ABSTRACT

Title of Thesis: SEASONAL VARIATION IN GOAT'S MILK COMPOSITION AND ITS EFFECT ON CHEESE QUALITY

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The composition of goat cheese was evaluated over a 12-month period to evaluate the influence of seasonal variation in goats' milk composition on variation in yield and composition of cheeses. Milk analysis included total fat, nonfat solids, and total protein. Yield was significantly correlated with milk composition. Cheese analysis included moisture content, water activity, crude lipid content, and ash content. Goat's milk was found to have significant variation in all parameters between seasons, with peak content in winter months. Significant differences were found in the compositions of cheeses, although not all followed the seasonal trends observed in milk. Correlations between milk and cheese compositions were evaluated but not found to be significant. Finally, an in-house environmental monitoring plan for *Listeria spp.* was evaluated using Hygiena® swabs. The in-house method was accurate in 78% of samples with no instances of false negatives.

SEASONAL VARIATION IN GOAT'S MILK COMPOSITION AND ITS EFFECT ON CHEESE QUALITY

By Stratton Thomas Wimsatt

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This thesis is dedicated to my parents, Andy and Ellen Wimsatt.

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Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

1.1 Overview of Cheese

1.1.1 Brief History of Cheese

Predating written language by about 2,000 years, the development of cheese was a crucial point in human history. It allowed Neolithic humans to preserve a nutrient dense food source for longer periods, allowing for longer-distance travel (Salque, 2013). The first cheeses were acid-coagulated and were hypothesized to have originated when clay milk-storage pots were left in the sun, leading to fermentation, acidification, and subsequent curdling of milk. The origin of rennet-coagulated cheese was likely the result of the discovery of cheese curds in lamb stomachs, either during slaughter or in using cured stomachs as a vessel for storing and transporting milk. Soon after the association between the young ruminants' stomachs and curdled milk was established, the process could be recreated using either epithelial scrapings or by reserving curds to be added to a new batch of milk (Kindstedt, 2012). In addition to its role as a preserved food, the development of cheese was also one of the early probiotic fermented foods. *Lactobacillus spp.* and *Lactococcus spp.* have since come to be understood as fundamental genera in human health through modulation of immune function and maintenance of gut health (Darby, 2019).

1.1.2 Characteristics of Milk and Cheeses in Modern Production

The choice of milk for cheesemaking has traditionally been a factor of the unique properties that the dairy animal imparts in her milk. For example, sheep's milk creates

firmer curds than cow's and goat's milk given that it has nearly double the amount of fat and casein protein (Jandal, 1996), and goat's milk produces cheese with a tangier flavor than cow's due to the higher representation of medium-chain fatty acids in total fat, even though the total lipid content is similar (Chilliard, 2003). In 2018 over 500,000 metric tons of goat cheese was produced worldwide, with about 250,000 metric tons produced in Africa and over 200,000 metric tons produced in Europe (FAO, 2021). Goat herding in the United States is less prominent than in Africa, Asia, and Europe; however, it has been on the rise, doubling in heads of livestock between 1983 and 2008 (FAO, 2021) (Miller, 2019).

Today, cheeses of all kinds are made using the same general technique. Pasteurized milk is inoculated with any ripening cultures followed by the coagulant (acid or rennet), which forms a protein gel. The gel is cut to allow water and whey protein to escape from the curd. The curd is then drained, salted, and formed into the appropriate shape for either immediate packaging or aging, where the surface can be inoculated with bacteria or filamentous molds (Hill, 2021). The unique varieties of cheeses come from the choices of ingredients and processing steps that distinguish them from one another.

The first distinguishing trait is whether the milk is acid-coagulated or enzyme-coagulated. Acid coagulation is generally reserved for fresh cheeses with a loose texture, while rennet coagulation is preferred for cheeses with more structure, due to the firmer curds produced from casein micelles. The term "rennet" traditionally has referred to the chymosin and pepsin enzymes found in calf, lamb, and goat kid stomachs. In addition, vegetable- and microbially-derived enzymes have gained popularity for their advantages

in terms of availability and economic scalability, as well as the ability to classify the final cheese products as suitable for a vegetarian diet (Wallace, 1922) (Thakur, 1993). Enzymatically coagulated cheeses can be further categorized by their moisture content, texture, and added ripening cultures: hard cheeses can contain 26%-50% moisture and are limited to surface-ripening molds, if any at all; semi-hard varieties will contain 42%-52% moisture and are usually inoculated with surface-ripening molds; semi-soft cheeses contain 45%-55% moisture and contain both surface-ripening molds and internal-ripening bacteria; and soft cheeses can contain 48%-80% moisture, and may or may not have any ripening inoculum (Varnam, 1994).

1.2 Seasonal Variation in Milk Production and Composition

1.2.1 Trends of Seasonal Variation in Milk Composition

The phenomenon of seasonal variation in volume and composition of milk has been previously observed in many breeds of goats and cows. Guo et al (2001) showed an inverse relationship between production volume and concentrations of fat, protein, and total solids in goat's milk collected from Vermont and New Hampshire, with peak richness occurring between November and February and peak production volume between March and July. Bhatta et al (2015) tracked the composition of goat's milk produced in the Purulia district of India, finding significant variation between hotter and more mild seasons. Heck et al (2009) reported cow's milk composition increasing in lipid and protein between June and January in the Netherlands. Overall milk density has also

been linked to variation between seasons, with all components seeing an increase in concentration in autumn (Parmar, 2020)

However, there are more possible influences than climate alone. Linzell et al (1973) observed an oscillating trend of volume of goat's milk produced, indicating higher volume output during longer, hotter summer months than during winter; however, this trend was also seen in goats kept in constant temperature and light cycle conditions, suggesting that the effect may be independent of seasonal conditions themselves. It was later observed in Australian dairy goats that the season in which the goats give birth, as well as the number of times the animal has previously given birth, had the most impact on volume and composition of milk (Zamuner, 2020). Auldist et al (1998) compared the composition of cow's milk from early-, mid-, and late-lactation periods, starting at different points in the season. They found that while there are some components of milk only vary with the changing of seasons, concentration of many components increases throughout the course of lactation. A similar increase in nutrient density in late lactation has also been observed in goat's milk by Kljajevic et al (2017).

Variation in the composition of bulk milk is to be expected, as it represents the combination of a herd of individual animals with room for intrinsic and environmental variation between animals. However, these kinds of deviations can impact the farmers and cheesemakers who depend on consistency to be able to predict the expected production level in any given season. One suggested alternative is to manufacture different dairy products when milk composition is more suitable (Chen, 2014), but this might not be possible or favorable for smaller manufacturers. The generation of a predictive model for estimating yield of chevre (a fresh goat's milk cheese) by correlating

it with individual constituents of goat's milk has been explored by Guo (2004), but it does not account for the known seasonal variation in milk composition. Curd yield can also be impacted by the concentrations of lipid and protein (Pazzola, 2019). Without accounting for the known variation, farmers and cheesemakers can risk unintended financial penalties, highlighting the need for a more robust yield model that includes predictable variation (Pirisi, 2007).

1.2.2 Means of Standardizing Milk Against Seasonal Variation

Cheesemakers depend on a degree of uniformity in milk composition to be able to manufacture a consistent product in both quality and quantity year-round. Two ways to reduce the effect of milk variation and to create a standardized product are skimming cream and addition of nonfat milk powder (Hill, 2021). Commercial skimming is commonly achieved via cold or warm centrifugation. More thorough standardization could be achieved through membrane separation of milk. Membrane separation, also referred to as ultrafiltration, is recognized by the U.S. Food and Drug Administration as a viable way to produce standardized cheeses (FDA, 2017). There are many commercially available ultrafiltration units available. There are general guidelines for composition of milk based on the cheese to be produced, (Hill, 2021); however, these methods can require equipment that smaller artisanal manufacturers lack the space or funds to acquire. In lieu of large-scale standardizing equipment, smaller manufacturers may be able to achieve some degree of standardization if sufficient links between milk composition and cheese composition can be elucidated, as the present study intends to do.

1.3 Safety

1.3.1 Cheese as a Ready to Eat Product

As a ready-to-eat product with no kill step between manufacture and consumption, cheeses are susceptible to carry pathogens if not properly handled. Because of the refrigerated conditions of cheese manufacture and storage, *Listeria monocytogenes* is of unique interest. In the United States, six *L. monocytogenes* outbreaks have been linked to cheeses since 2012, resulting in 84 illnesses, 78 hospitalizations, and 12 deaths (CDC, 2021). This risk is largely mitigated by pasteurization and sanitation, as well as the fact that some subspecies of *Lactococcus lactis* (one of the most common ripening bacteria in cheese) to produce bacteriocins (Alegría, 2010). Although, due to the prevalence of *L. monocytogenes* to persist in the environment, manufacturers cannot rely on competitive inoculum and must take extra precaution in monitoring the facility for presence of *L. monocytogenes* in the production and holding areas. Per FDA industry guidance, manufacturers need to be able to identify areas with potential to acquire and spread contamination, adequate frequency of testing, and valid corrective actions if contamination is discovered. (FDA, 2017).

