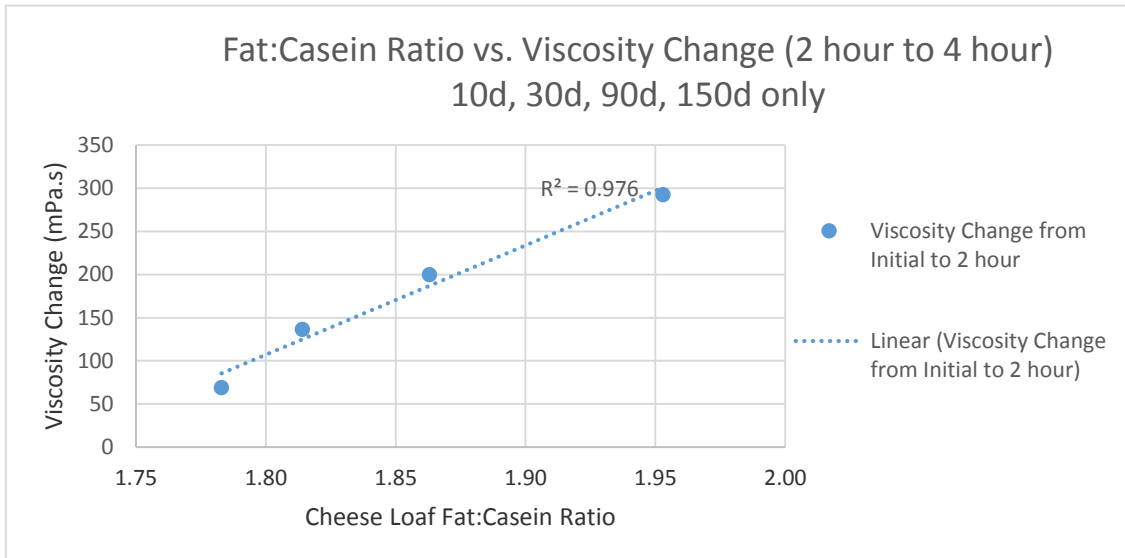


Figure 16. Plot of cheese loaf fat:casein ratio vs. viscosity change from 2 hour to 4 hour hold time points for 10d, 30d, 90d, and 150d cheese sauce treatments (linear)

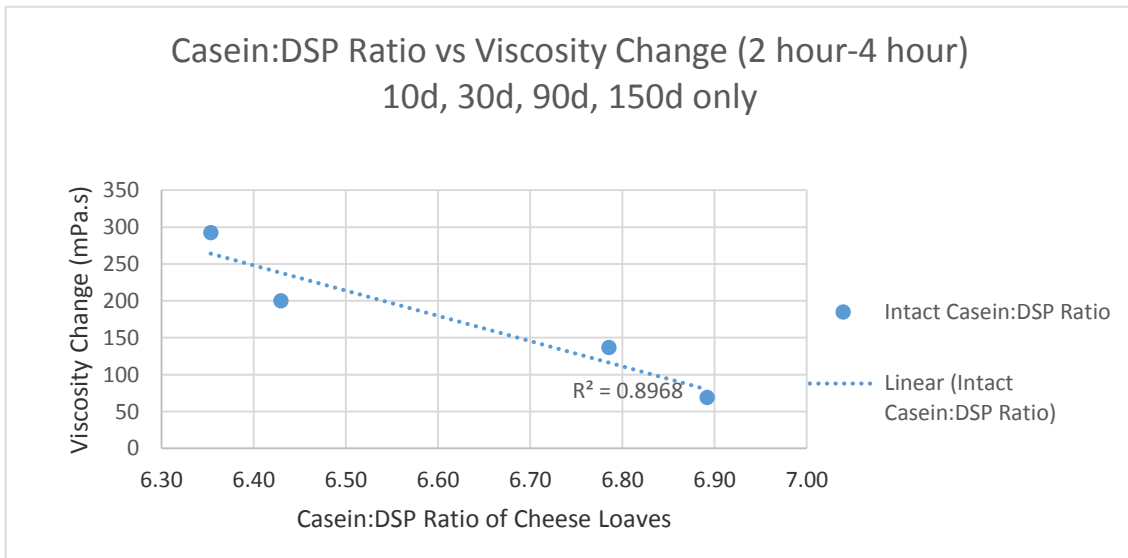


Figure 17. Plot of casein:DSP ratio of cheese loaves vs. viscosity change from 2 hour to 4 hour hold time points for 10d, 30d, 90d, 150d treatments (linear)

When including the 210d data from the 2 hour to 4 hour hold into each model, a decrease in viscosity change is observed as was between the initial and 2 hour time points (Figures 18 and 19) with the r^2 values also decreasing to 0.85 and 0.83 respectively.

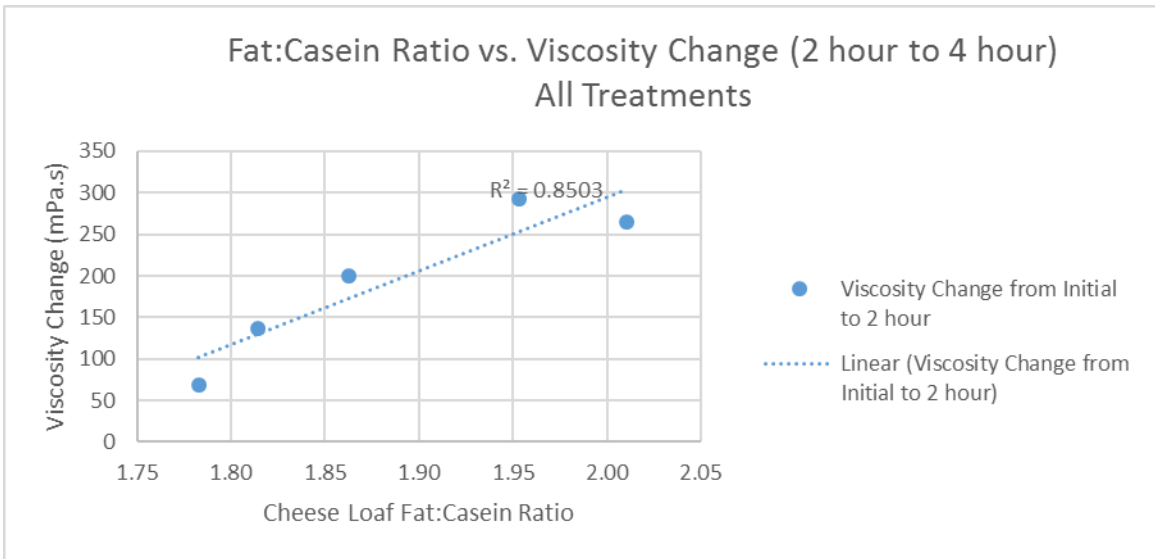


Figure 18. Plot of cheese loaf fat:casein ratio vs. viscosity change from 2 hour to 4 hour hold time points for all cheese sauce treatments (linear)

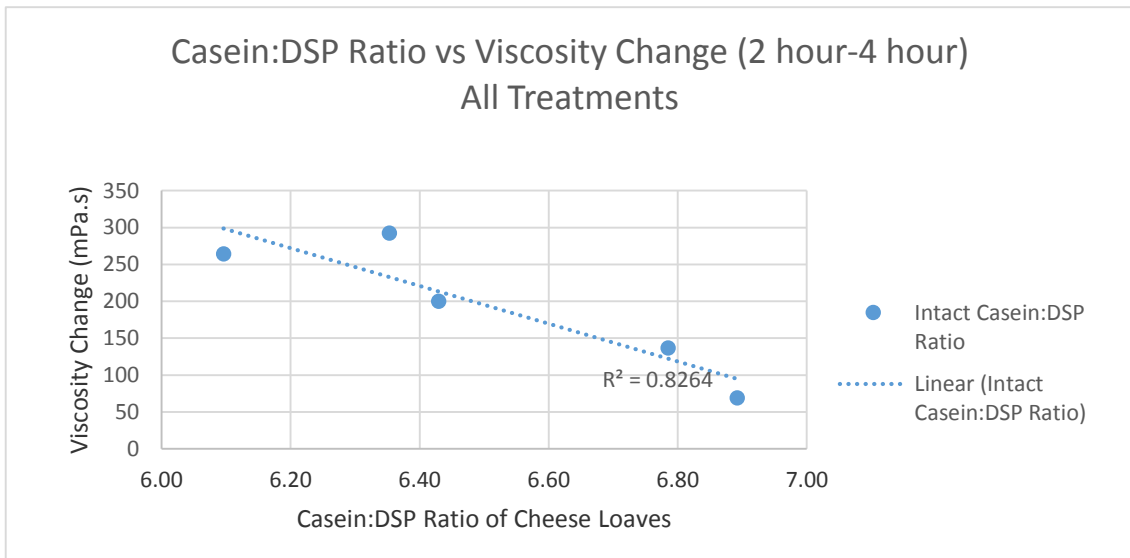


Figure 19. Plot of casein:DSP ratio of cheese loaves vs. viscosity change from 2 hour to 4 hour hold time points for all treatments

However, as observed between the initial and 2 hour hold points, there appears to be a polynomial relationship developing for both the fat:casein and casein:DSP ratios and viscosity. When analyzed as a 2nd order polynomial, the r^2 values increase to 0.99 and

0.90 for fat:casein and viscosity change and casein:DSP and viscosity change respectively for all treatments (Figures 20 and 21).

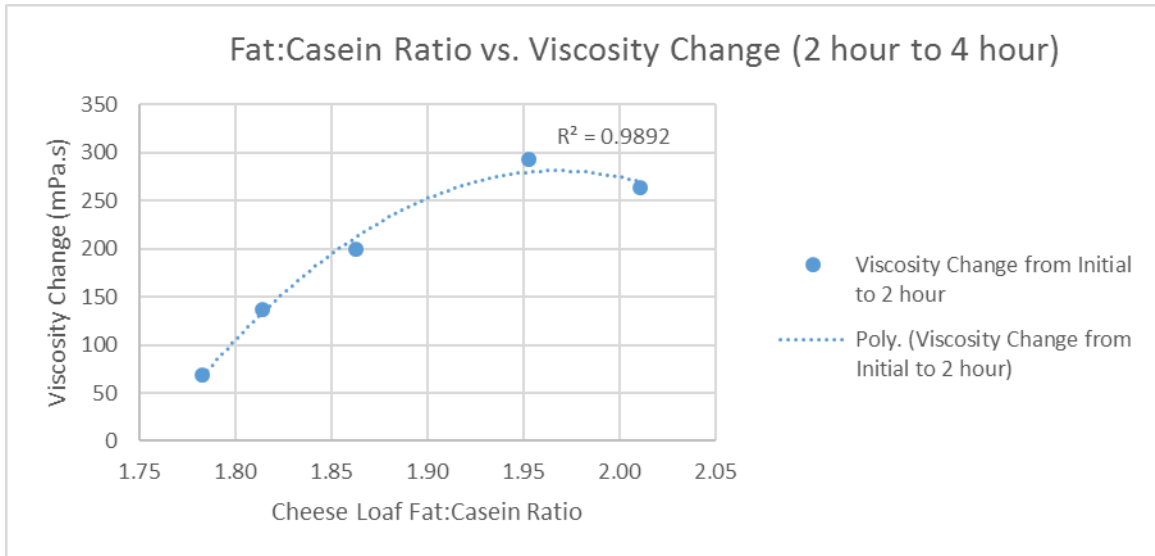


Figure 20. Plot of cheese loaf fat:casein ratio vs. viscosity change from 2 hour to 4 hour hold time points for all cheese sauce treatments (2nd order polynomial)

