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Influence of Change in pH on Whey Expulsion from Cheddar Cheese Curds made from Recombined Concentrated Milk

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INFLUENCE OF CHANGE IN pH ON WHEY EXPULSION FROM CHEDDAR CHEESE CURDS MADE FROM RECOMBINED CONCENTRATED MILK

by

Kanak Bulbul

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

of

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in

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ABSTRACT

Influence of Change in pH on Whey Expulsion from Cheddar Cheese Curds made from Recombined Concentrated Milk

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Utah State University, 2019

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Whey expulsion from cheese curd is influenced by temperature, pH, cut size and cooking. The objective of this research was to determine the extent to which pH drop prior to draining of whey influences cheese curd syneresis, cheese curd moisture before draining, and final cheese moisture when using concentrated milk. Recombined milk (7.5 kg) was prepared by mixing micellar casein concentrate (~9% casein), cream, and skim milk to 4% casein and casein-to-fat ratio of 0.68.

Four levels (0.5 times (X), 1X, 2X and 4X)) of a pH-controlled bulk starter culture were used to obtain different rates of pH change during cheesemaking where X is the normal amount of culture (0.5%) used in cheesemaking. Cheesemaking involved a typical cheddar make procedure with setting of prepared milk at 31°C, cutting of set curd after 30 min, cooking of cut curd to 38°C with 95 min set-to-drain time followed by cheddaring

with 85 min drain-to-mill time. Prior to cutting, the curd was overlaid with 750 ml of ultrafiltered milk permeate to minimize curd breakage upon stirring.

Initially there was rapid moisture loss from curd after cutting, followed by a linear pattern ($R^2 > 0.95$) until whey drainage. A faster drop in pH increased whey expulsion from the curd ($P = 0.0002$). With initial curd moisture levels at 5 min of 83.7% and 83.3% using 0.5X and 4X culture, respectively, curd moisture level of 75.0% and 72.8% respectively were obtained at 50 min (during cooking), and 65.5% and 58.5% respectively, at 95 min (at draining). As cheese make times were fixed, curd pH at draining was lower with faster acidification, the values being 6.5, 6.4, 6.1 and 5.8 for culture additions of 0.5X, 1X, 2X and 4X respectively. Cheese pH after 14 d of refrigerated storage was likewise affected with pH of 5.4, 5.3, 5.2 and 5.1, respectively. Mean cheese moisture contents were 37.8%, 37.1%, 37.2% and 35.2%, respectively. Cheese moisture and pH were correlated with drain pH ($R^2 = 0.48$ and 0.71, respectively). To conclude, the pH drop that occurs during cheesemaking increases rate and extent of whey expulsion and will produce cheese with lower moisture. To account for the increased buffering capacity of concentrated milk containing 4% casein, a drain pH of 5.9 to 6.0 would be required to obtain a cheese with d 14 pH of ~5.1.

(53 pages)

PUBLIC ABSTRACT

Influence of Change in pH on Whey Expulsion from Cheddar Cheese Curds made from Recombined Concentrated Milk

Kanak Bulbul

The Western Dairy Center at Utah State University funded this project to investigate cheese research using concentrated milks. Concentrated milk was provided by the South Dakota State University and starter culture for this study was prepared and donated by Vivolac Cultures Corporation, Greenfield, Indiana.

The project initiated as a continuation of a previous study on effects of protein concentration, coagulum cut size and set temperature on curd moisture loss kinetics while stirring during cheesemaking. It was aimed at determining the extent to which pH drop prior to draining and final cheese moisture when using microfiltered concentrated milk.

We performed twelve cheesemaking trials using recombined milk from micellar casein concentrate, cream and skim milk according to a modified cheddar cheese-make procedure. Four different levels of starter cultures were used to achieve different acidification rates for pH change during cheesemaking. The amount of starter culture added had significant effect on moisture of cheese at whey drainage, moisture and pH of cheese. Thus, it can be said that the pH drop that occurs during the cheesemaking increases rate and extent of whey expulsion.

Dedicated to my mother and father for all their sacrifices to ensure I receive the very best in my life

ACKNOWLEDGMENTS

I wish to render my humble submission to thank Dr. Donald J. McMahon who provided me the opportunity to be enrolled and work under the aegis of his celestial guardianship. I feel highly indebted for his generous support and guidance through my thick and thins. I am highly thankful to Dr. Marie Walsh and Dr. Silvana Martini for being considerate with their time and advice. I am thankful to Ram Raj Panthi for all the guidance on this project. I am thankful to Dave Irish, David Campbell, Dan Combe and Megan Armstrong for their help and co-operation during my cheesemaking schedules. I would also like to thank Tara B. Johnson for her day to day counseling as a forbearer and Kim Rasmussen for her kind support throughout the period of my master's program. I would like to thank my kind and loving parents for their constant upright support during all these years without which it could not have been possible to achieve my desired goal and my loving brothers Dr. Pavel Somavat and Dr. Romel Somavat for their perseverance, support and unconditional love. Last but not the least, I thank my friends Anusna Chakraborty, Avik Mukherjee, Keval Shah and Vaibhav Sahu for their support.

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LIST OF ABBREVIATIONS

ANOVA = Analysis of Variance

C:F = Casein to Fat ratio

HC-MCC = Highly Concentrated Micellar Casein Concentrate

GMP = Glycomacropeptide

IG = Immunoglobulins

IR = Infra-Red

LAB = Lactic Acid Bacteria

MCC = Micellar Casein Concentrate

MF = Microfiltration

MPC = Milk Protein Concentrate

MPI = Milk Protein Isolate

MWCO = Molecular Weight Cut Off

NF = Nanofiltration

RMD = Repeated Measures Design

RO = Reverse Osmosis

R^2 = Coefficient of Determination

UF = Ultrafiltration

INTRODUCTION

Cheesemaking has become an art and science during its course of long history from its probably accidental first production around 5,000 B.C. to its being a craft for thousands of years to its becoming a science with the start of standardization for cheesemaking during mid 1800s along with association of fields like chemistry, biochemistry, microbiology, enzymology, molecular genetics, flavor chemistry, rheology and chemical engineering (Fox et al., 2000).

Cheesemaking involves conversion of milk from liquid to semi-solid gel state which on cutting facilitates contraction of protein gel and expulsion of whey leading to formation of cheese curds over a number of steps which are ripened to get mature cheese. In cheddar cheesemaking, this conversion of liquid milk to gel state is facilitated by the use of proteolytic chymosin enzymes. The use of microbial starter cultures is a critical step in cheddar cheesemaking in a number of aspects: development of acid lowering the pH during cheesemaking enhancing curd contraction and whey expulsion, suppression of undesirable microbes which survived pasteurization, and development of flavor during ripening. Lactic acid bacteria (**LAB**) are the starter cultures used in most cheesemakings as they utilize milk sugar lactose to produce lactic acid and mesophilic LAB are used in cheddar cheesemaking.

Standardization of milk and cheesemaking process have facilitated increased cheese yields and improved factory throughputs. With the advent of membrane filtration technologies, ultrafiltered concentrated milks with the concentration of casein and whey protein fraction have replaced the use of whole milk in cheesemaking to a much extent for economic benefits. More recently, potential use of microfiltered (**MF**) concentrated milk in cheesemaking is of interest to facilitate to concentrate just the casein fraction of milk

proteins. The collected whey proteins can then be used to obtain various whey products such as whey protein concentrates and products □-lactalbumin, □-lactoglobulin, lactoferrin, glycomacropeptide, immunoglobulins etc. which have high dollar value when separated with membrane filtration before cheesemaking as compared to their separation from the whey released during cheesemaking.

The following study is a continuation of a recent study at the Western Dairy Center on the effects of temperature, cut-size and protein concentration on the whey expulsion during cheesemaking using milk by ultrafiltration (**UF**). It was observed (unpublished data) that whey expulsion during cheesemaking increases with increased temperature change during cheesemaking, smaller curd cut size, and higher protein concentration in the milk. In this study, the influence of rate of change in pH during cheesemaking using micellar casein concentrate (**MCC**) on whey expulsion, cheese moisture and cheese pH were studied. This will provide data to compare cheddar cheese manufacture using MF milk concentrate with and previous studies a baseline for future work on using MF milk at higher concentrations for obtaining a final desirable cheese pH and moisture.

HYPOTHESIS AND OBJECTIVES

Hypothesis

Increasing the rate of acidification when making cheese from concentrated milk will enhance whey expulsion so that cheddar cheese can be made to specifications of 36 to 38% moisture and pH 5.1 to 5.2.

