USING PREDICTIVE MODELS REGARDING *LISTERIA* IN COTTAGE CHEESE

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To Improve Hazard Analysis Critical Control Points

Introduction

When manufacturing foods for consumer consumption, food safety is of paramount concern. Historically, food sampling was used to determine presence of pathogens, but over time it was recognized that this approach was impractical for many reasons. With the advance of computing and the increase in computing power, several programs have been created that can model the potential growth of harmful organisms, like *Listeria monocytogenes* in foods under various specific food manufacturing conditions. By breaking down the steps in the food manufacturing process, and determining critical control points (CCP), a hazard analysis can be conducted to determine if there are any stages in a food manufacturing process where pathogens could develop, creating a potentially dangerous food product. This is called a Hazard Analysis of Critical Control Points, or HACCP. The application of comparative data from predictive models for survival and death of Listeria in cheese can help analysts to optimize the identification of the CCPs in HACCP by correlating the models to the effect of temperature, PH, and water activity (a_w) parameters. This paper analyzes the ComBase (U.S. Department of Agriculture [USDA] & University of Tazmania, 2017), Pathogen Modeling Program (PMP; USDA, n.d.-c), and the Food Spoilage and Safety Predictor (FSSP; National Food Institute [NFI], 2014) as risk assessment tools to predict growth and death rates of *L. monocytogenes* in Cottage cheese. By applying these predictive models to the temperatures, pH, and a_w found in the cottage cheese manufacturing process, it is postulated that a better control HACCP can be created.

Literature Review

Overview of Cheese Safety

When manufacturing cheeses of different types, safety is of a paramount concern as cheeses for the most part are intended for human consumption (Tenenhaus-Aziza, Daudin, Maffre, & Sanaa, 2014). Also, due to the nature of cheese production, the raw materials that are utilized to make cheeses are in the exact zone combination of temperature, pH, and a_w wherein certain bacteria like *L. monocytogenes* can flourish (Lahou & Uyttendaele, 2017; Rosshaug, Detmer, Ingmer, & Larsen, 2012; Tiwari, Walsh, Rivas, Jordan, & Duffy, 2014). When it comes to food safety, microbiologists primarily focus their efforts on making certain that any pathogenic microorganisms (like *L. monocytogenes*) are either killed or limited to an acceptable level in the resulting food product at specific points in time (Coroller, Kan-King-Yu, Leguerinel, Mafart, & Membré, 2012). To make certain that pathogenic microorganisms are sufficiently controlled in food products, key predictive modelling tools are used to forecast the amounts of the pathogens in the given conditions.

Three predictive models were used in this study to forecast the growth or survival rates of pathogens in the cheese production cycle. These models are known as ComBase, PMP and FSSP. ComBase is a useful predictive model that helps determine pathogen growth/inactivation within the dynamic variations of temperature, pH, and a_w (Psomas, Nychas, Haroutounian, & Skandamis, 2011). PMP is a polynomial predictive modeling tool that can predict the level of exposure of consumers to key pathogenic bacteria when they are eaten (Psomas et al., 2011; Van Gerwen & Zwietering, 1998). FSSP can be utilized to establish growth/no-growth predictions based on environmental circumstances of food manufacture (Tenenhaus-Aziza & Ellouze, 2015).

Overview of Cheese HACCP

As mentioned previously it is crucial while producing cheese to monitor the critical control points (CCP) that may occur during the cheese manufacturing process (Lahou & Uyttendaele, 2017). Critical control points represent points in food manufacturing processes where bacteria may be present (Nababan et al., 2017). The Hazard Analysis Critical Control Points (HACCP) process serves as proactive methodology that can assure, through predictive modeling, the safety of food manufactured (Stier, 2017). Software models such as ComBase, PMP, and FSSP are commonly used in the cheese HACCP process to make predictions on what levels of bacterial exposure are likely in manufactured products, including cheese, which improves food safety (Coroller et al., 2012; Łobacz, Kowalik, & Tarczynska, 2013; Stier, 2017; Tenenhaus-Aziza & Ellouze, 2015).

