MANUFACTURING, CHEMICAL, AND SENSORY PROPERTIES OF A HYBRID CHEDDAR CHEESE

By

Javier Abelardo Salas

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ABSTRACT

MANUFACTURING, CHEMICAL, AND SENSORY PROPERTIES OF A HYBRID CHEDDAR CHEESE

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Cheese derived from the milk of pasture fed cows contains a higher amount of CLA (c9, t11) than cheese derived from TMR diets. Although, using milk from pasture fed cows is a viable solution to increase CLA content in a cheese, formulating a hybrid cheese from the combination of cream from pasture fed cows and skim milk from TMR fed cows, provides an alternative for the manufacturing of a Cheddar cheese high in CLA. Three Cheddar cheeses were prepared for this study; one hybrid, one derived from a pasture diet, and one derived from a TMR diet. Using these cheeses, this study assessed the effect of hybridization across the following measures: chemical composition, consumer evaluation, texture profile analysis (TPA), and fat globule (FG) size assessment by confocal laser scanning microscopy. Hybridization was found to significantly influence protein, mineral, fatty acid composition, and sensory attributes. The protein, ash, and calcium composition of the hybrid cheese was higher and more similar to the TMR cheese. The fatty acid composition of the hybrid cheese matched that of the pasture cheese and was ~2.3-fold higher than the TMR cheese. The hybrid cheese received similar ratings for “liking” and “purchase intent” as the pasture cheeses which were higher than the same ratings of the TMR cheese. “Flavor intensity” of the hybrid cheese was found to be in between the pasture and TMR cheeses and may have been influenced by hybridization. The hybrid cheese had a “hardness” and “chewiness” that resembled more the TMR cheese. Finally, FG diameter of pasture, hybrid, and TMR cheeses was not found to be different, but this result was inconclusive due to large variations of FG size distribution, possibly influenced by testing conditions.

I would like to dedicate this work to my wife Carlee and son Nico, who were my rock throughout my journey as a student.

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KEY TO ABBREVIATIONS

TMR:	total mixed ration
CLA:	conjugated linoleic acid
c9, t11:	cis-9, trans-11
FA:	fatty acid
Ca:	calcium
P:	phosphorus
Mg:	magnesium
K:	potassium
Cl:	chloride
Na:	sodium
Fe:	iron
S:	sulfur
Cu:	copper
Zn:	zinc
Cit:	citrate
Insol Ca:	insoluble calcium
Sol Ca:	soluble calcium
Subsp:	subspecies
Lc.:	Lactococcus
pH:	power of hydrogen
g:	gram

mg:	milligram
N:	Newton
Hg:	mercury
MUFA:	monounsaturated fatty acid
PUFA:	polyunsaturated fatty acid
SFA:	saturated fatty acid
USDA:	United States Department of Agriculture
U.S.:	United States
CVD:	cardiovascular disease
LDL:	low-density lipoprotein
LAB:	lactic acid bacterium
CFR:	Code of Federal Regulations
MSU:	Michigan State University
W.K.:	Will Keith
rpm:	revolutions per minute
ºC:	degrees Celsius
h:	hour
min:	minute
s:	second
cm:	centimeter
TA:	titratable acidity
Kg:	kilogram
mL:	milliliter

L:	liter
wt:	weight
DI:	Deionized
HCL:	Hydrochloric acid
HNO_3:	Nitric acid
µg:	Microgram
AAS:	Atomic Absorption Spectrophotometer
W:	Watts
Vol:	Volume
FAME:	Fatty Acid Methyl Ester
GC:	Gas Chromatography
CATA:	Check-All-That-Apply
UCRIHS:	University Committee on Research Involving Human Subjects
FG:	Fat globule
TPA:	Texture Profile Analysis
SAS:	Statistical Analysis System
D:	diameter
Sqrt:	Square root
A:	Area
CLSM:	Confocal Laser Scanning Microscopy
2D:	Two Dimensional
SCFA:	Short Chain Fatty Acids
MCFA:	Medium Chain Fatty Acids

LCFA: Long Chain Fatty Acids

1.0 INTRODUCTION

The concentration of milk components, specifically fat and protein, are considered the most relevant factor for the commercial value of milk (Bijl et al. 2013); however, changes in the fatty acid composition of the milk influenced from the cow's diet has gained some interest for its potential nutritional and commercial value (Kliem et al. 2019). Two commonly practiced feed management systems in the United States that have shown to produce milk with different fatty acid composition are total mixed ration (TMR) and pasture-based diets (Moate et al. 2007; O'Callaghan et al. 2016b). Studies on these two feeding systems have shown that a pasture diet can increase the conjugated linoleic acid (CLA) content in milk (Couvreur et al. 2006; Hernández-Ortega et al. 2014; O'Callaghan et al. 2016b).

CLA isomers are inherently present in dairy and meat products but dairy products usually provide more than half of the total consumption in the human diet (Lawson et al. 2001). Some CLA isomers, like c9, t11 which is of particular interest to this work, have been found to have anticarcinogenic properties in animal studies (Kelley et al. 2007; Shokryazdan et al. 2015), and it has been suggested that they may also be beneficial to human health (Wahle et al. 2004; Kim et al. 2016). Additionally, an increase in CLA fatty acid is also associated with an increase on unsaturated fatty acids. This may provide an added nutritional and commercial advantage (Markey et al. 2017) to a Cheddar cheese product, especially since the United States Department of Agriculture Dietary Guidelines for Americans (USDA 2015) has recommended a decrease in the consumption of full fat cheese as a way to lower saturated fat intake in the diet of Americans.

Taking advantage of the common industry practice of the separation of the cream portion and skimmed portions of milk, cream high in CLA from pasture fed cows and skimmed milk from TMR fed cows will be combined to make a hybrid Cheddar cheese with a similar fatty acid composition to that of a Cheddar cheese derived from pasture fed cows. This approach of combining cream from pasture fed cows and skimmed milk from TMR fed cows to increase the CLA content in a Cheddar cheese has not been reported before. The proposed work of this research is to determine the potential compositional, texture, and microstructure (fat globule size) changes and consumer acceptability of a hybrid cheese.

2.0 HYPOTHESIS & OBJECTIVES

2.1 Hypothesis

This study hypothesizes that hybridization, the combination of cream from pasture fed cows and skim milk from TMR fed cows, is a suitable method to increase the unsaturated fatty acid and CLA (cis-9, *trans*-11) content in a Cheddar cheese. It is also expected that hybridization will impact sensory properties (liking, texture, flavor/texture intensity, and purchase intent) of a hybrid Cheddar cheese and it is hypothesized that hybridization will provide a hybrid Cheddar cheese with similar textural properties to a Cheddar cheese derived from a TMR diet.

2.2 Objectives

The research objectives of this study are the following: 1) assess compositional changes (proximate values, fat globule size, calcium) in a hybrid Cheddar cheese and compare them against Cheddar cheeses derived from pasture and TMR diets, 2) assess variations in a hybrid Cheddar cheese related to sensory and texture parameters and compare them against sensory and texture properties of cheeses derived from pasture and TMR diets, and 3) evaluate the suitability of making a hybrid Cheddar cheese high in CLA content by assessing its composition and consumer acceptability against that of cheeses derived from pasture and TMR diets.

3.0 LITERATURE REVIEW

3.1 Milk components and their importance to Cheddar cheese manufacturing

3.1.1 Lipids

Milk in the United States is legally recognized as the "lacteal secretion" of healthy cows. Milk is legally required to be free of colostrum, contain 8.25% of milk solids (no fat), 3.25% of milk fat and be pasteurized or ultrapasteurized (FDA 2018). Bovine milk is reported to have an average composition of 87.2% water, 3.7% lipids, 3.4% protein, 4.8% lactose, and 0.7% minerals (Fox et al. 2015a). Although, water is the largest component of milk, the most important milk components in terms of their nutritional importance and their processing functionality for Cheddar cheese manufacture are protein, lipids, lactose and minerals (Fox 2010).

As previously mentioned, the average lipid content of bovine milk is about 3.7%. Triacylglycerols make up most of the lipid content in milk (> 95%) with fatty acids ranging from 4-24 carbon atoms in length (Jensen 1995). The remaining lipids in milk are of a combination of mono- and di-glycerides, phospholipids, lipophilic vitamins, and cholesterol and ester cholesteryl (Fox 2010). Lipid content and composition is susceptible to changes in the cow's diet (Christian et al. 1999). Other factors that may influence lipid composition in milk are: breed of the cow, physiological differences between cows (between the same breed), differences in the lactation stage (early vs late lactation), and health of the cow (mastitis infections, etc.) (Fox 2010).

Milk lipids are different from other food lipids because they are encapsulated as fat globules inside a three-layered membrane. This three-layered membrane is primarily made up of a mix of proteins (proteins, glycoproteins), lipids (phospholipids, glycolipids), and enzymes (Keenan and Mather 2006). Milk fat globules normally tend to be of different sizes, however, factors like diet (Couvreur et al. 2007), breed, stage of lactation (Fleming et al. 2017) and individual differences among cows (Logan et al. 2014) may also influence their size. Although, it is lipid content and not lipid composition what is required to meet legal requirements for the manufacture of Cheddar cheese, lipid composition has potential to impact the texture of a Cheddar cheese (Bugaud et al. 2001c).

The texture attribute of Cheddar cheese is important factor that consumer consider when evaluating acceptability (Drake and Delahunty 2017). Two factors of fatty acid composition that have been reported to influence the texture of Cheddar cheese are 1) differences in the ratio of unsaturated and saturated fatty acids and 2) fat globule size (Michalski et al. 2005; Logan et al. 2014). Milk from pasture fed cows has shown to have a higher concentration of mono- and poly-unsaturated fatty acids than TMR milk (Bugaud et al. 2001a; Slots et al. 2009; Coppa et al. 2011b). The fatty acid ratio (saturated vs unsaturated FA) has been correlated by some authors with softer cheese texture (Bugaud et al. 2001c; Coppa et al. 2011a).

The primary factor that promotes softer texture of cheeses with higher ratios of unsaturated fat is that these unsaturated fatty acids are likely to be in the liquid phase at the time of consumption, which lead to a perceived softer texture (Coulon et al. 2004). In contrast, saturated fatty acids are likely to remain solid during consumption and produce a harder texture (Fox et al. 2017a). Thus, differences in the ratios of unsaturated and saturated fatty acids is likely to create texture differences.

Furthermore, fatty acid ratio may also influence cheese texture by affecting fat globule size and distribution in a cheese protein network, which may impact cheese texture by influencing the disruption patterns within the protein structure (Michalski et al. 2004; Logan et al. 2017). It has been reported that cheeses made from milk richer in CLA and unsaturated FA have smaller fat globules (Jones and others 2005; Collomb and others 2006). Cheeses with the same fat content but smaller fat globules distributed in the protein network may cause higher disruption of the protein network, which may weaken its structure and lead to softening of its texture (Logan and others 2017).

Another indirect effect on cheese texture related to fat globule size that has been reported is related to moisture content. Michalski et al. (2004) reported that manufacture of Emmental cheese with small fat globules resulted on significantly higher moisture content which was also correlated to a lower firmness of the cheese. Michalski et al. (2007) attributed the higher moisture retention of smaller fat globules to a larger fat globule surface area that contains a higher amount of glycoproteins which have an ability to bind water.

3.1.2 Protein

Most of the protein content in milk is made up of a combination of caseins (α_{s1}, α_{s2}, β , κ, γ) and whey proteins (α-lactalbumin , β-lactoglobulin) (Fox 2010). A complete protein composition of bovine milk is shown on Table 3.1.2.1. Casein proteins in milk are grouped together as a micelle complex and are able to be stabilized in suspension by κ-caseins (McMahon and Oommen 2013). Caseins are differentiated from whey proteins for having higher heat stability (~ 140 ºC) (Chandan and Kilara 2011). Casein micelles are susceptible to denaturation and aggregate to form a gel by enzyme activity on κ-casein (Fox et al. 2015a) and by milk acidification (Dalgleish and Corredig 2012). Enzymatic coagulation and acidification with lactic acid bacteria is the traditional method for the manufacture of a Cheddar cheese.

Denaturation and coagulation (gel formation) of both casein and whey proteins is also possible when high heat (~90ºC) and acidification by the addition of an acid are applied during processing (Fox and Guinee 2013). The heat-acid coagulation (non-enzymatic coagulation) allows for whey proteins to form disulfide links with caseins and is used for the manufacturing of Ricotta cheese (Chinprahast et al. 2015). However, high heat and the addition of an acidulant is not utilized for the manufacturing of Cheddar cheese and since linking of caseins with whey proteins is not promoted during Cheddar cheese manufacturing, most whey proteins remain in the aqueous phase (liquid whey) separated from the casein coagula.

Table 3.1.2.1. Proteins in bovine milk.

Proteins	Weight g/kg of milk
α-s_1-Casein	11.5
α-s_2-Casein	3.0
β-Casein	9.5
κ-Casein	3.4
γ-Casein	1.2
α-Lactalbumin	1.2
β-Lactoglobulin	3.1
Serum albumin	0.4
Immunoglobulin	0.8
Proteose-peptones	1.0
Total protein	35.1
Total casein	28.6
Whey protein	6.1

Adapted from Kailasapathy (2015)

Casein micelle complexes contain some of the minerals of milk, primarily calcium (Ca), phosphorus (P), and to a lesser extent magnesium (Mg) and potassium (K) (Gaucheron 2011). In addition to containing minerals, casein micelles also contain citrate (Cadwallader and Singh 2009). Holt (2004) found that the percentage of milk minerals and citrate (Cit) which form part of the micelle composition may be around 70% (Ca), 50% (P), 35% (Mg), 6% (K), and 7% (Cit). The remaining portion of these minerals and citrate is found in the aqueous phase of the milk.

The content and association of minerals is key to the stability of casein micelles (Bijl et al. 2013). Minerals like Ca, also known as water insoluble calcium (Insol Ca) when associated with casein micelles, and P contribute greatly to the coagulation process, and texture of a Cheddar cheese (Lucey and Fox 1993). Given their large association with caseins which constitute a large percentage of the total protein content in milk and cheese, changes in the casein content, also has a high potential to impact the concentration of those minerals associated with caseins (Bijl et al. 2013).

As mentioned before, casein proteins make up the majority of the protein composition and play a critical role for the production of Cheddar cheese. Related to Cheddar cheese production, casein content can impact Cheddar cheese yield and processability (Amenu and Deeth 2007). Small decreases in the casein content can have a significant economic impact on Cheddar cheese yield. It has been calculated that a 0.1% decrease in casein concentration may reduce cheese yield by 0.5 kg/100 kg milk (Guinee et al. 2001).

A decrease in protein content may also affect processability by affecting an optimal ratio of casein and fat of 0.64:072 (Amenu and Deeth 2007). A low ratio of casein content has potential to increase the coagulation time (Jõudu et al. 2008). In large manufacturing, it is common practice to adjust or standardize the protein content and fat content to the ratio mentioned above. However, standardizing for protein or fat usually has an additional cost to production and some small producers may not be able to standardize because of technical limitations. Thus, low casein content is not desired for Cheddar cheese production as it leads to a potential economic loss and challenges during Cheddar cheese manufacturing.

3.1.3 Lactose

Lactose is disaccharide made up of glucose and galactose and it is the primary carbohydrate in bovine milk (Holsinger 1997). Lactose content in milk tends to decrease as the lactation stage of the cow progresses (Fox et al. 2017b). Lactose also decreases when a cow has mastitis (udder infection) (Berglund et al. 2007). However, under normal conditions, day to day variation of lactose content is minimal compared with the variation of other milk components (protein and fat) (Forsbäck et al. 2010). Additionally, dairy practices where milk from cows at different lactations stages is commingled helps in decrease the lactose content fluctuations that may be presented on individual cows (Fox et al. 2017c).

Lactose is important for Cheddar cheese production as it is fermented to lactic acid once lactic acid bacteria (*Lactococcus* subsp. *lactis* and *Lc. lactis* subsp. *cremoris*) is added to the cheese milk (Banks 2011). Initial conversion of lactose to lactic acid decreases the pH in the milk and helps with the coagulation of casein proteins (Fox et al. 2015a). Although, most lactose is removed with the draining of the whey (~98%), the residual lactose is further fermented by the lactic acid bacteria (LAB) and contributes to a continual drop in the pH of the cheese curd (Shakeel-Ur-Rehman et al. 2004). The addition of salt to cheese curds slows further conversion of lactose to lactic acid by the LAB, however, large amounts of residual lactose may contribute to a further decrease in the pH of the cheese (Ong et al. 2017).

3.1.4 Minerals

Bovine milk contains around 20 minerals that are considered essential to human nutrition (de la Fuente and Juárez 2015). In terms of quantity, calcium, magnesium, phosphorus, potassium, sodium, and chloride make up the largest percentage of the total mineral content in bovine milk (Gaucheron 2011). Bovine milk also contains other minerals like sulfur, iron, zinc, copper, manganese, iodine, fluoride, selenium, cobalt, chromium, molybdenum, nickel, arsenic, silicon, and boron, however, these minerals are found in much smaller amounts than Ca, P, K, Mg, Cl, and Na (Flynn 1992). Table 3.1.4.1 shows the average mineral composition of some of the major minerals in bovine milk.

Table 3.1.4.1. Average composition of major minerals in bovine milk.

Mineral	mg/100g
Ca	122
Mg	12
P	119
K	152
Na	58
Cl	100
S	32
Fe	0.08
Cu	0.06
Zn	0.53

Table adjusted from Park et al. (2007)

The minerals of milk are heterogeneously distributed between the micellar and aqueous phases of milk. Some major minerals like Ca, P, and Mg and some minor minerals like zinc, manganese, iron, and copper are found in both phases (aqueous and micellar) (de la Fuente and Juárez 2015), while some others like potassium, sodium and chloride are found mostly in the aqueous phase (Gaucheron 2011). Calcium in the micellar phase is also known as water insoluble calcium (Insol Ca) (Metzger et al. 2001). Water insoluble calcium throughout this work will be referred to as Insol Ca.

Total calcium in cheese is the combination of Insol Ca and water soluble calcium (Sol Ca) (Lucey and Fox 1993; Hassan et al. 2004; Choi et al. 2008; Lee et al. 2010)(Fox and McSweeney 1998). The distribution and equilibrium of minerals in the micellar and aqueous phase in milk and cheese is highly sensible to pH changes during Cheddar cheese manufacturing (Gaucheron 2013; Fox et al. 2015b). As milk is acidified, either through fermentation or through the addition of acids, minerals in the micellar phase like Insol Ca, solubilize and migrate to the aqueous phase as Sol Ca (Mekmene et al. 2010). Changes of the mineral equilibrium of milk contribute greatly to coagulation and texture aspects of cheese and other dairy products (Tsioulpas et al. 2007; Lucey and Horne 2009).