Objectives of this study

1. Determine the effect of acidification rate on (a) change in pH and curd moisture during cheesemaking using recombined concentrated milk and (b)the final moisture and pH of the cheese.
2. Determine the relationship between the pH at which whey is drained from the curd and the pH of the cheese made from recombined MF concentrated milk.
3. Determine the acidification rate and drain pH required to manufacture cheese with 36 % to 38 % and pH of 5.1 - 5.2 from recombined concentrated milk containing 4% casein.

LITERATURE REVIEW

Membrane filtration

Membrane filtration includes microfiltration, ultrafiltration, nanofiltration (**NF**) and reverse osmosis (**RO**) that differ on membrane pore size (Table 1). This facilitates separation of specific components from a liquid stream based on the selective permeability of the membrane used (Moubois and Mocquot, 1975). For UF, pore size is described based on molecular weight cut off (**MWCO**) value of the membrane. Membrane filtration became an integral part of food industry during 1970s and UF in the dairy industry have become common practice to increase cheese plant productivity (Pouliot, 2008).

Ultrafiltration

Ultrafiltration refers to the medium pressure driven membrane filtration process where components with a molecular weight size of 1,000 to 200,000 Daltons are specifically retained. Ultrafiltration allows most dissolved components to pass through the membrane while the larger sized components are being retained by the membrane depending on the MWCO of the membrane used (Figure 1). The retentate stream includes proteins, fats, and bacteria. The permeate stream includes water, lactose, soluble minerals, non-protein nitrogen, and water-soluble vitamins (Mistry and Maubois, 1993). In the dairy industry, UF has diverse applications such as protein standardization of milk for making cheese and milk powders, protein concentration, and lactose reduction of milk (Maubois, 1989).

Table 1. Example of filtration membranes which can be used in dairy industry for various purposes, with pore size, retentate and permeate components.

Membrane type	Range (µm)	Retentate	Permeate
MF	0.1-5	Casein proteins, fat	Water, salt, lactose, vitamins, whey proteins
UF	0.001-0.1	Casein proteins, whey proteins, fat	Water, salt, lactose, vitamins, amino acids
NF	0.001	Casein proteins, whey proteins, fat, lactose	Water, salt, vitamins
RO	0.0001	Casein proteins, whey proteins, fat, lactose, vitamins, salt	Water

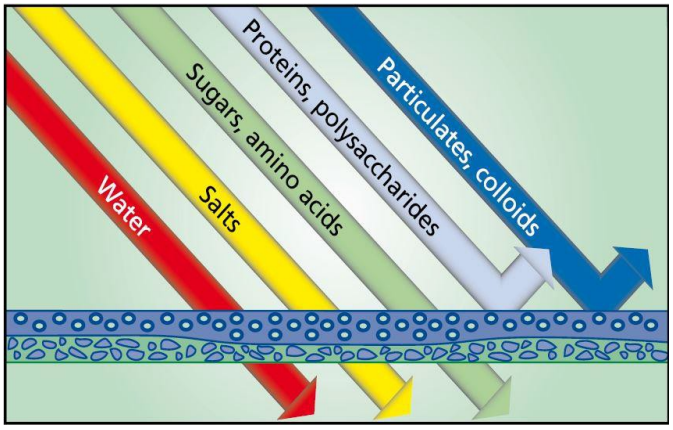


Figure 1. Schematic diagram showing retention of specific milk components when milk is filtered through an ultrafiltration membrane. Source: ‘’GEA Membrane filtration Publication,https://www.gea.com/en/binaries/membrane-filtration-ultrafiltration-nanofiltration-microfiltration-reverse-osmosis-gea_tcm11-34841.pdf, accessed on March 9, 2018’’.

Microfiltration

Microfiltration refers to low pressure driven membrane filtration process where filtration membranes with a size range of 0.1 to 5 microns are used. Microfiltration allows the dissolved non-aggregated components to pass through the membrane while most of colloidal components are retained depending on pore size of the membrane used (Figure 2). The retentate stream contains casein micelles, aggregated whey components, fat globules, and somatic cells (Mistry and Maubois, 1993). Application of MF in the dairy industry include bacterial reduction from skim milk, whey and brine, residual fat removal from whey after centrifugal separation, protein fractionation, and protein:casein standardization (Maubois, 1989).

When skim milk is the starting material, concentrated milk proteins manufactured using membrane filtration are of two types: milk protein concentrates (**MPC**) and milk protein isolates (**MPI**) and micellar casein concentrates (MCC) (McCarthy et al., 2014).

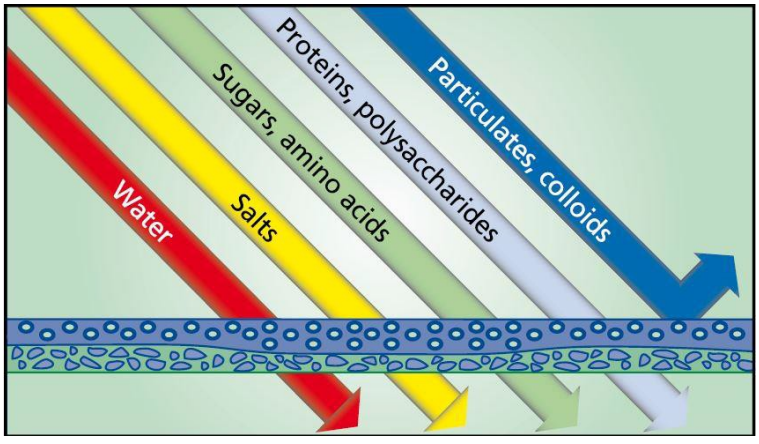


Figure 2. Schematic diagram showing retention of specific milk components when milk is filtered through a microfiltration membrane. Source: ''GEA Membrane filtration Publication,*https://www.gea.com/en/binaries/membrane-filtration-ultrafiltration*-nanofiltration-microfiltration-reverse-osmosis-gea_tcm11-34841.pdf, accessed on March 9, 2018''.

Table 2. Example of major milk components and approximate diameter and molecular weight.

Major milk components	Size (μm)	Approximate molecular weight (kD)
Fat globules	10 -1	3, 00, 000
Casein micelles	0.1- 0.01	10, 000, 000
Whey proteins	0.01 - 0.001	20 - 150
Lactose, milk salts etc.	0.001 - 0.0001	-

The MPC and MPI are manufactured using UF membranes with a size range of 0.001 to 0.1 micrometer. This concentrates both caseins and whey proteins in proportion similar to milk. In contrast, MCC is obtained by concentrating skim milk using MF membranes using pore size of 0.1 to 5 micrometer. Such MF membranes allow the passage of only non-colloidal whey proteins and smaller peptides such as those from □-casein, thus resulting in concentration of casein micelles in relation to whey proteins (Mistry and Maubois, 1993; Maubois and Olliver, 1997). Table 2 shows approximate diameter sizes of major milk components.

Cheesemaking using concentrated milks

The presence of increased amounts of whey proteins in cheese has been considered the reason for slow flavor development in cheeses made using highly concentrated UF milk containing 15% or more protein. Other issues are that there is an increased buffering capacity of UF milk and curd that can influence acidification rates during cheesemaking (El-Gazzar and Marth, 1991). The change in calcium phosphate content in UF retentate can result in textural defects like sandiness, firmness or crumbliness in UF cheeses (Kindstedt and Guo, 1998). However, as MF retentate cheese curds are similar to conventional cheese

curds because MCC has depleted amounts of whey proteins, fewer defects should be expected in MF cheeses in contrast to UF cheese (Papadatos et al., 2003). Thus, using MF concentrate for cheesemaking has advantages over using UF concentrate because only desirable casein protein is being concentrated. Using a liquid concentrate has advantages over using a dried powder as the protein remain hydrated which provides better functionality as drying results in loss of solubility (Baldwin and Truong, 2007). Also, milk protein powders can lose solubility during storage (Mimouni et al., 2010).

Increased protein concentration in milk can shorten the required curd treatment time thus decreasing the total processing time required in cheese making (Thomann et al., 2008). However, it can increase curd firmness (Lucisano et al., 1985) and increase whey expulsion for the curd (Peri et al., 1985). This can alter final cheese properties and quality. Membrane filtration technology has facilitated increased total factory output in cheese manufacturing and use of UF concentrated milk with up to 4 to 5 % protein has facilitated increased total throughput in the cheese industry (Ong et al., 2013a). However, cheese making using MF concentrates like MCC is not legally permitted in U.S. for standardized cheeses. However, MCC seems a promising substitute since desired caseins are concentrated (Marela et al, 2013) and there are fewer undesirable after effects of whey proteins such as slow flavor and texture changes during cheese aging and increased binding of denatured whey proteins to casein proteins. Use of MF milk concentrate in cheese making may increase existing cheese factory throughput, resulting in improved plant efficiency and decreased production costs (Papadatos et al., 2003). It has been suggested that recombined milk using highly concentrated MCC (**HC-MCC)** with up to ~11-12% casein can be used for cheesemaking

(Lu et al, 2016). Although there may be viscosity issues with normal milk and rapid curd firming being higher with high casein concentrations.