The HACCP process, as applied to cheese manufacturing, breaks the process of making the cheese down into all of the steps that are involved, then categorizes risk factors such as those of the physical and chemical conditions in the cheese itself (temperature, pH, a_w), potential secondary contaminant sources, and physical compartments that interact with the cheese during its' manufacturing and processing (Tenenhaus-Aziza et al., 2014). Of special interest in this process, is the breaking down of the cheesemaking steps on the chemical conditions previously stipulated, because some of these points could become critical points wherein pathogens like *L. monocytogenes* could flourish. According to Emmons and Tuckey (1967), during the approximately 5 hour process of manufacturing cottage cheese, temperatures can range anywhere from 72℃ to 32℃ and the pH can range anywhere from 4.6 to 4.8. Ryser, Marth, and Doyle (1985) noted that after manufacture, heat treatment and storage, the final pH of the cottage cheese ranged from 5.0 to 5.5.

Cottage Cheese Flow Chart and CCPs

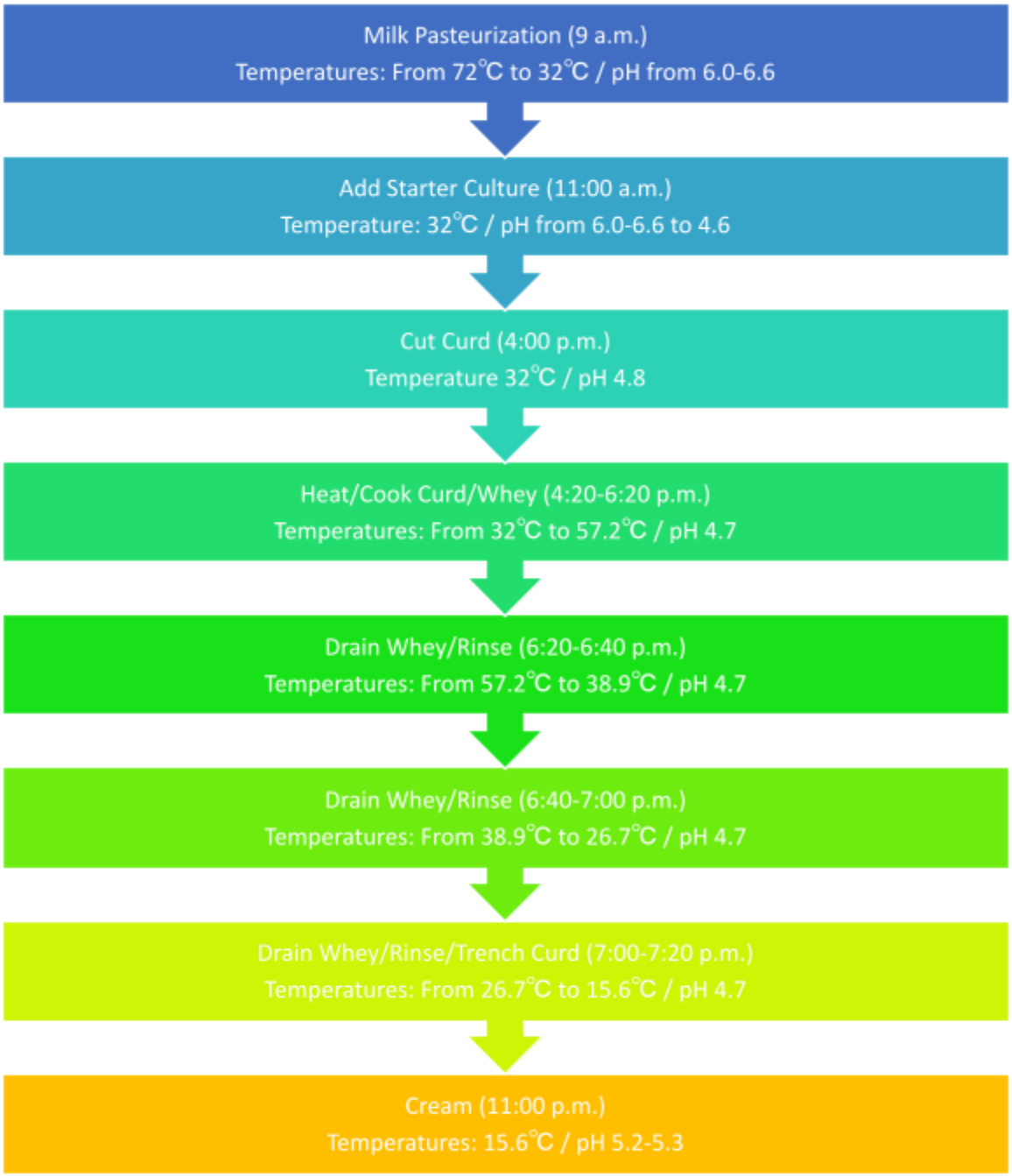

Table 1 – Cottage cheese flow chart with CCP (Daviau, Famelart, Pierre, Goudédranche & Maubois, 2000; Emmons & Tuckey, 1967; Ryser et al., 1985).