1.4 Research Objectives

While the effect of seasonal changes to goat's milk has been documented, there is still a need to connect that compositional variability with the final product. To achieve this, the following research objectives are:

- To investigate the year-round variability in composition of milk from goats raised in Maryland, West Virginia, and Pennsylvania. I hypothesize that there will be significant differences in composition between seasons.
- To correlate the measured components of goat's milk with cheese yield accounting for changes between seasons in composition. I hypothesize that there will be a significant difference between yield correlations of different seasons.
- To track composition of the finished cheeses throughout the year to observe for significant variation in cheese composition. I hypothesize that cheese composition will undergo significant changes during the sampling period.
- To investigate whether there exists a significant correlation between composition of milk and composition of cheese for lipid and nonfat solids. I hypothesize that there will be a significant correlation for each.
- To compare the efficacy of an in-house environmental monitoring against a PCR-based service for the detection of environmental *Listeria* species. I hypothesize that the in-house method will be equally as effective as commercial services.

Chapter 2: Materials & Methods

2.1 Milk Sample Collection and Analysis

2.1.1. Milk Sample Collection and Analysis

Goat's milk from seven farms located in Maryland, West Virginia, and Pennsylvania and stored at 4 °C in commingled tanks until pasteurized and manufactured into cheese. Milk samples were collected from February of 2019 through January of 2020. Chemical composition of raw milk received by the facility from the farms was measured using a LactiCheck™ RapiRead Ultrasonic-Spectroscopic Milk Analyzer (LC-02/RR). (Weber Scientific, Hamilton, New Jersey, USA). Milk samples were tested on site at the cheese manufacturing facility in Maryland on the days the milk was delivered. The parameters measured were lipid content, total protein content, and nonfat milk solids (NFS) since these three components make up most of the cheese matrix. Measurements were taken for deliveries from each farm individually as well as for milk from the comingled tanks and recorded as bulk average. Bulk averages were then categorized seasonally for analysis (Spring = March-May; Summer = June-August; Fall = September-November; Winter = December-February).

2.1.2. Yield Analysis

Cheese yield was measured and analyzed against milk composition to observe for correlation. Yield was calculated as a percentage using the equation:

$$\%\ \text{Yield} = \frac{\text{lb of Cheese Produced}}{\text{lb of Milk Used}} \times 100\%$$

2.2 Cheese Sample Collection and Analysis

2.2.1. Cheese Sample Collection

There were five cheeses sampled during this period, all of which were made from goat's milk, salt, rennet, and various ripening cultures. The first type was a fresh goat cheese (FGC), a soft cheese with a spreadable consistency. It was unique among those sampled in that it was not aged, rather, rennet-coagulated and strained in cheesecloth while lactic acid bacteria continued to ferment. The next style of cheese was a semi-soft, brie-style goat's milk cheese (BSC) which was aged to develop a bloomy white rind. An aged brie cheese (ABC) underwent extended aging to develop a free-flowing core consistency through proteolysis from fermentation. The only semi-firm cheese was a Basque-style, washed rind cheese (WRC) with a thin, orange rind. The last cheese style was semi-soft with a blue mold rind (BMR). Samples were shipped overnight on ice to University of Maryland where they were stored at 4 °C until further analysis. Sampling was limited to the production schedule of the different cheeses. Because of this, the durations of sampling periods were not uniform. All cheeses were provided by a manufacturer based in Maryland.

2.2.2. Moisture Analysis

Moisture content of cheeses was determined based on methods outlined by Bradley (2001). Samples were analyzed using an HE73 Moisture Analyzer (Mettler-Toledo, Columbus, Ohio, USA). Approximately 1 g of sample was placed into an aluminum tray which was then loaded into the analyzer. The sample chamber is heated to 110 °C and measures loss of mass to indicate moisture evaporation. The process ends

automatically when loss of mass is no longer detectable, and the moisture content displayed on the screen as a percentage automatically. Measurements were taken in triplicate for all samples to collect the average and standard deviation.

2.2.3. Water Activity Measurement

Water activity is a commonly measured parameter of pathogen growth; however, in fermented foods, it is also important in understanding the inoculum's growth environment. Water activity was measured using an HP-AW-A Water Activity Meter (Rotronic Instrument Corp., Hauppauge, New York, United States). Sample cups were filled halfway with cheese and placed into the sample chamber. Once loaded, water activity was automatically measured. Measurements were taken in triplicate for all samples to collect the average and standard deviation.

2.2.4. Freeze Drying

Samples were prepared for lipid and ash measurements via freeze drying in a freeze dryer (Harvest Right, North Salt Lake, Utah, United States). Samples were frozen for 24 hours before the drying process to protect the integrity of samples. To confirm completion, dried samples were tested in the moisture analyzer. Samples are considered successfully dried if the moisture content after drying was lower than ~0.05%. This value was selected since the manufacturer lists 0.05% as the lowest standard deviation of repeatability.

2.2.5. Crude Lipid Measurement

Lipid content was measured using the Soxhlet extraction method with petroleum ether as the solvent. Approximately 1 gram of freeze-dried sample is loaded into a

standard cellulose thimble (Cytiva, Marlborough, Massachusetts, United States) and placed in the extraction tube, and the 25 mL round bottom flask was filled with 15 mL of petroleum ether. Extractions were performed for two hours after the first solvent reflux was observed. After completion, the solvent was transferred to a glass vial. The vial was then submerged halfway in a heated water bath at 60 °C under a flow of compressed nitrogen gas to gently evaporate the solvent from the mixture, leaving behind only the extracted lipid. The final mass was weighed and used to calculate lipid content as a percent of dry basis and wet basis composition. Extractions were performed in triplicate for each sample to collect the average and standard deviation.

2.2.6. Inorganic Ash Measurement

Ash samples were initially prepared by incineration at 500 °C for 48 h in the laboratory's muffle furnace. However, when some samples required an additional 24 h in the furnace to reach completion, and the incineration time was changed to 72 h for the remainder of study. The procedure was determined to be complete when the remaining matter was uniformly white to light gray in color as described by Jorhem et al. (2000). The final mass was weighed and used to calculate inorganic ash content as a percent of dry and wet basis composition. Ash samples were intended to undergo inductively coupled plasma mass spectrometry (ICP-MS) to determine proportions of sodium, calcium, and iron, chosen for their roles in flavor, yield (Fagan, 2007), and rind formation (Monnet, 2012); however, the resources to achieve this within the project's budget was not found.

2.2.7 Correlating Milk Composition with Final Cheese Composition

To evaluate the effect of seasonal variation in milk composition on variation in the attributes of cheeses produced from that milk, quality parameters between the two were correlated using linear regression, and the significance of this relationship was statistically evaluated. The main parameters of interest for cheeses are fat, moisture content, and nonfat solids for their roles in flavor and quality.

2.3 Environmental Monitoring Analysis

2.3.1 Comparison of In-House Environmental Monitoring with Commercial Service

An in-house environmental monitoring method was developed around the manufacturer's existing sampling plan as a potentially more cost-effective method of pathogen detection. Ten sites were sampled monthly around the production and food handling zones of the facility. The in-house method was developed using InSite™ *Listeria* swabs (AOAC 121902) from Hygiena™ (Camarillo, CA, United States) and was compared against results the manufacturer's commercial provider of environmental testing, which used PCR to identify contaminants. Samples were collected by swabbing a 10 cm × 10 cm square with either the included swab (Hygiena™) or sterile sponge (Microbac™) from the same location. Samples from the in-house method were shipped overnight to University of Maryland, College Park where they were activated upon arrival and incubated at 37 °C for 48 h and observed for colorimetric indication of *Listeria spp.* Sponges for the commercial service were shipped overnight to Microbac Laboratories (Cranberry Township, PA, United States). Samples were collected for 12

months (November 2018 – January 2020), and locations where samples were taken were masked from researchers with numerical codenames. Samples of the enriched broth from presumptive positive InSite™ swabs were plated on PALCAM agar to confirm presence or absence of *Listeria spp.*, but no further speciation took place. Results were classified as a positive match, negative match, false positive or false negative. A false positive would denote a positive InSite™ result and negative PALCAM confirmation. A false negative would occur when a negative InSite™ result coincided with a positive PCR result.

