July 29, 2021 Dairy Spoilage: Organisms, Mechanisms, and Modeling

Request:

1. A literature review on (A) spoilage microorganisms (B) spoilage mechanisms in raw and pasteurized milk, and (C) factors associated with spoilage.
2. A list of microbiological spoilage models, highlighting those which focus on dairy and dairy-based data.

FRI Reply:

1A. With respect to **spoilage microorganisms in raw and pasteurized milk,** a number of comprehensive reviews on this topic have been published in recent years (Ledenbach and Marshall, 2009, Muir, 2011, Quigley et al., 2013, Erkmen and Bozoglu, 2016, Lu and Wang, 2017, Machado et al., 2017, Fusco et al., 2020, Martin et al., 2021). Some summary information follows.

- **Raw milk microorganisms**
  - Raw milk can be contaminated with a wide variety of microorganisms (Ledenbach and Marshall, 2009).
  - Immediately after milking, lactic acid bacteria (including *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Enterococcus*, and *Streptococcus*) are commonly found in milk (Wouters et al., 2002, Machado et al., 2017, Fusco et al., 2020). While many of these organisms play important functional roles in fermented dairy products (Wouters et al., 2002), these organisms may cause spoilage in milk if the milk is not kept cooled.
  - Once milk is cooled and refrigerated, the growth of psychrotrophs (including members of the genera *Bacillus*, *Micrococcus*, *Pseudomonas*, *Acinetobacter*, *Aeromonas*, and others) is favored, and they become the dominant microorganisms present (Muir, 2011, Quigley et al., 2013, YUAN et al., 2018a).
  - A recent review states that the microbiota of raw milk consists mostly of Gram-negative psychrotrophs (*Pseudomonas*, *Serratia*, *Aeromonas*, and *Enterobacter*) and Gram-positive spore-formers (*Bacillus*, *Aneurinibacillus*, *Brevibacillus*, and *Geobacillus* (Zhang et al., 2019).
  - Another review comments that "Storage of raw milk at refrigerator temperature for several days can lead to growth of psychrotrophic species of several bacterial genera: *Aerococcus*, *Bacillus*, *Lactobacillus*, *Leuconostoc*, *Microbacterium*, *Micrococcus*, *Propionibacterium*, *Proteus*, *Pseudomonas*, *Streptococcus*, coliforms, and others (Erkmen and Bozoglu, 2016).
  - "A wide variety of genera including Gram-negative genera (*Pseudomonas, Aeromonas, Alcaligenes, Acromobactor Acinetobacter, Flavobacterium, Chryseobacterium, Enterobacteriaceae* such as *Serratia, Hafnia, Klebsiella, Enterobacter and Rahnella*) and Gram-positive genera (*Bacillus, Clostridium, Corynebacterium, Micrococcus Streptococcus, Staphylococcus, Microbacterium, Lactococcus* and *Lactobacillus*) are frequently found in raw milk (Vithanage et al., 2016).
  - "Spore-forming bacteria in raw milk are predominantly *Bacillus* spp. (such as *B. cereus, B. licheniformis, B. megaterium,* and *B. subtilis*). *Clostridium* spp. are present in raw milk at low levels. Populations of spore-forming bacteria in raw milk vary seasonally. *Bacillus* and *Clostridium* spp. are at higher levels in the raw milk collected in winters than in summers, because in winters cows lie on spore-contaminated bedding materials and consume spore-containing silage' (Erkmen and Bozoglu, 2016).
  - *Pseudomonas* spp. are considered the most common cause of milk spoilage (Quigley et al., 2013). *Serratia liquefaciens* can also contribute to spoilage in raw milk (Bagliniere et al., 2017).
  - Because lactose is the main carbohydrate in milk, microorganisms which can hydrolyze lactose (organisms that have enzymes like lactase or beta-galactosidase) have an advantage over those which cannot (Erkmen and Bozoglu, 2016).

    - "The spoilage bacteria in raw milk are mostly aerobic Gram-negative psychrotrophic rods, such as *Alcaligenes*, *Flavobacterium*, *Pseudomonas*, and some coliforms. About 65–70% of psychrotrophic microorganisms in raw milk are *Pseudomonas* spp." (Erkmen and Bozoglu, 2016).

- **Pasteurized milk microorganisms**
    - "Pasteurization inactivates all pathogens and many spoilage microorganisms, but this treatment may also encourage the survival of thermoduric (such as *Bacillus*, *Lactobacillus*, and *Streptococcus*) and thermophilic spoilage microflora by destroying inhibitory chemicals and competing microorganisms, and activating spores" (Erkmen and Bozoglu, 2016).
    - Spoilage organisms associated with pasteurized milk includes psychrotrophs and spore-forming organisms. Thermostable enzymes released from bacteria which are killed during pasteurization may also be present and contribute to spoilage (Ledenbach and Marshall, 2009, Martin et al., 2021).
    - Sporeformers whose spores survive pasteurization include *B. cereus*, *B. weihenstephanensis, Paenibacillus, Psychrobacillus, Viridibacillus* (Muir, 2011, Martin et al., 2021). Some of these spores may originate in raw milk or may be introduced during processing (Barbano et al., 2006).
    - The most common post-processing microorganism in milk is *Pseudomonas* spp. (Martin et al., 2021).

