ABSTRACT

Food loss and waste is a major concern in the United States and globally, with dairy foods representing one of the top categories of food lost and wasted. Estimates indicate that in the United States, approximately a quarter of dairy products are lost at the production level or wasted at the retail or consumer level annually. Premature microbial spoilage of dairy products, including fluid milk, cheese, and cultured products, is a primary contributor to dairy food waste. Microbial contamination may occur at various points throughout the production and processing continuum and includes organisms such as gram-negative bacteria (e.g., *Pseudomonas*), gram-positive bacteria (e.g., *Paenibacillus*), and a wide range of fungal organisms. These organisms grow at refrigerated storage temperatures, often rapidly, and create various degradative enzymes that result in off-odors, flavors, and body defects (e.g., coagulation), rendering them inedible. Reducing premature dairy food spoilage will in turn reduce waste throughout the dairy continuum. Strategies to reduce premature spoilage include reducing raw material contamination on-farm, physically removing microbial contaminants, employing biocontrol agents to reduce outgrowth of microbial contaminants, tracking and eliminating microbial contaminants using advanced molecular microbiological techniques, and others. This review will address the primary microbial causes of premature dairy product spoilage and methods of controlling this spoilage to reduce loss and waste in dairy products.

Key words: *Pseudomonas*, sporeformers, fungi, contamination

INTRODUCTION

Over the last decade, there has been an increasing amount of attention paid to the issue of food loss and waste, both in the United States, as well as globally. The amount of food lost or wasted in the United States every year is estimated to exceed 130 billion pounds, or just over 30% of the total pounds available (Buzby et al., 2014). Dairy foods represent the third highest group in terms of dollar value of food lost and wasted at the retail and consumer level in the United States in 2010, with 17% of the total value lost ($165.6 billion), behind meat, poultry, and fish at 30% and vegetables at 19% (Buzby et al., 2014). The implications of food loss and waste are broad and include food and nutritional insecurity, environmental considerations, and economic losses for consumers and businesses (Papargyropoulou et al., 2014, Gunders and Bloom, 2017).

In addition to factors such as retail overstocking and discarding products that have surpassed their expiration or sell-by dates (Gunders and Bloom, 2017), an important contributor to dairy food waste is microbial spoilage. Although there are no estimates for how much food is wasted due to spoilage in the United States, in the United Kingdom approximately two-thirds of household waste is due to spoilage (Gunders and Bloom, 2017). Although some dairy foods contribute very little to food waste (e.g., extended shelf life products such as dried dairy powders and UHT fluid milk), many dairy foods such as conventionally pasteurized fluid milk, cheese, and cultured products are susceptible to contamination with spoilage microorganisms at various points throughout the production and processing continuum. These microorganisms include gram-negative and gram-positive bacteria and a broad range of fungal organisms (i.e., yeasts and molds; Ledenbach and Marshall, 2009). The mechanisms of dairy product spoilage vary by type of microorganism but typically include: (1) the production of extracellular enzymes that break down components including proteins, lipids, and lactose, producing off-odors, off-flavor, and body defects (e.g., coagulation in fluid milk); (2) visually detectable growth, most often by spoilage fungi; (3) production of pigments by bacterial and fungal contaminants; and (4) other metabolic processes (e.g., fermentation). When spoilage occurs, it renders all or a portion of the product inedible and results in dairy food waste.
Strategies for preventing or reducing dairy food waste due to spoilage should include a variety of approaches throughout the production process, including: (1) reducing transmission of spoilage microbes from environmental sources on the farm and from the processing facility into dairy products; (2) applying processing technologies to reduce or eliminate the presence of microbial contaminants; (3) controlling the outgrowth of spoilage microorganisms through biocontrol strategies; (4) preventing contamination through proactively monitoring and controlling spoilage organisms in raw ingredients and the environment; and (5) using data-driven decision making tools to optimize quality and reduce spoilage. Here we discuss the primary microbial agents responsible for dairy food spoilage and explore the diverse approaches that should be considered for use by the dairy industry to reduce microbial spoilage and resulting food waste.