Proposed uses of MCC

Micellar casein concentrate could be used for mozzarella cheese manufacture by adding added to whole milk to standardize the casein to fat ratio to approximately 1.2 (Garem et al., 2000). For cheddar cheese manufacture, an MF concentrate from skim milk could be recombined with cream and skim milk and standardized to a casein to fat ratio of approximately 0.68 (St-Gelais et al., 1995; Neocleous et al., 2002a, b). Rehydrated frozen MCC can be used for liquid food applications provided it is solubilized at temperatures of ~50° C as high casein concentrations lead to cold gelling (Lu et al., 2015).

Whey expulsion

During cheesemaking, concentration of casein in the milk gel is facilitated initially by cutting the gel or curd which allows whey to move outside the curd particles in response to the curd particle contraction. Cutting of curd increases the total curd surface area. Contraction of the curd particles results in rearrangements or restructuring of the casein matrix formed due to enzymatic coagulation (Jovanovic et al., 2004). Continued whey expulsion takes place during stirring and cooking of curd. The rate and extent of whey expulsion dictates a number of final chemical, rheological and organoleptic properties of manufactured cheese due to its direct influence on cheese moisture, time scale of production, protein and fat losses in whey (Castillo et al., 2000). Thus, control of whey expulsion allows a cheesemaker to better control the quality and biochemical properties of

the finished cheese which makes it an important factor to be considered during cheese making.

The rate and extent of whey expulsion is dependent on a number of factors such as milk composition, degree of milk concentration, milk homogenization, cooling of milk, pH, temperature, calcium equilibrium, concentration of casein, gel firmness, gel permeability, time and speed of cutting, time and speed of stirring, size of cut curd particles, curd washing, drying, molding, pressing, and salting of cheese (Walstra et al., 1985; Piyasena and Chambers, 2003). Early cutting of gel results in prolonged whey expulsion along with partial destruction of gel, and loss of milk fat in whey. Alteration of whey expulsion of cheese curds (Walstra et al., 1985).

Interaction of various factors have also been found to influence whey expulsion. Interaction of pH and concentration factor of MF have a significant influence on curd firmness and whey expulsion (Thomann et al., 2008). Higher protein content in cheese curds (5 - 6%) causes slower whey expulsion as compared to curd containing 4% protein although this is compensated for by removal of whey prior to cheesemaking as part of the concentration process. A larger cut size of curd facilitates slow whey expulsion as compared to a smaller cut size of cheese curd particles. Whey expulsion in cheese curds at higher set temperatures is faster as compared to cheese curds at lower set temperature when cooked to 37° C (Panthi, et. al, 2017). Having a larger change in temperature during cooking of the curd can compensate for slower whey expulsion at the lower set temperature. (Unpublished data, Panthi, R., Teagase, Moorepark, Ireland).

Curd pH is also a factor in determining whey expulsion of cheese curd particles. Also, pH is lowered during cheesemaking, whey expulsion is increased and as

concentration factor of MF increases, whey expulsion of cheese curd decreases (Daviau et al., 2000; Thomann et al., 2008). The lower the initial pH in cheesemaking, the whey expulsion is faster and continues to a greater extent (Fox et al, 2000) (Figure 3).

In this current study, the effect of change in pH during the cheesemaking process on whey expulsion from the cheese curd produced from milk with 4% casein was studied.

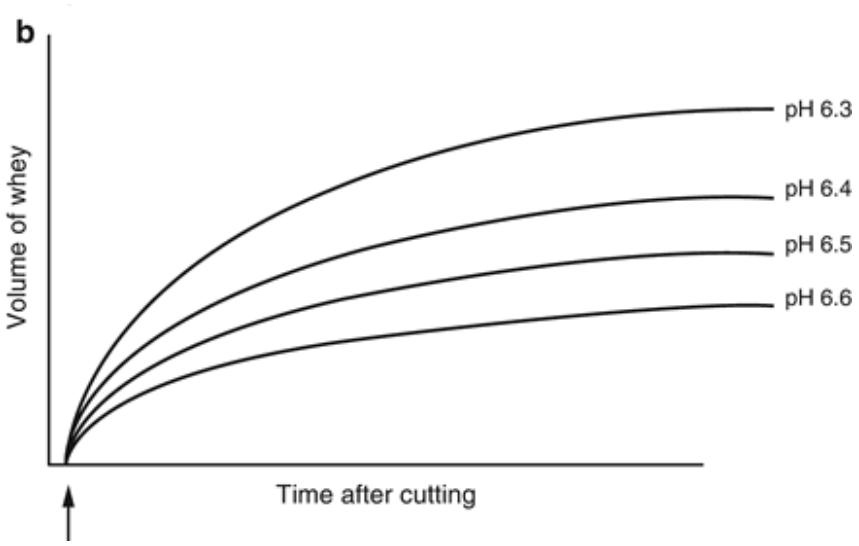


Figure 3. Effect of initial pH on the rate and extent of whey expulsion in cut or broken renneted milk gels made from whole milk. Source: Fox et al (2013).

MATERIALS AND METHODS

Materials and Milk Preparation

The MCC used in this study as casein source for cheese making was manufactured at the Institute for Dairy Ingredient Processing, South Dakota State University, Brookings. Skim milk was concentrated by MF followed by diafiltration to obtain MCC with casein level of approximately 9 to 10%. MCC was then frozen to -18°C and transported frozen to the Gary Haight Richardson Dairy Products Laboratory at Utah State University (**USU**), Logan, UT and stored at -18°C. Composition of MCC is shown in Table 3. The 9.4% of casein in MCC is ~3.5X of the casein in Holstein milk and was 92% of total protein compared to ~83% typically in milk.

Sufficient portions of MCC were held at □5°C for one day before use for cheesemaking and then thawed at □40°C and recombined with pasteurized cream as a source of fat (~35% fat) (Aggie Creamery, USU, Logan, UT) and pasteurized skim milk

Table 3. Composition of micellar casein concentrate

Rep #	Fat	Crude Protein	Lactose	Total Solids	True Protein	Casein
	----------(%)----------					
1	0.21	10.44	0.46	12.08	10.26	9.44
2	0.20	10.43	0.46	11.97	10.31	9.45
Mean	0.21	10.44	0.46	12.02	10.29	9.44
SD	0.01	0.00	0.00	0.08	0.04	0.01

(purchased from a local supermarket) to standardize the milk. A pH-controlled bulk starter culture MSM 9701 which is a multiple-mesophilic strain blend of Lactococcus lactis ssp. lactis and ssp. cremoris was prepared and donated by Vivolac Cultures Corporation, Greenfield, IN. Starter cultures were stored refrigerated upto 5 days and culture activity was measured prior to use. Chymosin rennet (Maxiren, ≥650 international milk clotting units/ml) was obtained from DSM Food Specialties USA Inc. (Eagleville, PA). The UF concentrated permeate used to overlay set curd before each curd cutting was produced by UF of whole milk (Aggie Creamery, USU, Logan, UT) stored frozen and thawed before use.

Samples of MCC, cream, skim milk and recombined milk prepared for cheesemaking were diluted and sent to Rocky Mountain Dairy Herd Improvement Association (North Logan, UT) laboratory for determination of fat, protein, lactose and total solid-not-fat contents by Infra-Red (**IR**) analysis.

Cheesemaking

Batches of milk containing 7.5 kg of concentrated milk was prepared by combining MCC (~40° C), cream (4° C) and skim milk (4° C) standardized to 4% casein and 5.8% fat (casein to fat ratio = 0.68) for twelve cheesemaking trials. Starter culture was added at 0.5 times (X), 1X, 2X and 4X its designated usage rate (based on activity) of 5 g/kg of milk. Cheesemaking was performed in triplicate using a cheese make procedure that was standardized to achieve set-to-mill time of 3 h.

The recombined concentrated milk was heated to 50° C to overcome effects of cold storage on its coagulation properties and cooled to 32° C. The required amount of starter culture was added and then 5 min later, 0.6 ml of rennet was added with dilution in 10 ml

cold water. Milk was stirred for 3 min then allowed to coagulate to produce a firm set (~30 min). Just prior to cutting, the curd was overlaid with 750 ml of UF permeate to facilitate easy stirring of curd particles in the vat and prevent their breakage and then the curd was cut using wire knives with a 6-mm spacing.