2.4 Statistical Analysis

2.4.1. Statistical Analysis

Statistical analysis for all measurements was conducted using Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, United States). For milk analysis experiments, significance of variation between mean values of seasons was determined using Welch's t-test to account for unequal variance. Where correlation coefficients were calculated, the significance of correlations was determined using a t-test for correlation. Variation between batches of cheeses was analyzed for significance using Welch's t-test to account for unequal variance. Significance was set to $\alpha = 0.05$ for all statistical analyses.

Chapter 3: Results

3.1 Milk Analysis

3.1.1. Seasonal Variation of Composition

Welch's t-test was employed to compare the means of the measured parameters of bulk milk between each season. Significant differences ($p<0.05$) were found between all seasons for all measured parameters (Figure 1). Bulk milk analysis results for fat, protein, and NFS were first plotted against the date of the milk delivery to observe visible trends in seasonal variance before performing t-tests. Figures 2a, 2b, and 2c demonstrate the trend, indicating that milkfat, protein, and NFS content are at their highest during winter and at their lowest during summer, in agreement with the literature. The abrupt changes to protein content in Figure 2c are the result of calibration of the milk analyzer, marked by vertical lines.

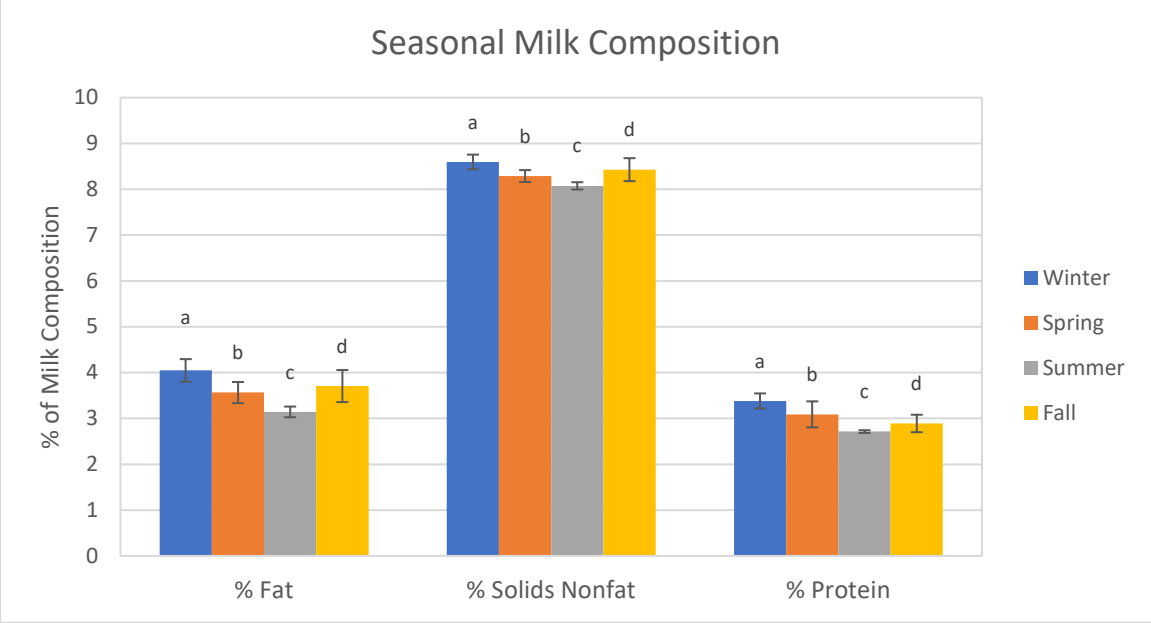


Figure 1 - Seasonal Averages of Milk Composition. Letters above columns indicate significance ($p<0.05$)

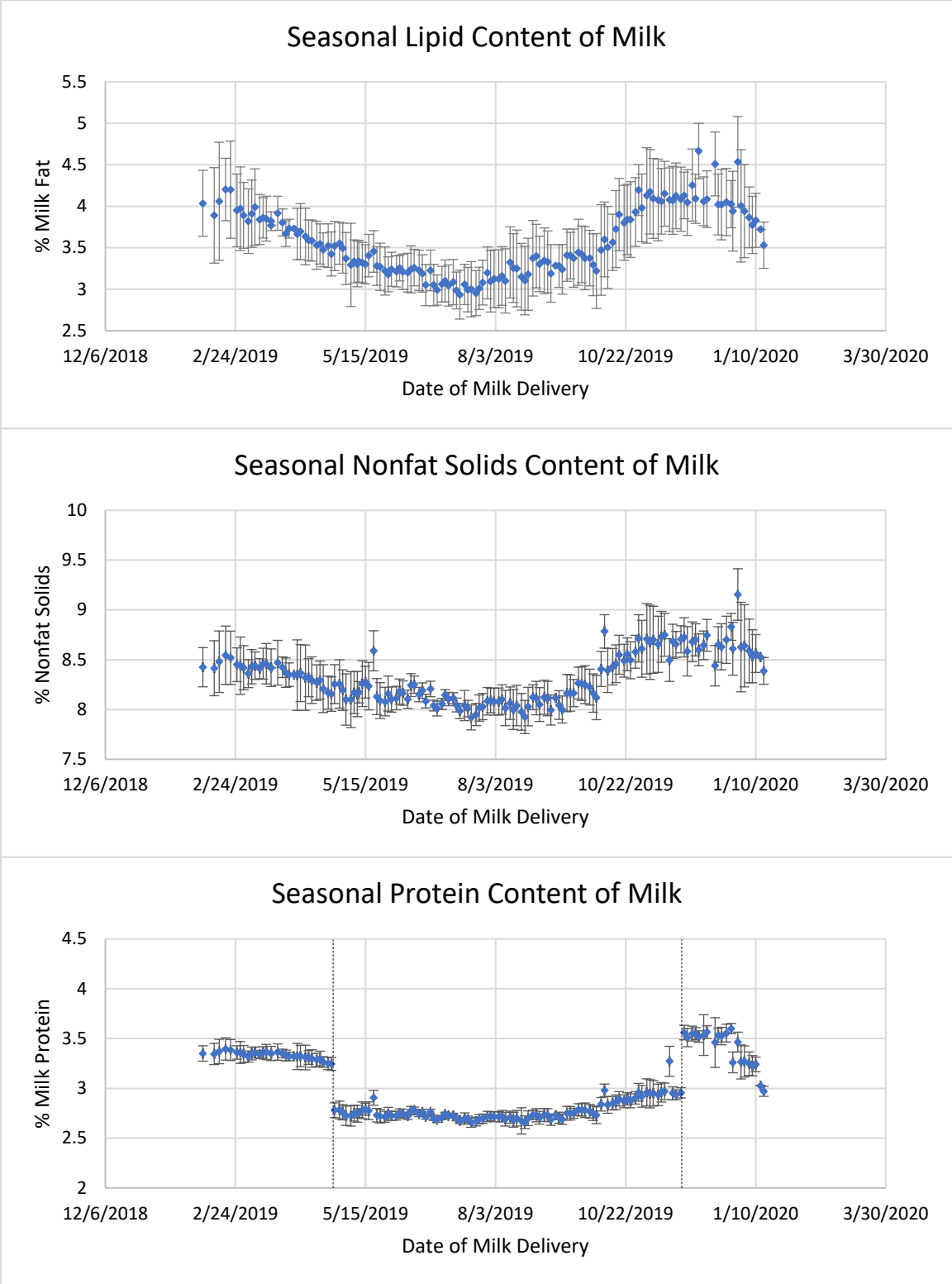


Figure 2a, 2b, and 2c (top to bottom) - Seasonal Trends of Milkfat (a), NFS (b), and Protein (c). Vertical markers in 2b indicate point of equipment calibration. Vertical lines in figure 2c represent equipment calibration points.

3.1.2. Yield Analysis

Yield variation was calculated by dividing the weight (in lbs.) of FGC produced by the weight (in lbs., converted from gallons) of milk used for the batch. Yield was then plotted against the date of milk delivery to observe if there was a similar trend. Figure 3 shows a similar seasonal pattern to the plots of fat, protein, and NFS content, although the trend is less pronounced. The variations in yield between seasons were found to be statistically significant ($p<0.05$) except between fall and winter. The only data collected for yield analysis come from the FGC batches, because it most closely resembles the curd which would be subject to other aging conditions to produce the other varieties.