**FLUID MILK SPOILAGE**

Fluid milk that is processed using HTST pasteurization, the primary method used in the United States, is an inherently perishable product with expected average shelf life of 14 to 21 d (Ranieri and Boor, 2009), which generally corresponds to the code or best-by date printed on the packaging. Bacterial spoilage of fluid milk occurs by 1 of 2 primary pathways: (1) recontamination after pasteurization in the processing facility, also known as post-pasteurization contamination (PPC), often with gram-negative bacteria such as *Pseudomonas* (Reichler et al., 2018), or (2) contamination at the farm level with psychrotolerant spore-forming bacteria that survive pasteurization in spore form and subsequently germinate and grow during refrigerated storage (Huck et al., 2008).

Gram-negative bacteria are common contaminants in raw milk, making up the majority of the raw milk microflora (Ternström et al., 1993). However, these organisms are heat labile, and reductions of at least 6 log are achieved using HTST pasteurization (e.g., a minimum of 72°C for 15s; Villaniel and de Jong, 2000). It should be noted that some psychrotolerant gram-negative bacteria in raw milk produce heat-stable enzymes that survive pasteurization, despite the destruction of the bacterial cell itself, and cause product deterioration over shelf life. Although it has been reported that very high bacterial concentrations (e.g., ~6.0 log cfu/mL) are necessary to produce these enzymes, Zhang et al., (2020) identified that even at much lower concentrations (e.g., ~4.0 log cfu/mL) some species of *Pseudomonas* can produce heat-stable enzymes that result in reduced quality product. However, this is likely to primarily affect products with extended shelf life (e.g., UHT milk) due to suboptimal enzymatic activity at refrigeration temperatures (Murphy et al., 2016). Hence, at levels below the Pasteurized Milk Ordinance (PMO) limit of 300,000 cfu/mL for comingled raw milk (FDA, 2017), psychrotolerant gram-negative bacteria not only remain at concentrations where production of heat-stable enzymes are not a major concern, but they are also effectively eliminated during the pasteurization process. Consequently, when gram-negative bacteria are found in pasteurized fluid milk, it typically is an indication that PPC has occurred. The most common microorganism responsible for PPC of fluid milk is *Pseudomonas* (Alles et al., 2018; Reichler et al., 2018). *Pseudomonas* is found ubiquitously in food processing environments, including dairy processing environments (Stellato et al., 2017), where they may form biofilms (Cherif-Antar et al., 2016). *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. More importantly in terms of food waste, it can grow to levels where product quality begins to deteriorate (i.e., approximately 1 million cfu/mL) only 7 to 10 d after pasteurization (Ranieri and Boor, 2009). In fact, PPC often occurs at low levels. Schröder (1984) reported contamination of milk occurred with 1 to 50 psychrotolerant gram-negative bacteria per 100 mL of fluid milk (Schröder, 1984), yet in theory, even contamination with 1 cell per container would cause product spoilage over refrigerated shelf life. Quality defects caused by *Pseudomonas* in fluid milk include off-odors, off-flavors, body defects, and pigment production (Dogan and Boor, 2003; Reichler et al., 2019). Beyond *Pseudomonas*, there are several other gram-negative organisms that cause PPC in fluid milk, including psychrotolerant coliform bacteria (*e.g.*, *Citrobacter*) and other members of the order Pseudomonadales (*e.g.*, *Acinetobacter*; Alles et al., 2018); however, these organisms represent a small proportion of the bacterial agents responsible for PPC in contemporary US fluid milk supplies. Approximately 50 to 60% of fluid milk that spoils in the United States due to microbial growth is a result of PPC (Alles et al., 2018; Reichler et al., 2018).