Predictive Modeling for Growth and Death Rates of *L. monocytogenes*

Predictive modeling for the growth and death rates of *L. monocytogenes* has been performed for a number of cheeses, including cottage cheese (Jesus et al., 2016; Østergaard, Eklöw & Dalgaard, 2014; Ryser et al., 1985; Schvartzman et al., 2011; Tenenhaus-Aziza & Ellouze, 2015), soft blue-white cheeses (Rosshaug et al., 2012), goat cheese (Chaix, Couvert, Guillaume, Gontard & Guillard, 2015), mold-ripened cheeses (Łobacz et al., 2013), smeared cheeses (Schvartzman et al., 2011), soft cheese (Psomas et al., 2011), and various other cheeses (Augustin et al., 2011; Couvert et al., 2010; Kapetanakou, Gkerekou, Vitzilaiou, & Skandamis, 2017; Lahou & Uyttendaele, 2017; Schvartzman, Belessi, Butler, Skandamis, & Jordan, 2010).

The predictive modeling process for death and growth rates of *L. monocytogenes* has also been conducted for a number of other food classes, such as meats (Augustin et al., 2011; Cornu, Billoir, Bergis, Beaufort, & Zuliani, 2011; Hereu, Bover-Cid, Garriga, & Aymerich, 2012; Karina, Julio, Leda, & Noemi, 2011; Koutsoumanis, Lianou, & Gougouli, 2016; Sheen, Hwang, & Juneja, 2011) and other foods (Coroller, Jeuge, Couvert, Christieans, & Ellouze, 2015; Coroller et al., 2012; Nunes, Alvarenga, de Souza Sant'Ana, Santos, & Granato, 2015; Van Gerwen & Zwietering, 1998; Zwietering, Jongenburger, Rombouts, Van, & van 'T Riet, 1990).

As Stier (2017) explains, while finished food testing was the standard that was initially applied to food safety, it was recognized long ago that testing of actual food was an insufficient form of prevention, and that other risk management systems needed to be developed. HACCP, with its focus on using predictive models, slowly became the standard and was codified into numerous laws, here in the United States and in other countries as well. However, one of the major drivers of predictive modeling came when large food processors, fast food restaurants and operators, and grocery chains insisted that food safety plans had to be instituted, or the producers

would lose the business. This final straw, combined with the U.S. Food and Drug Administration (FDA) support, has caused predictive modeling as a part of determining death and growth rates of various pathogens, including *L. monocytogenes*, to become the accepted practice in food safety.

Methods

Models to be Utilized

As was discussed in the literature review, the three predictive models that will be utilized for this study will be ComBase, PMP, and FSSP. These tools will be utilized to model the anticipated survival and growth of *L. monocytogenes* in Cottage Cheese during the production process. In this section, the specifics of how each of these predictive models work, as well as their strengths and weaknesses, will be discussed.

ComBase

ComBase is a predictive microbiology and risk assessment tool that was developed and is supported jointly by the University of Tasmania and the USDA (2017). Backed by a robust database of over 60,000 quantified microbial responses to the food environment, ComBase Predictor and Food Models combine to forecast growth and inactivation of various food-borne microorganisms. ComBase has been at the heart of many HACCP plans, and informs those who are designing food safety risk management plans. ComBase has a robust number of records regarding *L. monocytogenes* across many different food types and situations (Coroller et al., 2012).

As Lobaczet et al. (2013) explain, predictive microbiology models can fall into one of three general classifications. A primary model is one that will evaluate bacterial growth kinetics, which are defined as growth rate and lag phase duration, but only in the context of constant

environmental conditions. Secondary models bring into consideration environmental factors and describe how these can impact the food under consideration. Finally, tertiary models combine both the data in the primary models and influences of the secondary models. The ComBase Predictor software is considered a tertiary microbiology model. The added advantage that ComBase has that the others lack is that after conducting projections utilizing its' software model, it then compares the models that it has generated against its' database to validate the findings.