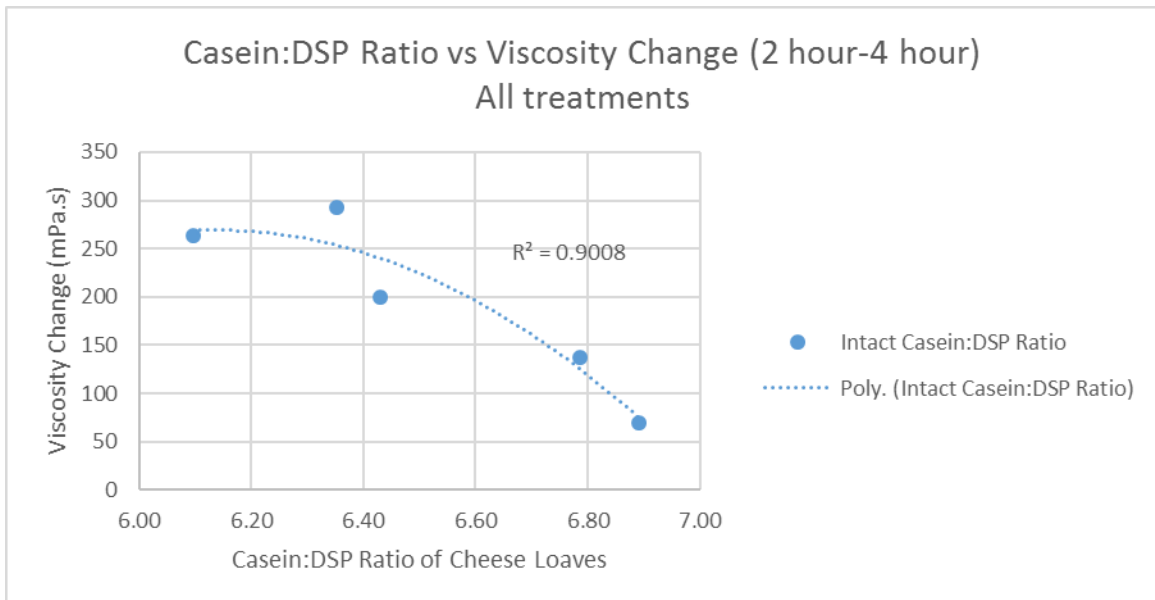


Figure 21. Plot of casein:DSP ratio of cheese loaves vs. viscosity change from 2 hour to 4 hour hold time points for all treatments (2nd order polynomial)

Assuming that the increase of viscosity in the cheese sauces over the hold time is related to some combination of casein-casein, casein-fat, and casein-phosphate interactions and the resultant complexes formed, it appears as though these interactions are significantly reduced or possibly at completion during the 2 hour to 4 hour hold time and a peak viscosity was achieved during this time. It is possible that a decrease in viscosity would occur if the cheese sauces were held beyond 4 hours as the protein-protein interaction could eventually override the protein-fat and protein-water interactions as was theorized in the previously mentioned studies by Kawasaki (2008) and Lee et al. (2003). However, because there was a lack of shear during the hold time, the time at which the protein matrix starts to disintegrate causing a change in the fat and water binding ability thus decreasing viscosity, may go beyond 4 hours, even at a high temperature. It would be interesting to see the effect of shear on cheese sauce viscosity change during the hold time as well as an extension of hold time and could be the subject of a future study. Moisture loss of the cheese sauces during the 2 hour to 4 hour hold was 0.39% or less (Table 13) for all treatments and was considered insignificant in terms of affecting viscosity change.

When looking at the overall change in viscosity over the total hold time (4 hour-initial) the changes were much higher in the 90d, 150d, and 210d compared to the 10d and 30d (Table 10) with the 150d having the highest increase at 717 mPa.s. As expected based on the trends from the previous hold times, there was an observed higher viscosity change as the fat:casein ratio increased and the casein:DSP ratio decreased for the first

four treatments (10d, 30d, 90d, and 150d) over the total hold time as shown in Figures 22 and 23 with r^2 values of 0.99 and 0.90 respectively.

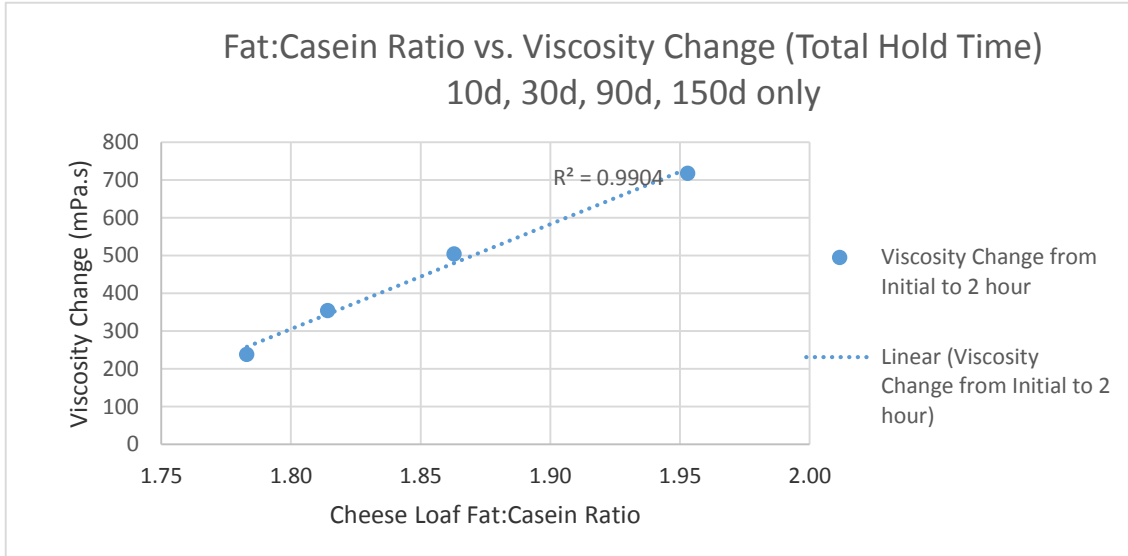


Figure 22. Plot of cheese loaf fat:casein ratio vs. viscosity change over total hold time (initial-4 hour) for 10d, 30d, 90d, and 150d cheese sauce treatments

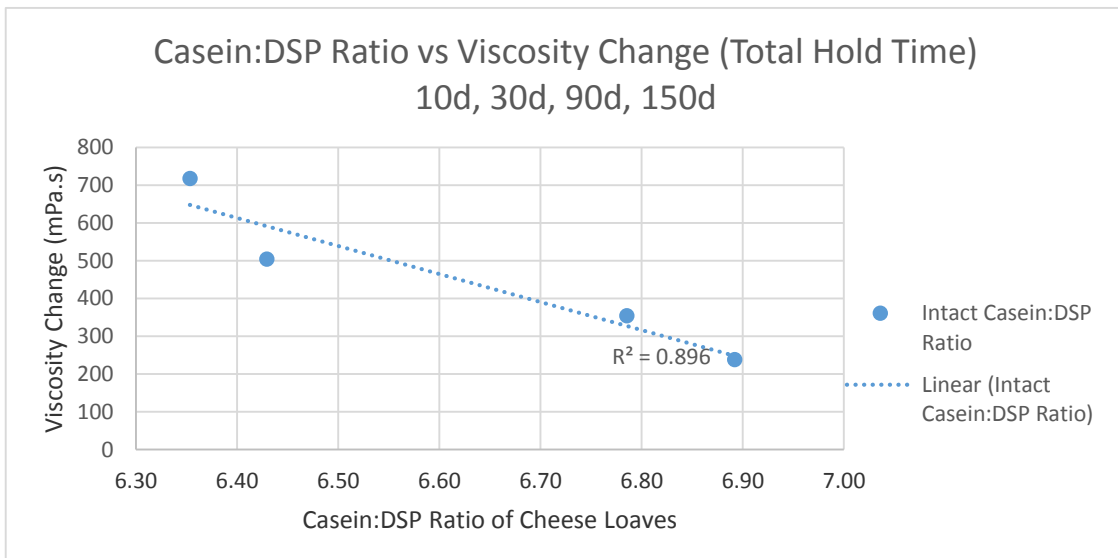


Figure 23. Plot of casein:DSP ratio of cheese loaves vs. viscosity change over total hold time (initial-4 hour) for 10d, 30d, 90d, 150d treatments

When including the 210d data over the total hold time the same decrease in viscosity change and r^2 values (0.63 and 0.61 respectively) in the models is observed as was in the previous hold time points (Figures 24 and 25).

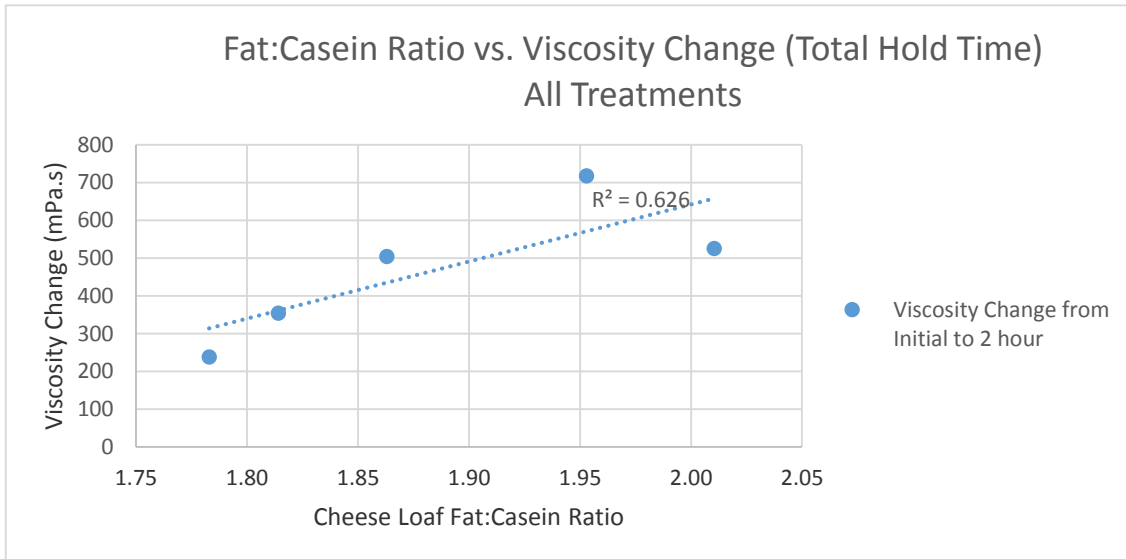


Figure 24. Plot of cheese loaf fat:casein ratio vs. viscosity change over total hold time (initial-4 hour) for all cheese sauce treatments