The remaining approximately 40 to 50% of fluid milk that reaches the PMO limit of 20,000 cfu/mL during refrigerated storage can be attributed to gram-positive bacteria, specifically spore-forming bacteria such as *Bacillus* and *Paenibacillus* (Ranieri and Boor, 2009; Alles et al., 2018; Reichler et al., 2018). In contrast to *Pseudomonas* 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 and Boor, 2009). Spores, the resistant structure formed by spore-forming bacteria, typically enter the fluid milk supply at the farm where they are found ubiquitously in the environment (e.g., soil, feed, bedding). They are then transferred into raw milk primarily during milking, survive pasteurization in spore form, and subsequently germinate and grow to spoilage levels during shelf life. Often psychrotolerant spores are present in bulk tank raw milk at very low levels; for example, a recent study of spore levels in environmental and milk samples from 17 New York State dairy farms found a mean psychrotolerant spore count of 0.57 cfu/mL (Martin et al., 2019). However, despite these low spore levels in bulk tank raw milk, and similarly to PPC, in theory, as few as 1 psychrotolerant spore per container of packaged fluid milk can grow to spoilage levels during refrigerated storage. Many psychrotolerant spore-forming bacteria produce lipolytic and proteolytic enzymes (Trmčić et al., 2015) capable of degrading the quality of fluid milk. Notably, Bacillus weihenstephanensis, a psychrotolerant spore-former in the Bacillus cereus group, is the causative agent of a defect known as sweet curdling, whereby coagulation occurs through proteolytic activity (Gopal et al., 2015). Indeed, Bacillus weihenstephanensis is one of the predominant spore-forming bacteria found in fluid milk that has reached bacterial levels capable of causing product defects (Masiello et al., 2014). Others include Paenibacillus, Psychrobacillus and Viridibacillus (Reichler et al., 2018).

It is worth noting that fluid milk spoilage occurs at various points throughout the shelf life of the product and is influenced by various factors, the most important being: (1) type and initial concentration of bacterial contaminants, (2) storage temperature, and (3) storage time. Fluid milk waste resulting from bacterial spoilage will be highly dependent on those factors. In general, however, fluid milk spoilage as a result of PPC occurs earlier in shelf life (i.e., 7 to 10 d after pasteurization, occurring before typical stated code or best-by-date), whereas spoilage occurring due to the growth of psychrotolerant spore-forming bacteria typically occurs near or after the stated shelf life of the product has elapsed (i.e., 14–21 d after pasteurization; Ranieri and Boor, 2009). Accurate shelf life dating and understanding corresponding consumer behaviors (e.g., what proportion of consumers continue to drink fluid milk beyond the labeled date) should also be considered when evaluating risk of consumer exposure to spoilage and corresponding food waste but will not be considered in depth in this review.

**CHEESE SPOILAGE**

In general, most cheeses are manufactured using starter cultures (i.e., lactic acid bacteria) to ferment lactose into lactic acid, thereby increasing the acidity of the milk and thus triggering coagulation and whey expulsion. The resulting intrinsic properties of many cheeses, namely low pH and water activity (a_w), inherently provide some protection against spoilage by many common bacterial contaminants. However, cheese products remain susceptible to growth of a variety of fungal species that can thrive under low pH and low a_w conditions. Fungi that are responsible for cheese spoilage are diverse and belong to a broad range of fungal genera, for example, Acremonium, Alternaria, Aspergillus, Aureobasidium, Botrytis, Cladosporium, Epicoccum, Eurotium, Exophiala, Fusarium, Gliocladium, Lecanicillium, Mucor, Penicillium, Rhizopus, and others (Garnier et al., 2017). In particular, the most common fungal genera found to cause cheese spoilage are Penicillium followed by Aspergillus (Kure and Skaar, 2019). These fungi cause a range of cheese defects including visual mycelial growth, pigment formation, and off-odors and off-flavors (Garnier et al., 2017). Yeast and molds in general are not heat resistant; therefore, in pasteurized cheese, raw milk fungal contamination is not a considerable source of spoilage. Sources of postprocessing fungal contamination commonly include contaminated air, brine, equipment, or ingredients (Garnier et al., 2017).