ComBase Predictor allows the user to create three types of models, growth, thermal inactivation, and non-thermal survival (University of Tasmania & USDA, 2017). Selectors are present for either a static model or a dynamic one, and the model can be calculated either for the influence of a_w or NaCl. The non-thermal survival model only supports a static mode. Based on the pathogen selected, the fields Phys. State, temperature (℃), pH, and A_w prepopulate, with A_w being preselected. The field Phys. State refers to the physiological state of the cell as a value between 0 and 1, with 0 meaning that there is no growth, and 1 meaning that there is no lag time, which would result in immediate growth (Mounier et al., 2010). It is important to note that ComBase Predictor has been criticized by some as overstating food risk danger (Couvert et al., 2010; Łobacz et al., 2013; Mounier et al., 2010). For this reason, Mounier et al. (2010) proposed adding a bias factor calculation which would provide an index indicating the average amount that the observed values were actually above the line of equivalence ($y=x$), and if this was so, it would reveal by how much the software was deviating.

Pathogen Modeling Program (PMP)

PMP, created and managed by the USDA (n.d.-c), is a tertiary microbiology prediction model. PMP, similar to ComBase Predictor, can accept numerous variables to calculate the level

of thermal inactivation of a pathogen, including temperature, pH, NaCl concentration, and concentration of sodium pyrophosphate. A log reduction value may also be added. For its' secondary prediction mechanism, PMP utilizes the Gompertz equation to calculate lag times (Valero, Carrasco, & García-Gimeno, 2012). PMP utilizes T ref temperature and Z-values which then are applied to an array of temperatures (Skandamis & Panagou, 2016).

According to the USDA (n.d.), oftentimes it is not possible with PMP to find the exact model that fits the food formulation, as is the case with cottage cheese. In that case, they suggest that the model closest to the formulation of the food one is trying to test be used. They note that this will provide more liberal estimations of growth or inactivation. They use the example of culture media (broth) samples, on which many of their models are based. The USDA (n.d.-a) explains that if the environmental parameters (temperature, pH, w_a) are set to the food that findings are desired for, then the results provided by PMP will over estimates both growth and inactivation.

Like ComBase Predictor, PMP is not without its' detractors. In a study evaluating *L. monocytogenes* growth in milk comparing the researcher's own results to those predicted by PMP, Kowalik et al. (2006) found that there were substantial discrepancies between the observed data in comparison with the data predicted by PMP. For example, in the PMP projections, the arrival of bacterium was significantly earlier than in their own observed samples. They recommended that a model be created for each type of food, which would render more accurate results.

Food Spoilage and Safety Predictor (FSSP)

The FSSP model is another model that has been successfully used in modeling pathogenic behavior with cottage cheese (Tenenhaus-Aziza & Ellouze, 2015). According to

Tenenhaus-Aziza and Ellouze (2015) while FSSP supports the same inputs as the other tools that have been discussed, such as temperature, pH, and a_w, they note that the tool can consider up to 12 parameters simultanously, enabling a much wider view of how the interaction of inputs could negatively impact food quality. Some of the inputs they noted in addition to those already mentioned include CO2, nitrite, organic acids such as acetic/diacetate, benzoic, citric, lactic and sorbic, and smoke inensity. The tool can also identify interactions between multiple inputs like a combination of *L. monocytogenes* and lactic acid bacteria (LAB).

In comparison to ComBase, FSSP has models they term as extensive that can predict simultaneous growth of *L. monocytogenes* in cottage cheese (National Food Institute, 2014). They also provide a growth and growth boundary model specific to *L. monocytogenes*. To improve the accuracy of the tool, it also permits uploading data already saved regarding observations seen in local testing facilites. FSSP is a tertiary predictive model, utilizing a logistic model with delay for the primary model, and simplified cardinal parameter type model for the secondary model. Recalling the earlier discussion, primary models evaluate bacterial growth kinetics, while secondary models evaluate environmental parameters' effects on growth kinetics (Łobacz et al., 2013). From all of the tools discussed thus far, FSSP is the only one to address its' validity as a testing tool, noting that it has been validated regarding growth/no-growth responses in challenge tests with inoculated food products (National Food Institute, n.d.).