During the processing of cheese, one aspect of cheese making influenced by initial calcium content is coagulation (Malacarne et al. 2014). Green and Manning (1982) describe coagulation as the stage where casein micelles begin to assemble in a random fashion to form a gel like structure where moisture and fat are entrapped. This gelling process in Cheddar cheese making is optimized by a pH decrease from lactic acid bacteria and warm milk temperature (~30°C) and begins with the first coagulation stage, which is triggered by the enzymatic (chymosin) clipping of glycomacropeptides (f 106-169) on κ-casein at their amino acid site of phenylalanine 105-methionine 106 (Fox et al. 2015a). Glycomacropeptides which are originally on the surface of κ-casein proteins are hydrophilic and help maintain casein micelles in suspension, but once most of them (>80%) are clipped from κ-casein, the casein micelle begins to aggregate and form a gel (Fox et al. 2017d).

Fox et al. (2015a) explains that although it is not well understood, Insol Ca plays a role in the coagulation stage. Fox et. al (2015a) explains that evidence that Insol Ca plays a role on the formation of a cheese coagulum is that a reduction of Insol Ca to significant levels tends to inhibit coagulation. In general, milk that exhibit good coagulation properties has been found to have a higher content of total and Insol Ca content as well as a higher content of P and Mg (Malacarne et al. 2014). Furthermore, the mineral content, in particular calcium, is not only relevant to coagulation. Coagulation also affects other aspects of manufacturing as optimal coagulation allows for faster cutting of coagulant and influences cheese texture (Johnson et al. 2001).

Related to the relationship of calcium content and cheese texture, Insol Ca content has been identified as an important factor to consider when evaluating cheese texture (Lucey and Fox 1993; Hassan et al. 2004). As mentioned previously, an important change of the ration of Inso Ca and Sol Ca happens first during the acidification of milk. The Insol Ca concentration in cheese and its correlation to cheese texture is directly influenced by the initial calcium content in the cheese milk and the rate and degree of acidification (Johnson and Lucey 2006; Lee and others 2010). The rate and degree of acidification during Cheddar cheese manufacturing have a strong influence on the equilibrium shifts of Insol Ca and Sol Ca, which in turn impact cheese texture by weakening the protein network of a cheese (Lucey and Fox 1993; Lee et al. 2005).

Although, there is strong evidence that Insol calcium plays a large role on the texture attribute of a cheese, total calcium content in general is also an indication of higher firmness in a cheese. Chevanan et al. (2008) was able to demonstrate that high levels of total calcium content of Cheddar cheeses (0.64-0.69 g/100g of cheese weight) had a significantly harder texture than lower total Ca content Cheddar cheeses (0.51-0.55 g/100g cheese weight). However, even though Chevanan et al. (2008) did not calculate Insol Ca, it is possible that Insol Ca was also higher in cheeses with higher total Ca content as Insol Ca content is correlated with total Ca content (Bijl et al. 2013).

An additional factor that also influences the solubilization of Insol Ca is ripening. Initially, most of the Ca that remains in the cheese is mostly in the form of Insol Ca associated with the protein matrix of the cheese, however, Insol Ca continues to solubilize as the ripening period of the cheese is extended (Hassan et al. 2004). Some work has shown that the most drastic change in calcium solubilization occurs between week 1 and week 4 of manufacture and it is heavily influenced by pH during production (Hassan et al. 2004). The solubilization of calcium during the ripening period also concurs with a softening of Cheddar cheese texture and there have been several reports that show a high correlation between solubilization of Insol calcium and softening of cheeses (Hassan et al. 2004; Lucey et al. 2005; O'Mahony et al. 2005). However, solubilization of Insol Ca may not be the only factor to affect cheese texture during ripening. Proteolysis also weakens the protein network of the cheese contributes to changes in cheese texture (Chevanan and Muthukumarappan 2008).

3.2 Factors that influence milk composition

Several factors influence milk composition. Milk components are most influenced by diet type and quality, breed of cow, genetic variations, and seasonal variations (Amenu and Deeth 2007). Diet type like pasture and TMR diets have a great impact on fatty acid composition and in some cases fat content as well (Kolver and Muller 1998; Couvreur et al. 2006; Moate et al. 2007)**.** Studies have shown pasture diets promote a higher production of mono- and poly-unsaturated fatty acids (MUFA and PUFA) as well as a higher concentration of conjugated linoleic (CLA) acid isomers (Couvreur et al. 2006; O'Callaghan et al. 2017; Gulati et al. 2018a). Couvreur et al. (2006) conducted a study with four groups of cows that were fed 0%, 30%, 60%, and 100% of grass. Couvreur et al. (2006) were able to show a direct increase of MUFA, PUFA, and CLA, in particular (*ci*s-9, *trans*-11), with an increase on the grass consumption of the cow.

There is evidence that suggests that even differences in the types of grass the cows consume can also affect the fatty acid composition (Bugaud et al. 2001a). Bugaud et al. (2001a) found that milk from cows on a mountain pasture diet and a valley pasture diet had a different fatty acid composition. However, even with some variations in the fatty acid composition from different types of pasture-diets, the general trend in fatty acid composition in milk from pasture diets, is a higher production MUFA, PUFA, and CLA (Bugaud et al. 2001a; Gulati et al. 2018b). The fatty acid composition in milk from a TMR diet, in contrast to milk from a pasture diet, tends to have a higher saturated fatty acid (SFA) composition, in particular palmitic acid (C16:0), and lower CLA content (O'Callaghan et al. 2017).

Another important factor that can also have an adverse effect on milk components is poor diet quality. This is most relevant for pasture fed cows as typical changes on the nutritional properties of the pasture or pasture availability may cause deficiencies in the nutrition of the cow which can translate to a decline in some milk components like fat and protein (Walker et al. 2004a; Knaus 2016). Typical compositional changes in milk from cows on a low-quality diet include a decrease in protein, mineral, and fat content (Christian et al. 1999; Walker et al. 2004a).

Milk from different breeds of cow can also produce compositional variations in milk. These compositional differences are most common in the fat content and to some extent on the protein content in the milk. Table 3.2.1 shows the average fat and protein content of different breeds of cows. An additional factor that influences milk composition is that even within cows of the same breed, there are potential genetic differences which may influence the composition of casein proteins (Williams 2002). These genetic differences are also likely to impact the manufacturing of Cheddar cheese, and it has been suggested that milk with higher concentrations of the B κ-casein variant may help increase Cheddar cheese yield (Amenu and Deeth 2007).

Another factor that has also been found contribute to seasonal variations of milk composition is the lactation period of cows. These changes are primarily due to physiological changes in the cow during the lactation period. As the lactation period of the cow progresses, dairy cows undergo physiological changes that influence fat, protein, and mineral content (Poulsen et al. 2015; Lin et al. 2017). These changes are most prominent in the fat content (Kay et al. 2005; Mesilati-Stahy and Argov-Argaman 2014) but to a smaller extent individual fatty acid composition and protein and mineral components can also be affected (Ostersen et al. 1997; Heck et al. 2009; Chassaing et al. 2016).

Table 3.2.1. Average fat and protein content of five different breeds of dairy cows.

Breed	Fat (g/100g of milk)	Protein (g/100g of milk)
Holstein	3.8	3.2
Ayrshire	4.0	3.3
Guemsey	4.8	3.7
Jersey	5.2	3.9
Brown Swiss	4.0	3.6

Adjusted from Amenu and Deeth (2007)

3.3 Total mixed ration and pasture feeding systems

Total mixed ration (TMR) and grass (pasture) based feeding systems are two of the most common feeding systems in the United States (Schingoethe 2017). A TMR diets is the most common feeding system in the United States and is particularly implemented with larger dairy farms (USDA 2014). A TMR diet may vary depending on feed availability but is primarily composed of a combination of silage (fermented grass or other plant matter), various types of grains, and some vitamins and minerals (Gulati et al. 2018b).

In contrast to TMR feeding systems, a pasture feeding system is more common for smaller scale dairy farming and is limited by seasonal availability of fresh grass (Martin et al. 2005). Some pasture feeding systems may include perennial ryegrass, meadow grass, orchard grass, smooth brome, Kentucky bluegrass, and other types of plants, like clover and weeds (Bargo et al. 2002; Gulati et al. 2018b). Different types of grass may be used for pasture systems and variation on the pasture type is mainly influenced by region (Washburn and Mullen 2014). Pasture feeding systems usually include an added supplementation composed of hay, grain, vitamins and minerals and may be increased or decreased depending on pasture availability (Gulati et al. 2018b)

Two main advantages of feeding a TMR diet to dairy cows is the ability to have better control on the quality of the feed throughout the lactation period of the cow and a higher milk yield (Schingoethe 2017). Pasture feeding systems also provide some advantages as this system has been found to lower methane (greenhouse gas) emission (O'Neill et al. 2011) and potentially lower the cost of feed for farmers (McCall and Clark 1999). Additionally, a pasture feeding system also provides the opportunity to modify the fatty acid composition by increasing MUFA, PUFA, and CLA content in milk and dairy products like Cheddar cheese (Couvreur et al. 2006; O'Callaghan et al. 2017).

3.4 Quality of Cheddar cheese

The overall quality rating of Cheddar cheese is given from the overall acceptability of cheese's texture, flavor, appearance and color (Muir 2010). The grading of a Cheddar cheese is mostly performed by a trained panel that is keen to identify specific attributes or defects related to flavor, texture, and color (Muir 2010). However, consumer testing with an untrained panel can also provide valuable information with regard to the overall quality of a Cheddar cheese (Caspia et al. 2006). The texture and flavor attributes of Cheddar cheese change through the ripening period of a Cheddar cheese, so ripening time should always be considered when comparing different Cheddar cheeses. Cheddar cheese is often labeled as mild, medium, or sharp, however, these definitions are subjective to some degree because they are not based on set legal requirements, ripening age, texture, or flavor profile (Drake et al. 2001).

Mild and medium Cheddar cheese texture are considered to have a firm texture that shows some resistance to deformation when force is applied (Fox et al. 2017a). In contrast to mild and medium Cheddar cheese, mature Cheddar cheese texture tends to have a higher propensity to fracture with the application of force (Fox et al. 2017a). In terms of flavor, some researchers have identified 27 descriptive terms for Cheddar cheese, and they may vary depending on the ripening age of the cheese (Drake et al. 2001).

Since there is not an exact definition for mild, medium, or mature Cheddar some researchers have investigated and identified some flavors that might help to defining these terms. Drake et al. (2008b) conducted a study to identify flavors that consumers associate with mild Cheddar cheese. They identified flavors associated with "milky/cooked, whey, brothy, and sour taste" as some of the most liked flavor/taste attributes for mild Cheddar cheeses (Drake et al. 2008b). Mature Cheddar cheese flavor is more associated with flavors and aroma described as "bitter, sour, sulfur, and brothy" (Caspia et al. 2006; Drake et al. 2008a).

The USDA classifies Cheddar cheese quality under four different grades. The grades in decreasing quality may be classified as AA, A, B, or C. A general description of the USDA's -

Table 3.4.1. Description of grade standards for Cheddar cheese as detailed by the United States Department of Agriculture.

U.S. Grade	Flavor	Body and Texture
AA	Fine and highly pleasing. May possess slight characteristic Cheddar cheese flavor. May possess a very slight feed flavor.	A plug drawn from the cheese shall be firm, appear smooth, compact, close, and should be slightly translucent, although it may have a few small mechanical openings. May possess limited sweet holes in accordance to the degree of curing but free from other holes.
A	Pleasing and free from undesirable flavors and odors. May possess feed, acid and bitter flavors within limited tolerances as the cheese ages.	A plug drawn from the cheese shall appear reasonable solid, compact, close and should be translucent although it may have a few mechanical openings but may not be large and connecting. May not have more than two sweet holes on a plug but free from other gas holes.
B	May possess certain undesirable flavors to a limited degree in accordance with the aging of the cheese.	A plug drawn from the cheese may be loose and open and may have numerous sweet holes, scattered yeast holes and other scattered gas holes; and may possess various body characteristics in accordance with degree of curing but pinny gas holes are not permitted.
C	May possess somewhat objectionable flavors and odors with certain increase tolerances in accordance with the degree of curing.	May be loose with large and connecting mechanical openings; possess various gas holes and body characteristics, with certain limitations and varying with the degree of curing. The cheese, however, shall be sufficiently compact to permit the drawing of a plug.

Adapted from USDA (2019)

standards for flavor, body, and texture of Cheddar cheese are provided above (Table 3.4.1) (USDA 2019). A specific description of each grade helps to maintain consistency of the quality of Cheddar to some degree cheese. However, in spite of efforts to standardize quality definitions of Cheddar cheese, differences may be found from product to product (Drake et al. 2008a).

3.5 Compositional difference in the milk of pasture and total mixed ration diets and their potential effect on the quality of Cheddar cheese

3.5.1 Lipid composition and its effect on Cheddar cheese quality

One of the main differences between milk from pasture and TMR fed cows is the fatty acid composition of the milk. The main influence these two diets have, on the fatty acid composition of the milk, is on the levels of MUFA, PUFA, and CLA (Jiang et al. 1996; Jahreis et al. 1997; White et al. 2001). Cows on pasture diets are able to produce milk with higher concentrations these fatty acids (Bugaud et al. 2001a; Walker et al. 2004b; Slots et al. 2009; Coppa et al. 2011b). Kelly et al. (1998) found that once the cows were 100% on a pasture diet, the CLA in the milk from the pasture fed cows doubled that of the milk from the cows on the TMR diet. Increasing MUFA, PUFA, and CLA and decreasing SFA in Cheddar cheese may be important for nutrition purposes as there is a potential health benefit from higher MUFA, PUFA, and CLA consumption (Livingstone et al. 2012).

Additionally, fatty acid composition may also be relevant for Cheddar cheese quality attributes like texture. Couvreur et al. (2006) found that higher unsaturated fatty acid content correlated with lower firmness of butter. Couvreur et al. (2006) suggested that the inherent lower melting property of unsaturated fatty acids and a smaller fat globule size in milk with higher unsaturated fatty acids likely contributed to the lower firmness of butter. Additional evidence to the potential influence of fatty acid composition on texture of dairy products was provided by

Bugaud et al. (2001b) who found that Abondance cheese that had a higher unsaturated fatty acid content also had a lower fracture strain.

However, there is some disagreement about the influence of fatty acid composition and texture, in particular as it relates to Cheddar cheese. In contrast to Couvreur et al. (2006) and Bugaud et al. (2001b) findings with the correlation of unsaturated fatty acids and softer texture of butter and Abondance cheese, O'Callaghan et al. (2017) found that a softer texture of Cheddar cheese higher in CLA and unsaturated fatty acids was only observed when the Cheddar cheese was analyzed at room temperature.

In regard to fat globule size and fatty acid composition, several studies have found that milk with a higher amount of CLA, MUFA, and PUFA has smaller fat globules (Wiking et al. 2004; Couvreur et al. 2006). Size of fat globules also has a potential to influence the texture of Cheddar cheese. Logan et al. (2017) was able to show that in Cheddar cheese manufacturing, fat globule size was correlated to the texture attributes of their Cheddar cheese treatments. Given the different melting properties of fatty acids and the different fat globule size found in milk with higher CLA, MUFA and PUFA fatty acids, it would be useful to consider the potential effect of fatty acid composition and fat globule size to better understand the texture attributes of the Cheddar cheese treatments in this study.

3.5.2 Protein and mineral composition

It has been previously mentioned that both pasture and TMR diets have the potential to influence protein and mineral content of milk. Regarding the potential effect of diet on protein and mineral composition, Gulati et al. (2018a) analyzed milk from a TMR and two different pasture diets for the manufacture of Mozzarella cheese and found that milk from cows on the two pasture diets contained a higher total protein and casein content than the milk from the TMR fed cows. The Ca, P, and Mg differences that Gulati et al. (2018a) found were directly correlated to the amount of protein content in the milk. Gulati et al. (2018a) did not find diet to have a significant effect on cheese quality.

However, in contrast to the higher protein content that Gulati et al. (2018a) found in milk from pasture fed cows, there is also evidence that a TMR diet may also produce a milk with a higher protein content than pasture fed cows. Kolver and Muller (1998) investigated the effect of a pasture and TMR diet on milk composition. Kolver and Muller (1998) found that milk from the TMR diet had a higher concentration of protein than the milk from pasture fed cows. Kolver and Muller (1998) accredited the lower protein content in the milk of pasture fed cows to a lower dry matter content of the pasture feed. The low dry matter intake of the pasture fed cows was in spite of the cows having a good quality pasture. Similar results of lower dry matter intake and lower protein content in pasture fed cows have also been observed by others (Bargo et al. 2002).

Although, Kolver and Muller (1998) did not test the potential effect diet had on cheese texture quality, it is possible that differences in the protein content of the milk could affect the texture properties of cheese since milk proteins (primarily caseins) play an important role in the formation of a cheese's structure and firmness attribute (Ong et al. 2017). Furthermore, milk proteins, in particular caseins which consist ~80% of the total protein content (Guinee et al. 2006), and some minerals like calcium, magnesium, potassium, phosphates, and sodium are closely interrelated and changes in protein composition could potentially also affect the mineral components, which was observed by Gulati et al. (2018a).

3.6 Sensory evaluation of Cheddar cheeses from TMR and pasture base diets

Although the nutritional improvement of foods has the potential to provide a great benefit for consumers, the benefit of a "better for you" food may only be in proportion to the consumer's enjoyment of all the organoleptic properties of the product. Common methods used to evaluate dairy products usually make use of trained or untrained panels. Trained panels are effective to identify or rate the intensity of specific organoleptic attributes whereas untrained ones provide an opinion that may be representative of the larger population (Drake and Delahunty 2017).

Most research about the consumer acceptability of Cheddar cheeses high in CLA that are derived from pasture fed cows and Cheddar cheeses derived from TMR fed cows seem to indicate a similarity in consumer acceptability (Khanal et al. 2005; O'Callaghan et al. 2017). Khanal et al. (2005) evaluated the consumer acceptability of Cheddar cheese from pasture fed cows that had higher CLA (3x) and unsaturated fatty acids than TMR cheese. Khanal et al. (2005) found that Cheddar cheeses from the TMR and pasture diets were not significantly different in color, flavor, texture, and overall acceptability. Similarly, O'Callaghan et al. (2017), who evaluated the acceptability of one Cheddar cheese from a TMR diet and two Cheddar cheeses from two different types of pasture diets, found that Cheddar cheeses from TMR and the pasture that was a composition of perennial ryegrass and white clover had similar scores for overall acceptability.