Beyond fungi, cheese spoilage may occur by bacterial contamination, especially in products with relatively high pH and a_w. Coliform bacteria, which are commonly used as indicators of hygienic conditions in cheese manufacturing, can produce gas in some styles of cheese causing a defect known as early blowing. For example, Enterobacter aerogenes, Escherichia coli, Klebsiella aerogenes (Alichanidis, 2007), C. braakii, C. freundii, K. oxytoca, and H. alvei (Tabla et al., 2016) have been associated with early blowing in cheese. This defect is typically found in brined white cheeses and is characterized by the presence of numerous small holes in the cheese curd that sometimes result in deformation of the cheese block and significant swelling (Alichanidis, 2007). In addition to coliforms, other gram-negative bacteria cause spoilage of cheese, namely Pseudomonas. Members of the Pseudomonas genus are unable to grow at pH lower than 4.5 (De Jonghe et al., 2011); however, in fresh and low-acid cheese products (e.g., mozzarella), these contaminants can thrive and cause several defects due to the production of enzymes such as proteases, lipases, pectinases, or lecithinase (Caldera et al., 2015), which result in flavor, odor, and body defects in cheese.
Another characteristic cheese defect caused by *Pseudomonas*, and in particular members of the *Pseudomonas fluorescens* group, is pigment formation. Martin et al. (2011) described queso fresco with blue and fluorescent pigments which were caused by *Pseudomonas fluorescens* biovar IV (Martin et al., 2011). Similar pigment defects caused by growth of *Pseudomonas* in fresh cheeses such as mozzarella and Burgos have also been reported (del Olmo et al., 2018).

In addition to gram-negative spoilage bacteria, certain gram-positive bacterial contaminants can cause spoilage in cheese. For example, nonstarter, heterofermentative lactic acid bacteria, such as *Lactobacillus brevis*, *Lactobacillus fermentum*, or *Lactobacillus wasatchensis*, can grow during cheese ripening and produce gas in cheese such as cheddar (Ortakci et al., 2015). The production of CO₂ occurs from the fermentation of residual lactose or galactose or by metabolism of citrate (Sheehan, 2007), resulting in an open cheese texture (slits or cracks) or blown wrappers, without any texture change in cheeses aged more than 3 mo (Laley et al., 1987). This defect occurs particularly in products aged at higher temperatures to facilitate faster ripening (Ortakci et al., 2015). Additionally, anaerobic gram-positive spore-forming bacteria of the genus *Clostridium* also cause a gas defect known as late blowing, especially in hard and semi-hard cheeses and more specifically, in brined salted cheeses (Sheehan, 2007). *Clostridium* spores germinate and grow during the aging process before achieving salt concentrations that limit microbial growth in the cheese loaf, as long as lactate is available as a substrate (Brändle et al., 2016). Slits, cracks, and irregular eyes are formed in the cheese due to the production of CO₂ and hydrogen gas (Doyle et al., 2015). *Clostridium tyrobutyricum* is the primary species of concern; however, there are several other *Clostridium* species that have been implicated in late blowing defects in cheese (Schöbitz et al., 2005; Gómez-Torres et al., 2019).

**YOGURT AND CULTURED DAIRY PRODUCT SPOILAGE**

Much as in the cheese products mentioned above, the types of microorganisms that cause spoilage of yogurt and other cultured dairy products (e.g., sour cream, buttermilk) are greatly affected by the intrinsic properties of the product itself, with pH playing the largest role. Many cultured dairy products have a pH below 4.6, which generally limits the growth of most bacterial contaminants. Food spoilage fungi (i.e., yeasts and molds) can grow under conditions that are normally prohibitive to bacterial growth (e.g., low pH, low aw), and many also grow at low temperatures, making them particularly well suited to growth in yogurt and other cultured dairy products. Hence, the primary organisms that cause spoilage of yogurt and other cultured dairy products are fungi.

Spoilage fungi isolated from yogurt and cultured dairy products represent a broad diversity of yeasts and molds reportedly spanning multiple fungal phyla (e.g., Ascomycota, Basidiomycota, and Mucoromycota), with yeasts often cited as the primary spoilage organism in these products (Ledenbach and Marshall, 2009). Indeed, Buehler et al. (2017) isolated 83 fungal isolates representing 22 different internal transcribed spacer (ITS) allelic types (i.e., unique subtypes based on the ITS sequencing region) from 30 yogurt products with *Torulaspora delbrueckii*, *Clavispora lusinae*, and *Penicillium* spp. representing the most common fungi in these samples. Other studies have also identified the spoilage yeasts *Debaryomyces*, *Rhodotorula*, *Kluyveromyces*, and *Candida* from yogurt (Rohm et al., 1992), along with identification of spoilage molds *Rhizomucor*, *Sistotrema*, and *Mucor* in yogurt (Buehler et al., 2017). Product defects caused by spoilage fungi in yogurt and other cultured dairy products include off-odors (e.g., yeasty odors), off-flavors (e.g., bitter), and body defects (e.g., gas production) through lactose fermentation, lipolysis, proteolysis, and other metabolic processes (Fleet, 1990, 1992). For example, spoilage yeasts may metabolize diacetyl in sour cream and buttermilk, leading to a yogurt-like flavor (Ledenbach and Marshall, 2009). Finally, in some cultured dairy products, spoilage may occur by gram-negative bacteria such as *Pseudomonas* or psychrotolerant coliform bacteria (Ledenbach and Marshall, 2009); although these represent a minor proportion of spoilage microorganisms in those products.