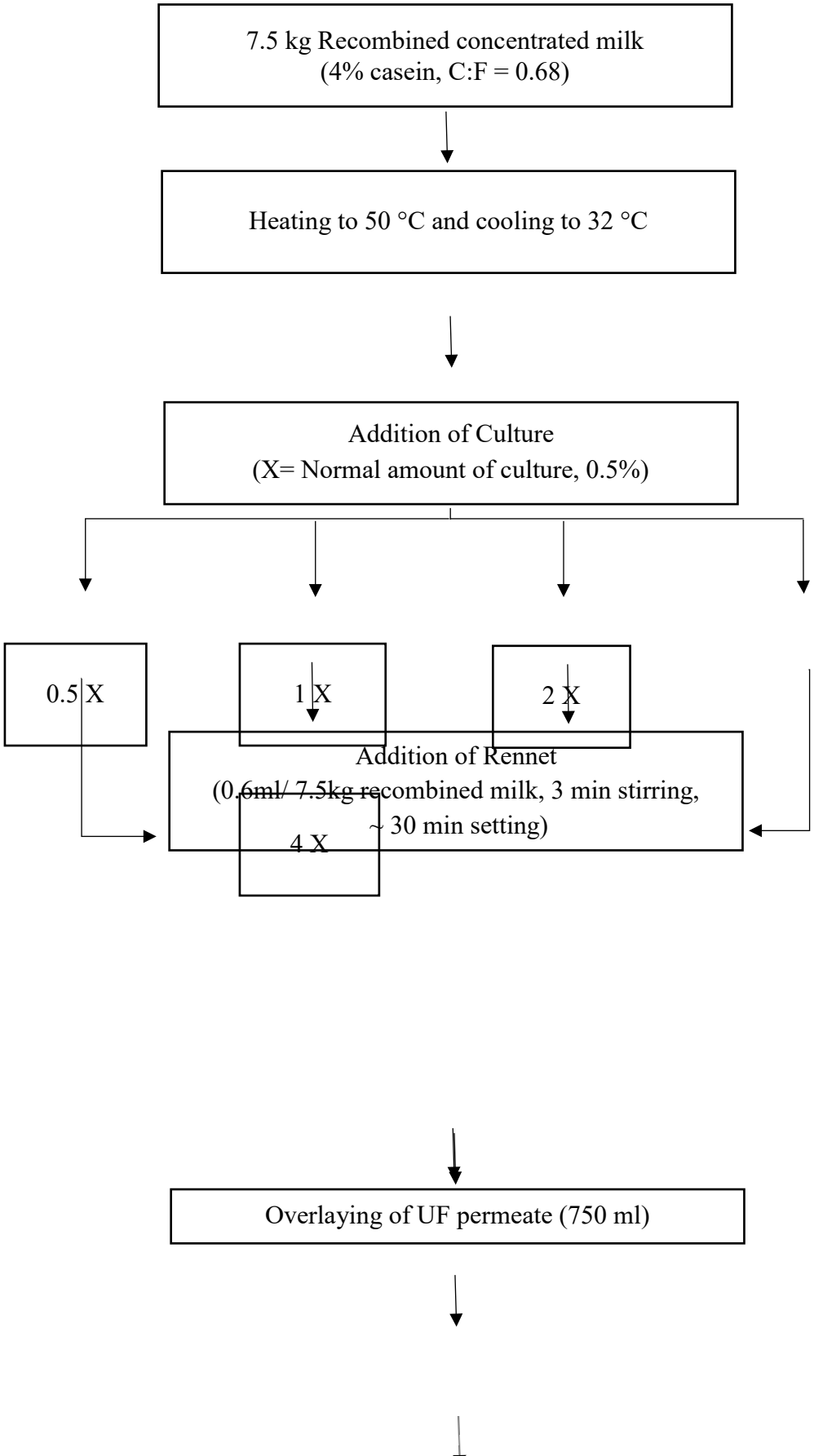
Cheese cutting involved first lengthwise cut by horizontal knife, sidewise cut by vertical knife followed by lengthwise cut by vertical knife. The cut curd was allowed to heal for 5 min and then stirred continuously for 30 min at 32°C. At 35 min after cutting, cooking of curd was done for 30 min to a temperature of 38.5°C with continuous stirring. The curd was then stirred continuously for next 30 min at 38.5°C and whey was drained at 95 min after cutting. After whey drainage, curd was packed and stacked on one side on the vat and was cut into two slabs after 10 min of packing. The two cut slabs were turned every 10 min for 30 min and then were stacked two high for the next 40 min with turning every 10 min. The temperature of curd slabs was maintained between 35 to 36.5°C. Cheese slabs were then milled manually and salted with salt level of 29 g/kg of curd over three applications 5 min apart for 15 min.

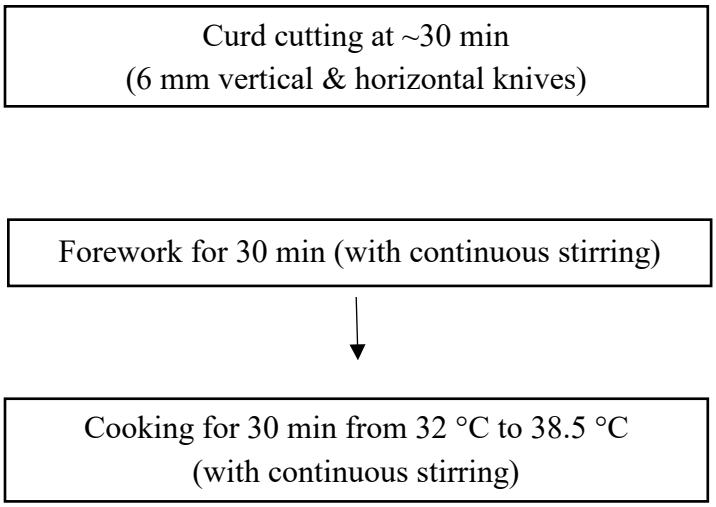
The salted curds were then hooped in round plastic hoops and pressed for 1 h at 170 kPa and 18 h at 410 KPa. The pressed cheese was then vacuum packaged in plastic bags and stored at 6° C. The cheesemaking process flow steps are given in Figure 4.

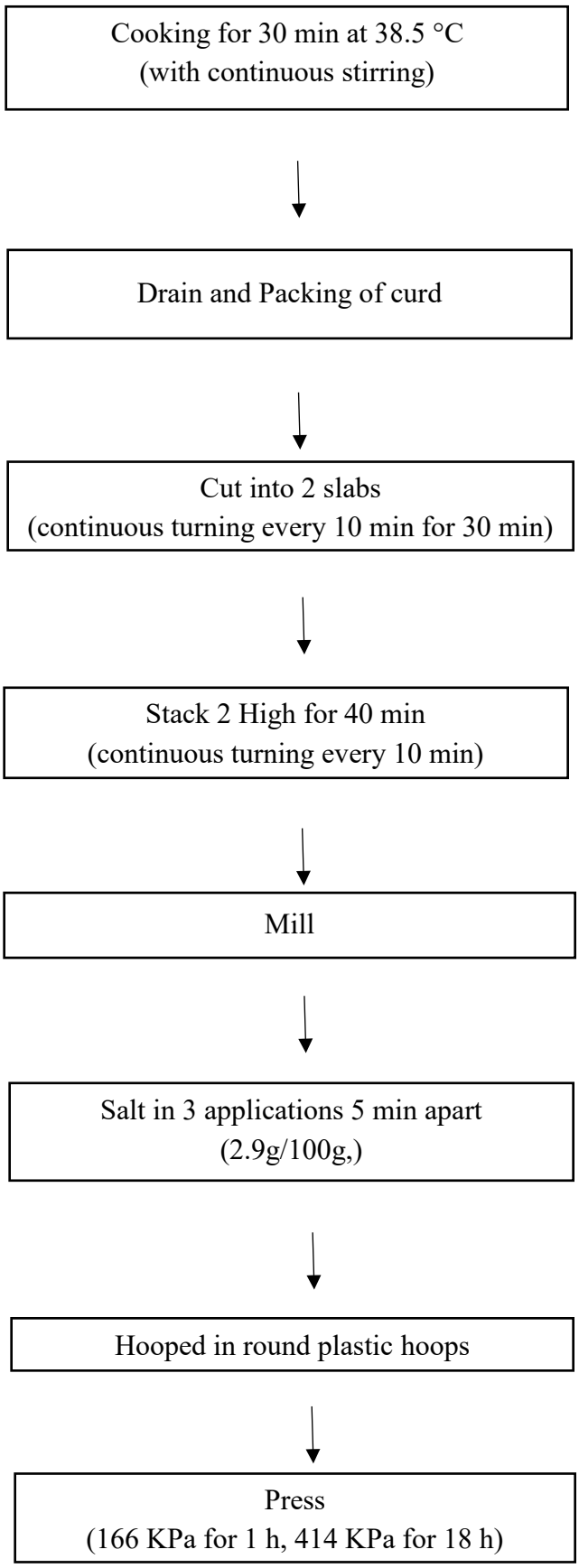
Curd and Cheese Analysis

Samples of cheese curd particles were collected for moisture analysis at 15 min intervals starting from 5 min after cutting the curd to the end of cooking i.e. during step 4 and 5 of cheesemaking process as shown in Table 4. Cheese curd samples were collected in pre-weighed aluminum sample dishes in duplicate and immediately weighed. Samples

were then dried in a force-air drying oven at 100° C for 18 h then weighed for final weight after drying. Rate of curd whey expulsion, measured as weight loss, were




