

Intense Pulsed Light Decontamination of Dairy Powders:
Effects on *Bacillus cereus* and *Bacillus licheniformis* Spores,
and Dairy Powder Functionality

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Dedication

- ◇ To my husband, for believing in me and my dreams
- ◇ To my mom and dad, for your unwavering love and support
- ◇ To my siblings, for enriching my life and making me the person I am today
- ◇ To my late bà ngoại, whom I miss dearly

Abstract

Intense pulsed light (IPL) is a novel technology used to decontaminate foods using only mild heat treatments. The importance and challenges presented by the application of IPL to inactivate mesophilic spores are investigated with a focus on *Bacillus cereus* and *Bacillus licheniformis* spores in nonfat dry milk, milk protein concentrate, and whey protein concentrate. IPL technology showed possible log reduction of spores in dairy powders, which were dependent on the spore type and not powder type or IPL exposure time. The effects of IPL-treated nonfat dry milk on quality, sensory, and functionality are presented in this study. The IPL-treated nonfat dry milk presented relatively little functional changes, however, some changes in sensory characteristics were undesired.

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List of Abbreviations and Acronyms

AMPI	Associated Milk Producers Inc.
a_w	Water activity
CAGR	Compound annual growth rate
CFR	Code of Federal Regulations
CFU	Colony forming unit
DI	Deionized
DPA	Dipicolinic acid
FDA	Food and Drug Administration
GMP	Good manufacturing practice
HPP	High-pressure processing
HTST	High-temperature-short-time
IPL	Intense pulsed light
ISi	Insolubility index
MPC	Milk protein concentrate
MPI	Milk protein isolate
NFDM	Nonfat dry milk
PEF	Pulsed electric field
SASP	Small acid-soluble proteins
SMP	Skim milk powder
Spp.	Species
TA	Titrateable acidity
TPC	Total plate count
UHT	Ultra-high temperature
USDA	United States Department of Agriculture
UV	Ultraviolet
WPC	Whey protein Concentrate
WPNI	Whey protein nitrogen index

General Introduction

Dairy powders are currently used as ingredients in foods for applications in confectionery, bakery, meat, or beverage products. These advantages include its stability and prolonged shelf-life compared to native dairy ingredients, low price as a by-product from cheese manufacturing, and functional properties in food product applications. Currently, the United States exports about half of the nonfat dry milk and skim milk powder they produce annually (Kent et al., 2016). The expanding growth in exporting markets for dry dairy powders highlights the importance on the control of the product quality and food safety. Despite the use of high-heat pasteurization with liquid milk, spore-forming bacteria will enter and persist in biofilms during the manufacturing process and lead to high levels of bacterial spores in the dried dairy powder end-product (Mansour et al., 1999). Ultimately, this creates concern in the food industry as it may influence product organoleptic and functional properties, shelf-life, and potentially lead to foodborne illnesses in humans. Fortunately, only a handful of Gram-positive and Gram-negative organisms can produce spores (Soni et al., 2016). *Bacillus cereus* and *Bacillus licheniformis* are mesophilic spore-forming bacteria, commonly isolated from raw milk, also in dried milk powders (Janstova and Lukasova, 2001).

Bacterial spores are resistant to radiation, heat, and chemical treatments, which makes conventional methods for control unreliable. Spores in dairy powders have strict tolerance levels set by the US Dairy Export Council, hence it is necessary to understand their proliferation and optimize processing parameters to minimize their survival in manufacturing equipment. The US Dairy council set the limit for mesophilic spores at no more than 1,000 CFU/g in dairy powders (Watterson et al., 2014). Importing countries have set stringent acceptance standards for milk powders and ingredients that are below the allowable levels of spores per gram as determined for each country. Therefore, new emerging (non-thermal) technologies for deactivation and control of bacterial spores are needed in the dairy industry for powdered foods and ingredients.

Intense pulsed light (IPL) is a non-thermal technology that emits pulses of high energy light and can be used to eliminate pathogenic and spoilage bacteria from foods, beverages, packaging materials, and processing environments. IPL technology has been

evaluated to kill numerous pathogens present on meat, fruits, vegetables, and liquids (Kramer et al., 2017). Specifically, IPL treatment has produced promising results for surface decontamination of cheese, as well as for spinach (Proulx et al., 2017; Agüero et al., 2016). Furthermore, Chen et al. (2018) determined that IPL treatment efficiently decontaminated *Cronobacter sakazakii* in non-fat dry milk. Choi et al. (2010) was able to achieve a 3 and 5 log reduction in infant powdered milk, and suggested the use of IPL to inactivate vegetative cells in liquid, paste, and powdered foods. However, there has been a paucity of research, on the effects of IPL on bacterial spores in foods particularly dairy powders.