**STRATEGIES TO AID IN REDUCING DAIRY PRODUCT SPOILAGE AND WASTE**

Strategies to reduce dairy product spoilage have long focused on preventing recontamination of processed products with spoilage microorganisms through a focus on cleaning and sanitation in the processing environment. For example, adequate cleaning and sanitation is necessary to prevent the development of biofilms in dairy processing equipment. Biofilms are a major source of dairy product contamination during processing (Knight, 2015), and once biofilms are established in processing equipment, they are a considerable challenge to eliminate (Bremer et al., 2015). Reducing microbial contamination during processing through cleaning and sanitization has been shown to have a dramatic effect of dairy product shelf life. For example, the shelf life (i.e., the number of days for the bacterial concentra-
tion to reach 20,000 cfu/mL of fluid milk in a study by Gruetmacher and Bradley (1999) was increased by more than 11 d (from 9 d to more than 21 d) following proper cleaning and sanitization with chlorine, and changing the sanitizing agent from chlorine to peroxyacetic acid increased the shelf life to more than 30 d (Gruetmacher and Bradley, 1999). These improvements should not be understated, as cleaning and sanitation programs have improved through advances in sanitary design of equipment and the use of novel chemical sanitizers. However, there is a need to explore additional strategies to reduce spoilage by microorganisms introduced at the farm and processing facility to further reduce food waste caused by premature spoilage. Here we discuss approaches that take advantage of the use of on-farm interventions, processing technologies, biocontrol strategies, and tools that provide information that can be used to reduce spoilage, including advanced molecular microbiology techniques and the application of mathematical modeling to improve product quality and reduce spoilage. A list of strategies that can be applied for different dairy products to reduce spoilage from specific groups of dairy spoilage organisms can be found in Table 1 and are discussed further here.

**ON-FARM INTERVENTION STRATEGIES**

The effect of raw milk quality on the shelf life and spoilage of processed dairy products has been extensively reviewed (Murphy et al., 2016). Somatic cells and total bacterial concentrations predominantly influence finished products by affecting yield and quality via heat-stable enzyme production. Here, we focus on strategies targeted toward spores in raw milk, as these are the primary microbial group that originate at the farm level, survive processing hurdles, and ultimately cause finished product quality deterioration in several dairy products (e.g., fluid milk and cheese). Bacterial spores are found ubiquitously in natural environments, specifically in the dairy farm environment, and enter raw milk primarily during milking (Martin et al., 2019). Several studies have investigated the role of farm practices and sources in the transmission of spores from environmental niches into raw milk, with bedding, feed, and parlor practices reportedly playing important roles (Vissers et al., 2006; Magnusson et al., 2007; Masiello et al., 2014; Martin et al., 2019; Murphy et al., 2019). To reduce the transmission of spores from dairy environments into bulk tank raw milk, thereby reducing finished product spoilage, intervention strategies have been investigated. For example, Magnusson et al. (2006) investigated the effect of premilking cleaning methods on teats experimentally contaminated with spores of *Clostridium tyrobutyricum* and *Bacillus cereus*. The authors found that cleaning teats with a moist towel followed by drying with a dry towel significantly reduced spore counts in raw milk (Magnusson et al., 2006). Similarly, Evanowski et al. (2020) applied a combination of interventions to reduce spore levels in bulk tank raw milk, including training milking parlor employees on enhanced teat-end cleaning and implementing a standard laundered towel cleaning protocol (i.e., laundering with detergent and chlorine bleach and fully drying). The authors concluded that the combination of interventions significantly decreased the mesophilic and thermophilic spore levels in bulk tank raw milk (Evanowski et al., 2020). The use

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<th>Product</th>
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<td>Molecular microbiological techniques</td>
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Table 1. Major microbial causes of dairy product spoilage and corresponding strategies to reduce spoilage
of simple, low-cost on-farm interventions, such as those discussed here, to reduce the concentration of spores in bulk tank raw milk is a viable strategy for reducing dairy food waste due to microbial spoilage. However, future studies are needed to further quantify the effect of these strategies on dairy spoilage.