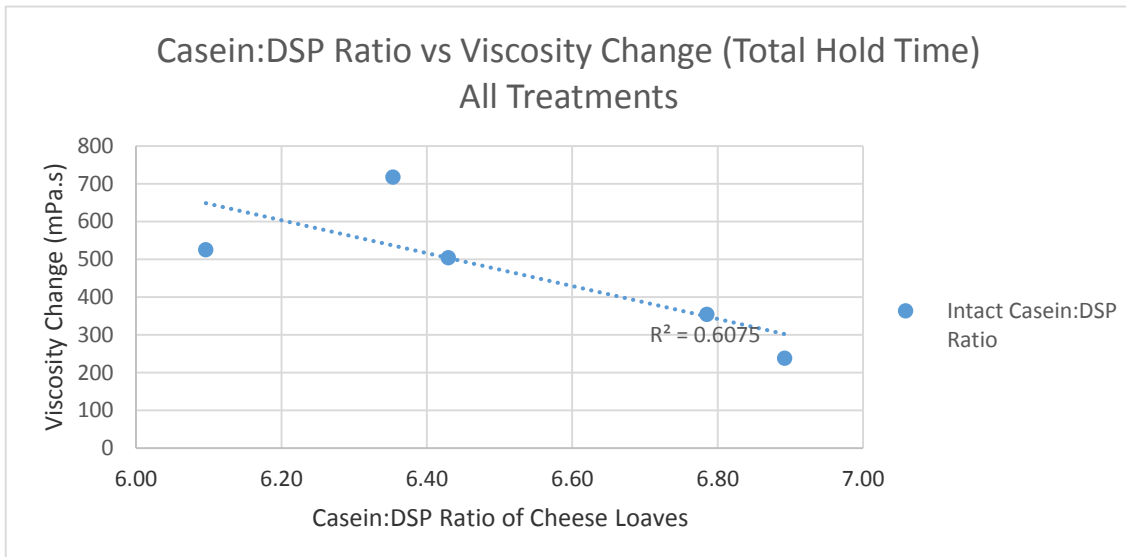


Figure 25. Plot of casein:DSP ratio of cheese loaves vs. viscosity change over total hold time (initial-4 hour) for all treatments

When analyzed as a polynomial relationship the r^2 values once again increase for the fat:casein and casein:fat ratio vs. viscosity change to 0.93 and 0.84 respectively (Figures 26 and 27).

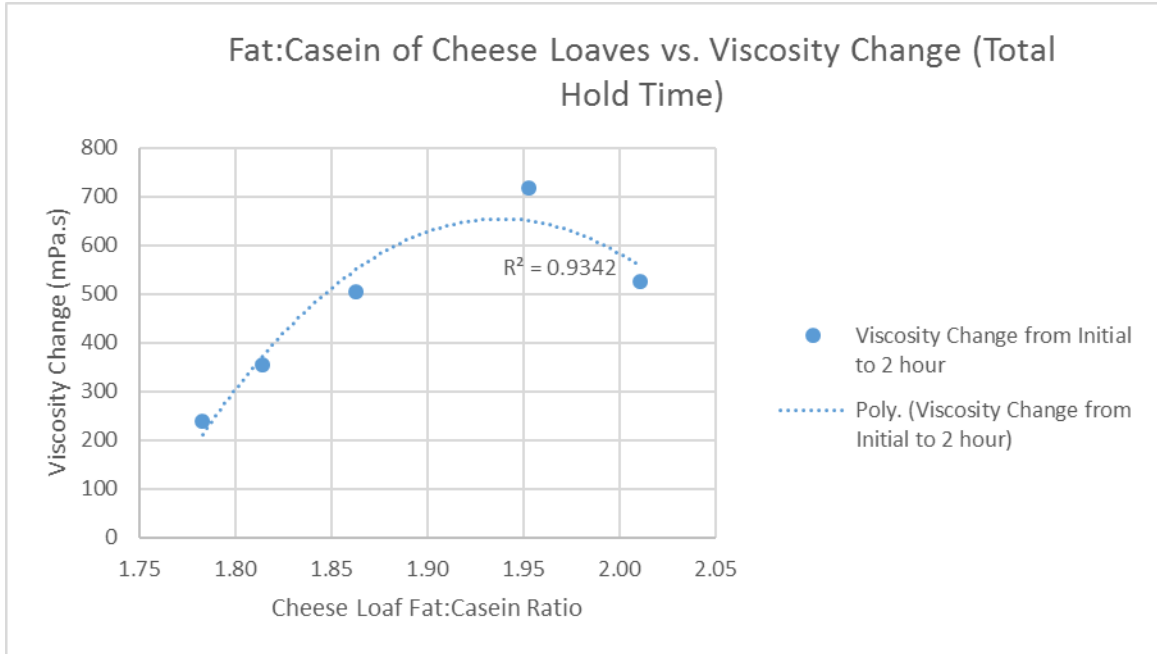


Figure 26. Plot of cheese loaf fat:casein ratio vs. viscosity change over total hold time (initial-4 hour) for all cheese sauce treatments (2nd order polynomial)

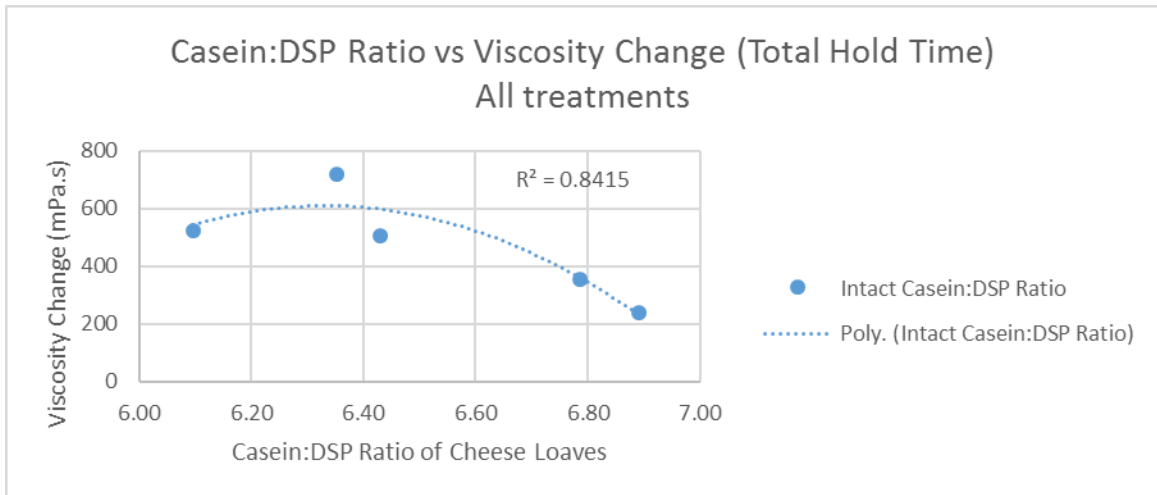


Figure 27. Plot of casein:DSP ratio of cheese loaves vs. viscosity change over total hold time (initial-4 hour) for all treatments (2nd order polynomial)

Moisture loss again did not play a role in the increased viscosity of the treatments from the initial or 2 hour hold time points as the results from the 4 hour hold time point testing were 0.65% or less difference from both the initial and 2 hour hold time points and considered to be an insignificant change (Table 13).

3.3.2.2 Methods of viscosity testing

The significant difference between the MVAG from the BV and RVA in terms of average viscosity (Table 6 and Fig. 5) is likely due to the lack of sensitivity of the MVAG to measure resistance. The MVAG is designed to measure dough rheology which is elastic and rubbery (Figure 28).



Figure 28. Brabender Micro Viscoamylograph spindle (Soon Suh and Jane, 2003)

Thus, the ability of the MVAG to pick up viscosity differences in a liquid cheese sauce with flow is diminished compared to viscometers intended to measure viscosity for more free flowing materials like the BV and RVA are. All the methods used for measuring viscosity in this study are considered rotational viscometers. For rotational viscometers, the viscosity is determined by measuring the torque needed to rotate an object (spindle or paddle) at a given speed. The higher amount of torque needed to rotate an object suggests that the material causes more resistance or drag against the rotating object and

thus is thicker or higher in viscosity compared to a material where less torque is needed to rotate an object at a set speed. Therefore, the range and sensitivity of torque that is able to be measured by a viscometer will directly affect the measureable viscosity range. This likely explains the lower viscosity results observed in the MVAG testing method as the torque sensitivity would be lower compared to the BV and RVA methods based on application usage.

The reason for the RVA average viscosity range (Table 9) being significantly higher ($P < 0.05$) than both the BV and MVAG is likely attributed to shear. Although the same rate (50 rpms) was used in all methods, there were differences in the viscosity measuring apparatus' design and method of spinning. The apparatus, or paddle, used to measure viscosity in the RVA has a large surface area (4.6 cm^2) relative to the container volume (77.12 cm^3) which houses the material being tested. This allows the RVA paddle to come into contact with most of the material when being tested enabling a high sensitivity for sensing minor viscosity changes within the material. Also, the paddle is shaped similar to a propeller (Figure 29) which along with the contact area to volume ratio may be creating a shear-thinning effect by causing a more consistent and intense disruption of the casein-casein and/or casein-phosphate interactions and networks that may be formed in the cheese sauce during the holding time.



Figure 29. Rapid Visco Analyser paddle (www.perten.com)

Figure 30 shows an example of a typical viscosity reading during a 5-minute sweep for all the testing methods.

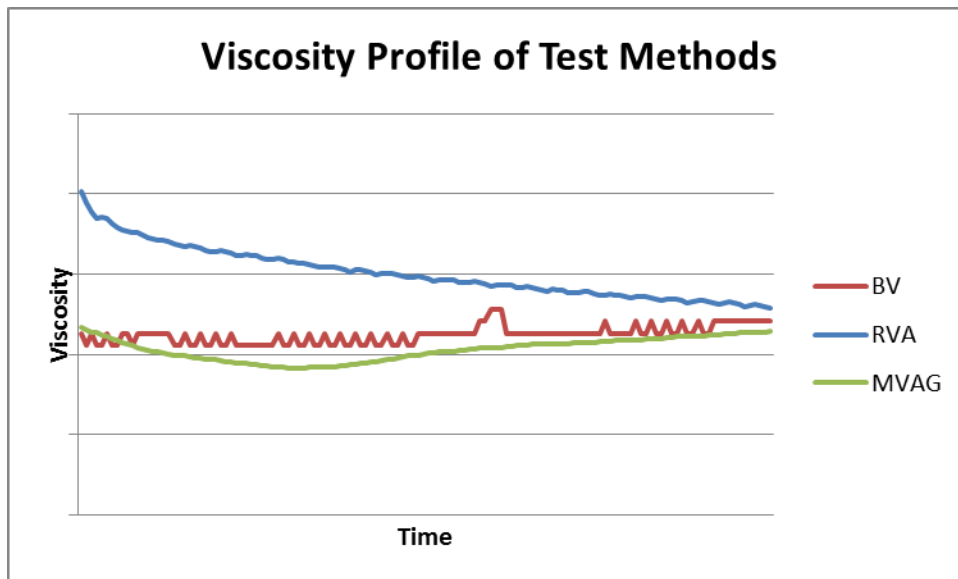


Figure 30. Typical viscosity profile of cheese sauce test for each testing method (BV, RVA, MVAG).

In this example, the shear-thinning of the RVA is apparent as the viscosity starts high and continually drops throughout the testing time while the other methods stay relatively

stable start to finish. Although the BV and MVAG both have minimal viscosity changes over the 5-minute time sweep, it is likely for different reasons. The BV measures viscosity through the use of an attached spindle with a small circular plate at the end (Fig. 31).