1B. Several different **mechanisms of spoilage** have been associated with different microorganisms in raw and processed milk.

- A variety of different types of microbial spoilage can occur in milk and dairy products, as shown in the following table:

Microbial Spoilage of Dairy Products

Product	Conditions	Spoilage Type	Spoilage Organisms	Enzymes	Metabolic Product	References
Milk, sour cream, yogurt	Temperature abuse during storage	Acid proteolysis	*Lactobacillus, Micrococcus, B. cereus*	Proteinases, lactase	Peptides, amino acids	(Nemeckova et al., 2010, Montanhini et al., 2013, Ribeiro et al., 2018b, Mehta et al., 2019)
Milk	During storage	Alcoholic flavor	Yeasts	Alcohol dehydrogenase	Ethanol	(Nakanishi and Arai, 1969, Roostita, 1994)
Pasteurized milk, cream	Refrigeration or higher storage temperatures	Bitter flavor, coagulation, off-odors	Psychrotrophic bacteria (*Pseudomonas* spp., *Chryseobacterium, Serratia* spp.), *Bacillus* spp.	Proteinases (AprX, Ser1, Ser 2, etc.), lipases	Bitter peptides, volatile organic compounds, fatty acids	(Adams et al., 1975, Deeth et al., 2002, Montanhini et al., 2013, Alothman et al., 2017, Bagliniere et al., 2017, Marchand et al., 2017)
Raw milk, higher pH cheeses	During storage	Fishiness	*Aeromonas hydrophila* (can also arise from a bovine polymorphism which causes abnormal secretion of trimethylamine)	Proteinases	Fish flavors	(Melas et al., 1999, Lundén et al., 2002, Erkmen and Bozoglu, 2016)
Milk, raw-milk cheese	Higher levels of esters were found in milk with increasing cell numbers, less aeration, inoculation with cells grown at 8 (vs. 20)°C, presence of ethanol	Fruity flavor	*P. fragi, Staphylococcus, Candida, S. cerevisiae, P. notatum, C. butyricum*	Esterase	Ethyl esters, lactones	https://www.uoguelph.ca/foodscience/book-page/characterization-flavour-defects-adsa (Reddy et al., 1969, Morales et al., 2005)
Milk, cheese	In cheese, the metabolic products giving these flavors develop within hours of the beginning of ripening and decline over months of ripening.	Caramel and malty flavor	*L. lactis* var. maltigenes, *Lactobacillus helveticus*	Oxidase	3-methylbutanal, furans and furanones	https://www.cheesescience.org/caramel.html https://www.uoguelph.ca/foodscience/book-page/characterization-flavour-defects-adsa (Morgan et al., 1966, Afzal et al., 2017, Meng et al., 2021)
Milk, processed cheese, cream, butter	Storage at refrigeration temperature	Putrefaction	*P. putrefaciens, Clostridium*	Proteinases	Peptones, amino acids	(Wolochow et al., 1942, Anonymous, 1944, Zant, 1957)
Milk, powdered milk	Storage of raw milk under refrigeration for days before heat treatment	Rancid flavor	Lipolytic bacteria, including psychrotrophs	Lipases	Free fatty acids such as butyric acid	(Andersson et al., 1981, Stead, 1986, Paez et al., 2006)
Milk, cheese, yogurt	Production of exopolysaccharide is affected by temperature, pH, etc.; often desirable in products such as yogurt and cheeses	Ropy texture	Lactic acid bacteria (LAB) including *Streptococcus thermophilus*	Polymerase	Exopolysaccharide	https://www.uoguelph.ca/foodscience/book-page/characterization-flavour-defects-adsa (Cerning et al., 1992, Broadbent et al., 2003)