Curd cutting at ~30 min
(6 mm vertical & horizontal knives)
Forework for 30 min (with continuous stirring)
Cooking for 30 min from 32 °C to 38.5 °C
(with continuous stirring)


Cooking for 30 min at 38.5 °C
(with continuous stirring)
Drain and Packing of curd
Cut into 2 slabs
(continuous turning every 10 min for 30 min)
Stack 2 High for 40 min
(continuous turning every 10 min)
Mill
Salt in 3 applications 5 min apart
(2.9g/100g,)
Hooped in round plastic hoops
Press
(166 KPa for 1 h, 414 KPa for 18 h)

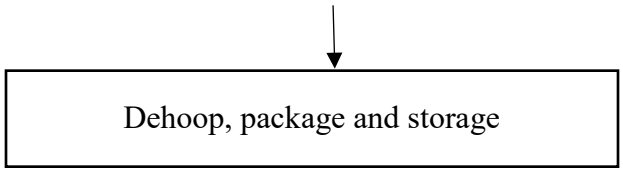


Figure 4. Flow of process steps for cheesemaking with different starter culture levels.

Table 4. Cheese Manufacture steps

	Cheesemaking Steps	Target Time	Min next	Target temp	Target pH	Actual pH	Comments
1	Add Starter	-0:35		32° C	6.7	( )	0.5% Bulk starter culture
2	Add Rennet	-0:30		32			0.6 ml DS Chymosin (diluted 1:20 with cold water)
3	Cut	0:00	5	32			6 mm wires. Add 750ml permeate before cutting.
4	Forework	0:05	30	32		( )	Stir slowly at first
5	Start Cook	0:35	30	32		( )	Heat slowly by schedule.
	End Cook	1:05 (65)	30	38.5		( )	Continue stirring
6	Start Draining	1:35 (95)	5	38.5	6.3	( )	Measure pH at 2:00 hours
7	Form curd into Pack	1:40 (100)	10	38.5			Drain & stir for total 5 min after draining, then form into pack at one end of vat
8	Cut and Turn	1:50 (110)	30	36.5		( )	Cut 2 slabs check that curd remains 35-36.5°C.
9	Stack 2 High	2:20 (140)	40	35		( )	Turn every 10 min. Measure pH
10	Mill	3:00 (180)	5	33	5.4	( )	Measure pH Curd - smooth and silky. Weigh curd slabs, then mill.
11	Salt	3:05 (185)	15				2.9g/100g curd salt over three applications 5 min apart
12	Hoop	3:20	10	32			
13	Press	3:30	60				24psi for 1hr. 60psi 18hrs.
14	Dehoop				5.1	( )	Dehoop.
15	Package			6°C			Vacuum pack and store at ~6°C.

measured for each 30-min period during forework, cooking of curd and after cooking.

Measurement of Cheese pH and Moisture

Cheese pH was measured after stomaching 20 g of grated cheese with 10 g of deionized water at 260 rpm for 1 min in Stomacher 400 (Steward, London, UK) using a glass electrode (Orion STAR A211 pH electrode, Hanna Instruments, Ann Arbor, MI) Moisture content of cheese was determined by microwave heating (Smart System 5, CEM Corp., Indian Trail, NC) at maximum temperature of 106 °F using 3 g of grated cheese.

Measurement of Cheese Fat and Salt

Fat measurements were done using a modified Babcock test (Richardson, 1985) using 9 g of grated cheese and transferring them to Babcock bottles. Salt measurements were by chloride analysis (model 926; Corning Scientific, Medfield, MA) after stomaching 5g grated cheese with 98.2 g of deionized water at 260 rpm for 4 min, filtering the slurry using Whatman No. 1 filter paper and analyzing the filtrate.

Measurement of Culture Activity

Culture activity test was measured prior to cheesemaking in order to determine the amounts of culture to be added. Ten milliliters of cold ultra-high temperature processed milk was placed in each of 4 test tubes alongwith 0.3 ml of culture being added to two of the tubes and incubated in a water-bath at 32° C for 2.5 hours. The pH of each tube was measured and average difference in pH between the cultured and blank was designated as culture activity (Personal communication, Randall Thunell, Vivolac Cultures Corp.). An

activity of 1.50 was considered as standard and a 0.5% addition to the cheese milk was used. With higher or lower activity, the amount added to the milk was adjusted proportionally.

Statistical Analysis

Curd moisture measurements during cheesemaking were considered as repeated measures design (**RMD**). Statistical analysis of the moisture data was performed using Statistical Analysis Software **SAS**, Studio university edition (SAS Institute, Cary, NC) for **ANOVA**. Post hoc means comparisons were made based on P-values ($\alpha = 0.05$) using Least Squares Means for Multiple Comparison with Tukey-Kramer adjustments and correlation coefficients calculated. Paired two Sample T-tests for means were done using Microsoft® Excel for Mac Version 16.15.

RESULTS AND DISCUSSION

Composition of Recombined Milk prepared for Cheesemaking

An example of preparation of milk for cheesemaking is shown in Table 5. Mean (±SD) composition of the recombined concentrated milk was fat 6.22% (±0.26%), protein 4.59% (±0.69%), lactose 3.75% (±0.08%) and total solids not fat 9.13 (±0.18%). Based on 92% of protein in MCC being casein and assuming casein was 83% of protein in skim milk and cream, the casein in the recombined milk was estimated at 4.3%. Compared to Holstein milk containing 3.1% protein, and hence 2.6% casein the recombined milk used for cheesemaking was equivalent to the casein concentration in milk concentrated to ~ 1.7X by UF.

Drain pH

The pH of the curd at draining was significantly influenced by level of culture addition ($P < 0.001$) with pH at draining based on culture level of 4X < 2X < 1X = 0.5X (Table 6).

Table 5. Example of milk components and amounts used to produce milk with 4% casein.

Components	Amount (kg)
Micellar Casein Concentrate	1.63
Cream	1.34
Skim Milk	4.53
Total	7.50

Based on previous cheesemaking experiences, when using a pH-controlled bulk starter culture with activity of 1.5, its addition at 5 g/kg to milk of normal concentration would provide sufficient acidification for the curd to reach pH 6.3 by ~135 to 140 min after adding the culture (data not shown). At which time the whey would be drained with the goal of producing cheddar cheese with pH 5.1 to 5.2 as the drainage of whey regulates the amount of lactose left in the curd controls amount lactic acid that can be generated during cheesemaking. This time corresponds to ~95 min after cutting which was the drain time used in this study to drain whey.

As the amount of culture increased from 1X to 4X the normal amount, there was faster acidification and hence the pH at time of draining was lower as shown in Figure 5.

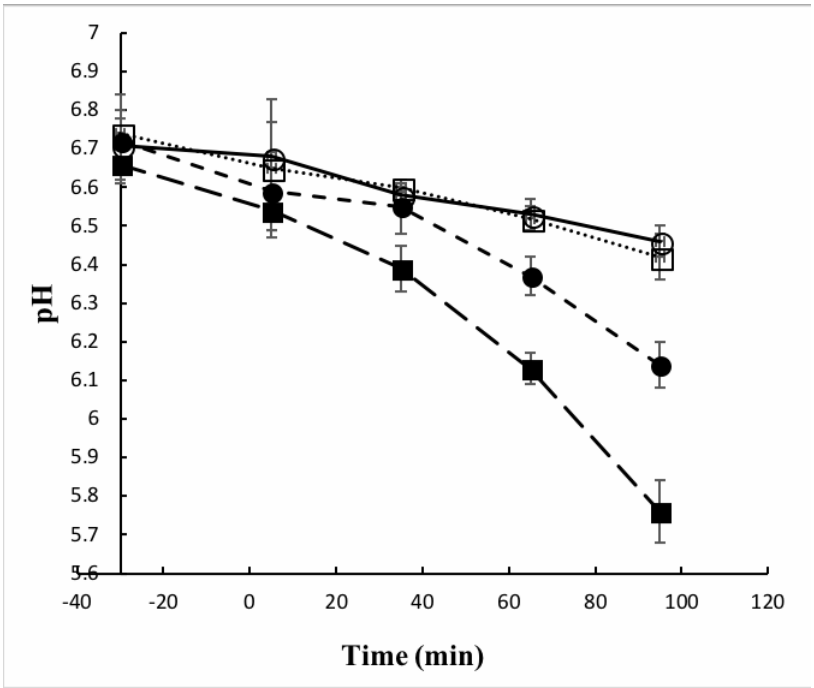


Figure 5. Change of pH from the start of culture addition in prepared milk till draining of whey during cheesemaking process when using starter culture at 0.5 (O —), 1 (□····), 2 (●- - -) or 4 (■ – –) times the normal amount.