Figure 31. Brookfield rotational spindle (www.coleparmer.com)

The significantly smaller spindle surface area to volume ratio (12.57 cm^2 to 2211.68 cm^3) and circular shape of the spindle compared to the RVA likely creates less shear in the cheese sauce, enabling a more stable and steady viscosity reading. The consistent viscosity readings of the BV method may also be due to a decreased effect of drag. The spindle used in the BV method was approximately 6.35 cm from the wall of the container holding the cheese sauce, while the paddles for the RVA and MVAG are within a few millimeters of the walls of the containers holding the cheese sauce. This basically eliminates any effect drag may have on viscosity measurements for the BV. While the MVAG has a similar spindle contact area to volume ratio and spindle to container distance as the RVA, the viscosity profile is relatively steady like the BV viscosity profile. This is likely a function of the decreased sensitivity for detecting viscosity

changes in free flowing materials compared to the other testing methods. As mentioned before, the MVAG is normally used to measure dough rheology and therefore viscosity changes within a free flowing material like cheese sauce may go undetected or be diminished compared to the BV or RVA. The MVAG also differs from the other methods in that the paddle or apparatus measuring the viscosity does not rotate. It is the container housing the cheese sauce that is rotated, causing the cheese sauce to flow against the paddle which measures the viscosity based on the force at which the cheese sauce is able to move the paddle. This method may also decrease shear and cause less disruption of the casein-phosphate network possibly being formed during the hold time, allowing for steady readings during the testing time. The smaller viscosity ranges during the testing time sweep (5 minutes) of the BV and MVAG suggest that similar viscosity results and trends can be obtained with a decrease in testing time. However, this may not be the case with the RVA as there is a consistent drop in viscosity over time.

When determining which method to use for measuring cheese sauce viscosity it is important to consider all the factors previously mentioned. While all the methods showed similar viscosity trends within the treatments, there were varying degrees in the viscosity values and profiles which may have a larger impact on measuring cheese sauces with viscosities outside the range of the ones in this study. Each method offers different capabilities and possibilities for viscosity measurement which enables an optimal selection to be made for obtaining different types of data being sought.

3.4 Conclusions

The blend age of the process cheese loaves treatments did have a significant impact on the viscosity of cheese sauces made from the loaves. The average viscosity at the initial time point had significant differences ($P < 0.05$) between the treatments and an overall trend of decreasing viscosity with an increase blend cheese age. This was attributed to casein content within the treatments as a similar trend of decreasing casein percentage of total protein was observed with increasing blend age. However, as the cheese sauces were held at a high temperature, new viscosity trends started to emerge. An overall trend of increasing viscosity over hold time was observed for all treatments. The main cause of this increase was likely a function of increased protein-protein interactions. However, another trend of significantly larger viscosity increases was observed in the 90d and 150d treatments compared to the younger aged treatments (10d, 30d) at both the 2 hour and 4 hour hold time points. This is hypothesized to be a function of casein-fat and casein-phosphate interactions as the more aged treatments had higher fat:casein and lower casein:DSP ratios than the younger blend age treatments. The data within this study shows a potential relationship between the ratios of fat:casein and casein:DSP with viscosity change and that an optimal ratio of fat:casein and casein:DSP exists in a process cheese loaf as it relates to sauce viscosity changes since the 210d treatment had lower viscosity changes at each time point despite higher fat:casein and lower casein:DSP ratios than the 150d treatment. There was not enough data to confirm a significant relationship between these ratios and sauce viscosity increase, but exploring

these ratios and their effect on sauce viscosity changes over time in more detail could be the subject of a future study.

While significant differences ($P < 0.05$) were found between the different methods (BV, RVA, MVAG) of viscosity testing for both average viscosity and viscosity range values, the overall trends within the treatments were similar. The differences in the viscosity values obtained from the different methods were likely a function of the different types of apparatus and measurement methods used within each piece of equipment. These differences may enable many different avenues of exploration for determining viscosity trends in cheese sauces or other similar materials.

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Appendix

Process Cheese Loaves Chemical Compositional Data

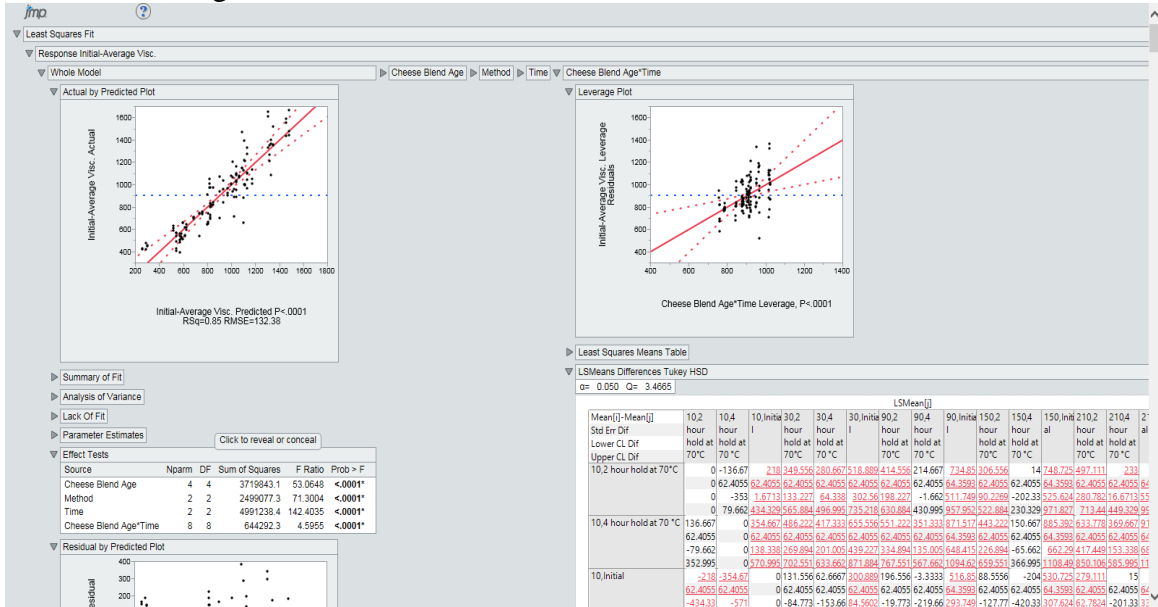
Batch #	Cheese Blend Age	Rep.	Fat (%)	Casein (%)	NPN (%)	Protein (%)	True Protein (%)	Moisture (%)	Calcium mg/100 g	Sodium mg/100 g	pH	Salt (%)	Lactose (%)
1	10	1	30.97%	16.92%	0.64%	19.09%	18.45%	40.15%	576	1410	5.47	1.94%	3.27%
2	10	2	30.67%	16.91%	0.63%	19.05%	18.42%	40.83%	568	1370	5.46	1.91%	3.18%
3	10	3	30.68%	17.06%	0.64%	19.23%	18.59%	40.55%	577	1380	5.46	1.91%	3.12%
AVERAGE			30.77%	16.96%	0.64%	19.12%	18.49%	40.51%	574	1387	5.46	1.92%	3.19%
4	30	1	30.35%	17.19%	0.76%	19.70%	18.94%	40.66%	596	1390	5.48	1.95%	2.83%
5	30	2	30.81%	17.25%	0.78%	19.69%	18.91%	40.44%	600	1370	5.47	1.95%	2.89%
6	30	3	31.00%	17.25%	0.79%	19.74%	18.95%	40.31%	600	1400	5.45	1.94%	2.87%
AVERAGE			30.72%	17.23%	0.78%	19.71%	18.93%	40.47%	599	1387	5.47	1.95%	2.86%
7	90	1	29.82%	16.06%	1.22%	19.22%	18.00%	41.01%	582	1390	5.47	1.91%	3.29%
8	90	2	30.05%	15.96%	1.23%	19.19%	17.96%	40.99%	577	1390	5.48	1.91%	3.39%
9	90	3	29.96%	16.20%	1.23%	19.40%	18.17%	40.92%	588	1390	5.44	1.92%	3.25%
AVERAGE			29.94%	16.07%	1.23%	19.27%	18.04%	40.97%	582	1390	5.46	1.91%	3.31%
10	150	1	30.89%	15.85%	1.74%	19.54%	17.80%	40.38%	579	1420	5.46	2.00%	2.58%
11	150	2	31.20%	16.02%	1.75%	19.76%	18.01%	40.22%	577	1400	5.49	1.98%	2.65%
12	150	3	30.97%	15.78%	1.81%	19.55%	17.74%	40.53%	585	1420	5.48	2.02%	2.54%
AVERAGE			31.02%	15.88%	1.77%	19.62%	17.85%	40.38%	580	1413	5.48	2.00%	2.59%
13	210	1	30.20%	15.08%	2.19%	19.55%	17.36%	40.76%	582	1420	5.48	2.02%	2.71%
14	210	2	30.80%	15.24%	2.19%	19.79%	17.60%	40.35%	577	1420	5.51	2.04%	2.70%
15	210	3	30.92%	15.40%	2.16%	19.75%	17.59%	40.54%	596	1410	5.51	2.02%	2.66%
AVERAGE			30.64%	15.24%	2.18%	19.70%	17.52%	40.55%	585	1417	5.50	2.03%	2.69%

Cheese Sauce Moisture Data

Batch #	Cheese Blend	Age	Rep.	Actual Sauce Moisture			Moisture Loss %			Average Sauce Moisture			Average Moisture Loss %		
				Initial	2 hr.	4 hr.	Initial-2 hr	2-4 hr	Total	Initial	2 hr.	4 hr.	Initial-2 hr	2-4 hr	Total
1	10	1		54.87%	55.23%	54.82%	0.36%	-0.41%	-0.05%						
2	10	2		55.21%	55.23%	54.94%	0.02%	-0.29%	-0.27%						
3	10	3		55.16%	54.99%	54.72%	-0.17%	-0.27%	-0.44%	55.08%	55.15%	54.83%	0.07%	-0.32%	-0.25%
4	30	1		55.53%	55.64%	55.28%	0.11%	-0.36%	-0.25%						
5	30	2		56.78%	56.89%	56.45%	0.11%	-0.44%	-0.33%						
6	30	3		55.11%	55.15%	54.78%	0.04%	-0.37%	-0.33%	55.81%	55.89%	55.50%	0.09%	-0.39%	-0.30%
7	90	1		55.78%	55.41%	55.31%	-0.37%	-0.10%	-0.47%						
8	90	2		55.74%	55.75%	55.26%	0.01%	-0.49%	-0.48%						
9	90	3		55.92%	55.83%	55.51%	-0.09%	-0.32%	-0.41%	55.81%	55.66%	55.36%	-0.15%	-0.30%	-0.45%
10	150	1		55.32%	55.28%	54.85%	-0.04%	-0.43%	-0.47%						
11	150	2		55.08%	55.03%	54.73%	-0.05%	-0.30%	-0.35%						
12	150	3		54.79%	54.84%	54.71%	0.05%	-0.13%	-0.08%	55.06%	55.05%	54.76%	-0.01%	-0.29%	-0.30%
13	210	1		55.41%	54.50%	54.36%	-0.91%	-0.14%	-1.05%						
14	210	2		55.03%	54.91%	54.67%	-0.12%	-0.24%	-0.36%						
15	210	3		54.84%	54.36%	54.30%	-0.48%	-0.06%	-0.54%	55.09%	54.59%	54.44%	-0.50%	-0.15%	-0.65%

Statistical Analysis

Average Cheese Sauce Viscosity of each Test Method-Least Square Fit with Tukey HSD-Cheese Blend Age*Time