It is important to note that O'Callaghan et al. (2017) found that the Cheddar cheese from the other pasture diet, which consisted only of perennial ryegrass, had lower scores for appearance and texture liking as well as lower acceptability than the Cheddar cheeses from the TMR and other pasture diet (ryegrass/white clover). Although, O'Callaghan et al. (2017) did not elaborate on the reason why the Cheddar cheese from cows under the perennial ryegrass diet produced lower scores for liking of appearance, texture, and overall acceptability, it is possible that the different pasture composition in the cow's diet may have influenced the sensory and quality attributes of these cheeses.

Nevertheless, related to the influence of diet on potential variations of Cheddar cheese sensory attributes, differences in cheese composition do not necessarily translate to lower liking and acceptability of cheese. Work by Jones et al. (2005) about the sensory evaluation of Caerphilly cheese high in CLA and unsaturated fatty acids and conventional Cheddar from a TMR diet, showed that although cheeses with the higher CLA and unsaturated fatty acid content had a significantly softer texture than the cheeses from the TMR diet, the overall acceptability and flavor rating was similar to the scores the TMR cheeses received.

3.7 Dairy fat and human health

Dairy products have been a very important part of the American diet (Weinberg et al. 2004; Fulgoni et al. 2007). Their richness in macronutrients (proteins, lipids) and micronutrients (calcium, potassium magnesium and even some vitamins) help consumers meet their daily nutritional needs (Nicklas et al. 2009). One dairy product of increasing commercial and nutritional importance in the United States (U.S.) is Cheddar cheese. Its sales have almost doubled since the 1980's (Johnson and Lucey 2006) and its market share is one of the highest of total cheese sales in the U.S. (Agarwal et al. 2011).

As consumption of Cheddar cheese continues to rise, interest in its nutritional impact on consumers has also increased because of its high content of saturated lipids (Putnam 1999; USDA 2015). Dietary Guidelines for Americans set by the USDA (2015) recommend limiting saturated fat consumption to less than 10% of the total daily calories. One suggestion from the

USDA (2015) dietary guide is to decrease saturated fat consumption is to substitute products rich in saturated fat with products rich in unsaturated and polyunsaturated fat. Cheddar cheese high in CLA, from a pasture diet, may help consumers lower the saturated fat consumption since the fatty acid profile of milk from pasture fed cows has been found to have less saturated fat and more unsaturated fatty acids (Bugaud et al. 2001a, b; Walker et al. 2004a; Slots et al. 2009; Coppa et al. 2011b). The higher unsaturated fatty acid content of a Cheddar may be of special interest to consumers of cheese as the USDA's (2015) guidelines have also specifically recommended to lower cheese consumption because of its high saturated fat content.

Consuming a Cheddar cheese with a lower saturated fat content might allow consumers to eat more Cheddar cheese and still meet the dietary guidelines recommended by the USDA. Furthermore, although extended research has not been conducted on human populations about the potential health benefits of consuming a Cheddar cheese high in CLAs, some work seems to indicate that increasing consumption of Cheddar cheese high in CLA does not affect negatively cardiovascular disease risk (CVD) risk factors, like low-density lipoprotein (LDL) cholesterol or plasma triacyl-glycerides (Huang et al. 1994; Ritzenthaler et al. 2005). The low risk of increasing CVD factors from consuming a Cheddar cheese high in CLA combined with the potential to lower the saturated fat intake of Cheddar cheese while also providing potential anticarcinogenic health benefits, are three potential advantages that Cheddar cheese and dairy products high in CLA and lower in SFA may be able to provide to consumers.

3.8 Conjugated linoleic acid in dairy products

Conjugated linoleic acid is a general term used for all different isomers of linoleic acid (Kay et al. 2004). Conjugated linoleic acid (CLA) *cis*-9, *trans*-11(c9, t11) is the major CLA isomer found in dairy products as it accounts for more than 80% of the total CLA isomers in milk (Chin et al. 1992; Jensen 2002). CLA is produced as a biohydrogenation intermediate of linoleic and α-linolenic fatty acids and also by enzymatic conversion of vaccenic acid to CLA (Kay et al. 2004; Prandini et al. 2011). The CLA discussed in this work, unless specified, refers to *cis*9, *trans*11 (c9, t11) CLA isomer.

Early in its identification, CLA was found to show anticarcinogenic properties in animal studies (Pariza and Hargraves 1985; Ha et al. 1987). Since then, other research in animal studies have confirmed that CLA has anticarcinogenic properties against different types of cancers (Ip et al. 2002; Voorrips et al. 2002; Chen 2003; Kelley et al. 2007). Dairy products are considered to be the highest dietary source of CLA and increasing their concentration in dairy products like Cheddar cheese could be of interest for consumers (Huang et al. 1994; Lawson et al. 2001; Kelsey et al. 2003).

Based on animal studies, the recommended amount of CLA to provide a health benefit to consumers is ~600mg/day (Ens et al. 2001). In a study conducted by Ens et al. (2001) about the CLA consumption in a small Canadian population, they found that the average CLA consumption was ~95mg/day. Although, daily consumption of CLA in the U.S. may vary from that of the Canadian population, the findings by Ens et al. (2001) suggest that a regular diet may not be able to provide the recommended CLA amount calculated to provide potential health benefits. Based on the limitation regular diets have in providing 600mg/day of CLA, increasing CLA content of dairy products could help consumers meet the recommended 600mg/day of CLA. Cheddar cheese high in CLA, being a concentrated form of a dairy product, could provide a particular advantage to consumers as it can provide a higher concentration of CLA than other dairy products like milk or yogurt.

3.9 Approaches to making Cheddar cheese high in conjugated linoleic acid

Nutritional improvement of dairy products is an ongoing area of research. Increases of CLA in Cheddar cheese have been done primarily by increasing the CLA content of cheese-milk (Khanal et al. 2005; Allred et al. 2006; Mohan et al. 2013; O'Callaghan et al. 2017) and also by the use selected lactic acid bacteria (Pandit 2009; Mohan et al. 2013). Increase of CLA in cheese milk, in addition to pasture feeding, can also be done by supplementing a cow's diet with PUFA rich foods (Khanal et al. 2005; Allred et al. 2006). However, although some of the approaches of supplementing the cow's diet with PUFA rich foods has successfully increased CLA without adverse effect on other milk components or milk yield, there is also some evidence that PUFA rich diets of cows may decrease fat and protein content in the milk (Baer et al. 1996; Hostens et al. 2011) .

The use of selected *Lactococcus lactis*, which is a lactic acid producing bacteria (LAB) has also been reported to increase CLA content through fermentation (Kim and Liu 2002; Pandit 2009). The mechanisms of this are not clearly understood, but Pandit (2009) proposed that specific enzymes released from the cell walls of the LAB may be responsible. One thing to consider about the use of LAB to increase CLA content is that, there is an indication that CLA isomer production by LAB may be different than the one produced through diet modifications of cows. Through the selection of high CLA *Lactococcus lactis* bacterium, Pandit (2009) was able to show an increase of CLA production through LAB fermentation, however, about 60% of the CLA produced by the *Lactococcus lactis* bacterium in Pandit's work was reported to be the CLA isomer *cis*-9, *trans*-11, and 21% was the CLA isomer *trans*-10, *cis*-12.

The 60% of c9, t11 CLA that Pandit (2009) was able to produce in her work was lower than the ≈90% typically found in dairy products from pasture fed cows (Chin et al. 1992; O'Shea et al. 1998; Prandini et al. 2007). The difference in ratios of CLA isomers produced from bovine feeding and LAB fermentation may be of significance as *cis*-9, *trans*-11 CLA and other CLA isomers are reported to potentially have different health effects (Shokryazdan et al. 2015).

3.10 Significance

Higher concentrations of CLA, UFA, and PUFA in milk can provide an opportunity to make Cheddar cheese with a lower saturated fatty acid profile. However, production of milk from pasture fed cows is seasonal and this may limit production of a Cheddar cheese high in CLA since CLA content is directly correlated to the amount of grass a cow eats in its diet, and grass availability changes throughout the year (Washburn and Mullen 2014). Taking advantage of common industry practices of the separation of the cream portion and skimmed portions of milk, pasture-cream and TMR-skimmed milk could be combined to make a hybrid Cheddar cheese high in CLA. This approach of combining pasture-cream and TMR-skimmed milk provides an option to expand production of a Cheddar cheese high in CLA and unsaturated fat.

This method of making a hybrid Cheddar cheese with cream from pasture fed cows and TMR skim milk may also provide an additional advantage. The enrichment of CLA and unsaturated fatty acids with cream and not with fatty acids from other sources (plant) meets the requirement of standard of identity of Cheddar cheese and does not require special labeling which may be of significance for some consumers (CFR 2017).The investigation of potential compositional changes in the hybrid Cheddar cheese will help assess effects on quality of the hybrid Cheddar cheese from the combination of cream and skim milk from cows under two different diets.

4.0 MATERIALS & METHODS

4.1 Separation and standardization of cheese-milk

Milk from TMR fed cows was obtained from the Michigan Milk Producers Association (Ovid, MI, USA) and milk from pasture fed cows was provided by MSU W.K. Kellogg Biological Station. The diet of pasture fed cows consisted of 80% grass. The remaining 20% of the diet of the pasture fed cows was a combination of high protein pellets and fermented silage. Cheese-milk from both sources was pasteurized prior to separation and standardization. After the cheese milk was pasteurized it was separated into the skim milk and cream portions. To achieve and effective separation of cream and skimmed milk, the milk from pasture and TMR fed cows was warmed to about 38 ºC. The warmed milk was then poured into a mechanical cream separator at 2,500 rpm. Once the skimmed milk and cream were separated, measurements of the fat content were carried out on the skimmed and the cream portions by the Babcock procedure (Nielsen 2010) to confirm that effective separation of cream from the skim milk portion had been achieved.

After obtaining the fat content of the skimmed milk and cream, the required amount of cream and skimmed milk to standardize the cheese-milk was calculated algebraically. Three different types of cheese-milk were standardized for the manufacture of three different Cheddar cheese treatments. The three types of cheese-milk were the following; 1) milk from pasture fed cows, 2) milk from TMR fed cows, and 3) a hybrid cheese-milk which was the combination of cream from pasture fed cows and skim milk from TMR fed cows. These three different types of cheese-milk will be referred as pasture milk, TMR milk, and hybrid milk, respectively. All of the cheese-milk was standardized to a fat content of 3.5%. The fat content of the standardized cheese-milk was also measured to confirm that the desired fat content had been achieved.

4.2 Manufacture of Cheddar cheese

Three Cheddar cheese treatments were manufactured for this study utilizing the standardized cheese-milk (pasture, TMR, and hybrid). The Cheddar cheeses manufactured from the pasture, TMR, and hybrid milk will be referred as pasture, TMR, and hybrid cheese, respectively. All Cheddar cheeses were manufactured following the procedure used by the MSU Dairy Plant. About 189 L of each type of milk (pasture, TMR, hybrid) was used to make each batch of cheese.

The starter culture used to make all Cheddar cheese was a combination of *Lactotoccus lactis subsp. Lactis* and *Lactococcus lactis subsp. Cremoris* (DVS 980, CHR Hansen, Hoersholm, Denmark) which was used at 1% wt/wt. Culture was added once the cheese-milk reached 32 ºC and then incubated for 45 min at which time 6 mL of annatto were added. Following the addition of annatto, 13 mL of rennet (Chy-Max, Chr. Hansen) diluted in 508 mL DI water were added. The cheese-milk for each cheese was then allowed to coagulate for 30 min at 32 ºC. Once the cheese-milk had coagulated (≈ 30 min), the curd was cut with wire knives. The cut curd was allowed to heal for 30 minutes before proceeding with the cooking of the curd. The curd was cooked for 1 h at 38 ºC. The whey was drained from the vat following the cooking of the curd. The Cheddaring process was followed by the draining of the whey. For this step the curd was matted and cut in about 15 cm x 50 cm slabs which were rotated on their side every 15 min to maintain their temperature at 35 ºC. The TA was monitored at the beginning of the Cheddaring process and it was stopped when the TA had reached 0.62%. After reaching the desired TA the Cheddar slabs were milled by hand.

Salting was carried out in three stages. The total of salt (table salt) added was 450 g. After the last addition of salt, the curd was placed in 9 kg hoops and pressed overnight for 12 h at

276 kPa. The pressed cheese was then removed from the hoops and placed in bags which were vacuum sealed and stored at 8 ºC for 14 weeks.

4.3 Compositional analysis of Cheddar cheese

4.3.1 Proximate analysis for fat, protein, moisture, fat, and ash content

The fat content analysis was performed with the Babcock procedure (Nielsen 2010). First, 9 g of cheese were placed in Babcock bottles and then 10 mL of 60 ºC deionized (DI) water was added. This was followed by digestion with of sulfuric acid (17.6 mL). To promote the complete separation of the fat from the rest of the milk components, the bottle with skim milk and sulfuric acid were shaken for 5 minutes and then placed in a centrifuge for an additional 5 minutes.

To complete the separation of fat, the bottles were filled with warm (60 °C) DI water to the base of the bottle's neck and then centrifuged for a second time for 2 minutes. After the second centrifuged cycle, the bottles were filled with warm (60 °C) DI water right below the last reading number of the bottle's neck and then centrifuged for a third time for 1 minute. The bottles were then placed in a water bath for 60 °C for 5 minutes to allow for the fat to completely settle. At the end of the 5 minutes measurements of the fat content were performed.

Total protein content for each treatment was determined by Eurofins DQCI laboratories (Horsham, PA, USA) in duplicates using the Kjeldahl method. For the moisture analysis, 2 g of sample were placed in pre-dried aluminum pans and pre-dried at atmospheric pressure for 30 min. Condensation that had accumulated on the door of the oven was removed. After the pre-drying step, the vacuum was applied at 25 Hg for 4 h. At the end of the 4 h, the samples were removed and weighed to calculate the moisture content. For ash analysis, 5 g of sample were weighed in acid (3 HCl: 1 HNO_3) treated crucibles. The samples were ashed in a muffle furnace at 525 ºC for 12 h. The samples were then removed and weighed to calculate the ash content.

4.3.2 Fatty acid analysis

The fatty acid analysis of the cheese and the milk was performed by the Michigan State University Dairy Lipid Nutrition Program laboratory. The total lipid content of cheese and milk was extracted with an adapted protocol of Hara and Radin (1978) using *n*-hyxane/isopropanol (3:2 vol/vol). To prepare the fatty acid methyl esters (FAME), lipids were mixed and agitated with a mixture of 2.5 mL of *n*-hexane, 2.5 mg of lipids and 0.5 mL of 0.5 *M* sodium methoxide solution in methanol for 5 min. Next, sodium bisulfate (1 g) was added. The vial was then subjected to 5 min of vortex agitation. After vortexing, the samples were centrifuged at 6,000 x g for 5 min. Following centrifugation, the supernatant containing the FAME was extracted and placed in a 2-mL GLC vial for analysis. Analysis of FAME was performed by a gas chromatograph (GC-2010 Plus; Shimadzu, Koto, Japan) equipped with a split injector (1:100 split ratio), flame ionization detector and a 100-m fused-silica column (CP-Sil 88 WCOT; 0.25 mm i.d. x 0.2-µm film thickness; Varian Inc., Lake Forest, CA). The GC conditions were those described by Lock et al. (2013). The injection volume of samples was 1 µL and the carrier gas was hydrogen and with a flow rate of 1 mL/min.

The hydrogen flow rate for the flame-ionization detector (FID) was 40 mL/min and the rest of the FID gases were purified air at 400 mL/min and nitrogen makeup gas at 30 mL/min. The temperature for the injector and detector was kept at 250 °C and the oven program for the oven was the following: 4 min holding time at 40 °C, programmed at 13 °C/min, with a 27 min hold at 175 °C, following with an increase in temperature of 4 °C/min and a 35 min hold at 215

°C. The software (GC solution software version 2.32.00; Shimadzu) used for integration and quantification of fatty acids was based on FID response. The identification of fatty acids including CLA (c9, t11) was by comparison of retention times with FAME standards (GLC-reference standard 463, GLC reference standard 481-B, and conjugated octadecadienoic mixture #UC-59-M from Nu-Check Prep Inc., Elysian, MN; Supelco 37 component FAME mix, *cis/trans* FAME mix, and PUFA No. 3 mix from Supelco Inc., Belleforte, PA).

4.3.3 Fat globule analysis by confocal laser scanning microscopy

The microstructure analysis of Cheddar cheese was performed with an Olympus FluoView FV1000 (Olympus America, Inc, Center Valley, PA) confocal laser scanning microscope configured on a fully automated inverted IX81 microscope. Imaging experiments were performed with a 20x objective (NA 0.5). Cheese samples for microscopic measured 10x10x2 mm and were cut at 4 °C. Samples were stained for fat as described by Auty et al. (2001). Fluorescent dye solution was prepared by combining Nile Red (Sigma-Aldrich) in 10 mL of polyethylene glycol (mol. Wt. 200 Da; Sigma-Aldrich). The final concentration of Nile Red was 0.02 g/L. Samples were stained for 10 min before analysis. The excitation wavelength applied was 488 nm for Nile Red dye (fluorescence for fat).

4.3.4 Image analysis of confocal laser scanning micrographs of fat globules

Measurement of fat globule (FG) diameter was performed as described by Everett (1995). The area and circularity of fat globules was first measure with ImageJ software (version:2.0.0-rc-69/1.52i). The area and circularity measurements were done on 2D CLSM images. Analysis of images was automated with a Macros setting with code to measure area and circularity. The range of particles that were selected for measurements was from 5 μm^2 to infinity. The diameter was calculated from the area measurements (D= $sqrt(\frac{A}{\pi}) * 2$) and the sphericity was calculated from fat globule circularity (*Sphericity*= *Circularity*^{2}). Three replicates of each treatment were used for image analysis.

4.3.5 Calcium and magnesium

For total calcium and magnesium analysis the cheese samples were dissolved to extract the mineral components using a Microwave Assisted Acid Digestion procedure. The cheese samples (1 g) were first pre-digested in 8 mL of nitric acid for 2 h in the microwave's pressure tubes. Samples then were digested in a Multiwave 3000 Modular microwave system (Anton Paar, Graz, Austria) with a microwave setting of 600 W, 160 °C, 13 bar, 30 min ramp time, and 10 min holding time. At the end of the digestion 2 mL of hydrogen peroxide (General Industrial Chemicals, East Hanover, NJ) were added. The samples were then transferred to a volumetric flask and brought to 25 mL volume. Further dilutions were required to get mineral concentrations within the standard curves. Dilutions for calcium and magnesium analysis included lanthanum at a concentration of 1000 μg/mL.