Beyond the contribution of spores that enter the dairy product continuum through raw milk at the farm level, there is also some evidence that microbial contamination and growth may occur during raw milk transportation from the farm to the processing facility, potentially leading to reduced finished product quality. For example, Huck et al., (2007) identified unique strains of spore-forming bacteria in raw milk collected from tanker trucks that were not identified in the raw milk at the farm, suggesting that the tanker truck itself may be a source of spores. Further, Teh et al. (2011) isolated a variety of bacterial genera from swabs of putative biofilms on internal surfaces of raw milk tanker trucks, including Bacillus and Pseudomonas. Importantly, the authors found that many of the isolates collected were able to produce heat-stable enzymes, highlighting the possibility that raw milk may become contaminated during transportation, not only with heat-resistant bacterial spores, but also potentially with heat-resistant enzymes (Teh et al., 2012), both of which may cause dairy product spoilage and waste.

**PROCESSING TECHNOLOGIES AND PRACTICES**

Technological strategies applied in dairy processing to improve product quality by removing bacterial contaminants, in particular bacterial spores (e.g., Bacillus, Paenibacillus, Clostridium, etc.), from either the incoming raw milk supply or the processed milk stream include bactofugation and microfiltration. Bactofugation, also known as bacterial clarification, is applied to separate components that have different densities using high-speed centrifugation (Damerow, 1970), with reported bacterial spore removal efficiencies between 90 and 98% (Gésan-Guiziou, 2010). This technology was first applied in the dairy industry as a method to remove spores of anaerobic Clostridium tyrobutyricum to prevent late blowing in cheese (Gésan-Guiziou, 2010). More recently, bactofugation has been applied to raw milk before HTST pasteurization to reduce bacterial cells including spores (Gésan-Guiziou, 2010), specifically those that lead to spoilage of fluid milk (e.g., Paenibacillus), although this application of bactofugation is less common (Goff and Griffiths, 2006). Alternatively, microfiltration uses a semi-permeable membrane with varying pore sizes to separate bacteria and milk components based on particle size. Microfiltration reportedly has higher levels of bacterial removal compared with bactofugation, with efficiencies that are between 99.1 and 99.99% (Gésan-Guiziou, 2010). In contrast to bactofugation, microfiltration has been more commonly applied to fluid milk products in combination with HTST pasteurization to extend shelf life and reduce spoilage (Elwell and Barbano, 2006). Indeed, Wang et al. (2019) report fluid milk shelf life exceeding 90 d at 6°C using high-quality raw milk and the combination of microfiltration and HTST pasteurization (Wang et al., 2019). Both bactofugation and microfiltration allow the dairy industry to reduce spoilage and quality defects without the addition of inhibitors of spore germination, such as nitrates, lysozyme, or nisin (Sheehan, 2011).

In addition to technologies that remove spoilage organisms as discussed above, process optimization to control the outgrowth of spoilage microorganisms also represents an important strategy to reduce dairy product spoilage. For example, Ranieri et al., (2009) demonstrated that fluid milk pasteurized at 85.2°C had significantly higher bacterial counts during refrigerated storage than fluid milk pasteurized at 72.9°C (Ranieri et al., 2009). A similar study found that in a commercial fluid milk processing facility, reducing the pasteurization temperature from 79.4 to 76.1°C resulted in reduced bacterial growth during refrigerated shelf life (Martin et al., 2012). In addition to the benefit of reduced spoilage and therefore, reduced food waste, lowering pasteurization temperature of fluid milk is easy to implement and requires less energy, conserving processor resources.