FSSP is also more sophisticated in its' modeling in that it allows a higher level of detail to be created in a single model. Like all of the other tools, one can enter standard inputs of a_w and pH. However FSSP takes the temperature parameter to a different level. As the National Food Institute (n.d.) indicates, FSSP supports temperature profiles, which allow for the

indication of temperatures for specified periods. For example, the profile may indicate that the product is at a temperature of 5ºC for 2 hours, at 12ºC for 24 hours, and 7ºC for 96 hours. By allowing for this type of variation, the prediction curves can be significantly more accurate. On top of that, the software allows you to use a few temperature profiles that it has built in, or you can upload temperature profiles from your own data. This makes the FSSP much better suited to situations like the cottage cheese manufacturing process, which goes through a wide range of temperatures over a 24-hour manufacturing period, not to mention storage after the product is manufactured.

Parameters for Growth Models

Step	Time (mins)	pH	Temperature
Milk Pasteurization	16s	6.0-6.6	72°C
Inoculate and incubate	100	6.5	32°C
Inoculate and incubate	100	5.5	32°C
Inoculate and incubate	100	5.0	32°C
Cut Curd	20	4.8	32°C
Heat/Cook Curd/Whey	120	4.7	32°C to 57.2°C
Drain Whey/Rinse	20	4.7	37°C
Drain Whey/Rinse	20	4.7	25°C
Drain Whey/Rinse/Trench Curd	20	4.7	15.6°C
Add Cream	220	5.2-5.3	15.6°C

Table 2 – Parameters for growth models (Daviau et al., 2000; Emmons & Tuckey, 1967; Ryser et al., 1985)

Results and Discussion

			Model Outputs for Predicted Growth Rates measured and calculated in log(cfu/g)/h		
Step	**Parameter(s) Modulated**	**Parameter Value**	**ComBase**	**FSSP**	**PMP**
Milk Pasteurization			N/A	N/A	N/A
Innoculate and Incubate	**pH** (Constants: Duration of 100 min & Temperature of 32 deg. C)	**1. 6.5** **2. 5.5** **3. 5.0**	0.494 0.3 0.185	N/A	0.42 0.26 0.156
Cut Curd	**None** (Constants: Duration of 20 min, pH of 4.8 and Temperature of 32 deg. C)		0.146	N/A	N/A
Heat/Cook Curd/Whey	**None** (Constants: Duration of 120 min, pH of 4.7 and Temperature of 37 deg. C)		-76.04	N/A	N/A
Drain Whey/Rinse/ Trench Curd	**Temperature in deg. C** (Constants: Duration of 20 min & pH of 4.7)	**1. 37** **2. 25** **3. 15.6**	0.136 0.089 0.033 (T)	N/A	0.12 0.055 0.03(T)
Add Cream	**Duration, Temperature, pH, and Inhibiting Factor**	**1. Duration of 220 min, pH of 5.2 to 5.3 and Temperature of 15.6 deg. C**	0.059*	N/A*	0.06*

Add Cream (C'td)		**2. Duration of 35 days, pH of 6.2 and Temperature of 5 deg. C** ***Uninhibited, Product 1 with 5 ppm Phenol smoke and 0 ppm Acetic Acid, Product 2 with 15 ppm Phenol smoke and 1500 ppm Acetic Acid** **3. Duration of 35 days, pH of 6.2 and Temperature of 5 deg. C** ***Product 1 with reduced Inhibiting Factor, Product 2 with enhanced Inhibiting Factor** **4. Duration of 15 days, pH of 6.2 and Temperature Profile 12 (1 day), 7 (4 days) and 5 (10 days) deg. C**	N/A N/A N/A	Product 1: 0.18 Product 2: 0 Product 1: 0.19(B)/ 0.08(A) Product 2: 0.19(B)/ -0.064(A) *Where B is before Peak Conc. of LAB, and A is After* 0.125 (during 5 deg. C section of	N/A N/A N/A

				temp. profile)	

Table 3 - Table Summary of Model Outputs for ComBase, FSSP & PMP simulations of *Listeria monocytogenes* growth rate during the 6 primary steps for Cottage Cheese Production (N/A indicates no model output available based on incompatible parameter requirements).
***measured and calculated in log(cfu/g)/day**

The predictive *Listeria monocytogenes* bacteria growth and death rate results for all models in the table above were extracted from the graphs and or tabular data obtained from the three different simulation models (ComBase, FSSP & PMP). The ComBase model directly provided the maximum growth rate in terms of log(cfu/g)/h while for the FSSP & PMP a tangent line was extrapolated in the LM bacterial growth phase after initial delay and prior to peak cell concentration at the time frame point with the greatest derivative (growth rate) and the linear slope was calculated at that point (or using the table values where provided).