The concentration of the standards for calcium and magnesium were 0, 0.25, 0.75, 1.25, 1.5, and 2 μg/mL. The calcium and magnesium stock solutions were prepared from a 1000 μg/mL concentrate solution. Calcium carbonate (Sigma-Aldrich) and magnesium metal strips (Sigma-Aldrich) were used to make the stock solutions. Lanthanum (1000 μg/mL) (Sigma-Adrich) was also added to the calcium and magnesium standards. In addition to the standards, reference material (0.25 g bovine liver; Standard Reference Material 1577b) was used to validate the accuracy of the atomic absorption spectrophotometer (AAS).

The digests were analyzed for calcium and magnesium by atomic absorption spectrophotometry (Varian SpectrAA 55B). For calcium analysis, the working conditions of the AAS were set at 422.7 nm wavelength and a 0.5 nm slit. The working conditions for magnesium were: 285.2 nm wavelength and 0.5 nm slit.

4.3.6 Soluble calcium analysis and water insoluble calcium calculation

The procedure for the determination of water-soluble calcium was similar to a procedure done by Metzger et al. (2001). This procedure was carried out by blending 1.5 g of graded cheese and 15 mL of DI water at 60 °C with a Polytron homogenizer (PT10-35, Kinematica, Switzerland). The blended mixture was then filtered with a Whatman #1 filter (Whatman International Ltd. Maidstone, England). The filtered extract was then further diluted to a concentration within the standard range (0-2 μg/mL). Lanthanum (1000 μg/mL) was also added to the dilution that was analyzed with AAS. The AAS settings were the same as settings previously described for total calcium analysis. The calcium results obtained through this procedure are described as soluble calcium.

Water-insoluble calcium was calculated by subtracting soluble calcium results of one of the cheeses from the total calcium that was found in that same cheese. The percentage of water-insoluble calcium was calculated by the following equation: (Water-insoluble calcium / Total calcium) * 100.

4.4 Sensory evaluation

Cheddar cheese samples were allowed to age for 14 weeks before the consumer evaluation test. The consumer acceptance panel (n=116) was recruited through an online recruiting service (SONA) at Michigan State University (MSU, East Lansing, Michigan, USA). The panel recruited for the consumer evaluation were adult (18 + years old) male and females of the MSU population and local area (student, staff and faculty) familiar with and consumers of Cheddar cheese. The sensory evaluation was performed at MSU's Food Science Sensory Laboratory. Participants were given a brief explanation about the study and were asked to sign a consent form (APPENDIX D) to meet the requirements of the University Committee on Research Involving Human Subjects at Michigan State University (UCRIHS).

Samples presented to panel participants were of the same size (3 cm cubes) and their temperature was ~5 °C at the time they were served. Samples were placed in plastic soufflé cups labeled with a randomized 3-digit code (pasture=314, TMR=491, hybrid=176**)**. The order of the samples was randomized for every participant throughout the study. Participants were also provided with salted crackers and water to clean their palate between tasting of samples. Panelist were asked to rate overall liking, overall cheese flavor liking, overall texture liking, with value 1= dislike extremely to value 9= like extremely and the middle value 5= neither like nor dislike. Panelist also evaluated Cheddar cheese flavor and texture intensity using a 9-point hedonic scale. Additionally, panelists were also asked check all that apply (CATA) questions about each sample. The CATA questions included 14 descriptive terms related to flavor and 7 descriptive terms related to texture (APPENDIX C). Finally, consumers were also asked to rate purchase intent using a 5-point scale where value 1= definitely would not buy to 5= definitely would buy. A 1-minute wait time between sample testing was enforced.

4.5 Texture profile analysis

The texture of the cheeses was determined by Texture Profile Analysis (TPA) using a TA-XT2i texture analyzer (Texture Technologies Corp., Hamilton, MA, USA). Cheese samples were collected with a cheese core sampler with a 1 cm diameter. Once the cores were collected their length was adjusted to 1 cm. Cheese samples were allowed to reach room temperature (25 °C) before analysis. The test conditions were similar to the ones used by Fang et al. (2016). The testing parameters set were the following; compression was set at 50% with a test speed of 1 $mm.s^{-1}$ and 11 technical replicate tests were performed for each cheese treatment including control.

4.6 Statistical analysis

Biological triplicates (n=3) of Cheddar cheese were manufactured in a randomized design. All of the analyzes of biological triplicates, except for TPA, protein, fatty acid, and sensory were performed with technical triplicates. TPA analysis was performed with 11 technical replicates and protein was done with duplicates of technical samples. Fatty acid analysis was performed with a single technical sample of each biological treatments (n=3) and a representative biological sample for each type of cheese treatment was selected for sensory analysis. All statistical models used the GLIMMIX procedure of S.A.S. (version 9.4, SAS Institute, Cary, NC). Differences of least square means were used to determine significant differences at $P < 0.05$ for the main effects of treatment and week and their interaction effects (treatment × week). SIMS software (SIMS, Berkley Heights, NJ, USA) was used to collect data for the consumer evaluation of cheeses at week 14. Consumer evaluation responses were analyzed by a mixed effects analysis of variance with consumers as a random effect using the

PROC GLIMMIX S.A.S. procedure (version 9.4, SAS Institute, Cary, NC) and significant differences ($P < 0.05$) among cheese treatments was analyzed using Chi square test.

XLSTAT-sensory® 2019 (Addinsoft; New York, N.Y., U.S.A.) for check-all-that-apply (CATA) analysis was used to evaluate significance ($P < 0.05$) of frequency of check-all-that-apply consumer responses for the attributes of flavor and texture with the Cochran's Q test. Contingency table is shown graphically with a correspondence analysis plot also created with the XLSTAT-sensory® 2019 software. Attributes in the CATA questionnaire that are reported are those that were identified by more than 10% of participants in at least one of the cheeses (pasture, TMR, hybrid).

5.0 RESULTS & DISCUSSION

Three Cheddar cheeses were developed for this study (hybrid, pasture and TMR). Using these three cheeses, this study assessed the effect of hybridization across several measures. The findings were organized as follows: their composition, consumer acceptability, texture profile analysis, and fat globule size. Although, the findings in this section includes a discussion of all the three cheeses across all four measures, this study focuses on the relative performance of the hybrid cheese.

5.1 Compositional analysis of Cheddar cheese

5.1.1 Proximate analysis for fat, protein, and ash content

Cheese samples were analyzed for total protein, moisture, fat, and ash content as detailed in the Materials and Methods section 4.3. Mean results with their standard deviation are shown on Table 5.1.1.1. Statistical results indicate that fat, and moisture content were not significantly different among cheeses, however, the moisture content of the pasture cheese was slightly higher than the moisture content of the TMR and hybrid cheeses. The only significant difference ($P < 0.05$) was for the protein and ash content of the pasture cheese. The pasture cheese had a significantly ($P < 0.05$) lower protein and ash content than both the TMR and hybrid cheeses.

Due that the cheese-milk was standardized to a fat content of 3.5% prior to cheese manufacturing, it was expected for all the cheeses to have a similar fat content. Fat content has shown to influence texture (Rogers et al. 2010). Due that the texture of the cheeses in this study is evaluated, one of the goals of this study was to eliminate fat content as a potential factor to influence cheese texture.

A similar protein composition was expected for the hybrid and TMR cheeses, due to the fact that the hybrid and TMR cheeses contained the same skim milk portion, which contains most of the milk proteins, made up primarily of caseins (Bijl et al. 2013). Protein concentration was also likely to influence the higher ash concentration of the hybrid and TMR cheese.

Table 5.1.1.1. Least square means for proximate composition (fat, protein, moisture, and ash content) of pasture, TMR, and hybrid Cheddar cheeses expressed as a percentage of cheese weight.

	Pasture	TMR	Hybrid	SEM[A]
Fat (%)	33.28 [a]	33.11 [a]	33.06 [a]	0.08
Protein (%) *	22.9 [b]	24.82 [a]	25.53 [a]	0.57
Moisture (%)	34.21 [a]	32.52 [a]	29.85 [a]	1.72
Ash (%)	2.91 [a]	3.32 [b]	3.03 [b]	0.17

[a, b] Means within a row with the same letter superscript are not significantly different ($P < 0.05$); n=3.
[*]Protein, n= 2.
[A]SEM = Standard error of the mean.

Caseins, which comprise most of the protein content in cheese, exist as micellar complexes and contain a significant amount of the major milk minerals like calcium (~70%), phosphate (50%), and magnesium (30%) (Barbano and Sherbon 1984; McMahon and Oommen 2013; Bijl et al. 2013). Although, calcium and phosphate decrease during Cheddar cheese manufacturing, a higher protein content in cheese most often also correlates with a higher ash content (Poulsen et al. 2015).

Given that proteins are the primary building element of cheese structure (Lucey and Kelly 1994), differences in the protein content of the cheeses in this study may contribute to their texture quality. A general trend that is observed is that with a higher protein content the firmness of a cheese also increases (Soodam et al. 2014; Fox et al. 2017a).

Although, not statistically significant ($P < 0.05$), it is worth noting the higher moisture content of the pasture cheese. This may have also been influenced by a lower protein content. It has been reported that protein content, which was significantly lower ($P < 0.05$) for the pasture cheese, can influence the final moisture content of a cheese. Guinee et al. (2006) reported an inverse relationship of protein content and moisture content, with moisture content decreasing as they increased the protein content of their cheese-milk. They observed moisture changes even when they made small protein increases (3.3 to 3.6%) (Guinee et al. 2006). A similar trend was observed in this present study with the TMR and hybrid cheeses. These cheeses were observed to have a lower moisture content and higher total protein content than the pasture cheese.

Although, the moisture content of the cheese from the pasture diet falls within the permissible limit for the moisture content of Cheddar cheese (39% by weight) (CFR 2018), it is worth mentioning that this component also has potential to impact cheese texture. Everard et al. (2006) analyzed rheological properties of different commercial Cheddar cheese and found that a softer texture correlated with a higher moisture content. Everard et al. (2006) noted that even small moisture increases (2%) were found to correlate with a less firm cheese. Additionally, a higher moisture content may also promote a higher degree of proteolysis (Upreti and Metzger 2006) which may further decrease the firmness of a cheese (O'Mahony et al. 2005).

In continuation with the assessment of moisture content of the cheese treatments in this study, it was also considered that potential differences in the size of fat globules, due to the fatty acid composition, could have influenced moisture content. Milk from pasture fed cows, which has a different fatty acid composition than milk derived from a TMR diet, has been found to have a smaller fat globule size (Couvreur et al. 2007). Work related to the influence of fat globule size on cheese quality attributes has found correlations of higher moisture content in cheeses manufactured with milk that contains smaller fat globules due to the larger surface area of fat globule membrane that the small globules provide and also due to the membrane's ability to bind water (Goudédranche et al. 2000; Michalski et al. 2004).

However, considering that the hybrid cheese had a slightly lower moisture content despite of having the same type of fat as the pasture cheese, and likely the same fat globule size as well, it is likely that protein content was the responsible factor for slight differences in moisture content.

5.1.2 Fatty acid analysis

Milk samples collected at the time of the manufacturing of the cheese as well as Cheddar cheese samples collected during week 1 and week 14 of ripening time were analyzed for CLA content as well as other fatty acids. Table 5.1.2.1 represents the calculated least square means of the fatty acid composition of milk and cheeses at week 1 and week 14 from production.

Comparison of short chain fatty acids (SCFA) and medium chain fatty acids (MCFA) shows to be significantly higher ($P < 0.05$) for TMR milk and cheese. The combination of SCFA and MCFA constituted the majority of the saturated fatty acids (SFA) and caused TMR milk and cheese to have a significantly higher ($P < 0.05$) concentration of SFA. Similar observations about higher SCFA and MCFA in milk from a TMR diet and lower SCFA and MCFA in a pasture diet have been made by others. Kelly et al. (1998) analyzed the FA composition of cows transitioning from a TMR diet to a pasture diet and noted that once cows had fully transitioned to a pasture diet, the SCFA and MCFA content in their milk had decreased. Kelly et al. (1998) also reported that the decrease in SCFA and MCFA was compensated with an increase of long chain fatty acids (LCFA; C18:0 >), which was also observed in this study.

A higher concentration of LCFA was found in pasture and hybrid milk and cheese. This contributed to the larger proportion of monounsaturated fatty acid (MUFA) in the pasture and hybrid milk and cheese. Polyunsaturated fatty acid (PUFA) content was significantly higher for pasture milk and cheese ($P < 0.05$). The PUFA content of the hybrid milk was in between pasture and TMR milk, however, the PUFA content of the hybrid cheese showed a small increase. This small increase of PUFA in the hybrid cheese was found to be significant ($P < 0.05$), however, due to the small increase, this finding is not likely to be biologically significant.

Fatty acid analysis was performed primarily to investigate potential changes on the concentration of CLA in the hybrid Cheddar cheese from the process of combining the cream derived from the pasture diet and the skim milk derived from the TMR diet. Statistical analysis showed that the conjugated linoleic acid (CLA) concentration of the hybrid milk and cheese as well as the CLA concentration of the pasture milk and cheese were not statistically different from each other. The CLA concentration of the TMR cheese and milk was significantly ($P < 0.05$) lower than the pasture and hybrid cheese and milk. The Cheddar cheese in all of the treatments showed a similar CLA content to the cheese-milk that was used for its manufacture. The CLA content was not significantly affected by the process of hybridization nor by the ripening period.

Compared to the TMR cheese, the pasture and hybrid cheese had ~2.3-fold increase in CLA content, which was similar to what O'Callaghan et al. (2017) reported in Cheddar cheese from a pasture diet. Based on animal studies where CLA has been found to provide health benefits, Ens et al. (2001) recommend a 600 mg of CLA/day to observe similar effects in -

Table 5.1.2.1. Least square means of fatty acid triglyceride content of cheese-milk and Cheddar cheeses derived from pasture and TMR diets and a hybrid formulation composed of cream from pasture fed cows and skim milk from TMR fed cows.

Fatty Acid	LS Means, 1g/100g of Fatty Acids									SEM[A]
	Milk			Cheese week 1			Cheese week 14			
	Pasture	TMR	Hybrid	Pasture	TMR	Hybrid	Pasture	TMR	Hybrid	
Σ SCFA C4 to C14[B]	19.36 ^{b}	24.61 ^{a}	19.47 ^{b}	19.48 ^{b}	24.72 ^{a}	19.54 ^{b}	19.37 ^{b}	25.06 ^{a}	19.54 ^{b}	1.63
Σ MCFA C15 to C17[C]	31.64 ^{b}	40.69 ^{a}	31.69 ^{b}	31.76 ^{b}	40.78 ^{a}	31.76 ^{b}	31.63 ^{b}	40.46 ^{a}	31.68 ^{b}	1.20
C18:0	13.93 ^{a}	9.06 ^{b}	13.94 ^{a}	13.69 ^{a}	8.87 ^{b}	13.70 ^{a}	13.65 ^{a}	8.74 ^{b}	13.63 ^{a}	0.83
C18:1 4-10t	31.34 ^{b}	40.80 ^{a}	31.39 ^{b}	31.45 ^{b}	40.89 ^{a}	31.45 ^{b}	31.45 ^{b}	40.55 ^{a}	31.34 ^{b}	1.29
C18:1 t11	2.60 ^{a}	0.744 ^{b}	2.71 ^{a}	2.64 ^{a}	0.74 ^{b}	2.72 ^{a}	2.65 ^{a}	0.79 ^{b}	2.71 ^{a}	0.10
C18:1 t12	0.25 ^{b}	0.34 ^{a}	0.25 ^{b}	0.25 ^{b}	0.33 ^{a}	0.25 ^{b}	0.25 ^{b}	0.33 ^{a}	0.25 ^{b}	0.01
C18:1 c9	26.51 ^{a}	18.46 ^{b}	26.38 ^{a}	26.37 ^{a}	18.37 ^{b}	26.32 ^{a}	26.61 ^{a}	18.27 ^{b}	26.40 ^{a}	2.06
C18:1 11-13c	0.45 ^{a}	0.40 ^{b}	0.44 ^{a}	0.45 ^{a}	0.40 ^{b}	0.45 ^{a}	0.46 ^{a}	0.41 ^{b}	0.46 ^{a}	0.01
C18:1 c14/t16	0.28 ^{a}	0.27 ^{a}	0.27 ^{a}	0.28 ^{a}	0.27 ^{a}	0.27 ^{a}	0.27 ^{a}	0.26 ^{a}	0.27 ^{a}	0.01
C18:2 c9, c12 (n-6)	1.88 ^{b}	2.30 ^{a}	1.90 ^{b}	1.90 ^{b}	2.31 ^{a}	1.93 ^{b}	1.90 ^{b}	2.34 ^{a}	1.93 ^{b}	0.04
C18:3 c9, c12, c15 (n-3)	0.59 ^{a}	0.38 ^{b}	0.58 ^{a}	0.60 ^{a}	0.37 ^{b}	0.59 ^{a}	0.60 ^{a}	0.38 ^{b}	0.59 ^{a}	0.03
CLA c9, t11	1.01 ^{a}	0.42 ^{b}	0.96 ^{a}	0.98 ^{a}	0.41 ^{b}	1.02 ^{a}	0.99 ^{a}	0.43 ^{b}	1.03 ^{a}	0.07
C20:2 c11, c14 (n-6)	0.04 ^{a}	0.03 ^{a}	0.03 ^{a}	0.03 ^{a}	0.03 ^{a}	0.03 ^{a}	0.03 ^{a}	0.02 ^{a}	0.03 ^{a}	>0.01
C20:3 c8, c11, c14 (n-6)	0.07 ^{b}	0.11 ^{a}	0.07 ^{b}	0.06 ^{b}	0.11 ^{a}	0.07 ^{b}	0.07 ^{b}	0.11 ^{a}	0.07 ^{b}	>0.01
C20:4 c5, c8, c11, c14 (n-6)	0.10 ^{b}	0.16 ^{a}	0.07 ^{b}	0.10 ^{b}	0.16 ^{a}	0.08 ^{b}	0.10 ^{b}	0.16 ^{a}	0.09 ^{b}	0.01
C22:4 c7, c10, c13, c16 (n-6)	0.02 ^{b}	0.03 ^{a}	0.01 ^{b}	0.01 ^{b}	0.03 ^{a}	0.01 ^{b}	0.01 ^{b}	0.03 ^{a}	0.01 ^{b}	>0.01
Σ LCFA C18 to C22[D]	48.84 ^{a}	34.09 ^{b}	48.72 ^{a}	48.52 ^{a}	33.80 ^{b}	48.47 ^{a}	48.74 ^{a}	33.72 ^{b}	48.53 ^{a}	2.67
Σ SFA[E]	62.45 ^{b}	71.70 ^{a}	62.66 ^{b}	62.42 ^{b}	71.68 ^{a}	62.52 ^{b}	62.15 ^{b}	71.57 ^{a}	62.37 ^{b}	1.94
Σ MUFA[F]	33.55 ^{a}	24.60 ^{b}	33.40 ^{a}	33.50 ^{a}	24.54 ^{b}	33.38 ^{a}	33.71 ^{a}	24.53 ^{b}	33.50 ^{a}	2.03
Σ PUFA[G]	3.72 ^{a}	3.36 ^{b}	3.66 ^{ab}	3.72 ^{a}	3.37 ^{b}	3.72 ^{a}	3.76 ^{a}	3.41 ^{b}	3.73 ^{a}	0.11

$^{a, b, c}$ Means within a row with the same letter superscript are not significantly different ($P < 0.05$); n= 3.
[A] SEM = Standard error of the mean.
[B] Short-chain fatty acids: (C4-C14).
[C] Medium-chain fatty acids: (C15-C17).
[D] Long-chain fatty acids: (C18-C22).
[E] Saturated fatty acids.
[F] Mono-unsaturated fatty acids.
[G] Poly-unsaturated fatty acids

humans. Considering an average fat content of 33% for all cheeses, pasture and hybrid cheese would provide ~330 mg/100 g of cheese consumed, which is more than half of what Ens et al.