Cheese Blend Age*Time Leverage, P<.0001

Least Squares Means Table

LSMeans Differences Tukey HSD

alpha = 0.050 C = 3.4665

LSMean[]	10.2 hour hold at 70 °C	10.4 hour hold at 70 °C	10 initial	30.2 hour hold at 70 °C	30.4 hour hold at 70 °C	30 initial	90.2 hour hold at 70 °C	90.4 hour hold at 70 °C	150.2 hour hold at 70 °C	150.4 hour hold at 70 °C	150 initial	210.2 hour hold at 70 °C	210.4 hour hold at 70 °C	210 initial
Mean[]-Mean[]	0	-136.67	218	249.556	280.667	318.889	414.556	214.667	734.85	306.556	14	748.725	497.111	233
Std Err Df	0	62.4055	62.4055	62.4055	62.4055	62.4055	62.4055	62.4055	62.4055	62.4055	62.4055	62.4055	62.4055	62.4055
Lower CL Df	0	-136.67	218	249.556	280.667	318.889	414.556	214.667	734.85	306.556	14	748.725	497.111	233
Upper CL Df	0	-136.67	218	249.556	280.667	318.889	414.556	214.667	734.85	306.556	14	748.725	497.111	233
10.2 hour hold at 70 °C	0													
10.4 hour hold at 70 °C	136.667	0												
10 initial	-218	-218	0											
30.2 hour hold at 70 °C	-318.889	-318.889	-318.889	0										
30.4 hour hold at 70 °C	-414.556	-414.556	-414.556	-414.556	0									
30 initial	-414.556	-414.556	-414.556	-414.556	-414.556	0								
90.2 hour hold at 70 °C	-214.667	-214.667	-214.667	-214.667	-214.667	-214.667	0							
90.4 hour hold at 70 °C	-734.85	-734.85	-734.85	-734.85	-734.85	-734.85	-734.85	0						
150.2 hour hold at 70 °C	-306.556	-306.556	-306.556	-306.556	-306.556	-306.556	-306.556	-306.556	0					
150.4 hour hold at 70 °C	-14	-14	-14	-14	-14	-14	-14	-14	-14	0				
150 initial	-14	-14	-14	-14	-14	-14	-14	-14	-14	-14	0			
210.2 hour hold at 70 °C	-748.725	-748.725	-748.725	-748.725	-748.725	-748.725	-748.725	-748.725	-748.725	-748.725	-748.725	0		
210.4 hour hold at 70 °C	-497.111	-497.111	-497.111	-497.111	-497.111	-497.111	-497.111	-497.111	-497.111	-497.111	-497.111	-497.111	0	
210 initial	-497.111	-497.111	-497.111	-497.111	-497.111	-497.111	-497.111	-497.111	-497.111	-497.111	-497.111	-497.111	-497.111	0

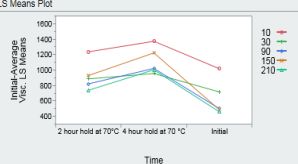
Ratio Prob > F
0643 <.0001*
3004 <.0001*
4035 <.0001*
5955 <.0001*

20 1800

LSMean[]	150.2 hour hold at 70 °C	150.4 hour hold at 70 °C	150 initial	210.2 hour hold at 70 °C	210.4 hour hold at 70 °C	210 initial
Mean[]-Mean[]	0	-136.67	218	249.556	280.667	318.889
Std Err Df	0	62.4055	62.4055	62.4055	62.4055	62.4055
Lower CL Df	0	-136.67	218	249.556	280.667	318.889
Upper CL Df	0	-136.67	218	249.556	280.667	318.889
150.2 hour hold at 70 °C	0					
150.4 hour hold at 70 °C	136.667	0				
150 initial	-218	-218	0			
210.2 hour hold at 70 °C	-318.889	-318.889	-318.889	0		
210.4 hour hold at 70 °C	-414.556	-414.556	-414.556	-414.556	0	
210 initial	-414.556	-414.556	-414.556	-414.556	-414.556	0

Level	Least Sq Mean
10.4 hour hold at 70 °C	A 1372.667
10.2 hour hold at 70 °C	A B 1236.000
150.4 hour hold at 70 °C	A B C 1222.000
90.4 hour hold at 70 °C	B C D 1021.3333
10 initial	C D 1016
210.4 hour hold at 70 °C	D 1003.0000
30.4 hour hold at 70 °C	D 965.3333
150.2 hour hold at 70 °C	D E 929.4444
30.2 hour hold at 70 °C	D E 886.4444
90.2 hour hold at 70 °C	D E 821.4444
210.2 hour hold at 70 °C	E 738.8889
30 initial	E F 717.1111
90 initial	F G 501.1499
150 initial	G 487.2749
210 initial	G 459.8999

Levels not connected by same letter are significantly different.

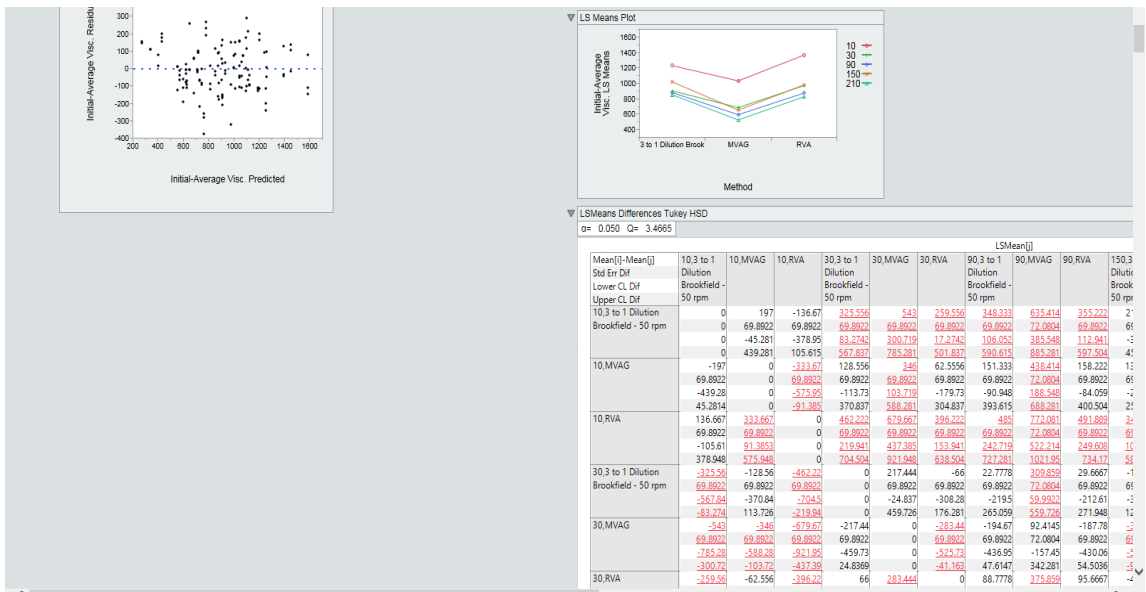
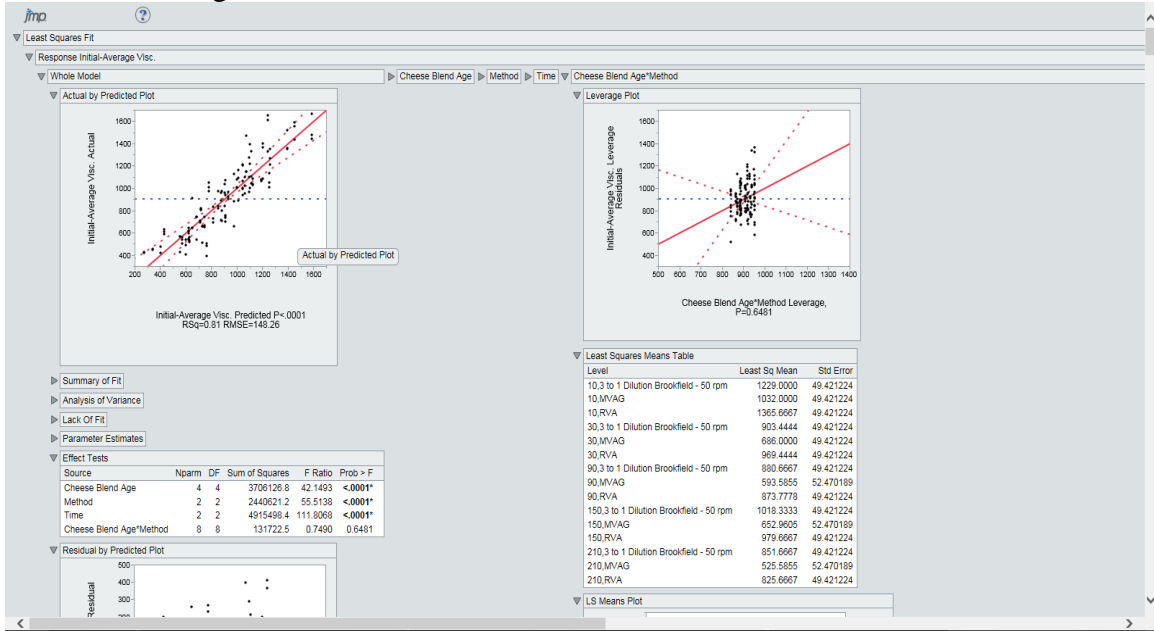


Cheese Sauce Viscosity of each Test Method-Least Square Fit with Tukey HSD-Cheese Blend Age*Method*Time



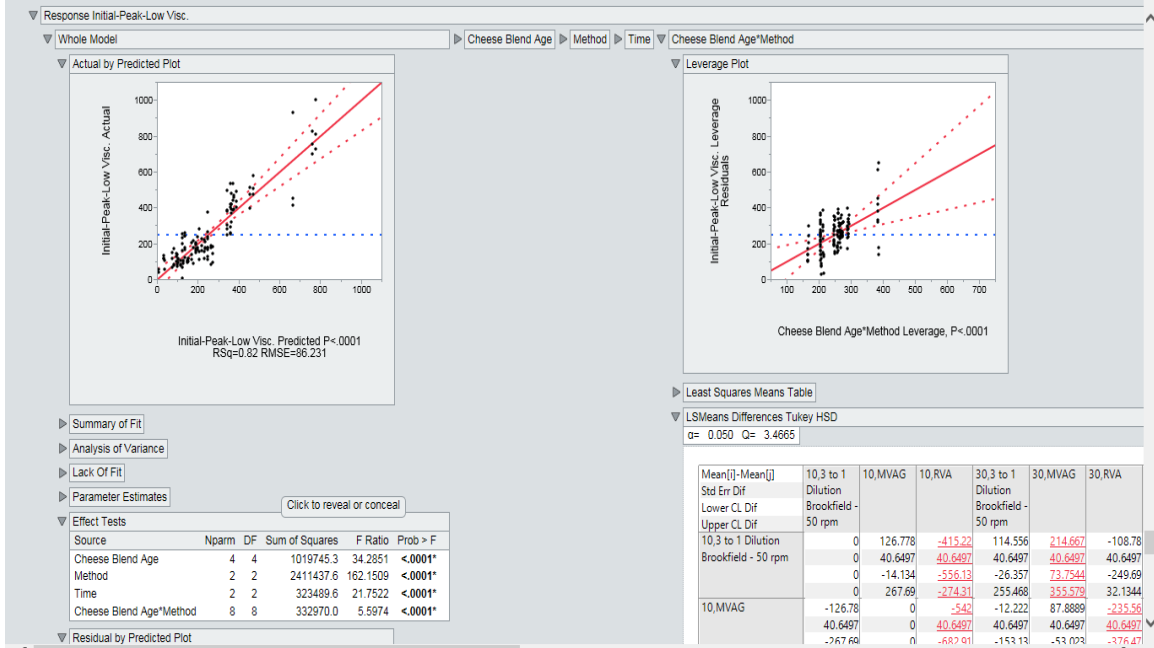
150,MVAG,2 hour hold at 70°C	J	K	L	M	N	O	673.0000
90,MVAG,2 hour hold at 70°C	J	K	L	M	N	O	617.0000
30,MVAG,Initial	J	K	L	M	N	O	608.6667
150,RVA,Initial	J	K	L	M	N	O	608.3333
90,RVA,Initial	K	L	M	N	O		585.6667
90,3 to 1 Dilution Brookfield - 50 rpm,Initial	L	M	N	O			536.0000
210,RVA,Initial	L	M	N	O			534.0000
210,MVAG,2 hour hold at 70°C	M	N	O				518.3333
210,3 to 1 Dilution Brookfield - 50 rpm,Initial	N	O					476.6667
150,3 to 1 Dilution Brookfield - 50 rpm,Initial	O						459.0000
90,MVAG,Initial	N	O					450.5000
150,MVAG,Initial	N	O					446.5000
210,MVAG,Initial	O						422.0000