Overall in the cottage cheese manufacturing process, as confirmed by both ComBase and PMP modelling, the inoculation and incubation period was most susceptible to LM culture growth at approximately 0.49 and 0.42 log(cfu/g)/d, which is a growth rate approaching approximately 3 colony forming units daily at maximum rate. Furthermore, this occured at the least acidic pH value of 6.5 for the inoculation and incubation process for cottage cheese production. However, subsequently it will be shown that due to practical implications such as lag phase that the most vulnerable phases of cottage cheese production to Listeria growth for targeting of HACCP protocol control points are actually the draining of whey/rinsing and trenching of curds and addition of cream followed by refrigerated storage in particular.

Literature (External) and Internal 3 Model Results Comparison for each Step within the Cottage Cheese Manufacturing Process

Milk Pasteurization

Milk Pasteurization, the first step in the Cottage Cheese Manufacturing Process takes place for a brief duration of 16 s at temperatures ranging between 72 °C and pH 6.0 to 6.6. Under the conditions of a study conducted at the University of Wisconsin-Madison, two thirds of pasteurization trials conducted in the range of 71.7 to 73.9 °C for 16.4 s while none did for 76.4 to 77.8 °C at 15.4 s (although the number of trial runs was limited to three for the latter) using two direct plating and three enrichment procedures for comprehensiveness in the possible resuscitation of heat-injured LM cells (Doyle, Glass, Beery, Garcia, Pollard & Schultz 1987). The pH was sampled to range between 6.5 and 6.6 for nearly all the samples of milk tested prior to pasteurization using the plate heat exchanger, which is comparable to our study and within range (Doyle, Glass, Beery, Garcia, Pollard & Schultz 1987).

Hence, the U.S. FDA guidelines for 71.7 °C at 15 s pasteurization may not completely remove LM bacteria from milk, and in addition both pasteurization methods still possessed intracellular Polymorphonuclear Leukocyte containing LM cells up to 4.8 x 104 CFU/mL (Doyle, Glass, Beery, Garcia, Pollard & Schultz 1987). Furthermore it should be noted that the models are limited in terms of temperature range and do not have the predictive capacity for 72 °C, PMP is limited up to 37 °C in evaluation of the lag and growth phase durations by USDA.

Inoculation and Incubation

The subsequent step in production is the inoculation and incubation phase, which for cottage cheese occurs at 32 °C for a duration of 100 minutes with pH ranging from 5 to 6.5. The process for cheddar cheese from literature is very similar, utilizing an incubation temperature for the starter culture of 31.1 °C for a duration of approximately 85 minutes at the low end of the pH range having an average between 5 and 5.15 (Ryser & Marth 1987).. It is necessary to vary the acidity in predictive model trials because of acid development during incubation of pasteurized skim milk with *S. cremoris* starter cultures (two strains OS and CC6) to generate cottage cheese in which the pH declines from 6.65 to 4.70, and likewise for the cheddar cheese process from 6.35 to 5.50 (Ryser, Marth & Doyle, 1985; Ryser & Marth 1987).

The PMP and ComBase models both predict concentration log(CFU/g) values of approximately 3 for the different pH conditions at the end of the 100 min inoculation and incubation period because in all three instances, as reported in literature also, different strains of LM (Scott A, V7, etc.) are recovered similar in quantity to the pre-incubation inoculation with LM culture. This is because these strains of LM have lag phases of at least 2 hours to assimilate nutrients before replication and under the best PMP condition of pH 6.5 (most conducive to growth) this can be seen in the predictive model where the starter bacterial culture population remains stagnant for the first 2 hours before the growth phase begins (Ryser, Marth & Doyle, 1985). Therefore, no growth is generally expected until the stage of manufacture involving cooking or draining of the curd. Scott A and V7 LM strains failed to grow in an inoculation of pasteurized skim milk with 5 weight % starter culture incubated at 32.2 °C according to literature results, so while the growth rates may be the greatest of all steps described subsequently, they are

not actualized in terms of the inoculation and incubation stage being susceptible to Listeria growth as a result of the lag phase if originating from a contamination (Ryser, Marth & Doyle, 1985).