(2001) recommend. In contrast, a 100 g serving of TMR cheese would only provide 142 mg/100 g serving.

Hybrid milk and cheese maintained most of the fatty acid composition without significant differences to that of the pasture cheese-milk and cheese. Maintaining the integrity of the fatty acids, especially those of nutritional interest (CLA and other unsaturated fatty acids) by the process of hybridization is important as it shows that the hybridization process is a suitable method to produce a Cheddar cheese high in CLA content.

5.1.3 Imaging (fat globule analysis)

The fat globules of the treatments during week 1 and week 14 were examined using a confocal laser scanning microscope (CSLM) as detailed in section 4.3.3 and analyzed following the procedure described in section 4.3.4. Fat globules shown on Figure 5.1.3.1, are shown in red. Least square means of fat globule diameter and sphericity are shown in Table 5.1.3.1. Statistical analysis of the fat globule diameter was not significant ($P < 0.05$) among treatments. Sphericity was only significantly different ($P < 0.05$) for samples at week 14 of ripening.

Fat globule diameter and sphericity seemed to follow a similar positive trend, with larger diameter of the fat globules, the sphericity also increased (Table 5.1.3.1). However, this observation, more than an effect from treatment or ripening time, appears to have been caused by small variations on the testing conditions, possibly by slight temperature differences of samples during analysis. Even though, samples were maintained at ~4°C and the time to take the frames was standardized to about 10 min, the actual time in which some frames were taken may have had some small time variations which could have influenced the temperature and the state (solid/liquid) of the fat globules providing potentially skewed results on sphericity and fat globule diameter (Figure 5.1.3.2).

Truong et al. (2016) explain that the lipid state (solid or liquid) is an important factor to consider when studying dairy lipids and lipids in general. A more liquid physical state of the fat globules tends to favor a higher degree of sphericity as the liquid fat pushes evenly on every direction of the globule membrane which creates more of a sphere shape (Sphericity of 1= a perfect sphere) (Everett et al. 1995). Considering the influence of liquid or solid state of lipids on the sphericity of fat globules, it is possible that sphericity could have been influenced by test conditions.

Table 5.1.3.1. Least square means of diameter and sphericity of fat globules of pasture, TMR, and hybrid Cheddar cheeses analyzed at week 1 and week 14 of ripening period.

	Week 1			Week 14			SEM[A]
	Pasture	TMR	Hybrid	Pasture	TMR	Hybrid	
	LS Means of fat globules						
Diameter, µm	15.30 [a]	15.40 [a]	15.09 [a]	15.05 [a]	15.30 [a]	15.82 [a]	0.52
Sphericity	0.66 [b]	0.69 [a]	0.65 [b]	0.69 [c]	0.74 [a]	0.72 [b]	0.02

[a, b, c] Means within a row with the same letter superscript are not significantly different ($P < 0.05$).
Number of fat globules measured during Week 1: Pasture, n= 3179; TMR, n= 3846; Hybrid, n= 3309.
Number of fat globules measured during Week 14: Pasture, n= 3703; TMR, n= 3066; Hybrid, n= 2741.
[A] SEM = Standard error of the mean.

Related to lipid composition, the TMR cheese had a higher content of saturated fat which remains solid at higher temperatures than unsaturated fat. In contrast to the TMR cheese, the pasture and hybrid cheeses had a higher unsaturated fat content. However, if the fatty acid composition played a role in the lipid state and sphericity, a larger amount of fat globules in the TMR cheese would have been in a solid state and their sphericity would have likely been lower-

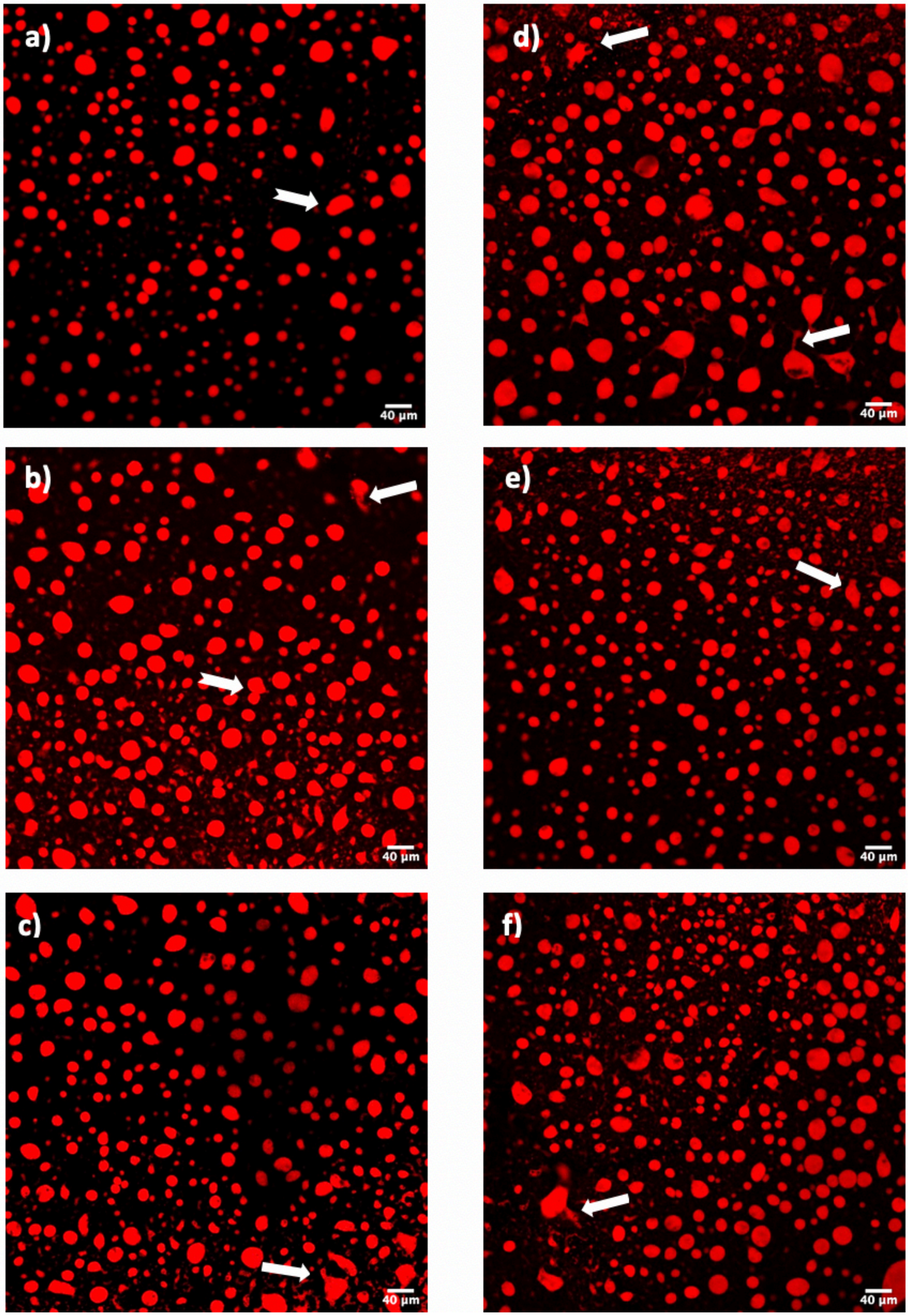


Figure 5.1.3.1. Confocal laser scanning micrographs of Cheddar cheese fat globules (shown in red). Cheeses at week 1 of ripening are: a) pasture, b) TMR, and c) hybrid. Cheeses at week 14 of ripening are: d) pasture, e) TMR, and f) hybrid. There was no significant difference ($P < 0.05$) for diameter or sphericity among samples during week 1 nor week 14. White straight arrows in micrographs b), c), d), e), and f) show free fat, and notched arrows in micrographs a) and b) show coalescing of fat globules.

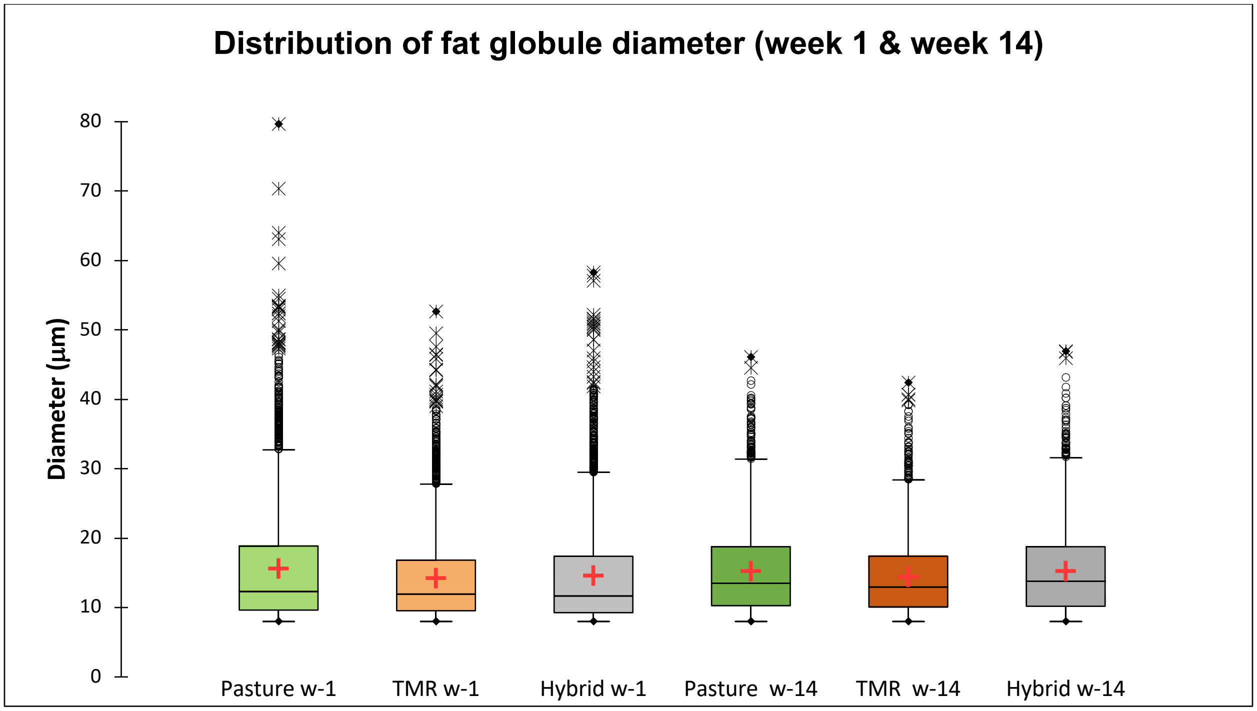


Figure 5.1.3.2. Size distribution of fat globule diameter of cheese samples during week 1 (w-1) and week 14 (w-14). Variation in size distribution of fat globules may have been influenced by testing conditions.

than the pasture and hybrid cheeses which had a higher unsaturated fatty acid content. Nevertheless, this was not the case for week 1 and week 14. Taking into consideration that the - opposite trend was observed during week 1 and week 14, with the TMR cheese (higher saturated fat) having larger sphericity than the pasture and hybrid cheeses, it is possible that testing conditions were the main factor that influenced the physical state of lipids in the fat globules.

Potential temperature variations during testing could have also affected size of fat globules. Although, Figure 5.1.3.2 shows the pasture cheese during week 1 having larger outliers than TMR and hybrid cheeses, pasture and hybrid cheeses follow a similar pattern of outlier distribution during week 1 and week 14, with these two cheeses having larger outliers than the TMR cheese during week 1 and week 14. This skewed distribution of fat globule diameter of the pasture and hybrid cheeses could have potentially affected the lack of significance finding of fat globule size. A likely factor to contribute to the distribution and number/size of outliers of fat globule diameter is that fat globules and melted free fat (Figure 5.1.3.2) in the samples could have coalesced with other fat globules or free fat and this could have potentially skewed results of fat globule diameter. However, given that the potential influence of test conditions on the sphericity and potentially on the size of fat globules was not anticipated, further analysis would need to be conducted to come to a more concrete conclusion

To reduce the potential coalescing of fat globules and free fat in cheese samples it is recommended that imaging of the samples is done in a standardized manner in the shortest amount of time. Additionally, in conjunction with CLSM imaging of fat globules in cheese, it would also be helpful to perform particle size measurements by laser light scattering (or similar technique) of fat globules in the milk prior to making the cheese as this analytical technique can help further understand and confirm potential differences of the fat globule size in Cheddar cheese.

5.1.4 Calcium and magnesium analysis

The purpose of analyzing the calcium content, both soluble (Sol Ca) and insoluble (Insol Ca), and magnesium was to understand whether the process of making a hybrid Cheddar cheese, which required the combination of ingredients from two different sources, could impact its calcium and magnesium composition. The mineral analysis for total calcium, water soluble and magnesium was carried out as explained in sections 4.3.5 and 4.3.6. The insoluble (Insol) calcium was calculated by subtracting the soluble (Sol) calcium content from the total calcium content. Calculated least square means are reported in Table 5.1.4.1.

Statistical analysis showed that calcium content was significantly ($P < 0.05$) affected by treatment. Calcium composition (Insol and Sol; shown as a % of total calcium content) were significantly affected by treatment and ripening time (week 1 vs week 14). The total calcium content of the hybrid cheese was in between TMR (highest; $P < 0.05$) and Pasture (lowest; $P < 0.05$) cheese, which suggests that calcium content is likely to be impacted by the hybridization process. The magnesium content was not significantly affected ($P < 0.05$) by hybridization or ripening period.

Given that the total calcium content in cheese can be considered as the sum of Insol and Sol calcium. Shifts in the concentrations of one of the calcium fractions (Insol and Sol; shown as percentages), which are inversely correlated, will correspond to a proportional change in concentration of the opposite fraction (see Figure 5.1.4.1). The Insol calcium content (% of total calcium) of the hybrid cheese resembled that of the pasture cheese the most, in spite of the hybrid cheese having a significantly ($P < 0.05$) higher total calcium content, which indicates that the proportion Insol calcium (%) of the hybrid cheese decreased significantly more in relation to its total calcium content. Both, hybrid and pasture cheeses had a significantly lower Insol calcium content (%) than the TMR cheese.

The overall decrease of Insol calcium (%) during ripening was expected to happen in all cheeses. It has been well established that when Insol calcium solubilizes or chemically unbinds from casein micelles during the ripening period it causes the Sol calcium content to increase (Hassan et al. 2004; O'Mahony et al. 2005; Lee et al. 2005). However, is worth highlighting the similarity of low Insol calcium concentration (%) of the hybrid and pasture cheeses during week 1 and week 14. Hybrid and pasture cheeses had comparably low Insol calcium content (%)

despite having been prepared with different types of skim milk and having different total calcium content.

It is possible that processing conditions influenced the low Insol calcium content of these cheeses (Lucey and Fox 1993; Lee et al. 2005). However, given that the hybrid and pasture cheeses were manufactured under the same conditions, suggests that other factors perhaps related to the compositional commonality between the hybrid and pasture cheeses could have influenced their low Insol calcium composition (%). Nevertheless, regarding this observation, there was not sufficient information gathered through this present work to determine the reason for the low Insol calcium content (%) of the hybrid and pasture cheeses.

Table 5.1.4.1. Least square means of calcium and magnesium composition of pasture, TMR, and hybrid cheeses at week 1 and week 14 of ripening period

	Pasture	TMR	Hybrid	SEM[A]
	LS Means of mineral content			
Week1, Total Ca, mg/100g	611.13 [c]	770.26 [a]	694.46 [b]	34.52
Week1, Sol Ca, mg/100g	199.13 [b]	140.77 [c]	261.88 [a]	21.14
Week1, Insol Ca, %	66.34 [b]	85.54 [a]	60.32 [b]	3.74
Week1, Mg, mg/100g	22.5 [a]	28.6 [a]	25.1 [a]	0.61
Week14, Sol Ca, mg/100g	404.57 [b]	408.54 [b]	505.66 [a]	21.14
Week14, Insol Ca, %	31.01 [b]	44.31 [a]	23.66 [b]	3.74
Week14, Mg, mg/100g	20.36 [a]	23.93 [a]	20.39 [a]	0.61

[a, b, c] Least Square means within a row with the same letter superscript are not significantly different ($P < 0.05$); n= 3. Samples were tested at week 1 (W-1) and week 14 (W-14) for total calcium (Total Ca), water soluble calcium (Sol Ca), and magnesium (Mg). Water insoluble calcium (Insol Ca) was calculated by subtracting soluble calcium from total calcium and is shown as a percentage of the total calcium content.
[A] SEM = Standard error of the mean.