To understand the relationship, if any, between fat, protein, and NFS content and ultimate yield, a linear regression was plotted for each relationship. Figures 4a–4c suggest that the lipid content of milk has a stronger correlation ($r=0.8605$) with yield than protein ($r=0.7656$), while NFS had the overall highest correlation ($r=0.8613$). All three correlation coefficients, however, were statistically significant ($p<0.05$).

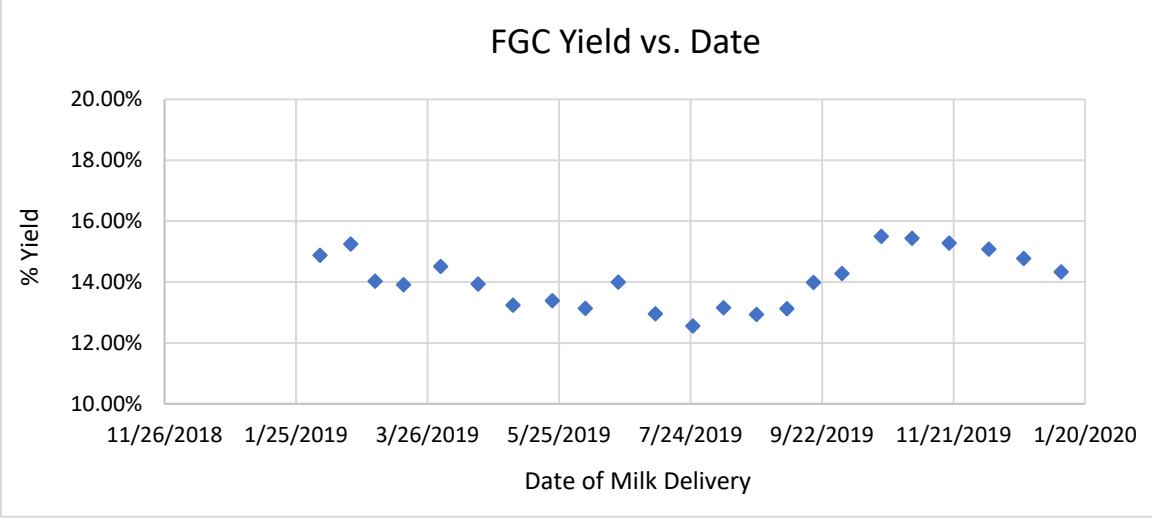
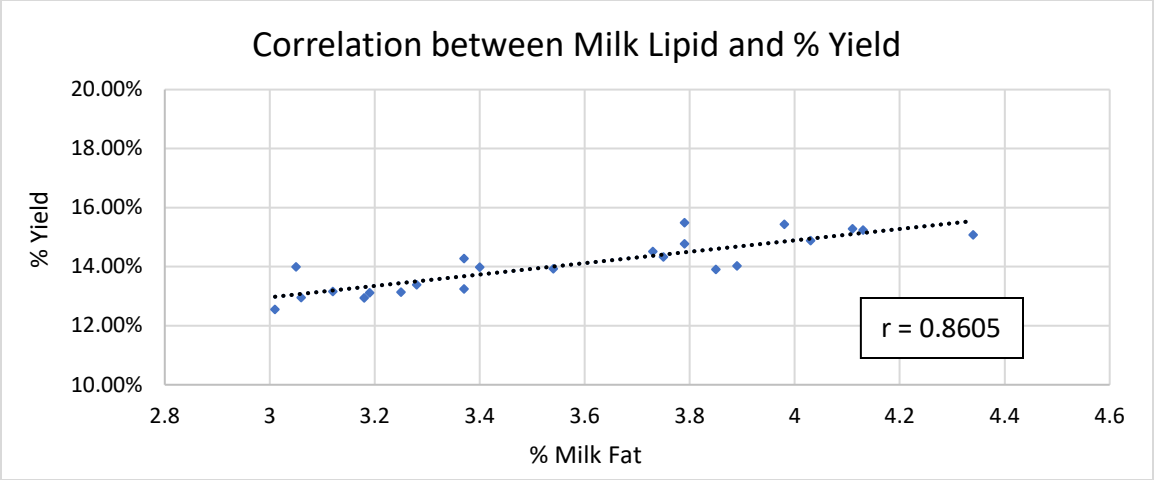
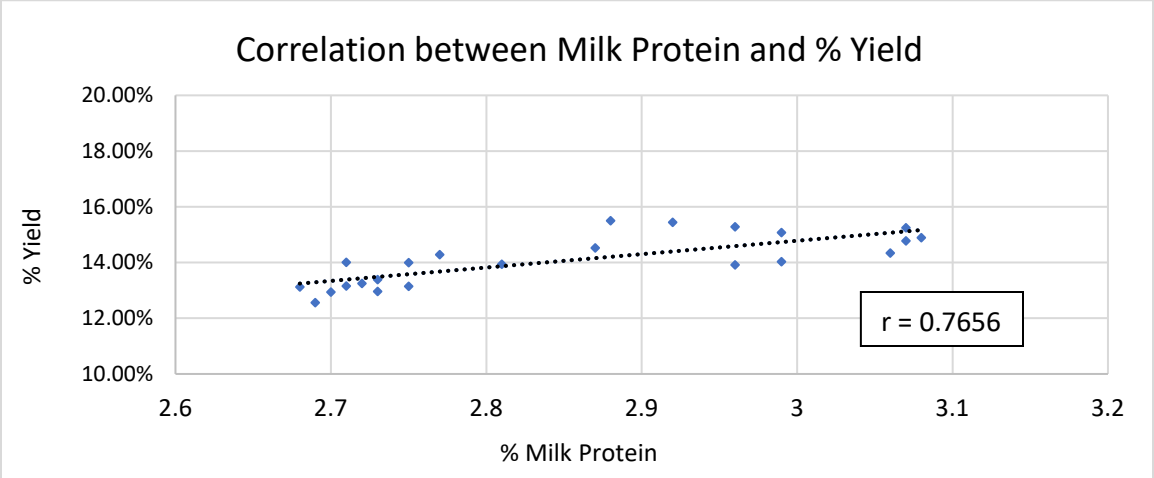


Figure 3 – Yield of Fresh Goat Cheese by Milk Delivery Date





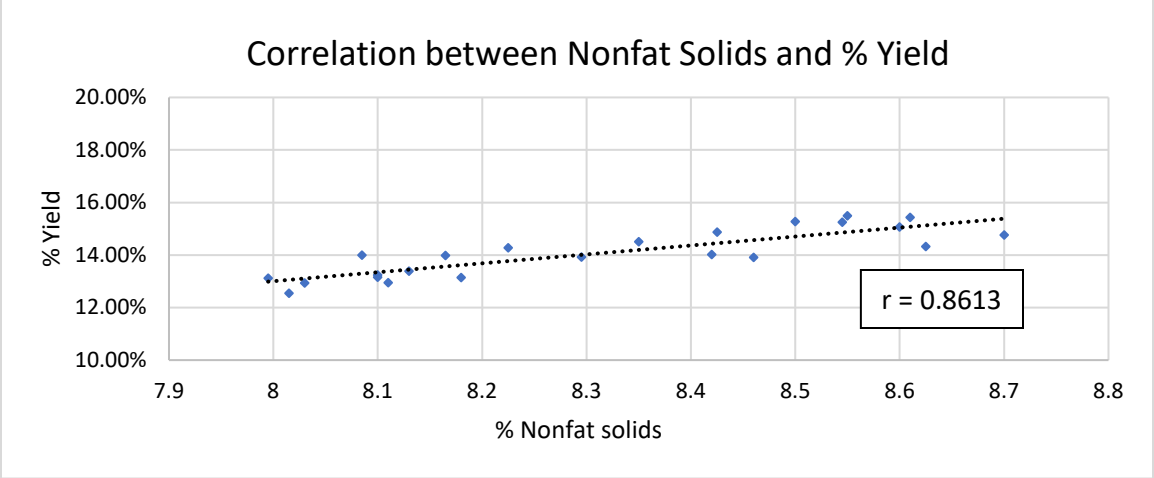


Figure 4a, 4b, and 4c (top to bottom) - Correlations between Milkfat, Protein, and NFS and Fresh Goas Cheese Yield.