In addition to the processing technologies and strategies outlined above, it should be noted that there are promising emerging technologies that, although not currently widely adopted or approved for use in dairy products, may represent viable options for reducing dairy product spoilage and waste in the future. For example, high pressure processing has been shown to provide similar log reduction of pathogens and spoilage organisms in milk as pasteurization (Stratakos et al., 2019; Liu et al., 2020) but may also provide additional benefits to consumers (e.g., retention of heat labile nutrients). Other nonthermal processing technologies have also been explored to inactivate microorganisms in dairy products, including pulsed electric fields (Bendicho et al., 2002; Walkling-Ribeiro et al., 2009), ultrasound (D’Amico et al., 2006; Barbosa-Cánovas and Bermúdez-Aguirre, 2010), irradiation (Matak et al., 2005; Choudhary and Bandla, 2012), and others (Barbosa-Cánovas and Bermúdez-Aguirre, 2010). Continued development of such nonthermal technologies...
and their use in concert with thermal technologies will provide additional strategies to reduce dairy product spoilage and waste.

**PRODUCT FORMULATION**

Manipulating product formulation has long been a method to prevent growth of spoilage microorganisms in dairy products. In particular, there is a long history of use of chemical preservatives (e.g., organic acids such as potassium sorbate) that are effective at controlling the outgrowth of spoilage bacteria and fungi in products such as cheese and cultured dairy (Zamani Mazdeh et al., 2017). Because consumer demand for preservative-free or clean-label dairy products has increased since the 1970s and 1980s, the use of these time-tested chemical preservatives has declined (Brockman and Beeren, 2011). Dairy manufacturers have reformulated their products using seemingly more natural strategies, namely the use of bioprotective cultures. Bioprotective cultures are defined as live microorganisms that are deliberately added to foods to control microbial growth without changing its technological and sensory qualities. They have 3 primary mechanisms of action: (1) displacement, (2) competition for nutrients, or (3) production of metabolites (Ben Said et al., 2019). The use of bioprotective cultures represents a preservative technique against yeast and molds in many food matrices and specifically in dairy, where the use of starter cultures for fermented products such as cheese and yogurt are already used. Lactic acid bacteria are the most common microorganisms used as bioprotective agents and have been granted “generally recognized as safe” status or “qualified presumption of safety” status in the United States and European Union, respectively. Production of metabolites is the most studied mechanism of action of bioprotective cultures, with several anti-fungal compounds identified, including organic acids, fatty acids, cyclodeptides, reuterin, hydrogen peroxide, and volatile compounds such as diacetyl (Sjögren et al., 2003; Crowley et al., 2013; Leyva Salas et al., 2017). *Lactobacillus* and specifically *Lactobacillus paracasei* and *Lactobacillus rhamnosus* have been reported to exhibit antifungal properties in yogurt against spoilage microorganisms (LaCainin et al., 2017). A recent study of these same 2 *Lactobacillus* strains in yogurt demonstrated effective inhibition of fungal contaminants through the competition of nutrients mechanism, namely competition for manganese (Siedler et al., 2020). Bioprotective cultures have also been developed for use against heterofermentative lactic acid spoilage bacteria, as well as gas-producing *Clostridium* species (Carmen Martínez-Cuesta et al., 2010).

The use of molecular microbiological methods in food microbiology has, until recently, been focused on identification, subtyping, and source tracking of bacterial pathogens. These techniques have changed considerably in recent years, becoming more discriminatory, faster, and less expensive to implement (Gerner-Smidt et al., 2019). The same technologies and methodologies used for food safety are increasingly being adopted to address contamination with spoilage microorganisms. In particular, the use of single-gene sequencing approaches represents a useful strategy for identification, subtyping, and source tracking of target spoilage organisms in dairy foods and associated environments (e.g., farm and processing facilities). For example, *rpoB*, the gene encoding for the β subunit of RNA polymerase, has been used to subtype aerobic spore-forming bacteria from farm environments, raw milk, distribution channels, processing facilities, and pasteurized fluid milk throughout shelf life (Huck et al., 2007, 2008). The use of *rpoB* sequencing has allowed dairy industry stakeholders, including producers and processors, to identify strategies to reduce levels of these organisms throughout the dairy product continuum, thereby reducing spoilage. For example, Miller et al. (2015) determined that isolates originating from raw milk and powder samples were significantly different by assigning *rpoB* allelic types to 1,949 aerobic spore-forming bacterial isolates collected from bulk tank raw milk and dairy powder processing facilities. The authors concluded that dairy powder processors should focus efforts to reduce aerobic spores at the farm and processing level based on the specific *rpoB* allelic types found in their product. Similarly, Buehler et al. (2019) used single-gene sequencing of the ITS region in dairy spoilage fungi to track fungal spoilage in 2 yogurt processing facilities. A total of 852 fungal isolates in the study originated from raw materials, in-process product samples, finished product samples, and environmental samples from 2 yogurt processing facilities, and the authors found that using ITS sequencing allowed for improved source tracking of fungal contamination throughout the processing continuum.