It is also important to note that the overall amount of Insol calcium (%) observed during week 14 in the hybrid and pasture cheeses was lower compared to what others have reported. Hassan et al. (2004), who investigated shifts in the Insol and Sol calcium (%) during ripening reported ~57% of Insol calcium in Cheddar cheese and a lower degree of Insol solubilization at the end of a 12-week study. One likely factor that contributed to a lower Insol calcium content observed in this study as compared to Hassan et al. (2004) is that cheeses in this present work (pasture, TMR, hybrid) were manufactured at lower pH than the cheese evaluated by Hassan et al. (2004). Hassan et al. (2004) reported a milling pH of about 5.44 and Lee et al. (2005) reported a milling pH of about 5.0. All of the cheese treatments in this study were milled at a titratable acidity of 0.63 which is a pH of approximately 4.7 (Emmons and Beckett 1984).

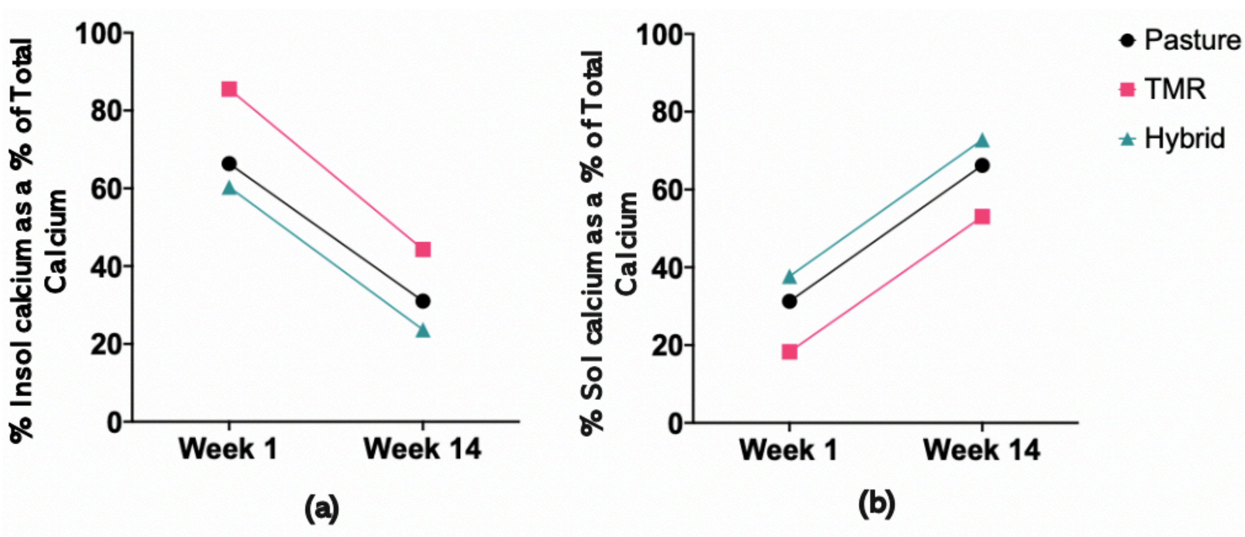


Figure 5.1.4.1. Shift of Insol (a) and Sol (b) calcium from week 1 to week 14 as a percentage of total calcium. Although the hybrid cheese contained the skimmed-milk of TMR it had a significantly lower ($P < 0.05$) Insol calcium content (figure a) during week 1 and week 14. The hybrid cheese also underwent the highest degree of Insol calcium solubilization ($P < 0.05$) and its Sol calcium content (figure a) was significantly higher than pasture and TMR.

Lee et al. (2005), who investigated the effect of pH on the shifts of Insol and Sol calcium, reported that a low pH during manufacturing correlated with a higher degree of Insol calcium solubilization and their lowest observed Insol calcium content was about 41%. The lower Insol calcium reported by Lee et al. (2005) is closer to the Insol calcium content (44.31%) found in the TMR cheese in this present work. Additional work by Lucey and Fox (1993) has also shown that Insol calcium solubilization is promoted to a higher degree when Cheddar curd is milled at a low pH. The initial lower pH at the point of milling the cheeses in this present study could have caused a higher degree of solubilization of the Insol calcium. Another potential reason to have influenced the low Insol content found in this study, although less significantly, is that cheeses were tested at week 14 which is a slightly longer ripening time than what Hassan et al. (2004) and Lee et al. (2005) reported.

Calcium content and the distribution of its fractions (Insol and Sol) are considered important factors to influence cheese texture. Chevanan and Muthukumarappan (2008), who studied the effect of calcium concentration on the elastic modulus, which is analogous to "hardness," were able to show that an increase in the total calcium content of Cheddar cheese correlated with an increase of "hardness." Similarly, other researchers have shown that higher Insol calcium content in cheese also correlates with a firmer cheese texture (Lucey et al. 2005; Lee et al. 2005; O'Mahony et al. 2006). Considering the findings by others with relation to the total calcium and Insol calcium content and its effects on cheese texture, it should be considered that the different total calcium concentrations and variations of Insol calcium in the pasture, TMR, and hybrid cheeses may be potential factors to influence their texture.

5.2 Sensory evaluation

Consumer acceptance testing was conducted at Michigan State University (see section 4.10). Table in Appendix B provides information about consumer demographic, consumer knowledge of CLA, income, gender, and race/ethnicity of participants. Most of the participants were white (74%) between 18-29 years old (54%), female (65%), and reported to be have previous knowledge about conjugated linoleic acid (84%) and 74% reported to consume Cheddar cheese at least once a week. The annual income reported by panelist was distributed as follows: <$10,000, 8%; $10,000-24,999, 18%; $25,000-49,999, 16%; $50,000-99,999, 29%; $100,000-199,999, 20%; >$200,000, 9%.

Consumer acceptability results are shown on Table 5.2.1. The pasture and hybrid cheese received significantly higher ($P < 0.05$) "overall liking," "flavor" and "texture liking" scores than the TMR cheese. "Flavor intensity" rating was significantly higher ($P < 0.05$) for the pasture cheese and significantly ($P < 0.05$) lower for the TMR cheese. The "flavor intensity" score of the hybrid cheese was in between the pasture and TMR cheeses. "Firmness intensity" was significantly higher ($P < 0.05$) for TMR cheese. Both, hybrid and pasture cheese received significantly higher ($P < 0.05$) scores for "purchase intent."

There were significant findings ($P < 0.05$) within the CATA questionnaire according to the Cochran's Q test (Table 5.2.2). Related to flavor/taste, the TMR cheese was identified by a higher number of consumers ($P < 0.05$) as being "sour" and "bitter." The pasture cheese was also identified by a similar number of consumers as the TMR cheese as being "sour" and "bitter." A significantly ($P < 0.05$) lower number of consumers identified the hybrid cheese as "sour" and "bitter" and a significantly higher ($P < 0.05$) number of consumers identified the pasture and hybrid cheeses as having a "buttery" taste. Related to texture responses from the CATA questionnaire, the TMR cheese was perceived by a larger number ($P < 0.05$) of consumers as "crumbly" and "firm" and a larger number of consumers ($P < 0.05$) identified the pasture and hybrid cheeses as "creamy" and "smooth."

Table 5.2.1. Means of consumer overall liking, specific liking, intensity rating and purchase intent means for Cheddar cheeses with standard deviation ($\pm$ in parentheses).

Attribute n= 116	Pasture	TMR	Hybrid
Overall liking	6.35 (1.71) ^{a}	5.51 (1.87) ^{b}	6.54 (1.60) ^{a}
Flavor liking	6.38 (1.86) ^{a}	5.22 (2.24) ^{b}	6.14 (1.93) ^{a}
Texture liking	6.71 (1.70) ^{a}	6.03 (1.98) ^{b}	7.11 (1.53) ^{a}
Flavor intensity	5.86 (1.79) ^{a}	5.29 (2.05) ^{b}	5.42 (2.04) ^{ab}
Firmness intensity	5.80 (1.95) ^{b}	6.82 (1.41) ^{a}	5.83 (1.93) ^{b}
Purchase intent *	3.27 (1.07) ^{a}	2.67 (1.30) ^{a}	3.35 (1.12) ^{a}

$^{a, b, c}$ Means within a row with the same letter superscript are not significantly different ($P < 0.05$). Liking attributes were rated on a 9-point hedonic scale with 1= dislike extremely and 9=liked extremely. Intensity attributes for flavor and firmness were also rated on a 9-point hedonic scale with the scale being 1=none and 9=strong for flavor and 1=soft and 9=firm for firmness.
* Purchase intent rating was done on a 5-point purchase probability scale with 1=would definitely not buy and 5= would definitely buy.
Cheese samples were analyzed at week 14 of ripening.

Correspondence analysis (CA) bi-plot (Figure 5.2.1) provides a summary of the association between cheeses (pasture, TMR, hybrid) and the attributes used in the Check-all-that-apply (CATA) questionnaire. The two axes F1×F2 of the CA analysis by-plot totaled 100% of cumulative inertia. The first dimension (F1) explained 87.7% of the data variability and the second dimension (F2) the remaining 12.27%. The pasture cheese showed a high correspondence (association) to the attributes "creamy," "buttery," "sharp," and "milky." The TMR cheese showed high association with the attributes "crumbly," "bitter," "fermented," "sour," and "firm" and the hybrid cheese showed a high association with the attributes “smooth,” “buttery,” “flat/low flavor,” “sweet,” and “chewy.”

Table 5.2.2. Frequency use of check-all-that-apply terms consumers used to describe flavor and texture attributes of pasture, TMR, and hybrid cheeses.

	Samples		
ATTRIBUTE	Pasture	TMR	Hybrid
FLAVOR			
Sour *	26 ^{ab}	34 ^{a}	18 ^{b}
Bitter *	15 ^{ab}	18 ^{a}	5 ^{b}
Buttery *	55 ^{a}	27 ^{b}	54 ^{a}
Fermented	13 ^{a}	18 ^{a}	9 ^{a}
Milky	54 ^{a}	39 ^{a}	51 ^{a}
Salty	26 ^{a}	21 ^{a}	27 ^{a}
Sharp	45 ^{a}	39 ^{a}	35 ^{a}
Sweet	14 ^{a}	14 ^{a}	19 ^{a}
Flat low/flavor	22 ^{a}	29 ^{a}	36 ^{a}
TEXTURE			
Chewy	33 ^{a}	33 ^{a}	44 ^{a}
Creamy *	69 ^{a}	29 ^{b}	58 ^{a}
Crumbly *	11 ^{b}	33 ^{a}	5 ^{b}
Firm *	64 ^{b}	84 ^{a}	59 ^{b}
Smooth *	61 ^{a}	29 ^{b}	67 ^{a}

* Indicates significant differences among samples according to the Cochran’s Q test ($P < 0.05$); n= 116.
[a, b] Samples sharing the same letter superscript are not significantly different according to the Cochran’s Q test ($P < 0.05$). Cheese samples were analyzed at week 14 of ripening.

The pasture and hybrid cheeses showed very similar ratings for most of the hedonic ratings with the exception of “flavor intensity” were hybrid cheese had a “flavor intensity” that was rated in between pasture and TMR cheeses. Given the higher “liking” and “purchase intent” scores and the “flavor intensity” similarity of pasture and hybrid cheeses (see Table 5.2.1), it may be possible that the intensity of certain flavor attributes like “buttery” was a driving factor in the consumer acceptability of the cheeses. The “buttery” attribute in this study had a higher frequency of identification in the pasture and the hybrid cheeses (Table 5.2.2). Work by Caspia et al. (2006) about the influence of sensory attributes on the consumer acceptability of 7, 9, and 12-month old Cheddar cheeses showed that cheeses with higher scores for the “buttery” attribute also received higher liking scores. Caspia et al. (2006) attributed taste as the main driver for consumer acceptability of Cheddar cheese.

As previously mentioned, a significantly higher ($P < 0.05$) number of consumers qualified pasture and hybrid cheeses as having a “buttery” flavor. The similarity of rating between “flavor liking” and “flavor intensity” of pasture and hybrid cheeses might also indicate that cream, being the common ingredient between these two cheeses, may have been the responsible factor for the higher “flavor liking” and “flavor intensity” of pasture and hybrid cheeses. Some “buttery” flavor compounds like methyl ketones are derived from fatty acids in cheese, which may be associated to the same type of fat in the hybrid and pasture cheeses (Singh et al. 2003).

Buttery and milky flavors have been reported as being a flavor attribute associated with young/mild Cheddar cheeses (Young et al. 2004). Work by Drake et al. (2008b) about the consumer preference of mild Cheddar cheese flavors found that “milkfat” (buttery) and “cooked/milky” flavor were some of the attributes present in the mild Cheddar cheeses most -

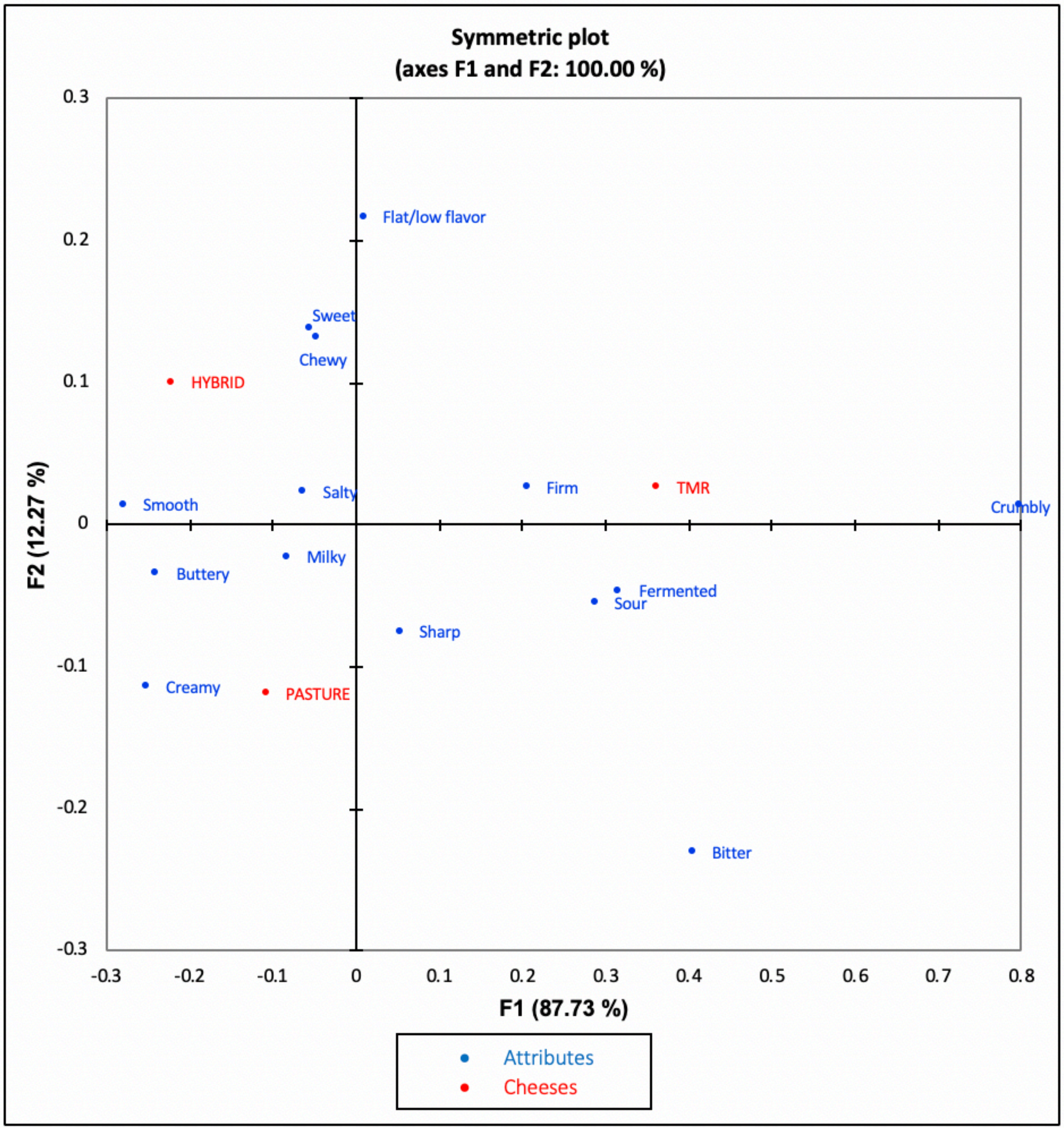


Figure 5.2.1. Correspondence analysis of the frequency identification of flavor and texture attributes associated with pasture, TMR, and hybrid cheeses from consumer Check-All-That-Apply (CATA) data. Cheese treatments are shown in red and flavor and texture attributes are shown in blue.

liked by consumers. It should be mentioned that hybrid cheese showed higher association (Figure 5.2.1) with "flat/low flavor" (not significant, Table 5.2.2), yet, it also was rated as having slightly higher “flavor intensity” than the cheese from the TMR diet (Table 5.2.1). It may be possible that the higher rating of “flavor intensity” was attributed to the higher association of certain flavors that consumers may have viewed as positive, like “buttery.”

Related to flavor/taste, the less perceived sour taste in the pasture and hybrid cheeses (Table 5.2.2) may have also played a role with consumer’s flavor liking preference. Drake et al. (2008b) characterized higher liked mild Cheddar cheeses as being less sour tasting. Although, the CATA questionnaire does not measure sourness intensity, the fact that a higher number of consumers identified the cheese from the TMR diet as being sour may indicate that the TMR cheese had a higher level of sourness.

Consumers were not asked about the aroma properties of cheeses. However, it is possible that the type of fat, which was the same for the pasture and hybrid cheeses, may have also influenced their aroma profile. Volatile compounds are common in Cheddar cheese and they have been known to influence flavor perception (O’Riordan and Delahunty 2001; Iwasawa et al. 2014). Work by Iwasawa et al. (2014) who evaluated the influence of volatile compounds showed that volatile compounds had a strong influence on “flavor intensity.” Some researchers have identified variations in the ratio or composition of some volatile organic compounds (VOCs) in pasture diets and conventional diets (Moio et al. 1996; Liu et al. 2018).

Some differences in the concentration of the VOCs in cheese have been attributed to higher concentrations of terpenes (Mariaca et al. 1997; Viallon et al. 1999; Bugaud et al. 2001a) and toluene (O’Callaghan et al. 2016a; Liu et al. 2018), which may be in higher concentrations in milk from pasture-based diets. For the most part, VOCs are considered to be lipophilic (Mariaca and Bosset 1997). This would mean that potential differences in the concentration of terpenes and other organic volatile compounds in the cheeses from the pasture diet and the hybrid cheese versus the cheese from the TMR diet could have been directly associated their fatty acid composition.

One thing to consider is that the flavor/aroma profile of hybrid cheese could have potentially been influenced by its fatty acid composition due that pasture diets may contain different flavor/aroma compounds in the cream (Buchin et al. 1998; Bugaud et al. 2001b). The influence of flavor and potentially aroma compounds in a hybrid cheese from a pasture cream may contribute positively to its liking since the ratings of flavor liking, overall liking, and purchase intent of the hybrid cheese were significantly higher than that of the TMR cheese.