3.2 Cheese Analyses

3.2.1. Moisture Analysis

Table 1. Moisture Content (%) of Cheeses

	Batch Number	***Milk Delivered***	***% Moisture ± SD***
BSC			
	11208	9/18/2019	$47.13^{ad} \pm 1.43$
	11252	9/30/2019	$52.21^{b} \pm 1.14$
	11494	12/11/2019	$52.93^{bc} \pm 1.31$
	11605	1/15/2020	$50.00^{abd} \pm 0.95$
	Average		50.57
ABC			
	10441	2/11/2019	55.65 ± 0.69
	10506	3/8/2019	54.19 ± 1.24
	10540	3/22/2019	56.98 ± 0.27
	Average		55.61
WRC			
	10424	2/4/2019	$38.47^{a} \pm 0.58$
	10440	2/11/2019	$41.77^{b} \pm 0.62$
	11445	11/25/2019	$45.00^{b} \pm 2.41$
	Average		41.75
BMR			
	10547	3/25/2019	$54.10^{a} \pm 0.39$
	11247	9/30/2019	$50.34^{b} \pm 1.11$
	11482	12/9/2019	$47.30^{ab} \pm 4.53$
	11528	12/23/2019	$48.79^{ab} \pm 3.46$
	Average		50.13
FGC			
	10437	2/7/2019	65.73 ± 1.17
	10625	4/18/2019	63.40 ± 0.66
	11200	9/18/2019	65.19 ± 2.61
	Average		69.47

Table 1 - Moisture Content of Cheeses.

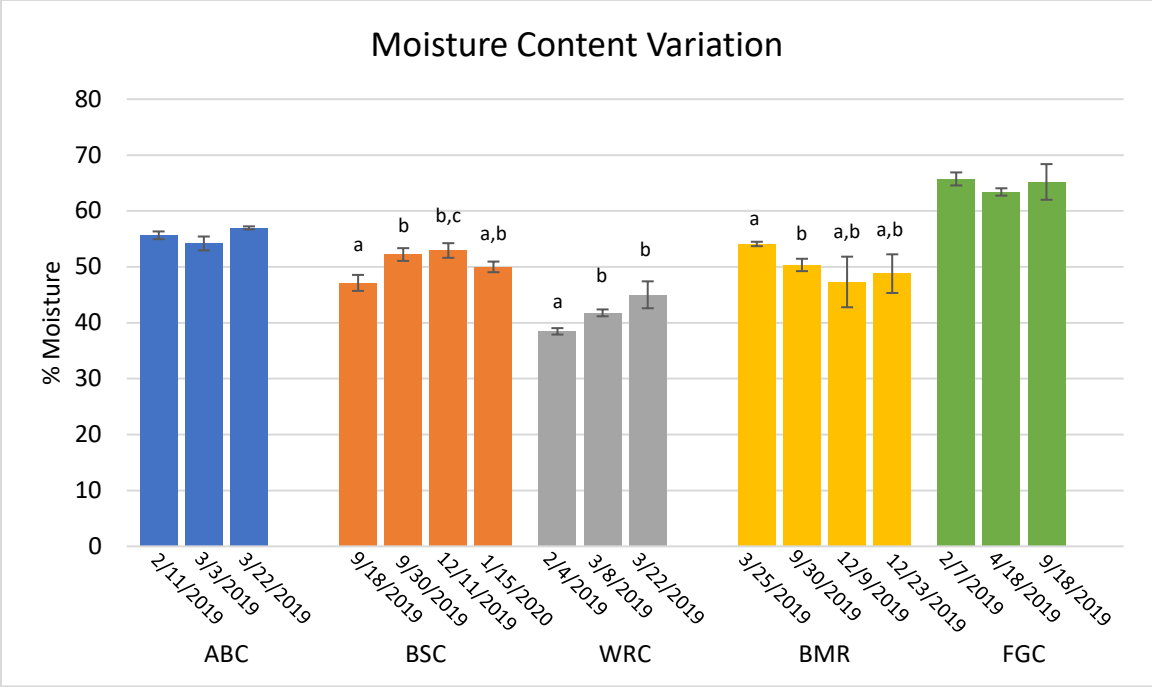


Figure 5 - Moisture Content of Cheeses. Dates under columns indicate delivery dates of milk used for cheese batch tested. Letters above columns indicate significance ($p<0.05$)

Welch's t-test yielded results indicating significant variation ($p<0.05$) in moisture content in three of the five cheeses. Moisture content significantly ($p<0.05$) increased from summer to winter for the BSC variety and decreased from spring to late summer for the BMR variety. A significant increase ($p<0.05$) in moisture was found during the duration of the winter season for the WRC variety, but it was not different from the fall measurement. No significant difference was recorded for the ABC or FGC varieties.

Final moisture of the cheeses affects quality perceptions because it directly impacts the concentrations of other flavor-determining components of the cheese in the form that consumers would experience. For measurements requiring the freeze-drying of cheese as preparation, the dry basis results are converted to wet basis using the equation:

$$Dry\ Basis\ \times [1 - \%\ Moisture(as\ a\ decimal)] = Wet\ Basis$$

3.2.2. Water Activity Analysis

Table 2. Water Activity of Cheeses

	Batch Number	*Milk Delivered*	$A_w \pm SD$
BSC			
	11208	9/18/2019	$1.000^{**} \pm 0.000$
	11252	9/30/2019	$0.933^{a*} \pm 0.007$
	11494	12/11/2019	$1.000^{**} \pm 0.000$
	11605	1/15/2020	$1.000^{**} \pm 0.000$
	Average		0.983
ABC			
	10441	2/11/2019	0.972 ± 0.012
	10506	3/8/2019	0.993 ± 0.001
	10540	3/22/2019	0.992 ± 0.001
	Average		0.986
WRC			
	10424	2/4/2019	$0.948^{a*} \pm 0.015$
	10440	2/11/2019	$0.860^{b*} \pm 0.037$
	11445	11/25/2019	$1.000^{c*} \pm 0.000$
	Average		0.936
BMR			
	10547	3/25/2019	$0.981^{a*} \pm 0.011$
	11247	9/30/2019	$0.912^{b*} \pm 0.006$
	11482	12/9/2019	$1.000^{**} \pm 0.000$
	11528	12/23/2019	$1.000^{**} \pm 0.000$
	Average		0.973
FGC			
	10437	2/7/2019	0.906 ± 0.015
	10625	4/18/2019	0.914 ± 0.038
	11200	9/18/2019	0.940 ± 0.021
	Average		0.916

Table 2 - Water Activity of Cheeses.

** = one group in the t-test was in violation of the maximum theoretical water activity*

*** = both groups in the t-test were in violation of the maximum theoretical water activity*

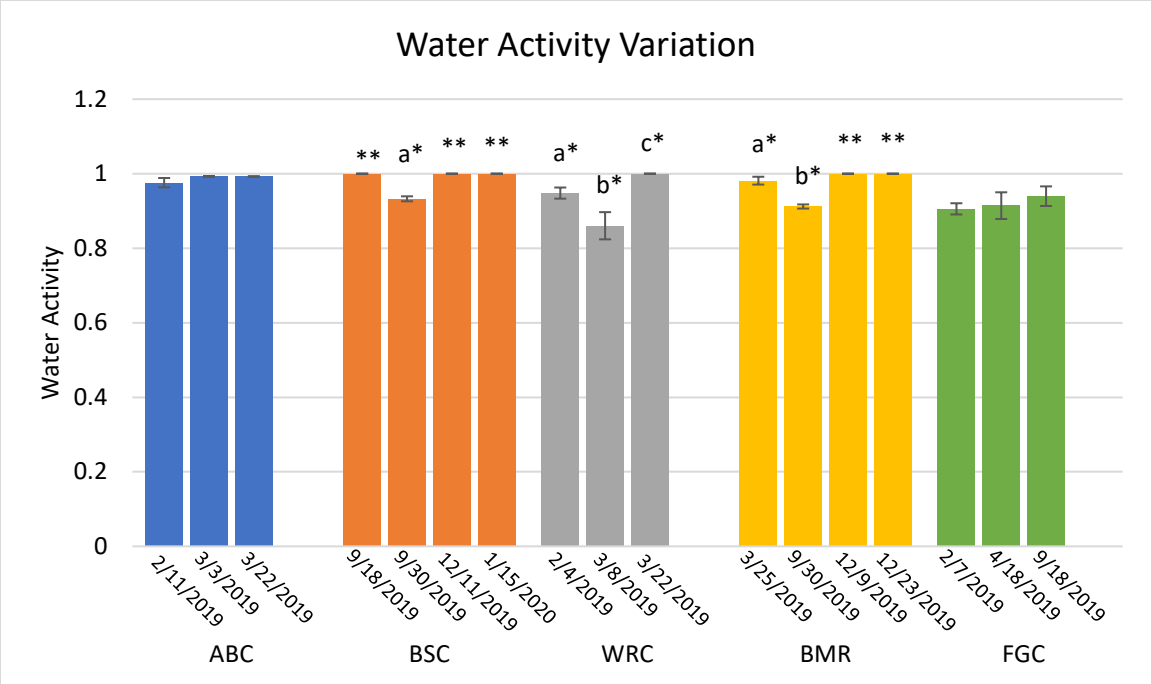


Figure 6 - Water Activity of Cheeses. Dates under columns indicate delivery dates of milk used for cheese batch tested. Letters above columns indicate significance ($p<0.05$)