Cheese Sauce Average Viscosity of all Time Points-Least Squares Fit with Tukey HSD-Cheese Blend Age*Method



Method		10.3 to 1 Dilution Brookfield - 50 rpm		10.MVAG		10.RVA		30.3 to 1 Dilution Brookfield - 50 rpm		30.MVAG		30.RVA		90.3 to 1 Dilution Brookfield - 50 rpm		90.MVAG		90.RVA		150.3 to 1 Dilution Brookfield - 50 rpm		150.MVAG		150.RVA		210.3 to 1 Dilution Brookfield - 50 rpm		210.MVAG		210.RVA	
LSMeans Differences Tukey HSD																															

Average Cheese Sauce Viscosity Range of Methods-Least Squares Fit with Tukey HSD- Cheese Blend Age*Method

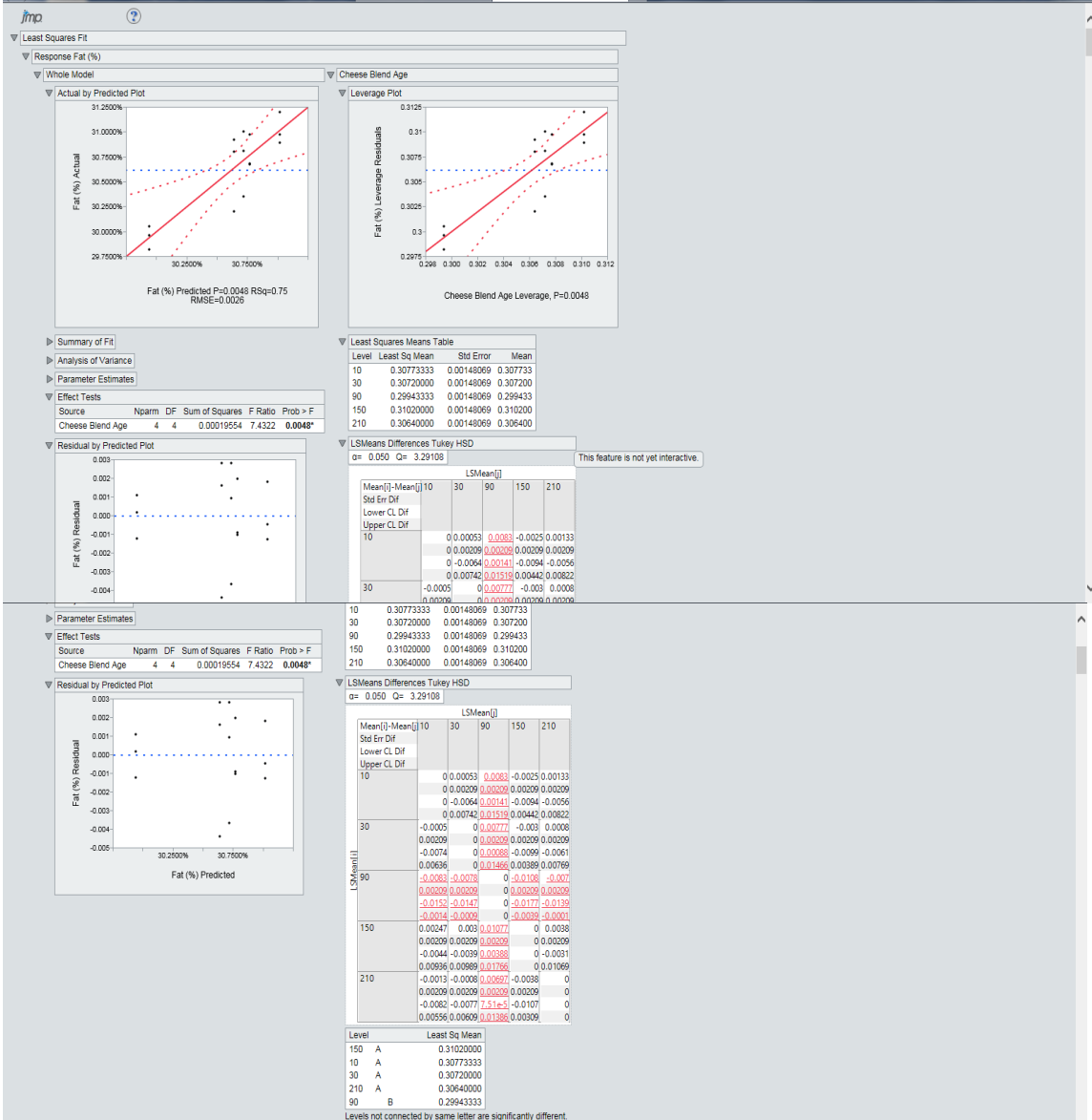


LSMeans Differences Tukey HSD
α= 0.050 Q= 3.4665

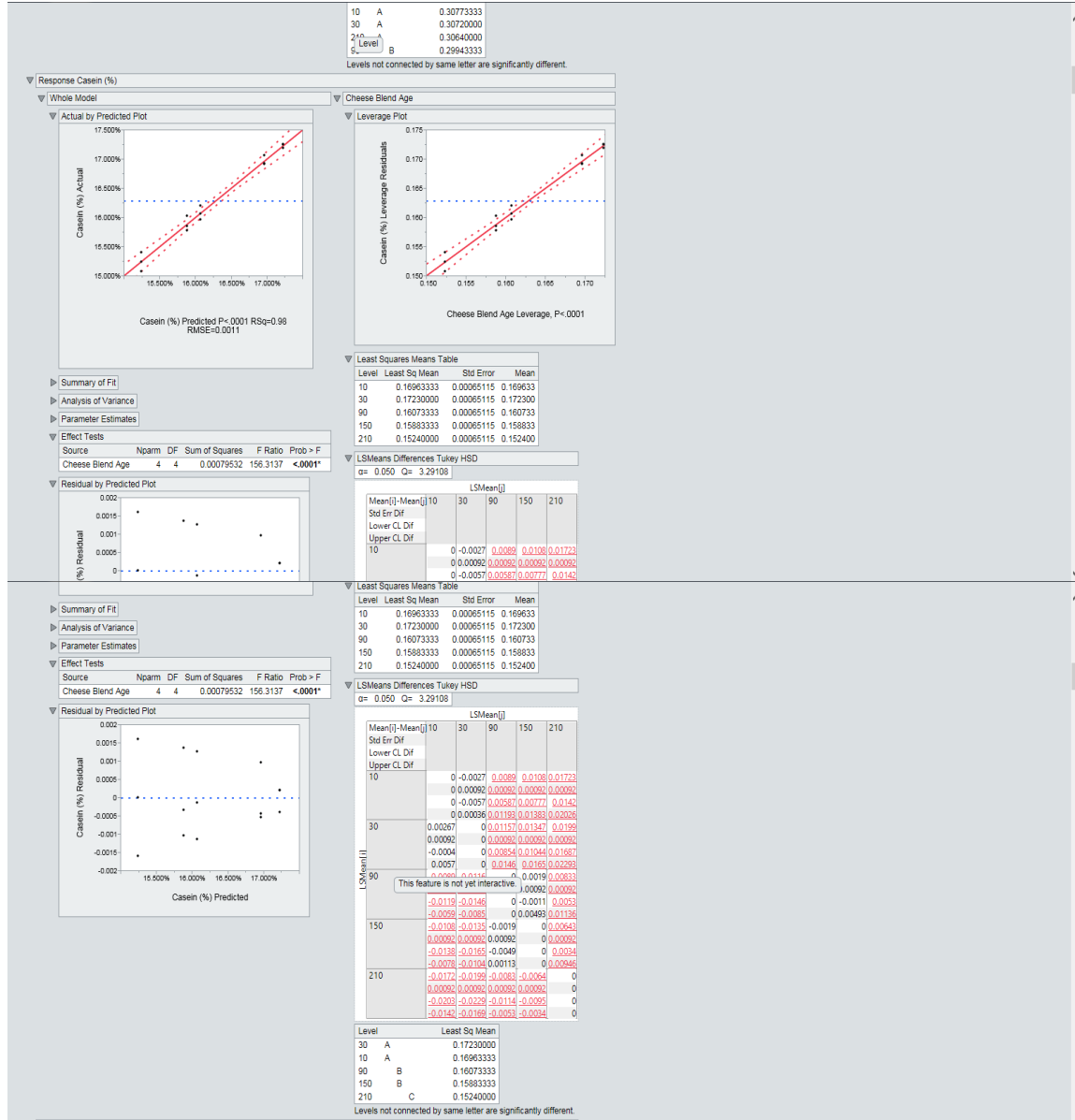
Mean[j]-Mean[i]	10,3 to 1 Dilution Brookfield - 50 rpm	10,MVAG	10,RVA	30,3 to 1 Dilution Brookfield - 50 rpm	30,MVAG	30,RVA	90,3 to 1 Dilution Brookfield - 50 rpm	90,MVAG	90,RVA	150,3 to 1 Dilution Brookfield - 50 rpm	150,MVAG	150,RVA	210,3 to 1 Dilution Brookfield - 50 rpm	210,MVAG	210,RVA
10,3 to 1 Dilution Brookfield - 50 rpm	0	126.778	-415.22	114.556	214.667	-108.78	165.556	247.35	-14	155.667	210.225	-25.556	175.333	239.85	1.55556
10,MVAG	-126.78	0	-542	-12.222	87.8889	-235.56	38.7778	120.572	-140.78	28.8889	83.4469	-152.33	48.5556	113.072	-125.22
10,RVA	40.6497	40.6497	0	40.6497	40.6497	40.6497	40.6497	41.9223	40.6497	40.6497	41.9223	40.6497	40.6497	41.9223	40.6497
30,3 to 1 Dilution Brookfield - 50 rpm	114.56	12.2222	-529.78	0	100.111	-223.33	51	132.794	-128.56	41.1111	95.6691	-140.11	60.7778	125.294	-113
30,MVAG	40.6497	40.6497	40.6497	0	40.6497	40.6497	40.6497	41.9223	40.6497	40.6497	41.9223	40.6497	40.6497	41.9223	40.6497
30,RVA	214.667	214.667	214.667	214.667	214.667	214.667	214.667	214.667	214.667	214.667	214.667	214.667	214.667	214.667	214.667
90,3 to 1 Dilution Brookfield - 50 rpm	165.56	165.56	165.56	165.56	165.56	165.56	0	274.333	356.127	94.7778	264.444	319.002	83.2222	284.111	348.627
90,MVAG	40.6497	40.6497	40.6497	40.6497	40.6497	40.6497	40.6497	0	40.6497	40.6497	40.6497	40.6497	40.6497	40.6497	40.6497
90,RVA	247.35	247.35	247.35	247.35	247.35	247.35	247.35	247.35	0	247.35	247.35	247.35	247.35	247.35	247.35
150,3 to 1 Dilution Brookfield - 50 rpm	155.667	155.667	155.667	155.667	155.667	155.667	155.667	155.667	155.667	0	155.667	155.667	155.667	155.667	155.667
150,MVAG	210.225	210.225	210.225	210.225	210.225	210.225	210.225	210.225	210.225	210.225	0	210.225	210.225	210.225	210.225
150,RVA	-25.556	-25.556	-25.556	-25.556	-25.556	-25.556	-25.556	-25.556	-25.556	-25.556	-25.556	0	-25.556	-25.556	-25.556
210,3 to 1 Dilution Brookfield - 50 rpm	175.333	175.333	175.333	175.333	175.333	175.333	175.333	175.333	175.333	175.333	175.333	175.333	0	175.333	175.333
210,MVAG	239.85	239.85	239.85	239.85	239.85	239.85	239.85	239.85	239.85	239.85	239.85	239.85	239.85	0	239.85
210,RVA	1.55556	1.55556	1.55556	1.55556	1.55556	1.55556	1.55556	1.55556	1.55556	1.55556	1.55556	1.55556	1.55556	1.55556	0