In terms of texture, higher “firmness intensity” did not seem to have influenced “texture liking” as the TMR cheese received a significantly higher ($P < 0.05$) score in “firmness intensity” but a significantly lower ($P < 0.05$) score in “texture liking” (Table 5.2.1). Instrumental measurements of texture by texture profile analysis (TPA) were also conducted in this study (Table 5.3.1). TPA of cheeses showed that hardness was higher for hybrid cheese than for TMR cheese during week 1 and week 14. However, firmness intensity of the hybrid cheese was rated lower ($P < 0.05$) by consumers during the sensory analysis. Additionally, the CATA response, showed that a lower number of consumers associated hybrid cheese as a firm cheese (Table 5.2.2). A possible reason for the lack of correlation of sensory rating of “firmness” and texture parameter of “hardness” is that cheese samples for sensory and TPA were tested at different temperature. Some researchers have found that the temperature of cheese samples has potential to influence TPA scores (O’Callaghan et al. 2017).

One possible explanation for the different results between sensory rating of “firmness intensity” and instrumental measurement of “hardness” is that a sensory evaluation is a more holistic approach to evaluate texture. Consumers may take into consideration other organoleptic properties of a cheese to judge “firmness” (Szczesniak 1987). Consumers may have also taken into consideration other texture attributes of the hybrid cheese into account, like “creamy” and “smooth,” which were higher for the hybrid cheese, to make a final judgement of its “firmness intensity.”

Additional evidence that consumers may have consider other factors to judge for firmness intensity is that, although pasture and hybrid cheeses had very similar sensory “firmness intensity” rating, hybrid cheese was rated slightly higher (not significant) in texture liking (Table 5.2.1) than the pasture cheese. It may be possible that although hardness of the hybrid cheese was higher, its fatty acid composition, different from that of the TMR cheese (Table 5.1.2.1), played a role as to how sensory panelists perceived its firmness.

5.3 Texture profile analysis

The texture of all cheeses (pasture, TMR and hybrid) was evaluated by texture profile analysis (detailed in section 4.5) during week 1 and week 14 of production. Cheese samples were analyzed at room temperature (25ºC). Least square means for the texture profile analysis (TPA) parameters of “hardness,” “springiness,” “cohesiveness,” “adhesiveness,” “resilience,” and “chewiness” are provided in Table 5.3.1. All parameters were significantly ($P < 0.05$) affected by the ripening period. Although “hardness” level decreased significantly ($P < 0.05$), differences in “hardness” among all cheeses followed a similar trend during week 1 and week 14. The hybrid cheese shared similar results with TMR and pasture cheese.

The hybrid cheese had “hardness” and “chewiness” level closer to the TMR cheese, although, “hardness” was significantly higher ($P < 0.05$) for the hybrid cheese. Both, hybrid and TMR cheeses showed significantly higher ($P < 0.05$) level of “hardness” and “chewiness” then pasture cheese during week 1 and week 14.The hybrid cheese showed higher similarity in "springiness" to pasture cheese during week 1 but during week 14 the "springiness" of the hybrid cheese resembled that of TMR cheese more.

Table 5.3.1. Least square means of the texture profile analysis results of pasture, TMR, and hybrid Cheddar cheeses

	Week 1			Week 14			SEM[A]
	Pasture	TMR	Hybrid	Pasture	TMR	Hybrid	
	LS Means of TPA measurements						
Hardness (N)	20.00 [c]	30.13 [b]	40.51 [a]	13.49 [c]	20.62 [b]	32.11 [a]	4.03
Springiness	0.85 [b]	0.93 [a]	0.84 [b]	0.73 [b]	0.85 [a]	0.82 [a]	0.03
Cohesiveness	0.61 [b]	0.82 [a]	0.61 [b]	0.47 [b]	0.71 [a]	0.56 [b]	0.07
Adhesiveness (N.s)	-0.04 [a]	-0.02 [a]	-0.02 [a]	-0.08 [a]	-0.08 [a]	-0.09 [a]	0.01
Resilience	0.33 [b]	0.46 [a]	0.36 [b]	0.25 [b]	0.36 [a]	0.30 [b]	0.04
Chewiness	10.83 [b]	21.53 [a]	21.79 [a]	4.16 [b]	12.24 [a]	14.58 [a]	1.46

[a, b, c] Means within a row with the same letter superscript are not significantly different ($P < 0.05$); n= 3.
[A] SEM = Standard error of the mean.

"Cohesiveness," "adhesiveness," and "resilience" were three parameters where the pasture and hybrid cheese shared a higher similarity. For "cohesiveness," "adhesiveness," and "resilience," both cheeses, pasture and hybrid, were significantly lower ($P < 0.05$) in those three parameters during week 1 and week 14. It is interesting to note that the TPA analysis revealed that the hybrid cheese shared some similarities with cheeses from the two different diets (pasture, TMR). Given that the hybrid cheese was found to have a shared compositional make-up of fatty acids with the pasture cheese (Table 5.1.2.1) and protein and mineral content with the TMR cheese (see Table 5.1.1.1), it is possible that its similarity in composition with pasture and TMR cheeses also influence a similarity with some texture parameters of these cheeses.

Changes of texture from week 1 to week 14 were due to the natural break down of proteins (proteolysis) in the cheese. Proteolysis of caseins is the main factor to have influenced changes of cheese texture during ripening because as Cheddar cheese ripens, caseins begin to break down weakening the structure of the cheese (Sousa et al. 2001; McSweeney 2004; Rogers et al. 2009). In addition to the protein breakdown, protein content was also likely to have influenced texture. TMR and hybrid cheeses were found to have a higher protein content (Table 5.1.1.1). Increasing concentration of protein levels in milk have been positively correlated to cheese "hardness" (Ong et al. 2013). Although, the texture of TMR and hybrid cheeses were significantly different during week 1 and week 14, these cheeses shared the similarity that they had significantly ($P < 0.05$) higher "hardness" than the pasture cheese. Thus, it is possible that a higher protein concentration helped increase the "hardness" of TMR and hybrid cheeses.

One additional factor related to protein content that might have also influenced "hardness" is the slight moisture content differences of the cheeses. TMR and hybrid cheese had a slightly lower moisture content than the pasture cheese. This higher moisture content in the pasture cheese was potentially attributed to its lower protein content (see section 5.1.1). Both factors combined may have lowered the "hardness" of the pasture cheese. Fox et al. (2017a) and Liu et al. (2008) have reported that components like high moisture content and lower protein content can decrease the structural rigidity (hardness) of cheese. Although, the moisture content of the pasture cheese was not significantly lower than the TMR and hybrid cheeses, the compounded effect of the lower protein and moisture content could have impacted its lower "hardness."

Related to other compositional differences and their influence on the texture profile of cheeses, the pasture and hybrid cheeses had a higher ratio of unsaturated fatty acids, which were expected to lower “hardness.” Some researchers have found that higher ratios of unsaturated fatty acids tend to lower the firmness of a cheese (Halmos et al. 2003; Coulon et al. 2004). Cheese samples in this study were tested at the same temperature (25 ºC) but due to the different melting points of unsaturated and saturated fats, the ratios of solid fat and liquid fat were likely to be different (de Hoog et al. 2011). However, in spite of its higher unsaturated fatty acid composition, the hybrid cheese was found to have a higher level of “hardness.” Additionally, another important component in cheese that has been known to play a role in cheese hardness is Insol calcium content. Several researchers have found a strong positive correlation between higher levels of Insol calcium content and “hardness” of a cheese (Pastorino et al. 2003; O’Mahony et al. 2005). However, the lower Insol calcium content of the hybrid cheese did not seem to affect its “hardness” compared to the other cheeses in this study.

A possible explanation for the higher hardness of the hybrid cheese than the TMR cheese which had a potentially lower amount of liquid fat and higher Insol calcium content, is that the TMR cheese may have had a higher level of “crumbliness.” Although, the TMR cheese had higher “cohesiveness” (Table 5.3.1) which may be indicative of a lower “crumbliness” of a cheese (Halmos et al. 2003), a larger number of consumers identified the TMR cheese on the CATA questionnaire as having a “crumbly” texture (Table 5.2.2) and a “crumbly” texture may be indicative of a weaker protein structure (Guinot et al. 2019). Halmos et al. (2003) who studied the effect of seasonality on the composition and texture variations of Cheddar cheese hypothesized that lower amounts of liquid fat in a Cheddar cheese limits its malleability and may enhance its easiness to fracture or crumble. However, further work is needed to confirm the correlation of fatty acid (liquid fat) and Insol calcium composition on the TPA parameter of "hardness" of a hybrid cheese.

6.0 CONCLUSIONS, LIMITATIONS & FUTURE WORK

6.1 Conclusions

This study shows that the hybridization, the combination of cream from pasture fed cows and skim milk from TMR fed cows, is a suitable method for the manufacture of a Cheddar cheese high in CLA c9, t11 isomer. To evaluate the suitability of manufacturing a hybrid Cheddar cheese high in CLA, the fatty acid profile and consumer acceptability measurements of the hybrid cheese were compared with those of pasture and TMR Cheddar cheeses. The hybrid cheese was found to have a similar fatty acid profile as the pasture derived cheese and a ~2.3-fold higher CLA content than the TMR cheese.

Further evidence of the suitability of manufacturing a hybrid Cheddar cheese was provided by the high consumer rating of “liking” and “purchase intent” the hybrid cheese received. The hybrid cheese was found to have a similar rating of “flavor intensity” and “flavor liking” as the pasture cheese, which suggests that the cream, being the common ingredient of these two cheeses, may have influenced their flavor profile.

Hybridization was found to alter the total calcium content and Insol calcium composition, which was expected to contribute to a softening of the texture of the hybrid cheese. However, this was not observed by instrumental analysis as the hybrid cheese had a highest level of “hardness” compared to the pasture and TMR cheeses.

The assessment of the fat globule diameter was not conclusive. There was evidence of potential coalescing of fat which could have skewed results. In order to make a better assessment of the potential influence of lipid composition on the fat globule size it is recommended to perform particle size analysis of fat globules in the milk prior to making cheese treatments to compare with microscopic measurements of fat globule diameter and to reduce and standardize testing time for microscopy analysis by confocal laser scanning microscopy.

Results from the current study suggest that hybridization may affect the total calcium and Insol calcium content of a hybrid cheese. However, this compositional change of the hybrid cheese was not found to have a negative contribution to its consumer acceptability.

6.2 Limitations & future work

Cheese is a complex food and its texture, flavor, and overall quality are influenced by several chemical and biochemical changes (McSweeney 2004; Murtaza et al. 2014). Something that was not investigated in this study was the degree of proteolysis (primary and secondary). Proteolysis has been positively correlated with cheeses with a crumbly texture and might help explain potential texture differences among cheese treatments (O'Callaghan et al. 2017). Related to primary proteolysis, some researchers have suggested that high levels of phosphopeptides, which are derived from β-casein hydrolysis during the early stages of the ripening period of a cheese may decrease Insol calcium levels (Lamichhane et al. 2019). Measuring levels of phosphopeptides might provide a deeper insight of the potential low Insol calcium content in a hybrid cheese. Furthermore, assessing other factors related to cheese ripening like pH changes throughout the aging period of the cheese and lipolysis may also provide additional information of potential changes in the texture and flavor profile of a hybrid cheese. Additionally, a detailed compositional analysis of the milk's casein content may also help explain potential changes in the composition of a hybrid cheese.

Future work may also include investigating and comparing compositional and quality attributes of two different types of hybrid Cheddar cheeses. One with high CLA content (pasture-cream and TMR-skim milk) and one with reversed composition (TMR-cream and pasture-skim milk). Comparing these two types of hybrid Cheddar cheese may help provide evidence of the potential contribution the cream derived from pasture fed cows has on the "flavor intensity" of a hybrid Cheddar cheese.

APPENDICES

APPENDIX A: Information for the online recruitment for the sensory evaluation of Cheddar cheese.

Information for online recruitment through SONA

The Department of Food Science and Human Nutrition is conducting a study about the consumer acceptability of different types of Cheddar cheeses.

The requirements to qualify are that you are 18 years or older and that you are a regular consumer of Cheddar cheese and do not have any allergy sensitivity to dairy products.

The risks of participation are similar to those of eating dairy products if you are dairy intolerant.

Compensation for completing of the 15-20 min study is an ice cream coupon for the MSU dairy store.

Principal Investigator:

Dr. Zeynep Ustunol
474 S. Anthony Hall
Food Science and Human Nutrition
Michigan State University, E. Lansing, MI 48824 E-mail: ustunol@msu.edu

Sensory Evaluation Leader

Javier Salas
237 Trout Building
Food Science and Human Nutrition
Michigan State University, E. Lansing, MI 48824 E-mail: salasjav@msu.edu

APPENDIX B. Consumer's responses about demographic questions.

Table 7.1. Demographical information of consumers (n=116) that participated in the consumer evaluation

	%
Age	
18-29	54
30-39	22
40 >	24
Frequency of cheddar cheese consumption	
More than once a week	46
Once a week	28
Once every two weeks	13
Once a month	10
Once every two months	2
Once every six months	1
Reported to know about conjugated linoleic acid	84
*Annual income	
<$10,000	8
$10,000-24,999	18
$25,000-49,999	16
$50,000-99,999	29
$100,000-199,999	20
>$200,000	9
Gender	
Male	34
Female	65
Other	1
Race/Ethnicity	
White	74
African American	3
Native Hawaiian or Other Pacific Islander	1
Asian	15
Hispanic or Latino	7

*Percentage reported about income is not of total number of participants (n=116). 16 participants chose not to report their income. Percentage shown is based on the remaining participants (n=100).

APENDIX C: Sensory evaluation questionnaire.

Cheddar cheese questionnaire

Cheddar cheese sample # ______

1. **Consider all characteristics (FLAVOR and TEXTURE) indicate your overall opinion by checking one box.**

□	□	□	□	□	□	□	□	□
Dislike extremely				Neither like nor dislike				Like extremely

- Comments: Please indicate WHAT in particular you LIKED about this product ( )
- Comments: Please indicate WHAT in particular you DISLIKED about this product ( )

2. **Cheddar Cheese Liking Questions of individual attributes**

Overall flavor

□	□	□	□	□	□	□	□	□
Dislike extremely				Neither like nor dislike				Like extremely

Overall texture

□	□	□	□	□	□	□	□	□
Dislike extremely				Neither like nor dislike				Like extremely

3. **Cheddar Cheese Specific Evaluation**

Overall Flavor Intensity Level

□	□	□	□	□	□	□	□	□
None								High

Overall Texture Intensity Level

□	□	□	□	□	□	□	□	□
Soft								Hard

4. **Check all that apply**

Flavor		**Texture**	
Sour	☐	Chewy	☐
Bitter	☐	Creamy	☐
Buttery	☐	Crumbly	☐
Fruity	☐	Firm	☐
Fermented	☐	Grainy	☐
Milky	☐	Smooth	☐
Moldy	☐	Weak/soft	☐
Nutty	☐		
Salty	☐		
Sharp	☐		
Sweet	☐		
Grassy	☐		
Cooked/heated milk	☐		
Flat/low flavor	☐		

5. **After sampling cheese #_____, how likely is it that you would buy a Cheddar cheese like sample # _____?**

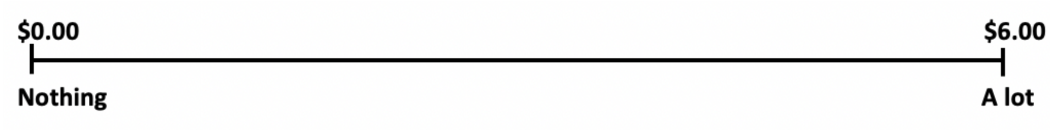
☐	☐	☐	☐	☐
Would definitely not buy		Might buy / might not buy		Would definitely buy

6. **Do you know what conjugated linoleic acid (CLA) is?**
   A. Yes → continue with 7
   B. No → Conjugated linoleic acid is a nutrient found in milk that has been shown to have potential health benefits like fighting cancer and reducing heart disease. Its amount in milk is directly influenced by the amount of time a cow spends out in pasture consuming grass. The more grass a cow has in its diet the more CLA in the milk (or dairy product).

7. **Rate how important it would be to see the CLA feature on a Cheddar cheese label?**

☐	☐	☐	☐	☐	☐	☐	☐	☐
Very unimportant				Neither unimportant nor important				Very Important

8. How much more would you be <u>WILLING TO PAY</u> for a Cheddar cheese with a high content of natural occurring CLA?



9. Demographic questions:

a) **How often do you eat Cheddar cheese?**

☐ More than once a week

☐ Once a week

☐ Once every two weeks

☐ Once a month

☐ Once every two months

b) **What is your household income?**

☐ <$10,000

☐ $10,000-24,999

☐ $25,000-49,999

☐ $50,000-99,999

☐ $100,000-199,999

☐ >$200,000

c) **What's your genre? M/F, other**

d) **Race ("X" those with which you identify):**

☐ White

☐ African American

- ☐ Native Hawaiian or Other Pacific Islander
- ☐ Asian
- ☐ American Indian or Alaska Native
- ☐ More than one race
- ☐ Unknown or not reported

e) **Ethnicity ("X" ONLY one with which you MOST CLOSELY identify):**

- ☐ Hispanic or Latino
- ☐ Not Hispanic or Latino
- ☐ Unknown or not reported

f) **What is your age?**

APPENDIX D: Consent form for participating in the sensory evaluation of Cheddar cheese.

Information about the Sensory Evaluation of Cheddar Cheese and Consent Form

1. EXPLANATION OF THE RESEARCH and WHAT YOU WILL DO:

INVITATION TO PARTICIPATE
You are invited to participate in this study to compares the texture and some flavor properties of a Cheddar cheese high in conjugated linoleic acid and unsaturated fatty acids.

TITLE OF THE RESEARCH
Analysis of bound calcium, microstructure, texture and consumer acceptability of a hybrid (Pasture/TMR) Cheddar cheese high in conjugated linoleic fatty acid **(make sure it matches with the title of the thesis)**

PURPOSE OF THE STUDY
This study will evaluate sensory attributes and overall acceptability of a Cheddar cheese high in conjugated linoleic acid and unsaturated fatty acids.

BASIS FOR SUBJECT SELECTION
Subjects selection is based on the ability of subjects to detect differences in sensory attributes of Cheddar cheese. Individuals with a cold, allergies or other condition that might limit their ability to identify sensory attributes of a Cheddar cheese will be excluded from participating. Participants **must be at least 18 years old**.