** = one group in the t-test was in violation of the maximum theoretical water activity*

*** = both groups in the t-test were in violation of the maximum theoretical water activity*

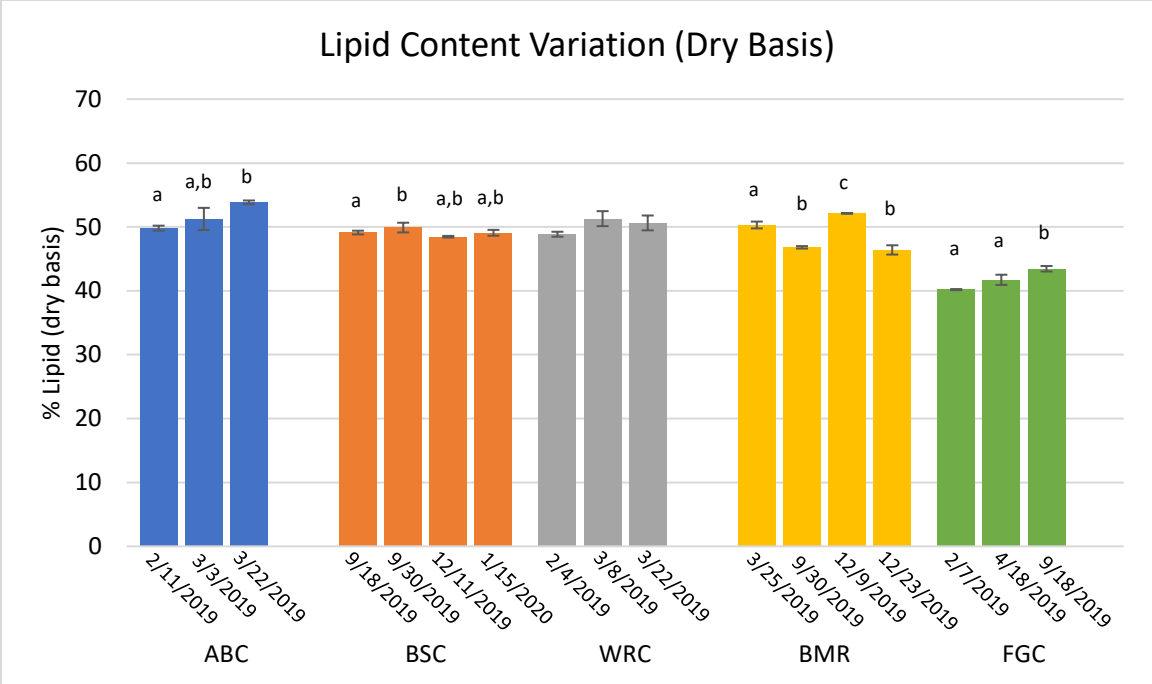
Three varieties of cheeses displayed at least one measurement above the theoretical maximum water activity of 1.000. These measurements were reduced to 1.000 and marked as unusable. The water activity meter was calibrated using standard solutions and confirmed with pure water, reading exactly 1.000; however, even after calibration, some samples would still read above 1.000 in all three runs of the triplicate. The source of the error was never discovered, but due to this error, the results of the BSC, WRC, and BMR varieties could not be evaluated. ABC and FGC did not encounter this error, but neither saw any significant change during the sampling period.

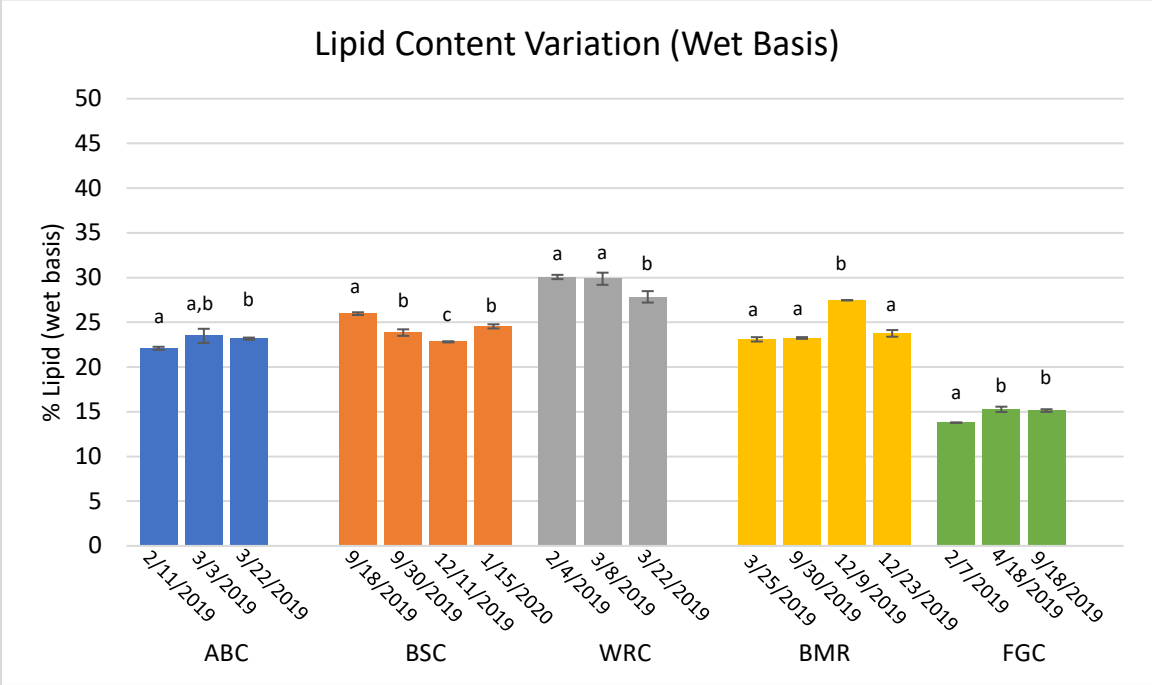
3.2.3. Lipid Content Analysis

Table 3. Lipid Content (%) (Dry and Wet Basis) of Cheeses

	Batch Number	*Milk Delivered*	*Dry Basis ± SD*	*Wet Basis ± SD*
BSC				
	11208	9/18/2019	$49.12^{a} \pm 0.29$	$25.97^{a} \pm 0.15$
	11252	9/30/2019	$49.90^{b} \pm 0.76$	$23.85^{b} \pm 0.36$
	11494	12/11/2019	$48.47^{ab} \pm 0.13$	$22.81^{c} \pm 0.07$
	11605	1/15/2020	$49.08^{ab} \pm 0.46$	$24.54^{b} \pm 0.23$
	Average		49.14	24.29
ABC				
	10441	2/11/2019	$49.81^{a} \pm 0.62$	$22.18^{a} \pm 0.17$
	10506	3/8/2019	$51.26^{ab} \pm 1.73$	$29.87^{ab} \pm 0.79$
	10540	3/22/2019	$53.86^{b} \pm 0.29$	$23.17^{b} \pm 0.12$
	Average		51.64	25.07
WRC				
	10424	2/4/2019	48.86 ± 0.39	$30.06^{a} \pm 0.24$
	10440	2/11/2019	51.30 ± 1.18	$30.56^{a} \pm 0.68$
	11445	11/25/2019	50.63 ± 1.16	$27.85^{b} \pm 0.64$
	Average		50.26	29.49
BMR				
	10547	3/25/2019	$50.31^{a} \pm 0.53$	$23.09^{a} \pm 0.24$
	11247	9/30/2019	$46.81^{b} \pm 0.19$	$23.23^{a} \pm 0.10$
	11482	12/9/2019	$52.11^{c} \pm 0.06$	$27.47^{b} \pm 0.03$
	11528	12/23/2019	$46.40^{b} \pm 0.73$	$23.76^{a} \pm 0.37$
	Average		48.91	24.39
FGC				
	10437	2/7/2019	$40.19^{a} \pm 0.09$	$13.80^{a} \pm 0.03$
	10625	4/18/2019	$41.72^{a} \pm 0.80$	$14.97^{b} \pm 0.30$
	11200	9/18/2019	$43.46^{b} \pm 0.43$	$15.13^{b} \pm 0.15$
	Average		40.80	12.53

Table 3 - Lipid Content of Cheeses (dry basis & wet basis).