Both the ComBase and PMP models resulted in very similar LM culture growth rates for all pH conditions trialed. The ComBase model, as expected and described previously, overstated the growth rates and thus subsequent food risks as a result of model bias, which could be seen in the slightly larger growth rate values compared to the PMP model. The increasingly acidic pH for both cottage and cheddar cheeses lowers the growth rate for the LM bacteria, with the percentage reduction in growth rate becoming more drastic the lower the pH (e.g. 1.0 pH drop from 6.5 to 5.5 resulted in growth rate drop of 39.3% while the subsequent 0.5 pH drop 38.4% for the ComBase outputs), which is logical since bacteria have an optimal pH point for growth around which growth drops exponentially (bell-curve). FSSP methods are suited to pH above 5 and temperatures below 25 °C such as the 5 °C storage temperature after cream addition and as such was not applied herein for incubation occurring at 32 °C (Ryser, Marth & Doyle, 1985).

Cut Curd

As stated previously, no LM bacterial growth is actually expected until after the cooking step due to lag phase growth characteristics. Nonetheless when environmental parameters of the model were set to the same temperature as during incubation (32 °C), for a shorter duration of 20 minutes at a slightly lower pH of 4.8 (than at the tail end of incubation) for cutting curd, the ComBase hypothetical max growth rate under those conditions was 0.146 log(CFU/g) per hour. This is less than the growth rate of 0.185 for the 5.0 pH simulated for incubation and agrees with the findings stated prior. Again FSSP is invalid; both pH and temperature exceed model bounds.

Heat Curd/Cook/Whey

The heating of intermediate cottage cheese curd product reaches temperatures of 57.2 °C (from 32); because of this FSSP predictive modelling is disqualified again. PMP is also disqualified since its maximum temperature constraint is 37 °C and the USDA states that PMP models are not suitable for determining lethality calculations via heat inactivation of bacteria (n.d. -c). Temperatures above 50 °C are considered lethal to LM cells ("Listeria Monocytogenes", 2013). At this temperature ComBase was tested (also out of its 37 °C upper limit) and yielded a very high maximum death rate or negative growth of 76.04 log (CFU/g) per hour, similar to the effects of the pasteurization step (if lag phase was not present). While this would imply a starter culture of 3 log (CFU/g) killed in under a fraction of a second which may be unrealistic (model performing out of boundaries), the general premise of high listeria death rate (>99%) is verified by literature in which LM cells were found to be below detectable limits of 100 or 10 per gram post curd from the 10^4 to 10^5 per gram of skim milk during inoculation after pasteurization cooking in cottage cheese manufacturing but were not completely eliminated (Ryser, Marth & Doyle, 1985).

Drain Whey/Rinse/Trench Curd

This phase of the cottage cheese manufacturing process takes place at temperatures ranging from 37 down to 15.6 °C, but a pH of 4.7 that disqualifies FSSP. The highest growth rate of 0.136 log (CFU/g)/h was taken at the highest temperature for draining whey and rinsing of 37 °C based on the conservative number taken from the ComBase model. This aligns precisely with literature in which the optimal temperature range for LM bacterial culture growth ranges

between 30 to 37 °C, and because the lag phase was completed at 2 h just prior to curd cooking, any strains surviving the cooking phase now have the potential conditions for real growth (Ryser, Marth & Doyle, 1985; “Listeria Monocytogenes”, 2013). The presence of LM after cooking and/or washing was verified via cold enrichment in Tryptose Broth in which 6 of 9 samples tested positive for Listeria in cottage cheese manufacturing (Ryser, Marth & Doyle, 1985).

Add Cream and Refrigeration Storage

Finally, where cream was added and cheese was placed in storage at refrigerated temperatures of 5 °C, the FSSP model could finally be used. During the 220 minute cream addition step at 15.6 °C and subsequent storage, growth rates were nearly double that of curd trenching in the previous step, probably due to less acidic (~5.2 rather than 4.7) pH and greater time allotted for growth according to ComBase and PMP modellings. However, FSSP allowed examination of the refrigeration storage stage for a high level of input parameter (up to 12) interaction as discussed in the methodology section previously. For our study, two cases were presented: Inhibiting Factor of Lactic Acid Bacteria (LAB) on other bacterial cultures (notably LM) in the cheese was enhanced/reduced versus a control where LAB growth did not affect LM growth (factor set to 1.0). Furthermore, the ppm of phenol smoke and acetic acid were tuned within the latter control. For a storage period of 35 days at 5 °C at pH 6.2, the control trial with greater concentration of phenol smoke and acetic acid at 1500 ppm, LAB exhibited a reduced maximum growth rate but eventually reached the same final population concentration, but the LM was only able to grow in the culture without acetic acid. Both LAB and LM cultures peaked and stabilized at approximately 16 days in the non-acetic acid containing control group. Hence

sufficient acetic acid concentration can prevent the start of the LM growth phase, but may alter flavour and other parameters of the cheese in an undesired way.