Process Cheese Loaves Chemical Composition-Least Square Fit with Tukey HSD- Cheese Blend Age

Fat%



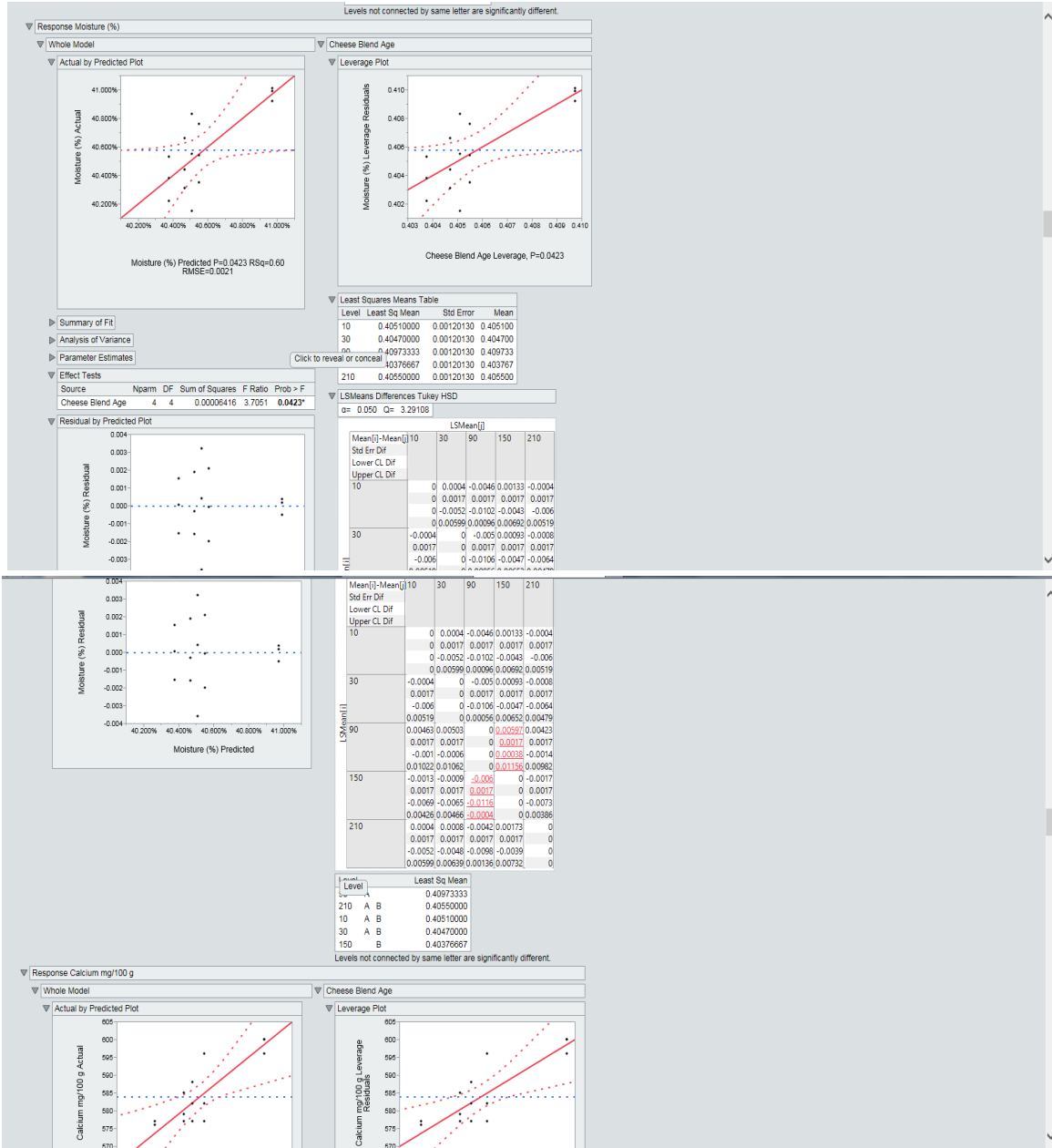
Casein%



Protein%



Moisture%



Calcium mg/100 g

30	A	B	0.4047000
150	B		0.40376667

Levels not connected by same letter are significantly different.

▼ Response Calcium mg/100 g

▼ Whole Model

▼ Actual by Predicted Plot

Calcium mg/100 g Predicted $P=0.0051$
 $RSQ=0.74$ $RMSE=5.9048$

▼ Summary of Fit

► Analysis of Variance

► Parameter Estimates

▼ Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Cheese Blend Age	4	4	1017.3333	7.2945	0.0051*

▼ Residual by Predicted Plot

► Analysis of Variance

► Parameter Estimates

▼ Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Cheese Blend Age	4	4	1017.3333	7.2945	0.0051*

▼ Residual by Predicted Plot

▼ Cheese Blend Age

▼ Leverage Plot

Cheese Blend Age Leverage, $P=0.0051$

▼ Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
10	573.66667	3.4091380	573.667
30	598.66667	3.4091380	598.667
90	582.33333	3.4091380	582.333
150	580.33333	3.4091380	580.333
210	585.00000	3.4091380	585.000

▼ LSMeans Differences Tukey HSD

$\alpha = 0.050$ $Q = 3.29108$

		LSMean[j]				
	Mean[i]-Mean[j]	10	30	90	150	210
10	Std Err DF					
	Lower CL DF					
	Upper CL DF					
	0	-2.5	-8.6667	-6.6667	-11.333	
	0	4.82125	4.82125	4.82125	4.82125	
	0	-40.867	-24.534	-22.534	-27.2	
	0	-3.1320	7.20046	9.20046	4.53379	
				0.63333	3.3333	13.6667
30			573.66667	598.66667	582.33333	580.33333
	Std Err DF					
	Lower CL DF					
	Upper CL DF					
	2.5	0	16.3333	18.3333	13.6667	
	4.82125	0	4.82125	4.82125	4.82125	
	9.13287	0	0.46621	2.46621	-2.2005	
	40.8671	0	32.2005	34.2005	29.5338	
90	8.66667	-16.333	0	2	-2.6667	
	4.82125	4.82125	0	4.82125	4.82125	
	-7.2005	-23.2	0	-13.867	-18.534	
	24.5338	-0.4662	0	17.8671	13.2005	
	6.66667	-18.333	-2	0	-4.6667	
4.82125	4.82125	4.82125	0	4.82125		
-9.2005	-34.2	-17.867	0	-20.534		
23.5338	-2.6667	13.6671	0	11.2005		
11.3333	-13.667	2.66667	4.66667	0		
4.82125	4.82125	4.82125	4.82125	0		
-4.5338	-29.534	-13.2	-11.2	0		
27.2005	2.20046	18.5338	20.5338	0		
Level	Least Sq Mean					
30	A	598.66667				
210	A	585.00000				
90	B	582.33333				
150	B	580.33333				
10	B	573.66667				

Levels not connected by same letter are significantly different.

▼ Response Sodium mg/100 g

▼ Whole Model

▼ Actual by Predicted Plot

1430

▼ Leverage Plot

1430

pH

Levels not connected by same letter are significantly different.

Response pH

Whole Model

Actual by Predicted Plot

pH Predicted $P=0.0764$ $R^2=0.54$ $RMSE=0.0157$

Cheese Blend Age

Leverage Plot

Cheese Blend Age Leverage, $P=0.0764$

Summary of Fit

Analysis of Variance

Parameter Estimates

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Cheese Blend Age	4	4	0.00289333	2.9324	0.0764

Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
10	5.4633333	0.00906765	5.46333
30	5.4666667	0.00906765	5.46667
90	5.4633333	0.00906765	5.46333
150	5.4766667	0.00906765	5.47667
210	5.5000000	0.00906765	5.50000

LSMeans Differences Tukey HSD

$\alpha=0.050$ $Q=3.29108$

Mean[]-Mean[]	LSMean[]				
	10	30	90	150	210
Std Err Df					
Lower CL Df					
Upper CL Df					
10					
	0	-0.0033	-9e-16	-0.0133	-0.0367
	0	0.01282	0.01282	0.01282	0.01282
	0	-0.0455	-0.0422	-0.0555	-0.0789
	0	0.03887	0.0422	0.02887	0.00554
30	0.00333		0.00333	-0.01	-0.0333
	0.01282		0.01282	0.01282	0.01282
	-0.0369		0	-0.0369	-0.0522
	-0.0369		0	-0.0369	-0.0522
	0.04554		0	0.04554	0.0322
90	8.9e-16		-0.0033	0	-0.0133
	0.01282		0.01282	0.01282	0.01282
	-0.0422		-0.0455	0	-0.0555
	0.0422		0.03887	0	0.02887
150	0.01333		0.01	0.01333	0
	0.01282		0.01282	0.01282	0.01282
	-0.0289		-0.0322	-0.0289	0
	0.05554		0.0522	0.05554	0.01887
210	0.03667		0.0333	0.03667	0.02333
	0.01282		0.01282	0.01282	0.01282
	-0.0055		-0.0089	-0.0055	-0.0189
	0.07887		0.07554	0.07887	0.06554

Source

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Cheese Blend Age	4	4	0.00289333	2.9324	0.0764

Residual by Predicted Plot

Residual by Predicted Plot

LSMeans Differences Tukey HSD

$\alpha=0.050$ $Q=3.29108$

Mean[]-Mean[]	LSMean[]				
	10	30	90	150	210
Std Err Df					
Lower CL Df					
Upper CL Df					
10					
	0	-0.0033	-9e-16	-0.0133	-0.0367
	0	0.01282	0.01282	0.01282	0.01282
	0	-0.0455	-0.0422	-0.0555	-0.0789
	0	0.03887	0.0422	0.02887	0.00554
30	0.00333		0.00333	-0.01	-0.0333
	0.01282		0.01282	0.01282	0.01282
	-0.0369		0	-0.0369	-0.0522
	-0.0369		0	-0.0369	-0.0522
	0.04554		0	0.04554	0.0322
90	8.9e-16		-0.0033	0	-0.0133
	0.01282		0.01282	0.01282	0.01282
	-0.0422		-0.0455	0	-0.0555
	0.0422		0.03887	0	0.02887
150	0.01333		0.01	0.01333	0
	0.01282		0.01282	0.01282	0.01282
	-0.0289		-0.0322	-0.0289	0
	0.05554		0.0522	0.05554	0.01887
210	0.03667		0.0333	0.03667	0.02333
	0.01282		0.01282	0.01282	0.01282
	-0.0055		-0.0089	-0.0055	-0.0189
	0.07887		0.07554	0.07887	0.06554

Level

Level	Least Sq Mean
210	A
150	A
30	A
90	A
10	A

Levels not connected by same letter are significantly different.