When culture was added at the 1X level to the milk, the curd pH at draining was only 6.42 by that time rather than 6.3 expected when using normal milk. This was attributed to the higher level of casein (~4% compared to 2.6%) which would increase the total buffering capacity of the curd and whey, so that less of the lactic acid H+ generated by the starter culture was available to lower curd pH. This was expected as using concentrated milks for cheesemaking, the amount of starter culture added should be in proportion tothe protein content of the milk (Ong et al., 2013a).

When the culture level was increased to 2X, there was faster acidification and the curd reached pH 6.1 to 6.2, and with 4X culture the drain pH was pH 5.7 to 5.8. Reducing the culture level to 0.5X had no effect on rate of acidification such that pH at draining was not significantly different between 0.5X and 1X culture treatments while it was significantly different between all the other levels with $P < 0.001$ (Table 7).

Curd moisture

Whey expulsion and corresponding curd moisture was influenced by cutting the curd, stirring of the curd, raising the curd temperature during cooking, time and the change in curd pH. Immediately after cutting, the curd was allowed to heal without stirring for 5 min. This facilitates forming of the outer surface of the curd particles and thus lessens curd breakage during stirring. During this healing period, there was only a slight (1 to 2%) drop in curd moisture (Figure 6).

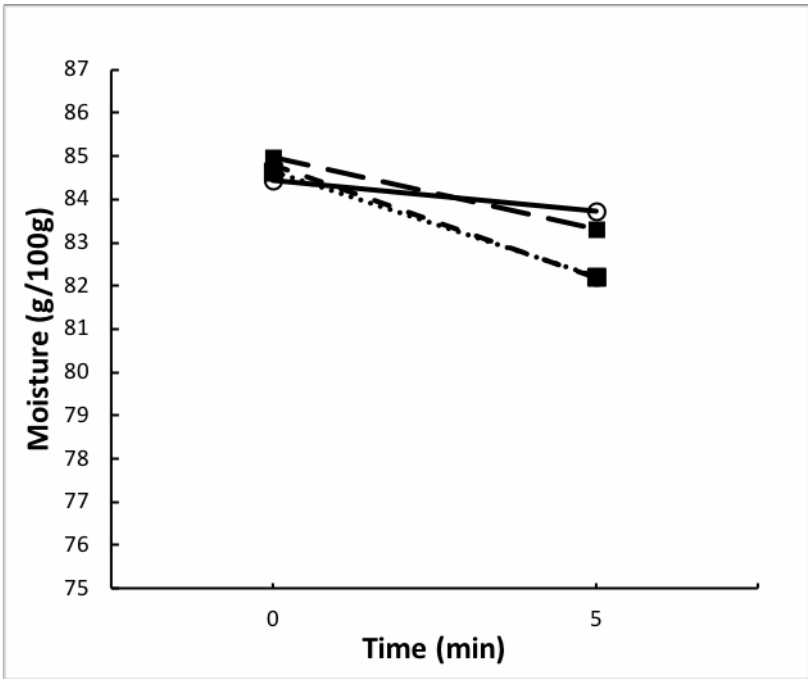


Figure 6. Curd moisture during 5 min healing period after cutting on culture levels of 0.5 (O —), 1 (□·····), 2 (●- - -) or 4 (■ – –) times the normal amount used in cheesemaking.

When the curd was stirred starting from 5 min, there was more rapid whey expulsion from the curd particles and concomitant drop in curd moisture (Figure 7). This was attributed to mechanical stirring of the curd as there was little change in pH over this short time period especially for 0.5X, 1Xand 2X culture additions. It has been previously shown (Panthi et al., 2017) that a power-law model can be used to explain whey expulsion from cheese curds under static conditions of constant temperature and pH. Curd moisture from 5 min to 35 min after cutting was fitted using power-law regression lines as shown in Figure 7 with values of 0.92, 0.98, 0.96 and 0.96 for culture levels of 0.5X, 1X, 2X and 4X respectively. Based on Paired Two Sample test for Means there were significant

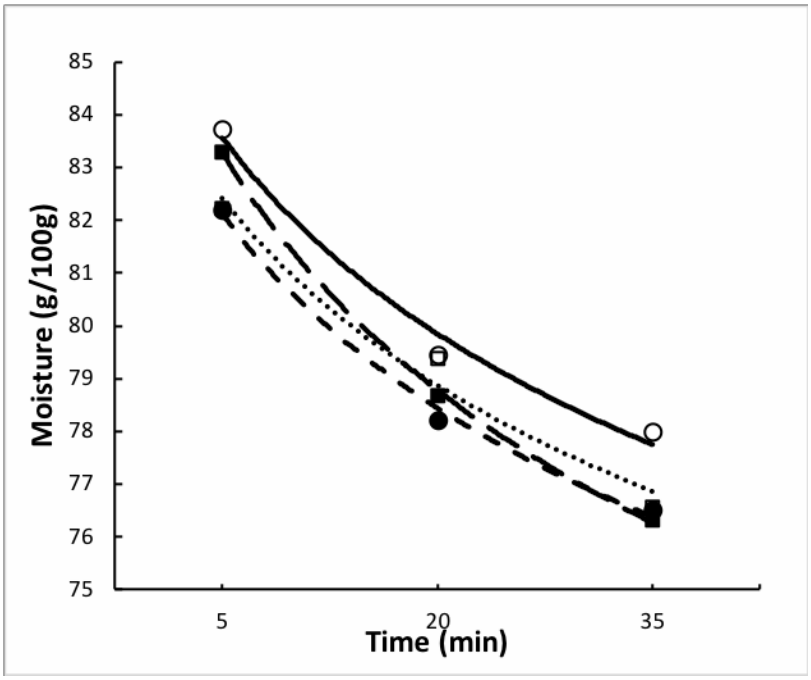


Figure 7. Curd moisture (and power regression lines) from 5 min after curd cutting to start of cooking based on culture levels of 0.5 (O —), 1 (□·····), 2 (●- - -) or 4 (■ – –) times the normal amount. □□□

differences ($P < 0.01$) in curd moisture for all treatments between 5 min and 20 min, and between 20 min and 35 min. The amount of drop in moisture during the first 15-min period was approximately double (Table 8) the amount of moisture lost during the second 15-min period (Table 9). Based on ANOVA and Least Significant Difference testing, there was a significant difference in curd moisture after 35 min between 0.5X and 4X culture levels but not between any of the other levels.

When curd temperature is increased during cooking (from 35 to 65 min), the drop in curd moisture no longer followed the power-law model and could be described by linear regression ($R^2 > 0.91$). This continued after the 38.5 C cook temperature was reached and throughout stirring until whey drainage at 95 min (Figure 8). If a cooking step had not been

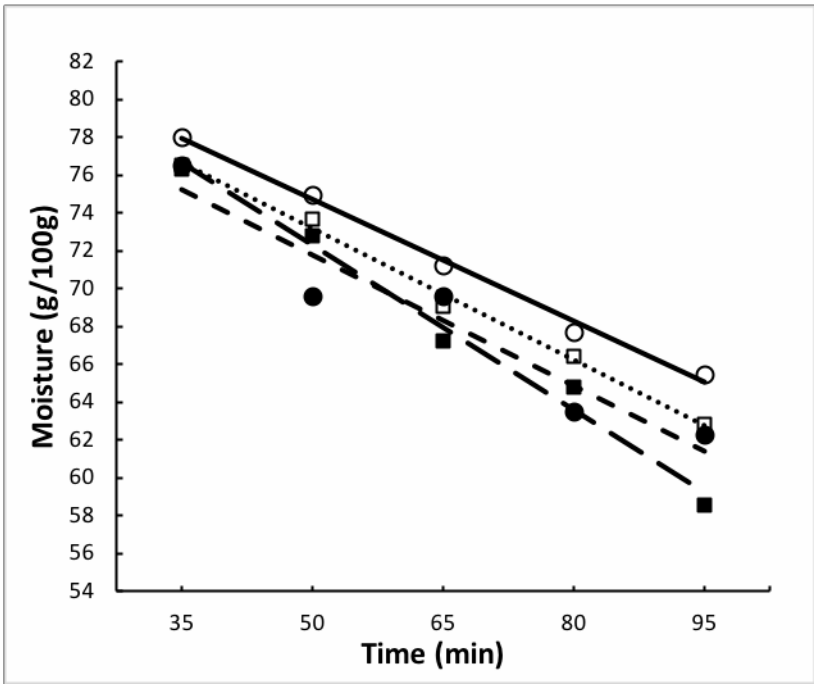


Figure 8. Curd moisture (and linear regression lines) during and after cooking when using starter culture at 0.5 (O —), 1 (□·····), 2 (●- - -) or 4 (■ – –) times the normal amount.

used, it would have been expected that the whey expulsion would become less and less and curd moisture would read a constant value. Including the cook step increases the importance of the hydrophobic effect on the protein network with the curd particles and induces curd shrinkage and further whey expulsion. The rate of decrease in curd moisture was faster with faster acidification as shown by curd moisture being significantly lower ($P = 0.001$) when using 4X culture levels compared to 0.5X culture levels. Because of

variations in the triplicate cheesemaking trials, there were not significant differences in curd moisture with the intermediate culture levels.