Figures 7a and 7b (top and bottom) – Lipid Content of Cheeses (Dry Basis and Wet Basis). Dates under columns indicate delivery dates of milk used for cheese batch tested. Letters above columns indicate significance ($p<0.05$)

Lipid content was measured on both a dry and wet basis because each offers different information about the cheeses. Wet basis lipid content is the mass percentage of lipid in a cheese as it would appear on a store shelf. Dry basis lipid content refers to the ratio of lipid to other non-water components. Dry basis lipid content is measured because it is a more robust indicator of variation in lipid content, showing proportionality of lipid to other solids, while the wet basis measurement can variation in lipid content caused by variation in moisture content.

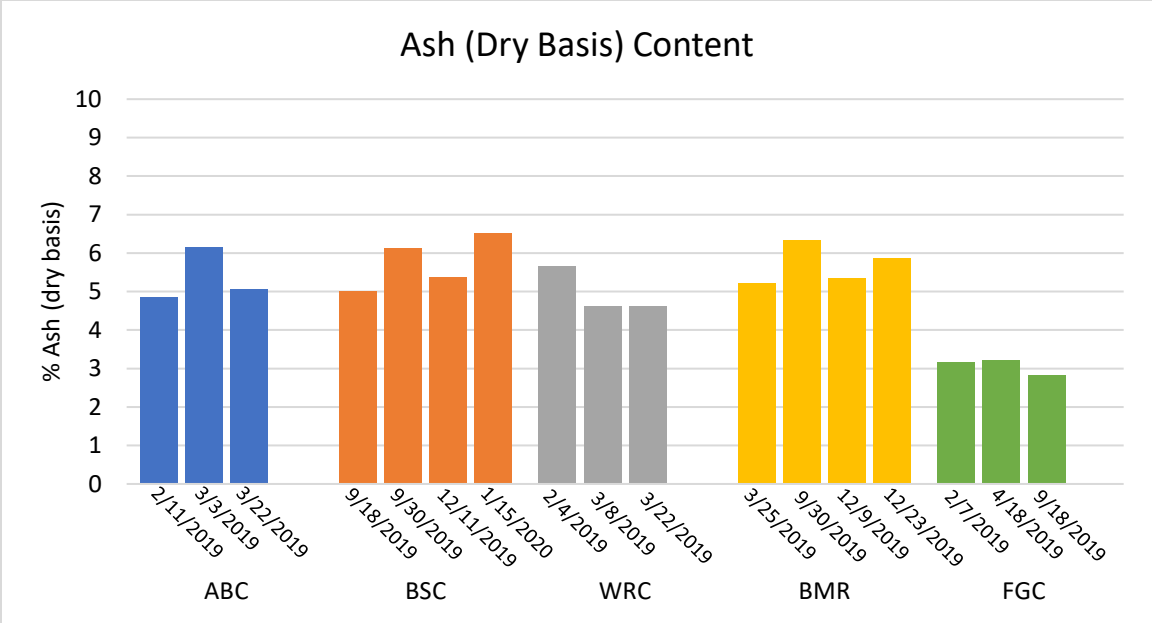
Dry and wet basis lipid variations are shown in Table 3 and Figures 7a-b. There was a significant ($p<0.05$) increase in dry basis lipid content of BSC from late summer to early fall as well as an increase for ABC from winter into spring. BMR saw a significant decrease in dry lipid content from spring into summer, then increased from summer to winter, while FGC Significantly increased between winter and summer. Wet basis lipid measurements generally followed the same patterns as dry basis, although proportionately affected by the moisture content, with significant variation ($p<0.05$) in all five cheese varieties.

3.2.4. Inorganic Ash Analysis (dry ant wet basis)

Table 4. Ash Content (%) (Dry and Wet Basis) of Cheeses

	Batch Number	*Milk Delivered*	*Dry Basis*	*Wet Basis*
BSC				
	11208	9/18/2019	5.01	2.65
	11252	9/30/2019	6.11	3.07
	11494	12/11/2019	5.36	2.56
	11605	1/15/2020	6.50	3.25
	Average		5.75	2.88
ABC				
	10441	2/11/2019	4.84	2.15
	10506	3/8/2019	6.15	2.82
	10540	3/22/2019	5.05	2.17
	Average		5.35	2.38
WRC				
	10424	2/4/2019	5.66	3.48
	10440	2/11/2019	4.62	2.96
	11445	11/25/2019	5.84	3.21
	Average		5.14	3.09
BMR				
	10547	3/25/2019	5.21	2.39
	11247	9/30/2019	6.32	3.14
	11482	12/9/2019	5.35	2.82
	11528	12/23/2019	5.86	3.00
	Average		5.69	2.84
FGC				
	10437	2/7/2019	3.15	1.08
	10625	4/18/2019	3.21	1.17
	11200	9/18/2019	2.81	1.09
	Average		3.55	1.04

Table 4 - Ash Content of Cheeses (dry basis & wet basis).



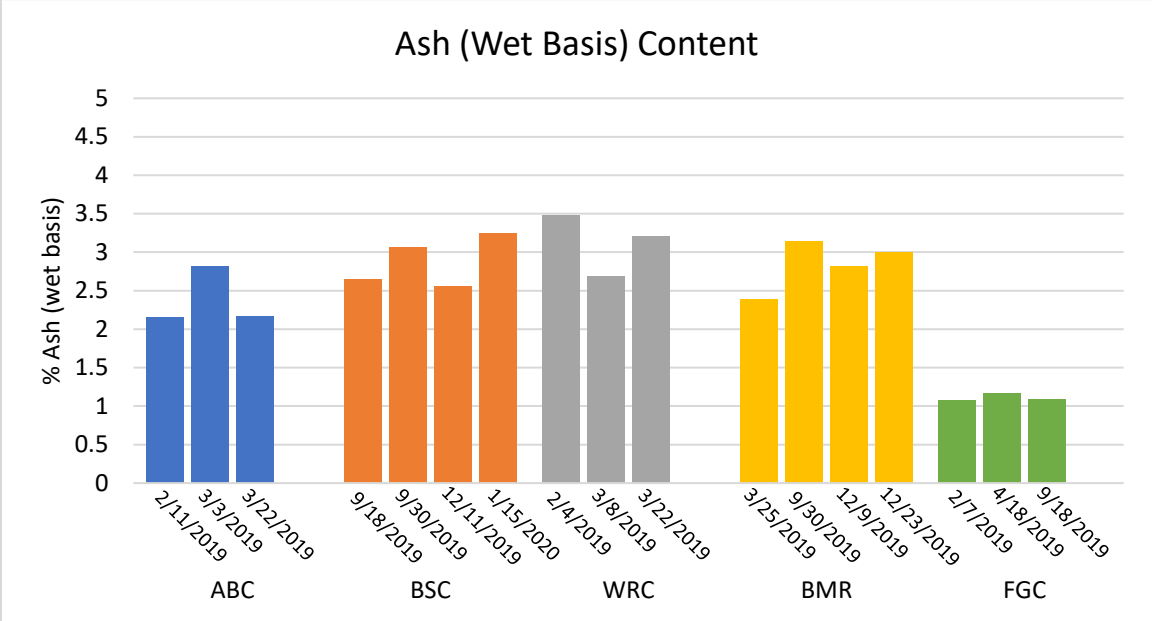


Figure 8a and 8b (top and bottom) – Ash Content of Cheeses (Dry and Wet Basis). Dates under columns indicate delivery dates of milk used for cheese batch tested.

Ash content of cheeses was not measured in triplicate, and statistical analysis could not be performed. While samples were intended to be analyzed for individual mineral composition through ICP-MS, a feasible means of accomplishing this was not identified during the experimental timeframe.

3.2.5. Correlating Milk Composition with Cheese Composition

Given the significance of variability of milk composition between seasons, the significance of the impact that milk composition has on final cheese composition was investigated. The only overlapping parameters measured between milk and cheese compositions were lipid content and NFS content (NFS was calculated by subtracting dry-basis lipid content from total dry weight, representing the rest of the cheese matrix). Although some correlations appeared to be strong ($r > 0.7$), none of the parameters showed any significant correlation between milk and any of the five cheeses when analyzed with a t-test. The correlation coefficients can be found in Table 5.

Table 5. Correlation Coefficients of Milk & Cheese Compositions.

	r (Fat, dry)	r (Fat, wet)	r (NFS, dry)	r (NFS, wet)
BSC	0.85094066	0.727873615	0.782815	0.491121
ABC	0.08	0.991866927	0.993932	0.336303
WRC	0.51681718	0.81178815	0.222486	0.805729
BMR	0.41521079	0.565862174	0.240624	0.236643
FGC	0.88396833	0.592368129	0.965039	0.493761

Table 5 - Correlation Coefficients of Milk & Cheese Compositions. Column 1: Milk Lipid vs Cheese Lipid (dry basis). Column 2: Milk Lipid vs Cheese Lipid (wet basis). Column 3: Milk NFS vs Cheese NFS (dry basis). Column 4: Milk NFS vs Cheese NFS (wet basis).