Product	Conditions	Spoilage Type	Spoilage Organisms	Enzymes	Metabolic Product	References
Milk	Desirable in products such as sour cream, butter, and cheese	Sour and acid flavor	*Lactococcus lactis*	Lactase	Lactate	(Suzuki et al., 1979)
Milk	Temperature of storage	Sour flavor at 10 to 40°C	LAB, coliforms, *Enterococcus*	Lactase	Lactate, acetate	https://foodsafety.foodscience.cornell.edu/sites/foodsafety.foodscience.cornell.edu/files/shared/documents/CU-DFScience-Notes-Dairy-Cultures-HomoHeteroferm-10-08.pdf
Milk	Temperature of storage	Sour flavor at 37 to 50°C	*L. thermophiles, S. thermophilus*	Lactase	Lactate, acetate	(Suzuki et al., 1979)
Cheeses, milk	Higher temperature storage	Amino acid degradation	*Yarrowia lipolytica, Lactobacillus buchnerii*	Histidine decarboxylase and other decarboxylases	Biogenic amines such as histamine and tyramine	(Garnier et al., 2017), https://www.cheesescience.org/histamine.html
Pasteurized and heat-treated milk	Refrigeration temperatures	Protein and fat breakdown, off-flavors (rancidity, bitterness)	Somatic cells, which are present in milk at increased levels when cows have mastitis	Native enzymes, including protease (plasmin) and lipase (lipoprotein lipase)	Peptides, fatty acids	(Barbano et al., 2006, Chove et al., 2013)
Evaporated milk	Room temperature	Flat-sour, coagulation	*Geobacillus stearothermophilus* and various *Bacillus* spp., *E. faecium*	Hydrolases (esterase, esterase lipase, lipase, etc.)	Lactic acid	(McKellar and Nicholsnelson, 1984, Kalogridou-Vassiliadou, 1992)
Mozzarella and other fresh cheeses	On surface of cheeses when exposed to air	Blue discoloration	*Pseudomonas fluorescens*	Proteolytic enzymes release aromatic amino acids which are precursors for pigments	Pigments such as indigoidine and pyocyanin	(del Olmo et al., 2018)
Milk, butter, sour cream, yogurt	Higher diacetyl levels in butters undergoing a fermentation process.	Buttery flavor	*Lactococcus lactis* ssp. diacetylactis and *Leuconostoc citrovorum*	Various bacterial metabolic pathways, bacterial citrate metabolism	Diacetyl (2,3-butanedione)	(Mallia et al., 2008, Cheng, 2010, Rincon-Delgadillo et al., 2012, Shibamoto, 2014)

- Some of the key types of spoilage are discussed below. Some useful reviews on this topic have been published (Erkmen and Bozoglu, 2016, YUAN et al., 2018a, Zhang et al., 2019).

- **Acidification:**
    - Lactic acid bacteria release acids, souring milk (Wouters et al., 2002, Erkmen and Bozoglu, 2016). When the pH of milk is reduced to ~5.5), the milk protein casein starts to aggregate. At a pH of 4.6, casein is insoluble (Muir, 2011).

- **Enzymatic (General Comments):**
    - Bacteria found in milk produce enzymes, including proteases, lipases, and phospholipases, that are released extracellularly and can interact with milk components such as proteins, lipids, and lactose, compromising the quality (odor, flavor, texture) of milk (Martin et al., 2021) (Erkmen and Bozoglu, 2016). (More specific discussion related to proteases, lipases, and phospholipases occurs later in this document).
    - "A notable feature of spoilage bacteria found in raw milk is their almost universal ability to produce extra-cellular degradative enzymes" (Muir, 2011).
    - Some of these enzymes (especially proteases) are very heat-stable and therefore survive pasteurization and other processing conditions (YUAN et al., 2018a).
    - Undesirable spoilage-causing enzymes are generally secreted during late log or stationary growth phases of psychrotrophic bacteria (Erkmen and Bozoglu, 2016, YUAN et al., 2018a).
    - The optimum temperature for protease production for *Pseudomonas* is 20°C, but protease can be made at relatively high levels at temperatures as low as 5°C (Erkmen and Bozoglu, 2016).
    - The majority (70%) of *Pseudomonas fluorescens* isolates possess both proteolytic and lipolytic activity, and many of the psychrotrophs found in raw milk possess both lipolytic and proteolytic activity (Muir, 2011), as shown in the table 27.6 from that reference.
    - Not all strains of a species have the same types of enzymatic activities, as shown in the table above and in other studies (Bagliniere et al., 2017).
    - Psychrotolerant spore-formers such as *Bacillus weihenstephanensis* (formerly a member of the *B. cereus* group) also produce lipolytic and proteolytic enzymes (Martin et al., 2021).
    - **Proteases (or Proteinases)**
        - "Psychrotrophic species of *Alcaligenes, Flavobacterium, Bacillus, Micrococcus*, and *Pseudomonas* can grow at low temperatures and produce extracellular proteinases (Erkmen and Bozoglu, 2016).
        - A study of Brazilian refrigerated raw milk identified many different bacterial species with proteolytic potential (*Lactococcus lactis, Enterobacter kobei, Serratia ureilytica, Aerococcus urinaeequi,* and *Bacillus licheniformis*). Some of these (*E. kobei, L. lactis, A. urinaeequi*) and others (*Acinetobacter lwoffii*) also had lipolytic activity (Ribeiro et al., 2018a).
        - Raw milk bacteria (Gram-negative psychrotrophs and Gram-positive spore-formers) produce zinc-metalloprotease in the serralysin subfamily (such as AprX) and in the serin subfamily (such as subtilisin and thermolysin). These proteases share similar spoilage mechanisms (Zhang et al., 2019).
        - Proteases can spoil milk via a variety of mechanisms:

        - "Proteinases hydrolyze casein in milk to liberate bitter peptides (putrid off-flavors). Proteolytic putrid off-flavors associated with lower molecular weight products such as ammonia, amines, and sulfides" (Erkmen and Bozoglu, 2016).
        - Proteolytic degradation of casein by bacterial enzymes can impact emulsification capacity of milk because casein's hydrophobic nature can stabilize fat droplets in solutions (Muir, 2011).
        - Heat-resistant proteases from psychrotrophic bacteria can lead to age gelation, in part through the cleavage of K-casein, leading to unstable micelles.
        - Proteolysis of casein micelles by *Pseudomonas fluorescens* contributes to the destabilization of UHT milk during storage (Gaucher et al., 2011), leading to sediment and formation of aggregates. The instabilities are related to decreases in the negative charge and hydration of casein micelles.
        - Similarly, *Serratia liquefaciens* destabilized UHT milk during storage, resulting in decreases in hydration and zeta potential of casein micelles and led to aggregates (Bagliniere et al., 2017).
        - "UHT milk is more sensitive to proteinase defects than raw milk due to either heat-inducing change in casein micelle structure or heat inactivation of proteinase inhibitors in milk" (Erkmen and Bozoglu, 2016) .
        - Peptides generated through proteolysis of AprX can lead to formation of a gel network (Zhang et al., 2019).
        - Proteolysis results in free amino groups. These free amino groups can potentially react with reductions sugars, as a result of Maillard reactions, and produce new aromatic compounds in milk during storage (Valero et al., 2001)
    - Some **specific proteases** have been identified and studied.
        - *Pseudomonas fluorescens* secretes the metalloprotease AprX, a member of the serralysin family, which is resistant to UHT treatment.
        - Other proteases and peptidases are also released by this bacteria in milk and may impact milk quality (Wang et al., 2021).
        - Some strains of *P. fluorescens* have the AprX gene but do not hydrolyze casein (and vice versa).
        - *Serratia liquefaciens* produces two extracellular proteases, Ser1 and Ser 2, which are members of the serralysin family (Bagliniere et al., 2017). Ser2 exhibited broad activity, and, like AprX secreted by *P. fluorescens*, could hydrolyze all four caseins (Bagliniere et al., 2017).
        - *Bacillus cereus* produces a heat-resistant protease that leads to rotten, bitter flavors and age gelation in milk (Yang et al., 2021).

- **Lipases**
    - Milk fat is primarily triglycerides, which are glycerol esters of fatty acids. The fatty acids generally range between 4 and 18 in terms of carbon length and are generally saturated (Muir, 2011).
    - The action of lipases on triglycerides releases glycerol and free fatty acids, which may result in rancid and bitter flavors. Oxidation of unsaturated fatty acids can result in formation of aldehydes, acids, and ketones (Erkmen and Bozoglu, 2016).

- **Phospholipases:**
    - "Several bacterial genera, well-represented in raw milk, including *Pseudomonas*, *Acinetobacter*, and *Bacillus*, are known to produce spoilage enzymes such as phospholipase C, which disrupt the milk fat globule membrane, and expose lipids to the endogenous milk lipase or to microbial lipases" (Munsch-Alatossava et al., 2018, Yuan et al., 2018b).
    - Psychrotrophic *Bacillus* spp. that survive pasteurization of milk produce the enzyme lectinase, which hydrolyzes phospholipids and cause aggregation of fat globules that adhere to container surfaces (Erkmen and Bozoglu, 2016).
- **Quorum sensing** can also play a role in bacterial spoilage of milk.
    - Psychrotrophic bacteria are frequently found in biofilms in tanks, pipes, etc. within dairy facilities, and proteolysis and lipolysis are regulated through quorum sensing (YUAN et al., 2018a).
    - Acylated homoserine lactones are commonly produced by Gram negative proteolytic psychrotrophic bacteria from cooled raw milk, and these compounds have been demonstrated to mediate communication between bacterial cells (Pinto et al., 2007).
- **Pigments**
    - "Blue mozzarella" from *Pseudomonas fluorescens*: quorum sensing may induce the production of blue pigment in cheese (del Olmo et al., 2018) and in fluid milk (Reichler et al., 2019).
    - Pink discoloration in cheese has also been noted (Martelli et al., 2020).
    - *Bacillus sporothermodurans* spoilage of milk can lead to a pink color change as well as other changes (Erkmen and Bozoglu, 2016)

1C. Factors Involved in Spoilage of Dairy Products

- **The numbers of microorganisms/temperature/time/when the spoilage does happen?**
    - Spoilage can be caused during microbial growth and/or by bacterial enzymes which persist after heat treatment or after microbial growth has stopped.
        - Spoilage affects organoleptic and/or physical properties of milk and can assessed in numerous ways, including gelling, presence of aggregates, flavor, phosphate test, size of casein micelles, non-casein nitrogen levels, presence of volatiles, levels of proteolysis, etc. (Valero et al., 2001, Gaucher et al., 2011, Muir, 2011, Machado et al., 2017).
        - Different strains of a single species (*Serratia liquiefaciens* and also various *Pseudomonas* spp.) can destabilize milk differently (Bagliniere et al., 2017).
    - It is not always the organism present at the highest level that is the driver of spoilage in a particular food, and neither the total viable cell count nor the number of specific spoilage organisms can be relied upon to predict sensory changes related to spoilage of a food (Braun and Sutherland, 2006).
    - However, numerous papers have identified numbers of microorganisms in milk products associated with spoilage, including the following:
        - "Most studies report that the end of shelf life is reached when microbial populations in pasteurized milk reaches 5.0 to 7.0 log CFU/mL. However, lower levels may cause sensory changes that can be detected by consumers as spoilage" (Ziyaina et al., 2018).