Based on curd moisture at 5 and 95 min, it was calculated that during this 90 min period 18, 19, 20 and 25%, respectively, of the total amount of moisture contained in the curd at 5 min after cutting was expelled as a function of stirring and heating the curd.

Influence of Whey Drainage pH on Moisture

The influence of whey drainage pH on cheese composition was studied for cheese curd moisture at drainage (95 min), cheese moisture, pH and calcium content after overnight pressing (d 1). For curd moisture at draining there was a significant difference (based on Least Significant Means) only between curd made using 0.5X culture level compared to 4X culture level ($P < 0.001$) Table 10. As shown in Figure 9, there was a significant positive correlation between pH at whey drainage and curd moisture at the time of whey draining. However, the R^2 was only 0.57. There was less variance from regression line at drain pH ~5.3 and ~6.2 than there was at pH 6.4 to 6.5.

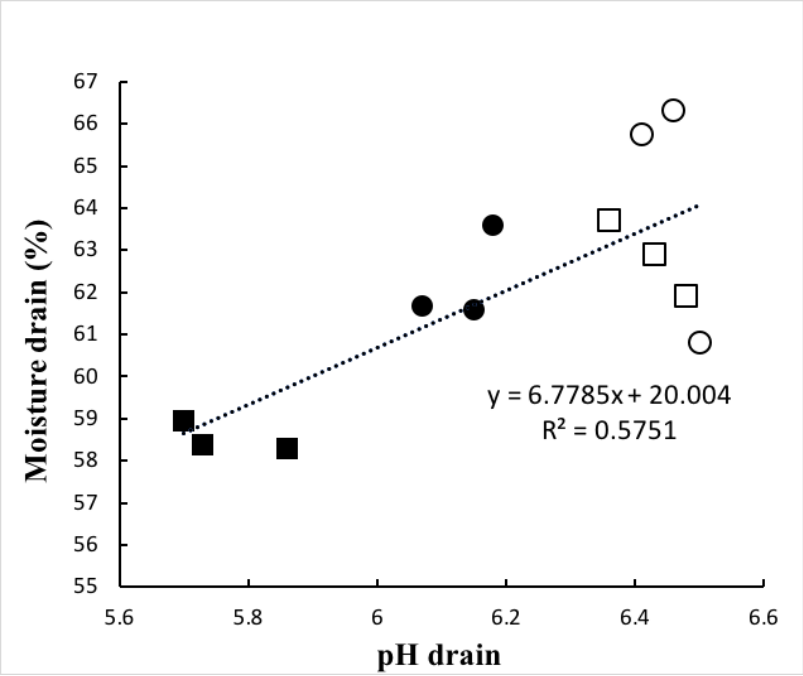


Figure 9. Effect of pH at time of whey draining (with linear regression) on cheese pH when using starter culture at 0.5 (O), 1 (□), 2 (●) or 4 (■) times the normal amount.

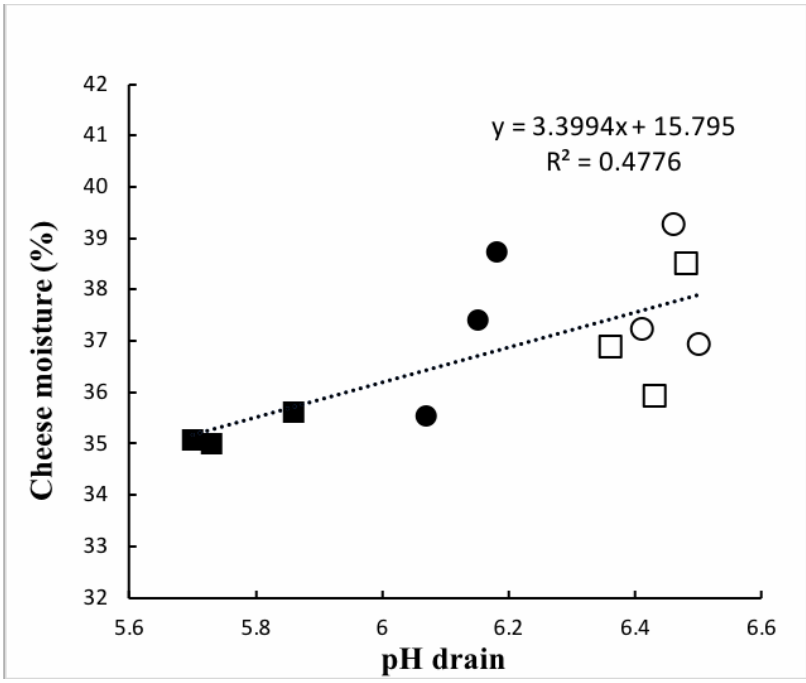


Figure 10. Effect of pH at whey draining (with linear regression) on moisture of cheese after pressing when using starter culture at 0.5 (O), 1 (□), 2 (●) or 4 (■) times the normal amount.

Likewise, there was also a positive correlation between mean cheese moisture and pH at whey drainage although R^2 was only 0.48 (Figure 10). Although analysis of variance showed no main effect of culture on cheese moisture of d 1 with P = 0.1335 (Table 11) there was a significant difference based on Least Square Means with P = 0.0325 between cheese moisture when using 0.5X culture levels compared to curd moisture when using 4X culture levels (Table 12). This significant difference between 0.5X and 4X culture levels indicates there is a decrease in cheese moisture with lowering of drain pH although there was more variance in cheese moisture when the drain pH was 6.4 to 6.5.

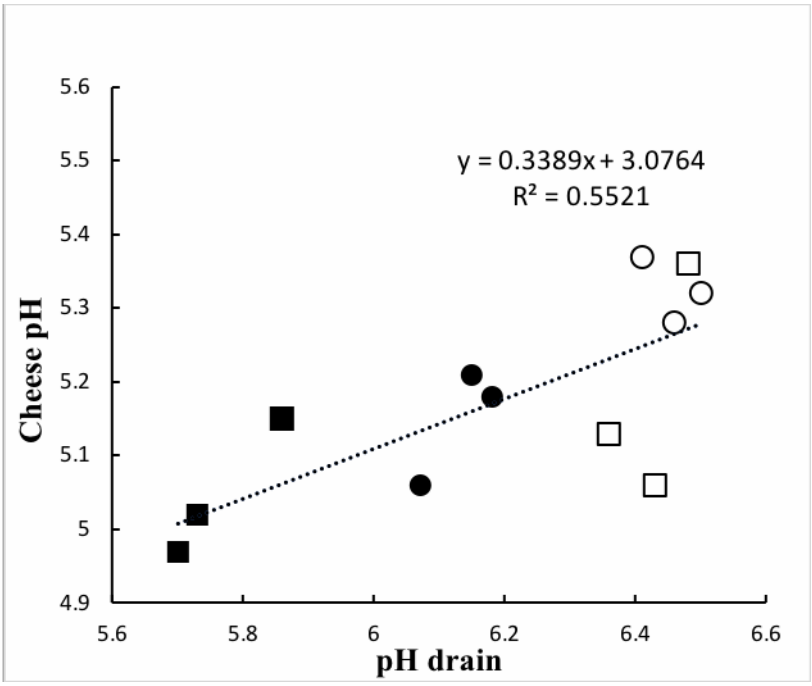


Figure 11. Effect of pH at whey draining (with linear regression) on final pH of cheese after pressing when using starter culture at 0.5 (O), 1 (□), 2 (●) or 4 (■) times the normal amount.

Influence of Whey Drainage pH on Cheese pH and Calcium

There was a significant positive correlation between curd pH at draining and cheese pH after pressing. Although the R^2 was only 0.55 (Figure 11). ANOVA showed statistically significant main effect of culture level on cheese pH with P = 0.0357 (Table 13). Based on Least Square Means, there was a statistically significant difference between cheese pH when using 0.5X culture level compared to cheese pH using 2X and 4X culture levels (Table 14). And also, when using 1X culture level compared to 4X culture level. Since calcium content in cheese is related to the calcium concentration in the whey and as calcium phosphate becomes more soluble at lower pH, it was expected that the cheese drained at lower pH would also have lower calcium levels.