Response Salt (%)

Whole Model

Actual by Predicted Plot

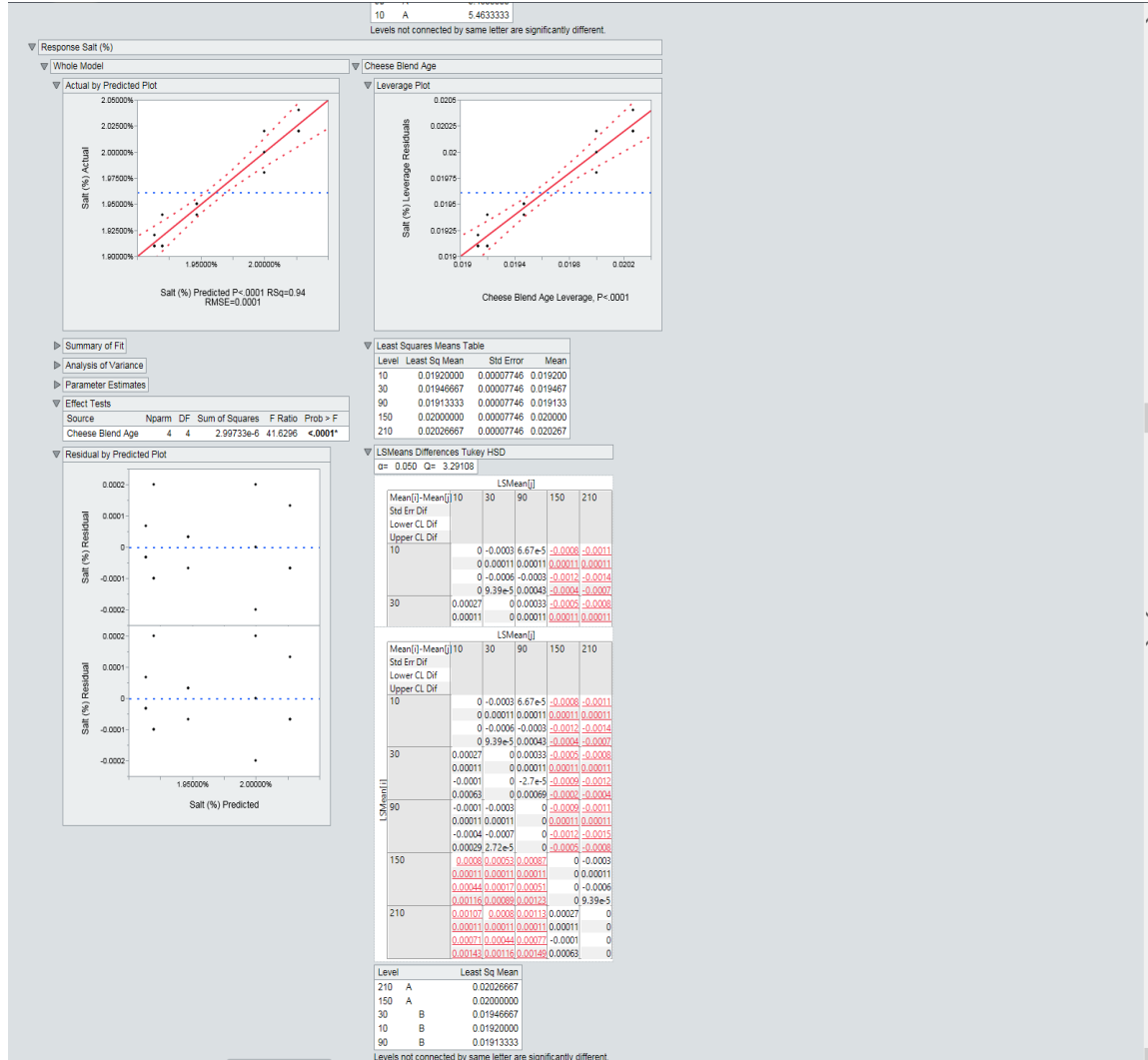
Salt Predicted

Cheese Blend Age

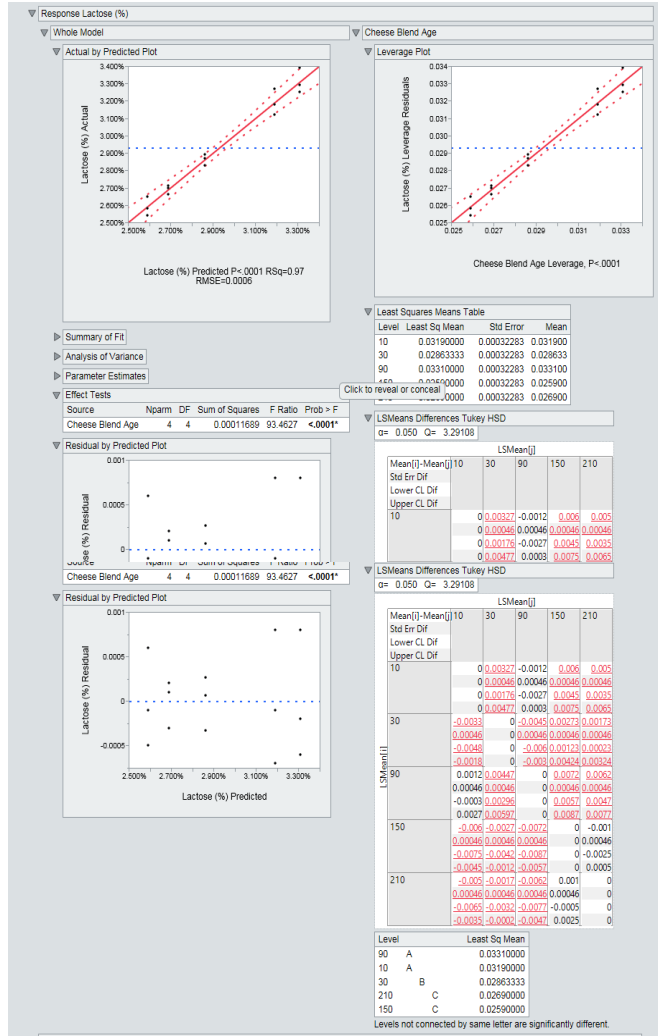
Leverage Plot

Cheese Blend Age Leverage

Salt%



Lactose%



Casein% of Protein

mpo

Response Casein % of Protein

Whole Model

Actual by Predicted Plot

Casein % of Protein Predicted $P < .0001$
RSq=1.00 RMSE=0.0026

Summary of Fit

RSquare	0.996889
RSquare Adj	0.995645
Root Mean Square Error	0.002844
Mean of Response	0.835749
Observations (or Sum Wgts)	15

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	0.02591085	0.006478	801.1426
Error	10	0.00038086	8.086e-6	Prob > F
C. Total	14	0.02599171		<.0001*

Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
10	0.88704915	0.00164171	0.887049
30	0.87417608	0.00164171	0.874176
90	0.83410755	0.00164171	0.834108
150	0.80968216	0.00164171	0.809682
210	0.77372941	0.00164171	0.773729

LSMeans Differences Tukey HSD

$\alpha = 0.050$ $Q = 3.29108$

		LSMean[j]				
Mean[i]-Mean[j]		10	30	90	150	210
Std Err Dif						
Lower CL Dif						
Upper CL Dif						
10			0.01287	0.05294	0.07737	0.11332
		0.00232	0.00232	0.00232	0.00232	0.00232
		0.00523	0.0453	0.06973	0.10568	0.14163
		0.02051	0.06038	0.08501	0.12096	0.15691
30		-0.0119		0.04007	0.06449	0.10045
		0.00232	0.00232	0.00232	0.00232	0.00232
		-0.0205	0.03245	0.05683	0.09281	0.12879
		-0.0652	0.04771	0.07213	0.10809	0.14394
90		-0.0529	-0.0401		0.02443	0.06038
		0.00232	0.00232	0.00232	0.00232	0.00232
		-0.0606	-0.0477	0.01678	0.05274	0.08870
		-0.0453	-0.0324	0.03207	0.06802	0.10335
150		-0.0774	-0.0645	-0.0244		0.03585
		0.00232	0.00232	0.00232	0.00232	0.00232
		-0.085	-0.0721	-0.0321	0.02831	0.06426
		-0.0697	-0.0569	-0.0168	0.04359	0.07924
210		-0.1133	-0.1004	-0.0604	-0.036	
		0.00232	0.00232	0.00232	0.00232	0.00232
		-0.121	-0.1081	-0.068	-0.0436	0.01991
		-0.1057	-0.0928	-0.0527	-0.0283	0.01546

LSMeans Differences Tukey HSD

$\alpha = 0.050$ $Q = 3.29108$

		LSMean[j]				
Mean[i]-Mean[j]		10	30	90	150	210
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		0.00232	0.00232	0.00232	0.00232	0.00232
		0.00523	0.0453	0.06973	0.10568	0.14163
		0.02051	0.06038	0.08501	0.12096	0.15691
30		-0.0119		0.04007	0.06449	0.10045
		0.00232	0.00232	0.00232	0.00232	0.00232
		-0.0205	0.03245	0.05683	0.09281	0.12879
		-0.0652	0.04771	0.07213	0.10809	0.14394
90		-0.0529	-0.0401		0.02443	0.06038
		0.00232	0.00232	0.00232	0.00232	0.00232
		-0.0606	-0.0477	0.01678	0.05274	0.08870
		-0.0453	-0.0324	0.03207	0.06802	0.10335
150		-0.0774	-0.0645	-0.0244		0.03585
		0.00232	0.00232	0.00232	0.00232	0.00232
		-0.085	-0.0721	-0.0321	0.02831	0.06426
		-0.0697	-0.0569	-0.0168	0.04359	0.07924
210		-0.1133	-0.1004	-0.0604	-0.036	
		0.00232	0.00232	0.00232	0.00232	0.00232
		-0.121	-0.1081	-0.068	-0.0436	0.01991
		-0.1057	-0.0928	-0.0527	-0.0283	0.01546

Level

Level	Least Sq Mean
10	0.88704915
30	0.87417608
90	0.83410755
150	0.80968216
210	0.77372941

Levels not connected by same letter are significantly different.

Residual by Predicted Plot

Casein % of Protein Residual

Casein % of Protein Predicted