        - "If the raw milk bacterial count is <25,000 CFU/mL, then the raw milk somatic cell count will be the most important determinant of shelf life in pasteurized extended shelf-life milk with respect to development of off-flavors when post-pasteurization bacterial growth is controlled (i.e., <500,000 CFU/mL) (Barbano et al., 2006).
        - **"**Milks in which *Ps. fluorescens* NCDO 2085 had grown to 5 x 10^7 and 8 x 10^6 CFU/ml gelled after 10-14 d and 8-10 weeks respectively, after UHT sterilization (140°C/3-5 s) and storage at 20°C (Law et al. 1977).
        - "Uninoculated low count milks or milks containing only 8 x 10^5 CFU/ml pseudomonads remained liquid for at least 20 weeks after sterilization, although a sediment gradually formed in the latter" (Law et al., 1977, Fairbairn and Law, 1986).
        - "*Pseudomonas* grows rapidly at refrigeration temperatures (e.g., 6°C), and even when it is introduced into fluid milk at low levels (e.g., <1 CFU/mL), it can grow to levels exceeding the PMO limit of 20,000 CFU/mL only 4 to 7 d after pasteurization… it can grow to levels where product quality begins to deteriorate (i.e., approximately 1 million CFU/mL) only to 10 days after pasteurization" (Martin et al., 2021).
        - "In contrast to and other post-pasteurization contaminants in fluid milk, spoilage resulting from the growth of gram-positive spore-forming bacteria occurs later in shelf life, reaching 20,000 CFU/mL approximately 14 to 21 d after pasteurization" (Ranieri et al., 2009, Martin et al., 2021).
        - "After addition of 0.05-1.0% of a 2-day-old culture of *P. fluorescens* strain 22F (incubated at 20°C, 5 X 10^9 bacteria/ml) to skim milk which was subsequently heated for 45 s at 142°C, the milk developed a bitter flavor within less than or equal to 1 wk and gelled within 0.3-5.0 wk... Milk with 0.001-0.01% culture became bitter within about 3-7 wk" (Driessen, 1976).
        - Inoculation of UHT milk with 1.3 x 10^3 CFU/mL *Pseudomonas* MC60 resulted in a bitter flavor after 4 days of incubation (Adams et al., 1975). Levels of protease in the milk were inversely related to the numbers of days to spoilage, with levels of protease greater than 19 units/ml leading to spoilage in less than three days.
        - In pasteurized milk, a standard plate count exceeding 10 million CFU/mL results in unacceptable flavor defects related to bacterial growth and metabolism.
        - Lactic acid bacteria spoilage can cause a sour odor when the microbial population exceeds 10^6 cells/ml in fluid milk (Erkmen and Bozoglu, 2016).
        - Microbiological assessment of raw milk does not predict the shelf-life of commercially pasteurized fluid milk (Martin et al., 2011)
    - Some studies have assessed time and temperature combinations related to spoilage of milk products, although these values are dependent to some extent on the specific strains present, the levels of these bacteria present initially, the amounts and activity of protease and other enzymes that are present, etc.
        - "Generation time of psychrotrophic *Pseudomonas* spp. in milk is 8–12 h at 3°C and 5–10 h at 5°C. These growth rates are sufficient to cause spoilage within 5 days even if the milk initially contains only one *Pseudomonas* per milliliter" (Erkmen and Bozoglu, 2016).
        - "*Pseudomonas fluorescens* and *Chryseobacterium* spp. increased volatile organic compounds (a sign of milk spoilage) in UHT milk during cold storage at 4.5°C for 26 days" (Alothman et al., 2017, Odeyemi et al., 2020).

        - *Geobacillus stearothermophilus* causes spoilage in evaporated milk within 29 to 223 hr after contamination, depending on the temperature profile under which it was stored (Kakagianni et al., 2016).

- **Are the enzymes produced at end of log phase or all the time?**
    - Undesirable spoilage-causing enzymes are generally secreted during late log or stationary growth phases of psychrotrophic bacteria (Erkmen and Bozoglu, 2016, YUAN et al., 2018a).
    - Lipase production by *Pseudomonas* spp. is produced in the late exponential and stationary phases of growth (Erkmen and Bozoglu, 2016).
    - One study found that proteolytic activity was detected during mid- to late-exponential phase for five *Pseudomonas* strains (Nicodème et al., 2005).
    - A recent paper suggests that production of protease occurs in log phase but is highest in stationary phase, but this paper also states that maximum thermostable protease expression occurs when psychrotrophic strains reach late exponential phase (10^7 to 10^8 cfu/mL) (Morandi et al., 2021).
    - *Pseudomonas* protease production was strongly temperature dependent, with very low levels of activity produced at 30°C compared to 20°C (Nicodème et al., 2005), while another paper reported even lower production of *Pseudomonas* protease at 4°C than at 30°C.
    - Some of the interplay between factors such as temperature and growth state for proteolytic activity of AprX are discussed in a recent review (Zhang et al., 2019).