There was a slight correlation between calcium content of the cheese and drain pH (Figure 12) but the R^2 was only 0.35. However, it was not statistically significant with P = 0.3255 (Table 16). This trend agrees with Kiely et al. (1992) who observed decrease in calcium content of Mozzarella cheese from 0.83% when the whey was drained at pH 6.4 to 0.75% and 0.69% when the whey was drained at pH 6.15 and 5.9 respectively.

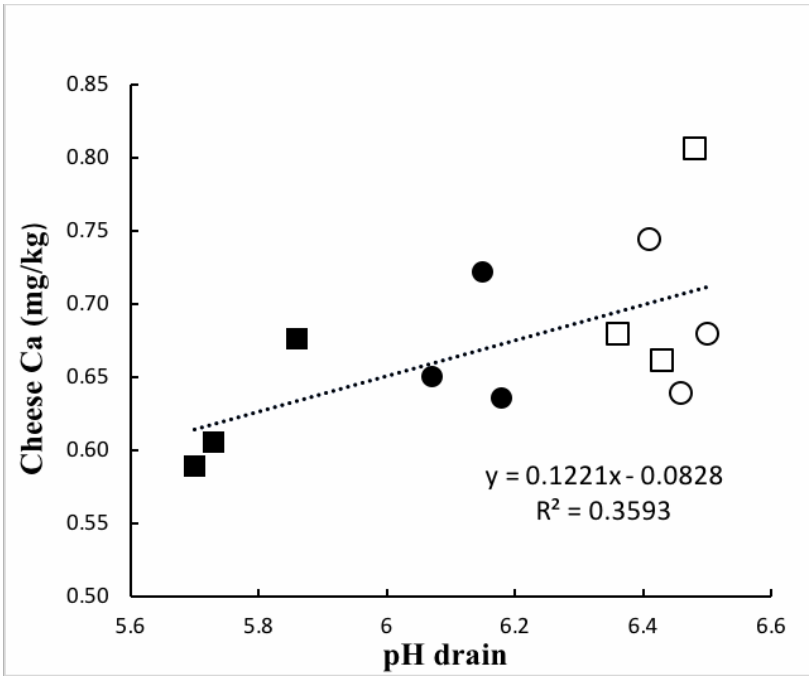


Figure 12. Effect of pH at whey draining (with linear regression) on final calcium content of cheese after pressing when using starter culture at 0.5 (O), 1 (□), 2 (●) or 4 (■) times the normal amount**.**

CONCLUSION

Use of starter cultures at 0.5, 1, 2 and 4 times the normal amount produced different rates of change of pH during cheesemaking when concentrated milk reconstituted from MCC, cream and skim milk so that the milk contained ~3.8% casein was used and did have a significant effect on moisture of cheese curds at whey drainage. The maximum moisture loss from the cheese curds at whey drainage with 4 times the normal amount of culture demonstrates that increased acidification during the cheesemaking process resulted in increased loss of moisture form the cheese curds at whey drainage.

Also, the use of 4X amount of culture for cheesemaking produced the cheese with lowest moisture content and pH. Thus, it can be concluded that the pH drop that occurs during cheesemaking increases rate and extent of whey expulsion and will produce cheese with lower moisture. Understanding the effect of rate of change in pH during cheesemaking with use of concentrated milk, on the whey expulsion would facilitate the optimization of cheesemaking processes using concentrated milks to achieve a desirable pH (5.1 to 5.2) and moisture level (36 to 38%) of the final cheddar cheese.

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APPENDICES

APPENDIX A.

Effect of culture amounts on pH drain
The GLM Procedure

Table 6. ANOVA for effect of culture amounts on pH drain

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.93222500	0.31074167	77.04	<.0001
Error	8	0.03226667	0.00403333		
Corrected Total	11	0.96449167			

Table 7. Least Squares Means for effect of culture amounts on pH drain

Least Squares Means for effect Culture Pr > |t| for H0: LSMean (i) = LSMean (j) Dependent Variable: pHdrain				
i/j	1 (1X)	2 (2X)	3 (4X)	4 (0.5X)
1 (1X)		0.0005	<.0001	0.5383
2 (2X)	0.0005		<.0001	0.0002
3 (4X)	<.0001	<.0001		<.0001
4 (0.5X)	0.5383	0.0002	<.0001	

APPENDIX B.

t-Tests

Table 8. t-Test: Paired Two Sample for Means for moisture at 5 min and 20 min.

	Variable 1	Variable 2
Mean	82.8591667	78.925
Variance	0.59426944	0.34807778
Observations	4	4
Pearson Correlation	0.38285842	
Hypothesized Mean Difference	0	
t Stat	10.208361	
P(T<=t) one-tail	0.00100177	
t Critical one-tail	2.35336343	
P(T<=t) two-tail	0.00200355	
t Critical two-tail	3.18244631	

Table 9. t-Test - Paired Two Sample for Means for moisture at 20 min and 35 min.

	Variable 1	Variable 2
Mean	78.925	76.8541667
Variance	0.34807778	0.5933213
Observations	4	4
Pearson Correlation	0.60570581	
Hypothesized Mean Difference	0	
t Stat	6.62453146	
P(T<=t) one-tail	0.00350307	

t Critical one-tail	2.35336343	
P(T<=t) two-tail	0.00700613	
t Critical two-tail	3.18244631	

APPENDIX C.

Analysis of Variance – Moisture loss from Cheddar Cheese Curd

Table 10. Culture Time Least Square Means Adjustment for Multiple Comparisons

Culture	Time	_Culture	_Time	Standard Error	DF	t Value	Pr > |t|	Adj P
1	95	2	95	1.2748	48	2.09	0.0420	0.9337
1	95	3	95	1.2748	48	2.52	0.0152	0.7233
1	95	4	95	1.2748	48	5.47	<0.0001	0.0005
2	95	3	95	1.2748	48	0.43	0.6700	1.0000
2	95	4	95	1.2748	48	3.38	0.0015	0.1928
3	95	4	95	1.2748	48	2.95	0.0049	0.4246

APPENDIX D.

Effect of culture amounts on moisture of cheese
The GLM Procedure

Table 11. ANOVA for effect of culture amounts on moisture of cheese

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	11.29189167	3.76396389	2.50	0.1335
Error	8	12.04520000	1.50565000		
Corrected Total	11	23.33709167			

Table 12. Least Squares Means for effect of culture amounts on moisture of cheese

Least Squares Means for effect Culture Pr > |t| for H0: LSMean (i) = LSMean (j) Dependent Variable: Moisture				
i/j	1 (1X)	2 (2X)	3 (4X)	4 (0.5X)
1 (1X)		0.9102	0.0979	0.4987
2 (2X)	0.9102		0.0818	0.5701
3 (4X)	0.0979	0.0818		0.0325
4 (0.5X)	0.4987	0.5701	0.0325	

APPENDIX E.

Effect of culture amounts on pH of cheese
The GLM Procedure

Table 13. ANOVA for effect of culture amounts on pH of cheese

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.15795833	0.05265278	4.69	0.0357
Error	8	0.08973333	0.01121667		
Corrected Total	11	0.24769167			

Table 14. Least Squares Means for effect of culture amounts on pH of cheese

Least Squares Means for effect Culture Pr > |t| for H0: LSMean (i) = LSMean (j) Dependent Variable: pH_cheese				
i/j	1 (1X)	2 (2X)	3 (4X)	4 (0.5X)
1 (1X)		0.1811	0.0256	0.5074
2 (2X)	0.1811		0.2391	0.0629
3 (4X)	0.0256	0.2391		0.0089
4 (0.5X)	0.5074	0.0629	0.0089	

APPENDIX F.

Effect of culture amounts on calcium content of cheese
The GLM Procedure

Table 15. ANOVA for effect of culture amounts on calcium content of cheese

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.01345692	0.00448564	1.35	0.3255
Error	8	0.02659286	0.00332411		
Corrected Total	11	0.04004979			

Table 16. Least Squares Means for effect of culture amounts on calcium content of cheese

Least Squares Means for effect Culture Pr > |t| for H0: LSMean (i) = LSMean (j) Dependent Variable: pH_cheese				
i/j	1 (1X)	2 (2X)	3 (4X)	4 (2X)
1 (1X)		0.3547	0.0863	0.5708
2 (2X)	0.3547		0.3592	0.7058
3 (4X)	0.0863	0.3592		0.2097
4 (0.5X)	0.5708	0.7058	0.2097	