Subsequently when the inhibiting factor was adjusted a pivot-point in growth rate could be seen in the LM growth corresponding to where the LAB reached population saturation. Up to the saturation point both increased and reduced inhibition effect of LM by LAB cells resulted in identical growth rates by day 16, after which the reduced inhibition group continued growing (slightly slower) and the enhanced inhibition group of LM experienced a death rate in their population where the LM culture died essentially entirely by day 34 in the model. Hence inhibitory effects on LM growth are only effective when a stabilized mature LAB population and not during the growth phase.

In both creamed and uncreamed samples of cottage cheese at 3 °C in the literature, LM Scott A. and V7 strains were recovered (as supported by our PMP, ComBase and control FSSP models), and Scott A. strains in particular were found by direct plating and count in a greater number of samples in the creamed samples believed to be due to the higher pH conducive to growth and bacterial cell repair (Ryser, Marth & Doyle, 1985). Lactic Acid Bacteria strains fail to produce enough acid to sustain pH below 5.9 (hence setting of 6.2 to 6.3 is acceptable for our study) failing to kill LM via acidic conditions (LM can grow at pH of down to 4.0) and are believed to suppress growth of LM via peptidic toxins known as bacteriocin, which increase during the exponential growth phase and then stabilize for LAB (Coelho, Silva, Ribeiro, Dapkevicius & Rosa, 2014; “Listeria Monocytogenes”, 2013). A three-log unit reduction in LM cell count was observed using a lacticin-481 producing strain of *L. lactis* (Coelho, Silva, Ribeiro, Dapkevicius & Rosa, 2014).

With respect to the variable temperature profile examined using FSSP, it is trivial to note that temperature reductions reduced growth rate (concentration slope) for LAB. Since as mentioned above, LAB has a suppressive effect on LM growth, LAB growth rates can be manipulated via temperature to control LM size.

Limitations

It should be noted that there were limitations to this study. As was mentioned previously, PMP does not have models for cottage cheese in particular. The USDA (n.d.-c) recommends when using PMP for foods that do not have a model in their system to use the nearest equivalent along with the settings that are appropriate for the particular environment. With that, the temperature, pH, and a_w were entered in the tool, and the findings were generally consistent with the other tools.

Another limitation that existed in all three tools was the maximum temperature permissible. In ComBase, the maximum permissible temperature to enter is 40℃. PMP allows a maximum temperature of 37℃. FSSP allows a maximum temperature of 25℃. These limitations can be partly explained in the fact that these tools were designed for different purposes. In the description accompanying PMP, the USDA (n.d.-b) notes that they are measuring for changes generally from 37℃ down in order to determine how long the lag phase is (that is, how long it will take for the cells to start growing) and growth phase. According to Todar (2012), the optimum growth range for *L. monocytogenes* is 30-37℃. Even with the limitations of these various tools, the findings agree and are consistent with the science that they are supposed to be predicting.

Conclusion

As was presented at the outset of this discussion, the purpose of this paper was to analyze the comparative data presented by three major predictive models on the survival and death of *L. monocytogenes* in cottage cheese to help analysts to determine and optimize identification of the CCPs in the HACCP of cottage cheese manufacturing. This was done by corelating the various models and their responses to the conditions of temperature, pH, and aw during the manufacturing process. The ComBase, PMP, and FSSP predictive models were compared to determine what findings they would predict regarding the various CCP in the Cottage Cheese manufacturing process. The findings of the three tools were considerably similar, lending credence to the fact that these models are doing a reliable job of predicting microbiological risk in foods under specific conditions. In addition, the findings are consistent with manually conducted studies on food safety, which created direct experiments on the various steps in the manufacturing of cottage cheese and physically measured the findings at each step. If there is any caveat in the findings of this study as well as those referenced in this study it is this: if any of these predictive tools are guilty of anything, it is over predicting food risk. Due to these and many other findings, then, it is highly unlikely based on this experiment as well as the studies of many others that potentially dangerous development of pathogens in a normal manufacturing operation would be missed or under predicted. The consistency of these tools with each other, in addition to with manual studies on the same topic, confirms the fact that the predictive models tested are reliable and valid for the purpose intended, and that they can be beneficial in helping to create better HACCP programs.

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