- **Are some products more at risk than others? If so why?**
    - Different dairy products may have different types of spoilage organisms (see table below, adapted from (Ledenbach and Marshall, 2009).

Dairy Product	**Spoilage Concerns**
Raw milk	Many different microorganisms
Pasteurized milk	Psychrotrophs, spore-forming bacteria, microbial enzyme degradation
Dried milk	Microbial enzyme degradation
Butter	Psychrotrophs, enzymatic degradation
Cultured buttermilk or sour cream	Psychrotrophs, coliforms, yeasts, lactic acid bacteria
Cottage cheese	Psychrotrophs, coliforms, yeasts, molds, microbial enzyme degradation
Yogurt (including yogurt beverages)	Yeasts
Cream cheese, processed cheese	Fungi, spore-forming bacteria
Soft/fresh cheese	Psychrotrophs, coliforms, fungi, lactic acid bacteria, microbial enzyme degradation
Ripened cheese	Fungi, lactic acid bacteria, spore-forming bacteria, microbial enzyme degradation

    - Some microbial spoilage risks can be predicted based on the pH, water activity, storage temperature, microbial environment, processing methods, storage conditions, etc.
        - Yeasts grow well at the lower pH of cultured dairy products (Ledenbach and Marshall, 2009).
        - Molds grow well on the surface of cheeses when oxygen is available. Penicillium spp. and Cladosporium can also grow at low oxygen levels (Ledenbach and Marshall, 2009).
        - Cream cheeses can be spoiled by heat-resistant molds such as *Byssochlamys nivea*, which can grow at reduced oxygen levels (Ledenbach and Marshall, 2009)
        - Extensive proteolysis that occurs during aging of ripened cheeses results in the release of amino acids and an increased pH, which favors the growth of

clostridia such as *Clostridium tyrobutyricum* (Klijn et al., 1995, Ledenbach and Marshall, 2009).

- Lipases tends to partition into cream and can hydrolyze fat in butter (Stead, 2009).

2. Predictive Growth Models Related to Milk Spoilage

The following table presents basic information microbiological spoilage modeling programs/databases which might be of interest:

Name	Food Products	Microorganisms	Comments	Website
ComBase	Many, including pasteurized milk, cheese and other dairy	Many, including aerobic spoilage organisms, *Bacillus* spoilage bacteria, Pseudomonads, spoilage yeasts	The ComBase Browser searches a database of kinetics of spoilage organisms and pathogens in broth and food. The data come from the scientific literature or were produced by miscellaneous institutions. The ComBase models give predictions from models based on selected data of the ComBase database as a function of environmental factors such as temperature, pH, and water activity in broth. There is also a premium version of ComBase.	https://www.combase.cc/index.php/en/
Food Safety and Spoilage Predictor	Originally focused on seafood, it contains a generic model to predict microbial shelf life and a model for cottage cheese	A variety of spoilage organisms	This is an expanded version of the Seafood Spoilage and Safety Predictor from Technical University of Denmark	http://fssp.food.dtu.dk/
Pathogen Modeling Program	Various food types	Pathogens	USDA program, limited to pathogens rather than spoilage organisms	https://portal.errc.ars.usda.gov/
Forecast from Campden BRI	Not clear if their models would cover dairy	Various spoilage organisms, including *Pseudomonas*, *Bacillus*, yeasts, LAB, etc.	The models are proprietary and require you to work with Campden to use them	https://www.campdenbri.co.uk/services/predictive-microbiological-models.pdf
Sym'previus	Multiple	Many spoilage organisms, including *Pseudomonas*, *Bacillus* spp., etc.	European, subscription-based tool	http://symprevius.eu/en/
Pasteurized Milk Shelf Life Calculator	Pasteurized milk	Pseudomonads	Simple calculator predicts when spoilage will occur based on plate count	https://www.dairyscience.info/newCalculators/milk.asp

Several older papers (Rowe, 1993, Griffiths, 1994) discusses various predictive models used for assessing the quality and safety of raw milk, pasteurized milk, and cottage cheese. A recent paper (Possas et al., 2021) specifically reviews models developed for microbial pathogens in cheeses. It discusses software tools integrating predictive models developed in cheeses. There are several models listed in Table 3 of this paper (including ComBase Premium, GroPIN, and Dairy Product Safety Predictor) that my web browser will not let me access. Several other papers review predictive microbiological modeling used in the dairy industry in a more general context (Roupas, 2008, McMeekin et al., 2010). One article in the popular press does a decent job of explaining some of the difficulties and new strategies for assessing and predicting food spoilage, but does not discuss dairy products.