In addition to single-gene sequencing approaches, metagenomics is another emerging technology that is becoming increasingly accessible to the dairy industry with potential applications in the area of spoilage prevention and waste reduction. In contrast to single-gene sequencing tools, whereby a gene from single bacterial isolate is sequenced, metagenomics is the sequencing
of single genes (e.g., 16s) or entire genomes (e.g., shotgun metagenomics) from potentially diverse microbial communities inhabiting a common environment (Yeung, 2012). Metagenomics tools have been proposed for use as a powerful raw ingredient monitoring tool, whereby deviations in the expected microbial population of raw ingredients alert processors to potential lot-to-lot inconsistencies, which may result in reduced quality or spoilage in the finished product (De Filippis et al., 2017). Metagenomic tools have also been used to monitor microbial contaminants in dairy processing environments, which are an important source of spoilage and pathogenic microorganisms (Bokulich and Mills, 2013; Stellato et al., 2015; Calasso et al., 2016). Further, the use of metagenomics to understand the role of microbial communities can be instrumental in identifying sources and transmission pathways of quality deteriorating microbes (Kable et al., 2016; Vermote et al., 2018; McHugh et al., 2020). Although the use of metagenomics is not currently widespread in the US dairy industry, these tools promise to revolutionize our understanding of microbial populations throughout the dairy product continuum and how these communities affect finished product quality and spoilage.

**MATHEMATICAL MODELING TO PREDICT SHELF LIFE AND OPTIMIZE RESOURCE MANAGEMENT**

With the increasing diversity of strategies available to dairy industry stakeholders to improve product quality and reduce spoilage, the implementation of predictive mathematical modeling tools will be important to enable data-driven decision making throughout the dairy product continuum. Predictive models, such as Monte Carlo simulations, allow users to model complex systems, such as product spoilage, by accounting for the normal variation within factors important to microbial growth (e.g., microbial contamination level, population composition; Poschet et al., 2003). These models not only allow for prediction of shelf life and product spoilage, but they importantly allow users to predict how implementation of intervention strategies will affect these outcomes. For example, Buehler et al. (2018a) used Monte Carlo simulations to predict fluid milk spoilage by psychrotolerant aerobic spore-forming bacteria (e.g., *Paenibacillus*) using data on initial spore concentration, prevalence of spore types, and experimentally determined growth parameters for the most prevalent strains. The authors also modeled how the implementation of microfiltration and reduced storage temperature would affect fluid milk spoilage (Buehler et al., 2018a). In another study by Buehler et al. (2018b), Monte Carlo simulations were used to estimate yogurt spoilage by fungal contaminants and assess the use of 2 fungal control strategies (i.e., using a shortened distribution chain and reduced storage temperature) on the number of consumers who would be exposed to spoiled yogurt. Using these tools to predict the effect of various spoilage control strategies, the dairy industry will be able to ensure that resources are devoted to the best methods.

**CONCLUSIONS**

As the dairy industry strives to reduce waste, a major consideration should be reducing microbial spoilage. Using the approaches outlined here, dairy industry stakeholders may adopt one or multiple strategies to control and reduce spoilage by key microbial populations throughout the farm-to-consumer continuum. Importantly, it will be critical for stakeholders to use tools that not only reduce spoilage but also conserve economic, employee, and environmental resources to ensure a sustainable dairy industry for the future.

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