3.3 Environmental Monitoring Analysis

3.3.1 Comparison of In-House vs. Commercial Environmental Monitoring

The results of the InSite™ swabs were compared with the results of swabs sent to Microbac and classified as either a positive match, negative match, false positive, or false negative. Over the 12 months of samples collected, the rate of accuracy (combined true positive, true negative) between the two methods was 78.33%, with a false positive rate of 21.67%, and a false negative rate of 0.00%.

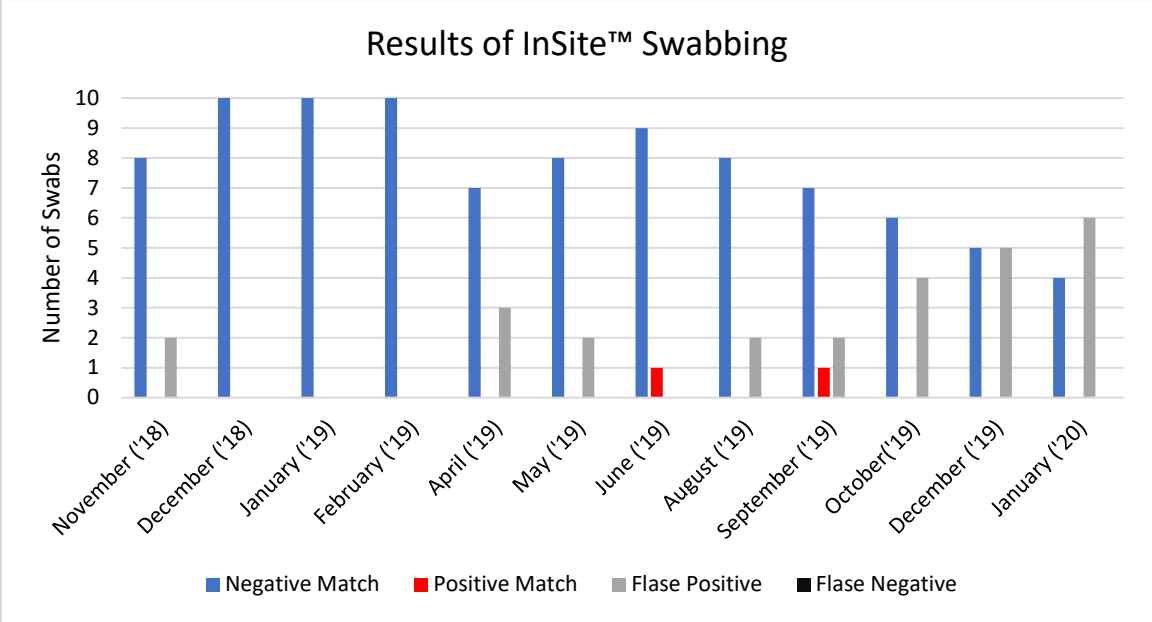


Figure 99 - Comparison of Environmental Monitoring Methods.

Chapter 4: Discussion

Milk Composition Analysis

Throughout the 2019 sampling period, significant variation in goat's milk composition was observed between seasons. Milkfat, total protein, and NFS were measured because of their roles in pricing, yield prediction, quality impact, and all exhibited a trend of higher concentrations in the winter which declined through summer and rebounded into the fall. These results were in agreement with literature findings (Guo, 2001) (Bhatta, 2015) (Heck, 2009).

Yield also varied between seasons in the same pattern as milk composition, with significant variation ($p<0.05$) between all seasons except for the transition from fall to winter. Yield was also compared with individual components of milk to show significant correlations ($p<0.05$) between yield and milkfat, total protein, and NFS. Because the milk analyzer counts total protein content (including both casein and whey), and whey is mostly expelled from the matrix during curdling, a more robust test to measure strictly the casein content of milk could be adopted in search of a stronger indicator for yield prediction. One such method is the use of near-infrared transmission spectroscopy, as detailed by Laporte (1999), and could be used to compare casein content of milk and cheese (Stocco, 2019). Rough estimates of casein content of goat's milk can be used, but don't account for variation throughout the year (Ceballos, 2009).

Analysis of Cheese Composition

Five varieties of cheeses made from the goat milk in this sampling period were also measured for seasonal variation in moisture content, water activity, lipid content, and

ash content. Moisture content showed significant changes between seasons in three of the five varieties measured. The cheeses which were not significantly affected were the fresh cheese and the aged brie-style. The aged brie was only able to be sampled from late winter into early spring due to schedule of production, which may account for its lack of significant variation. The fresh cheese, however, was sampled in winter, spring, and summer, so its lack of significant variation is less explainable.

Water activity was measured for the roles it plays in safety and in creating an environment favorable to microbes. During the sampling period, however, the instrument used to measure water activity malfunctioned and began giving readings of over 1.000, which would be the value for pure water. The reason behind this malfunction was never isolated, as the device was calibrated multiple times and gave normal readings for samples other than cheese. One hypothesis for why it occurred is that the live ripening cultures were actively respirating, leading to an artificially high vapor pressure. But because of this, the results of this test cannot be used to infer anything regarding seasonal variation in water activity among the cheeses.

Lipid content was measured for its role in flavor development and product texture. Dry-basis lipid content was a stronger measure of variation of lipid proportion itself, while wet-basis lipid content represents the actual product and accounts for variation due to moisture content variation. This effect is illustrated by the fact that four of the five cheeses underwent significant seasonal changes in dry-basis lipid content, but all cheeses significantly varied in wet-basis lipid content. Another notable finding was that the direction of change between seasons was inconsistent with milk lipid variation. The brie-style cheese and fresh goat cheese had their highest dry-lipid proportion in the

summer and lowest proportion in the winter. The blue-mold rind cheese, however, followed the trend observed in milkfat variation, giving its highest readings in winter and lowest in summer. While it is unclear what caused the discrepancy, it may indicate that seasonal variation of milk is not the driving factor for variation in cheeses.

Ash content was the only parameter not measured in triplicate and was not able to be evaluated statistically. Observationally, the ash content of cheeses did vary, but did not follow any noticeable pattern. Future experiments measuring ash would be able to offer more insights into any compositional changes that may be present. As noted, identification of minerals through ICP-MS was initially planned for the measurement of individual minerals of interest but was not possible during the time of sampling.

The correlation between milk and cheese compositions for lipid and nonfat solids (dry and wet basis) was also investigated. Neither parameter showed significant correlation with milk composition for any of the five cheeses, even though some correlations were strong ($r>0.7$). This could be an effect of the small sample size of cheeses used for the t-test ($n=3$ and $n=4$). Future research could improve on this by using a larger sample size.

Comparison of Environmental Monitoring Methods

The comparison of an in-house environmental monitoring procedure against a commercially provided service resulted in reasonably accurate results, with over 78% of samples matching the commercial service. While the rate of false positive results from the in-house method was generally low (21.67%), it became more frequent after October 2019. It is uncertain what was the cause for this spike; but given that the manufacturer of

the test indicates certain strains of *Enterococcus spp.* can produce a false positive, it is possible that higher than normal amounts of *Enterococci* were present in the sampled area in the final three months of testing. Additionally, there were zero instances of false negative, which could potentially lead to an outbreak scenario.

Chapter 5: Conclusion

Goat cheese is a diverse category of products, including fresh and soft to firm, aged cheeses. With the noted seasonal variability in goat's milk composition, there lies a need to better understand how these trends affect products downstream from milk. This research was intended to quantify seasonal variation in quality parameters of five cheese products and their connection to variation in quality parameters of the milk used. Variation in milk composition was in agreement with previous findings. Cheese compositions were also found to vary significantly between batches across seasons, but no trends in this variation were observed. Recommendations were also provided to the manufacturer based on the findings of this research, including the implementation of milk standardization to account for the decline in nutrient abundance noticed in the summer months.

More research is needed to address the limitations in the present study. A method for casein-specific protein measurement could allow a more accurate yield model. Future research should include only cheese varieties which are produced year-round, allowing for the tracking of composition of cheeses from all seasons. Having more samples would also allow for a more robust statistical analysis of correlations between milk composition and cheese composition. Finally, eliminating the error in water activity measurement and identifying a source for mineral analysis would help expand the scope of these results.

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