In addition to these databases, many publications in the literature have described mathematical models for predicting the growth of specific spoilage organisms in various dairy products, including this non-exhaustive list of reports:

Food	**Organisms**	**Model Description**	**Reference**
Milk (UHT)	*E. coli* BR and LAB (a fast starter culture)	This model predicts the simultaneous growth of LAB and *E. coli* in milk stored at ~12 to 30°C.	(Acai et al., 2016)
Milk and ham	*Listeria monocytogenes* and *Lactobacillus plantarum*	Growth predictions of the two organisms were validated in milk and ham.	(Aryani et al., 2016)
Skim milk	Psychrotolerant spore-formers (*Bacillus* and *Paenibacillus*	The study identified growth parameter data needed to reliably predict shelf life of fluid milk due to psychrotolerant spore formers	(Buehler et al., 2018b)
Greek yogurt	Yeasts and molds	This study modeled the effect of protective cultures on the growth of yeasts and molds in Greek-style yogurt.	(Buehler et al., 2018a)
UHT milk (whole and low fat)	Pseudomonas spp. and *Hafnia alvei*	A modified Gompertz equation was used to model the growth of these organisms during 14-days storage at 7°C	(Chen et al., 2011)
Reconstituted milk	*Bacillus cereus* growth and toxin formation	This study describes the simultaneous *Bacillus cereus* growth and cereulide formation, in culture medium and cereal-, dairy-, meat-, and vegetable-based food matrices under a wide range of temperatures (from 9 to 45°C)	(Ellouze et al., 2021)
Milk	*Pseudomonas fragi*	"Both the Arrhenius and square root model fit the lag phase and the growth rate data of the microbe at constant temperature very well. Both would be needed for shelf-life prediction. Significant negative history effects for growth rate and significant positive effects on lag phase of *P. fragi* were found in a single stepwise temperature shift which may be experienced in some distribution."	(Fu et al., 1991)
Whey	*Bacillus subtilis*	A model was developed which describes the kinetics of growth and the differentiation of vegetative cells into spores within dairy whey.	(Gauvry et al., 2019)
Yogurt	12 different fungal species	The effect of storage temperature (0 to 40°C) and inoculum size (10^1 to 10^5 spores) on the mycelium growth kinetics of 12 fungal species on yogurt were monitored and used to develop growth models.	(Gougouli et al., 2011)
Pasteurized milk	Psychrotroph counts	An equation was developed which allowed prediction of shelf-life of pasteurized milk at any constant storage temperature.	(Griffiths and Phillips, 1988)
Evaporated milk	*Geobacillus stearothermophilus*	A model was developed to provide realistic predictions for evaporated milk spoilage by describing the growth of *G. stearothermophilus*.	(Kakagianni et al., 2016, Kakagianni and Koutsoumanis, 2018)
Stainless steel surfaces in whole milk	*Geobacillus* spp., *Anoxybacillus flavithermus*	Modeling was used to describe biofilm formation by thermophilic bacilli in milk.	(Karaca et al., 2021)
Pasteurized milk	Gram-negative spoilage organisms	A Monte Carlo simulation model was developed to predict spoilage of pasteurized milk due to post-pasteurization contamination by Gram-negative bacteria.	(Lau et al., 2020)

Food	**Organisms**	**Model Description**	**Reference**
Grana cheese	Fungal growth and mycotoxin production and release	"Available predictive models fitted fungal growth on the cheese rind well, but validation was not possible for mycotoxins because they were produced in a very narrow T range."	(Leggieri et al., 2020)
Pasteurized Milk	*Pseudomonas fluorescens*	A model was developed which predicts the shelf life of pasteurized milk, presumed to contain a small initial population of spoilage organisms, during storage with temperature fluctuations.	(Lin et al., 2016)
Cheese-based Greek appetizers (tyrosalata and tyrokafteri)	Lactic acid spoilage bacteria	Predictive models were developed and validated to predict the growth of LAB spoilage bacteria in cheese-based Greek appetizers.	(Manios et al., 2009)
Milk and cottage cheese	Psychrotolerant pseudomonads	Models included the effects of temperature, pH, salt, lactic acid, and sorbic acid and were validated in raw milk, heated milk, and cottage cheese.	(Martinez-Rios et al., 2016)
Yogurt with fruit	Natural contamination (including LAB, yeasts, and mold)	A model was developed to quantify shelf life of yogurt with fruit during storage at 5 to 20°C.	(Mataragas et al., 2011)
UHT milk	*E. coli* and *S. aureus* 2064 co-cultivation	A model describing the growth of these two organisms together in milk is developed.	(Medved'ova et al., 2020)
Cheese	*Mucor* spp.	A model describing the growth of Mucor spp. in cheese is presented.	(Morin-Sardin et al., 2016)
Milk and other dairy products	Listeria monocytogenes	Models developed in skim milk were then tested in a range of dairy products including cheddar, Camembert, and cottage cheeses, pasteurized and UHT milk, cream, butter	(Murphy et al., 1996)
Milk and milk-based products	Pseudomonads	These classic papers present a model for pseudomonads growth and validates it for a variety of foods, including raw milk and cream under both constant and fluctuating temperatures.	(Neumeyer et al., 1997a, Neumeyer et al., 1997b)
Soft cheese	Lactic acid bacteria and *Listeria* spp.	In this report, the competition between LAB and listeria in a soft cheese was modeled.	(Panebianco et al., 2021)
UHT milk	Pseudomonads	While improving upon an earlier model, this paper also discusses many of the sources of bias and error when predictive models are applied to real food products (vs. broth).	(Pin et al., 1999)
Pasteurized milk stored in residential refrigerators	*Listeria monocytogenes* and *Pseudomonas putida*	Predictive models were developed for growth of a pathogen and a spoilage organism in pasteurized milk under conditions simulating those of a consumer.	(Rodriguez-Martinez et al., 2020)
Dairy and other foods	Probiotic, spoilage, and pathogenic bacteria	This paper is unfortunately in Polish, but the abstract is in English. It describes the development of a database of predictive models ("ProgBaz SGGW") for probiotic, spoilage, and pathogenic bacteria in foods, including dairy foods.	(Rosiak et al., 2019)
UHT milk	Lactic acid bacteria and *Geotrichum candidum* (co-cultivation)	The growth characteristics of milk inoculated with LAB and a microscopic fungus was described in this report.	(Šipošová et al., 2020)
Pasteurized whole milk	Aerobic plate count	This study found that microbial counts and other quality measures could be used to determine the shelf life of pasteurized milk under various storage conditions.	(Ziyaina et al., 2018)
Pasteurized milk	*Bacillus cereus*	This older paper used predictive microbiology to examine the growth of *B. cereus* in pasteurized milk.	(Zwietering et al., 1996)