EXPLANATION OF PROCEDURES
You will be asked to sit at a booth and taste a number of numerically coded cheese samples. You will be provided with water for rinsing your mouth between samples. The tasting exercise will take a maximum of 25 minutes of your time, depending upon your speed of tasting. You will use a sensory evaluation questionnaire form to record responses concerning specific product attributes. Tasting will occur in Sensory Evaluation/Human Studies Laboratory located in Room 102 of the G. Malcom Trout (Food Science) Building.

2. YOUR RIGHTS TO PARTICIPATE, SAY NO, OR WITHDRAW:
Participation in this research project is completely voluntary. You have the right to say no. You may change your mind at any time and withdraw. You may choose not to answer specific questions or to stop participating at any time. Whether you choose to participate or not will have no affect on your grade or evaluation.

POTENTIAL RISKS
The cheese samples to be evaluated contain the following ingredients: milk, cultures, annatto, rennet, and sodium chloride (table salt). All of these ingredients are USDA and/or FDA approved for use in foods intended for human consumption and are being used at USDA/FDA approved levels. Each product was produced in a safe and wholesome manner according to USDA and/or FDA regulations. These products samples pose no adverse health risk upon digestion, provided

the subject has not been identified as being susceptible to an allergic reaction to the previous listed product ingredients. If you believe there is a potential of an allergic reaction upon ingesting the test products, or you believe that participating will violate religious or cultural beliefs, notify the on-site sensory evaluation coordinator and/or principal investigator immediately. You will be released from participating in the study.

ASSURANCE OF CONFIDENTIALITY
Any information obtained in connection with this study that could be identified with you will be kept confidential by ensuring that all consent forms are securely stored, and your privacy will be protected to the minimum extent allowable by law. All data analyzed will be reported in an aggregate format that will not permit associating subjects with specific responses or findings.

3. COMPENSATION FOR BEING IN THE STUDY:
After you have completed your sensory testing session and turned in your sensory ballot, you will be offered a choice of treats (i.e., candy or ice cream coupon) for your time and effort.

POTENTIAL BENEFITS
There are no direct benefits gained from participation in this study. However, your participation provides valuable data for the development of cheese enriched with conjugated linoleic and unsaturated fatty acids. Information obtained from this study will be published in appropriate scientific journals to expand our current knowledge in enhancing the health value of cheese.

4. CONTACT INFORMATION FOR QUESTIONS AND CONCERNS:

If you have any question, please do not hesitate to contact the on-site sensory evaluation leader and/or the principal investigator. You are voluntarily deciding to participate in this study today. Your signature certificates that you have decided to participate after having read the information provided above and that you had an adequate opportunity to discuss this study with the principal investigator and have had all your questions answered to your satisfaction. You will be given a copy of this consent form for to keep upon request.

Sensory Evaluation Leader

Javier Salas
237 Trout Building
Food Science and Human Nutrition
Michigan State University, E. Lansing, MI
48824 E-mail: salasjav@msu.edu

Principal Investigator

Dr. Zeynep Ustunol
474 S. Anthony Hall
Food Science and Human Nutrition
Michigan State University, E. Lansing, MI
48824 E-mail: ustunol@msu.edu

If you have questions or concerns about your role and rights as a research participant, would like to obtain information or offer input, or would like to register a complaint about this study, you may contact, anonymously if you wish, the Michigan State University's Human Research Protection Program at 517-355-2180, Fax 517-432-4503, or e-mail irb@msu.edu or regular mail at 4000 Collins Rd, Suite 136, Lansing, MI 48910.

5. INFORMED CONSENT:

Your signature below means that you voluntarily agree to participate in this research study.

SIGNATURE OF SUBJECT	DATE
______________________	_________________

APPENDIX E: IRB exemption for the evaluation of Cheddar cheese.

MICHIGAN STATE
UNIVERSITY

EXEMPT DETERMINATION

October 19, 2018

To: Zeynep Ustunol

Re: **MSU Study ID:** STUDY00001393
Principal Investigator: Zeynep Ustunol
Category: Exempt 6
Exempt Determination Date: 10/19/2018

Title: Consumer evaluation of a Cheddar cheese high in conjugated linoleic and unsaturated fatty acids

This study has been determined to be exempt under 45 CFR 46.101(b) 6.

Principal Investigator (PI) Responsibilities: The PI assumes the responsibilities for the protection of human subjects in this study as outlined in Human Research Protection Program (HRPP) Manual Section 8-1, Exemptions.

Continuing Review: Exempt studies do not need to be renewed.

Modifications: In general, investigators are not required to submit changes to the Michigan State University (MSU) Institutional Review Board (IRB) once a research study is designated as exempt as long as those changes do not affect the exempt category or criteria for exempt determination (changing from exempt status to expedited or full review, changing exempt category) or that may substantially change the focus of the research study such as a change in hypothesis or study design. See HRPP Manual Section 8-1, Exemptions, for examples. If the study is modified to add additional sites for the research, please note that you may not begin the research at those sites until you receive the appropriate approvals/permissions from the sites.

Change in Funding: If new external funding is obtained for an active study that had been determined exempt, a new initial IRB submission will be required, with limited exceptions.

Reportable Events: If issues should arise during the conduct of the research, such as unanticipated problems that may involve risks to subjects or others, or any problem that may increase the risk to the human subjects and change the category of review, notify the IRB office promptly. Any complaints from participants that may change the level of review from exempt to expedited or full review must be reported to the IRB. Please report new information through the study's workspace and contact the IRB office with any urgent events. Please visit the Human Research Protection Program (HRPP) website to obtain more information, including reporting timelines.

Office of Regulatory Affairs
Human Research Protection Program

4000 Collins Road
Suite 136
Lansing, MI 48910

517-355-2180
Fax: 517-432-4503
Email: irb@msu.edu
www.hrpp.msu.edu

APPENDIX F: Certificate of analysis for total protein content of pasture, TMR, and hybrid Cheddar cheeses.

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Eurofins DQCI Horsham

702 Electronic Drive
Horsham, PA 19044
+215 355 3900
DQCIEastInterco@eurofinsus.com

Michigan State University

Zeynep Ustunol
469 Wilson Road
Toom 204
East Lansing, MI 48864

Analytical Report

AR-19-TF-002780-01

Client Code: TF0001443

Received On: 03/06/2019
Reported On: 03/18/2019

Eurofins Sample Code:	153-2019-03060141	**Sample Registration Date:**	03/06/2019
Client Sample Code:	06/26/18	**Condition Upon Receipt:**	acceptable
Sample Description:	Pasture Cheddar	**Sample Reference:**	Set 1
Sampling Date	03/04/19 12:00		
Sampled By	Client		
Sampling Temperature	4°C		

QV063 - Total Protein Kjeldahl Duplicate	**Reference**	**Accreditation**	**Analysis Completed**	**Sub**
	AOAC 991.20	A2LA 3500.01	03/18/2019	√

Parameter	**Result**
Crude Protein (Nx6.38) 1	22.97 %
Crude Protein (Nx6.38) 2	23.02 %
Average Crude Protein	22.99 %

Subcontracting partners:

Eurofins DQCI (Mounds View), MN

Respectfully Submitted,

Robert Kurz
Chemistry Supervisor

Results shown in this report relate solely to the item submitted for analysis. | Any opinions/interpretations expressed on this report are given independent of the laboratory's scope of accreditation. | All results are reported on an "As Received" basis unless otherwise stated. | Reports shall not be reproduced except in full without written permission of Eurofins Scientific, Inc. | All work done in accordance with Eurofins General Terms and Conditions of Sale: www.eurofinsus.com/terms_and_conditions.pdf | √ Indicates a subcontract test to a different lab. Lab(s) are listed at end of the report. For further details about the performing labs please contact your customer service contact at Eurofins. Measurement of uncertainty can be obtained upon request.

Eurofins DQCI Horsham

Eurofins DQCI Horsham

702 Electronic Drive
Horsham, PA 19044
+215 355 3900
DQCIEastInterco@eurofinsus.com

Michigan State University

Zeynep Ustunol
469 Wilson Road
Toom 204
East Lansing, MI 48864

Analytical Report

AR-19-TF-002779-01

Client Code: TF0001443

Received On: 03/06/2019
Reported On: 03/18/2019

Eurofins Sample Code:	153-2019-03060142	**Sample Registration Date:**	03/06/2019
Client Sample Code:	06/26/18	**Condition Upon Receipt:**	acceptable
Sample Description:	Pasture Cheddar	**Sample Reference:**	Set 2
Sampling Date	03/04/19 12:00		
Sampled By	Client		
Sampling Temperature	4°C		

QV063 - Total Protein Kjeldahl Duplicate	Reference	Accreditation	Analysis Completed	Sub
	AOAC 991.20	A2LA 3500.01	03/18/2019	√

Parameter	Result
Crude Protein (Nx6.38) 1	23.58 %
Crude Protein (Nx6.38) 2	23.71 %
Average Crude Protein	23.65 %

Subcontracting partners:

Eurofins DQCI (Mounds View), MN

Respectfully Submitted,

Robert Kurz
Chemistry Supervisor

Eurofins DQCI Horsham

Eurofins DQCI Horsham

702 Electronic Drive
Horsham, PA 19044
+215 355 3900
DQCIEastInterco@eurofinsus.com

Michigan State University

Zeynep Ustunol
469 Wilson Road
Toom 204
East Lansing, MI 48864

Analytical Report

AR-19-TF-002778-01

Client Code: TF0001443

Received On: 03/06/2019
Reported On: 03/18/2019

Eurofins Sample Code:	153-2019-03060143	**Sample Registration Date:**	03/06/2019
Client Sample Code:	06/26/18	**Condition Upon Receipt:**	acceptable
Sample Description:	Pasture Cheddar	**Sample Reference:**	Set 3
Sampling Date	03/04/19 12:00		
Sampled By	Client		
Sampling Temperature	4°C		

QV063 - Total Protein Kjeldahl Duplicate	**Reference**	**Accreditation**	**Analysis Completed**	**Sub**
	AOAC 991.20	A2LA 3500.01	03/18/2019	√

Parameter	**Result**
Crude Protein (Nx6.38) 1	23.56 %
Crude Protein (Nx6.38) 2	23.97 %
Average Crude Protein	23.77 %

Subcontracting partners:

Eurofins DQCI (Mounds View), MN

Respectfully Submitted,

Robert Kurz
Chemistry Supervisor

Eurofins DQCI Horsham

Eurofins DQCI Horsham

702 Electronic Drive
Horsham, PA 19044
+215 355 3900
DQCIEastInterco@eurofinsus.com

Michigan State University

Zeynep Ustunol
469 Wilson Road
Toom 204
East Lansing, MI 48864

Analytical Report

AR-19-TF-002777-01

Client Code: TF0001443

Received On: 03/06/2019
Reported On: 03/18/2019

Eurofins Sample Code:	153-2019-03060144	**Sample Registration Date:**	03/06/2019
Client Sample Code:	06/26/18	**Condition Upon Receipt:**	acceptable
Sample Description:	TMR Cheddar	**Sample Reference:**	Set 1
Sampling Date	03/04/19 12:00		
Sampled By	Client		
Sampling Temperature	4°C		

QV063 - Total Protein Kjeldahl Duplicate	Reference	Accreditation	Analysis Completed	Sub
	AOAC 991.20	A2LA 3500.01	03/18/2019	√

Parameter	Result
Crude Protein (Nx6.38) 1	25.45 %
Crude Protein (Nx6.38) 2	25.48 %
Average Crude Protein	25.47 %

Subcontracting partners:

Eurofins DQCI (Mounds View), MN

Respectfully Submitted,

Robert Kurz
Chemistry Supervisor

Eurofins DQCI Horsham

Eurofins DQCI Horsham

702 Electronic Drive
Horsham, PA 19044
+215 355 3900
DQCIEastInterco@eurofinsus.com

Michigan State University

Zeynep Ustunol
469 Wilson Road
Toom 204
East Lansing, MI 48864

Analytical Report

AR-19-TF-002776-01

Client Code: TF0001443

Received On: 03/06/2019
Reported On: 03/18/2019

Eurofins Sample Code:	153-2019-03060145	**Sample Registration Date:**	03/06/2019
Client Sample Code:	06/26/18	**Condition Upon Receipt:**	acceptable
Sample Description:	TMR Cheddar	**Sample Reference:**	Set 2
Sampling Date	03/04/19 12:00		
Sampled By	Client		
Sampling Temperature	4°C		

QV063 - Total Protein Kjeldahl Duplicate	**Reference**	**Accreditation**	**Analysis Completed**	**Sub**
	AOAC 991.20	A2LA 3500.01	03/18/2019	√

Parameter	**Result**
Crude Protein (Nx6.38) 1	23.63 %
Crude Protein (Nx6.38) 2	23.65 %
Average Crude Protein	23.64 %

Subcontracting partners:

Eurofins DQCI (Mounds View), MN

Respectfully Submitted,

Robert Kurz
Chemistry Supervisor

Eurofins DQCI Horsham

Eurofins DQCI Horsham

702 Electronic Drive
Horsham, PA 19044
+215 355 3900
DQCIEastInterco@eurofinsus.com

Michigan State University

Zeynep Ustunol
469 Wilson Road
Toom 204
East Lansing, MI 48864

Analytical Report

AR-19-TF-002775-01

Client Code: TF0001443

Received On: 03/06/2019
Reported On: 03/18/2019

Eurofins Sample Code:	153-2019-03060146	**Sample Registration Date:**	03/06/2019
Client Sample Code:	06/26/18	**Condition Upon Receipt:**	acceptable
Sample Description:	TMR Cheddar	**Sample Reference:**	Set 3
Sampling Date	03/04/19 12:00		
Sampled By	Client		
Sampling Temperature	4°C		

QV063 - Total Protein Kjeldahl Duplicate	**Reference**	**Accreditation**	**Analysis Completed**	**Sub**
	AOAC 991.20	A2LA 3500.01	03/18/2019	√

Parameter	**Result**
Crude Protein (Nx6.38) 1	24.89 %
Crude Protein (Nx6.38) 2	25.03 %
Average Crude Protein	24.96 %

Subcontracting partners:

Eurofins DQCI (Mounds View), MN

Respectfully Submitted,

Robert Kurz
Chemistry Supervisor

eurofins | **Eurofins DQCI Horsham**

Eurofins DQCI Horsham

702 Electronic Drive
Horsham, PA 19044
+215 355 3900
DQCIEastInterco@eurofinsus.com

Michigan State University

Zeynep Ustunol
469 Wilson Road
Toom 204
East Lansing, MI 48864

Analytical Report

AR-19-TF-002774-01

Client Code: TF0001443

Received On: 03/06/2019
Reported On: 03/18/2019

Eurofins Sample Code:	153-2019-03060147	**Sample Registration Date:**	03/06/2019
Client Sample Code:	06/25/18	**Condition Upon Receipt:**	acceptable
Sample Description:	Hybrid Cheddar	**Sample Reference:**	Set 1
Sampling Date	03/04/19 12:00		
Sampled By	Client		
Sampling Temperature	4°C		

QV063 - Total Protein Kjeldahl Duplicate	**Reference**	**Accreditation**	**Analysis Completed**	**Sub**
	AOAC 991.20	A2LA 3500.01	03/18/2019	√

Parameter	**Result**
Crude Protein (Nx6.38) 1	25.95 %
Crude Protein (Nx6.38) 2	26.08 %
Average Crude Protein	26.02 %

Subcontracting partners:

Eurofins DQCI (Mounds View), MN

Respectfully Submitted,

Robert Kurz
Chemistry Supervisor

Eurofins DQCI Horsham

Eurofins DQCI Horsham

702 Electronic Drive
Horsham, PA 19044
+215 355 3900
DQCIEastInterco@eurofinsus.com

Michigan State University

Zeynep Ustunol
469 Wilson Road
Toom 204
East Lansing, MI 48864

Analytical Report

AR-19-TF-002773-01

Client Code: TF0001443

Received On: 03/06/2019
Reported On: 03/18/2019

Eurofins Sample Code:	153-2019-03060148	**Sample Registration Date:**	03/06/2019
Client Sample Code:	06/25/18	**Condition Upon Receipt:**	acceptable
Sample Description:	Hybrid Cheddar	**Sample Reference:**	Set 2
Sampling Date	03/04/19 12:00		
Sampled By	Client		
Sampling Temperature	4°C		

QV063 - Total Protein Kjeldahl Duplicate	**Reference**	**Accreditation**	**Analysis Completed**	**Sub**
	AOAC 991.20	A2LA 3500.01	03/18/2019	√

Parameter	**Result**
Crude Protein (Nx6.38) 1	24.33 %
Crude Protein (Nx6.38) 2	24.65 %
Average Crude Protein	24.49 %

Subcontracting partners:

Eurofins DQCI (Mounds View), MN

Respectfully Submitted,

Robert Kurz
Chemistry Supervisor

Eurofins DQCI Horsham

Eurofins DQCI Horsham

702 Electronic Drive
Horsham, PA 19044
+215 355 3900
DQCIEastInterco@eurofinsus.com

Michigan State University

Zeynep Ustunol
469 Wilson Road
Toom 204
East Lansing, MI 48864

Analytical Report

AR-19-TF-002772-01

Client Code: TF0001443

Received On: 03/06/2019
Reported On: 03/18/2019

Eurofins Sample Code:	153-2019-03060149	**Sample Registration Date:**	03/06/2019
Client Sample Code:	06/25/18	**Condition Upon Receipt:**	acceptable
Sample Description:	Hybrid Cheddar	**Sample Reference:**	Set 3
Sampling Date	03/04/19 12:00		
Sampled By	Client		
Sampling Temperature	4°C		

QV063 - Total Protein Kjeldahl Duplicate	**Reference**	**Accreditation**	**Analysis Completed**	**Sub**
	AOAC 991.20	A2LA 3500.01	03/18/2019	√

Parameter	**Result**
Crude Protein (Nx6.38) 1	24.99 %
Crude Protein (Nx6.38) 2	25.13 %
Average Crude Protein	25.06 %

Subcontracting partners:

Eurofins DQCI (Mounds View), MN

Respectfully Submitted,

Robert Kurz
Chemistry Supervisor

APPENDIX G: Calcium and magnesium content of reference material (bovine liver) for the validation of the atomic absorption spectrophotometer.

Table 7.2. Calcium and magnesium content of bovine liver (BL, Reference Material 1577b; Institute of Standards and Technology).

	Calcium (μg/0.5 g of BL)	Magnesium (μg/0.5g of BL)
Reference content	58.00	300.5
Experimental content	64.74 ± 1.00	290.51 ± 2.89

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