No predictive models of spoilage of dairy products were identified that were based primarily on enzymatic activity rather than microbial growth (although, of course, enzymatic activity generally is related to microbial growth).

Additional information regarding modeling and model validation

Several papers review general information about modeling spoilage related to microbial growth in food matrices and may be of interest (Rowe, 1993, Braun and Sutherland, 2006, Membré and Dagnas, 2016, Mermelstein, 2018).

Since many predictive models are developed in matrices (such as broth) that are different, to some extent, to the food product of interest, models may not be able to correctly predict the growth of microorganisms in foods (Pin et al., 1999, Aryani et al., 2016). Growth of microorganisms in broth is unhindered by competition from other microorganisms and inhibitory substances or structure in the food, so models developed in broths are often thought to represent worst-case scenarios and are those conservative or “fail-safe” (Braun and Sutherland, 2006). However, some components of food such as fats might serve a protective function for microbes, potentially making models based on broth “fail-hazardous” (Braun and Sutherland, 2006). Food matrices also contain microenvironments which may alter microbial growth in certain regions. Some attempts have been made to accommodate the microarchitecture of food in models (Wilson et al., 2002). Good growth curves of microorganisms are easier to obtain in liquid milk products in comparison to cream, butter, and cheese, in part due to the viscosity or semi-solid nature of these products in comparison to milk and the potential for uneven distribution of microbes in these matrices (Murphy et al., 1996).

Most models that are developed for food safety are designed to be “fail-safe”, and such conservative approaches to modeling may be less useful when assessing spoilage (Braun and Sutherland, 2006).

Models of bacterial growth do not typically predict the lag phase duration as well as they predict the growth phase, and lag phase predictions are less amenable to modeling (Braun and Sutherland, 2006).

Validation of predictive models

Validation of predictive models involves comparing the predictions of a model (often developed in growth media) vs. growth data measured in a food product (Aryani et al., 2016). Some detailed descriptions of the processes used to validate general predictive models of microbial growth in foods are found in the literature (McClure et al., 1994, Murphy et al., 1996, Braun and Sutherland, 2006).

Validations can be made with controlled experimental data from challenge studies and with data from the scientific literature. For validation testing, foods can be inoculated with known concentrations of a microorganism (McClure et al., 1994) or may be allowed to spoil “naturally “ without inoculation (Braun and Sutherland, 2006) during storage at known temperatures. The change in number of microorganisms was assessed at various times and used to calculate kinetic growth parameters. Literature data often lack sufficient information for detailed analysis, but values of generation time or 1000-fold increases in numbers may still be useful. Comparison of the predicted vs. experimental/literature data can be used to determine where the model is valid and where the model should not be used. (McClure et al., 1994, Braun and Sutherland, 2006), and various statistical analyses may be used to judge model performance (Braun and Sutherland, 2006).

An older paper specifically discusses validation of predictive models of food spoilage organisms (Pin et al., 1999) and sources of error within models.

Validity of models at extreme temperatures

Simple chemical reactions typically follow Arrhenius kinetics, in which logarithm of the reaction rate is linearly related to the reciprocal of the absolute temperature. Theoretical attempts to model temperature dependence of more complicated biological reactions such as microbial growth using Arrhenius kinetics may work within a narrow temperature range but tend to fall apart outside of that range (Kavanau, 1950, Ratkowsky et al., 2005). To model these more complicated systems such as microbial growth, empirically derived models (which may or may not be based initially on mechanistic-based models) have been developed.

Empirically derived models are thus designed to fit data over a specific temperature range, and their performance outside of these ranges is unknown. Furthermore, at the extreme edges of the range for which the model has been validated, fewer data points may have been available for testing, and there is more chance that small variations in other factors may reduce the efficacy of the model at that temperature (K. Glass, personal communication).

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