An IPL apparatus has been developed in collaboration with four multidisciplinary Professors and lab research groups with expertise in Food Engineering, Microbiology, Metabolomics, Sensory, and Extension have established a center for IPL research on foods at the University of Minnesota. The University of Minnesota has recently developed this technology to work with external stakeholders on dry food matrices with the goal of ensuring food safety while preserving sensory and physical attributes of powdered foods and ingredients. Ultimately, IPL technology is a promising food control method that may decontaminate and/or reduce levels of bacterial spores in dry milk powders for applications on other dry food matrices.

Chapter 1

1. A Literature Review on *Bacillus* Spores and Dry Dairy Powders

1.1 Introduction on Powdered Products (Recent Recall History)

In December 2017, an outbreak in France from the foodborne pathogen *Salmonella* Agona occurred in children younger than six months old (Jourdan-da Silva et al., 2018). This outbreak was traced back to five different infant powdered milk products that were distributed to 66 countries, and subsequently recalled. Similarly, in July 2018, a recall occurred in the United States for *Salmonella* contamination of whey powder ingredients supplied by Associated Milk Producers Inc. (AMPI). The companies affected, Mondelez Global LLC and Pepperidge Farm, voluntarily recalled a variety of products containing whey powder in the food products due to the potential contamination of *Salmonella* (FDA, 2018).

Similarly, a wheat flour-related recall occurred in 2016, leading the United States Food and Drug Administration (FDA) to recall 5 flour products dating back to 2015 and linked to customers of General Mills, which were contaminated with Shiga toxin-producing *Escherichia coli* O121 and O26 (FDA, 2017). Additionally, on November 7, 2018 and January 14, 2019, a recall was initiated by Conagra Brands' on Duncan Hines Cake Mixes tainted with *Salmonella* Agbeni (FDA, 2019a).

In 2019, within the span of 5 weeks, recalls due to foodborne pathogens occurred by four manufacturers related to flour or baking mixes. The first recall was announced on May 22, 2019 and expanded on May 23, 2019 by ALDI and ADM Milling Co. linked to ALDI's Baker's Corner Flour (FDA, 2019b). It was determined that the flour was contaminated with *E. coli* O26. A customer of ADM Milling Co., King Arthur Flour, also initiated a recall of bags of unbleached all-purpose flour on June 13, 2019. On June 14, 2019, Hometown Food Company, another customer of ADM Milling Co., recalled bags of Pillsbury Best Bread Flour. Finally, on June 21, 2019, Brand Castle, LLC, recalled brownie and cookie mixes that contained contaminated flour in the mixes. This affected several brands and was also linked to ADM Milling Co.

Outbreaks due to the foodborne pathogens shiga-toxin producing *E. coli* and *Salmonella* in powdered foods are increasing, however, biological contamination may go unreported if they are not related to clinically reported foodborne illnesses. In addition, spore-forming bacteria are resistant to heat treatments used in processing, and bacterial spores can lead to downstream spoilage and in some cases foodborne disease in packaged dry goods. Spore-forming bacteria are not always associated with food recalls, but are still a concern in the food industry due to their occurrence in high numbers in foods processing facilities and this may directly impact on the value of food products. Specifically, due to the strict tolerance levels set by the US Dairy Export Council of less than 1,000 CFU/g for mesophilic spores, the new technologies for the control and reduction of spores in dairy powders are warranted (Miller et al., 2015).

1.1.1 Spore Physiology

A vegetative cell is the physiological state of all bacteria cells during logarithmic and stationary phases of growth, whereas, some bacteria can also produce bacterial spores from vegetative cells as a mode of survival under unfavorable growth or external environmental conditions. Bacterial spores are generated from vegetative cells of bacteria and released from the mother cell at the end of the sporulation process (Matthews et al., 2017). The anatomy of a spore consists of seven layers: exosporium, coats, outer forespore membrane, cortex, germ cell wall, inner forespore membrane, and core (Figure 1.1).

The sizes of the spores and thickness of each layer will vary among different species. *Bacillus* spores were found to be approximately 1.1 to 1.5 μm in major-axis length and 0.7 to 1.3 μm in minor-axis length (Liang et al., 2014). The outer layer of a spore, called the exosporium, consists of a cascade of different proteins, which can contain greater than 50 proteins, and those found on the extracellular spore coat are called glycoproteins (Setlow, 2006). Spore glycoproteins are responsible for its ability to attach to surfaces, act as a filter to molecules, and may detoxify potential damaging chemicals. The spore coat also provides protection from lytic enzymes. Underneath this layer is the

cortex that is composed of peptidoglycan, and functions to protect the core from chemical damage. Underneath the cortex is the germ cell wall, followed by the inner membrane, which also creates a barrier for the core against chemical damage. The inner membrane contains phospholipids similar to those found in the cytoplasmic membrane found in vegetative cells. The core contains ribosomes, inert enzymes, and small acid-soluble proteins (SASP) bound to DNA. All the layers provide the armor that is required for the spore to lie dormant for extended periods.

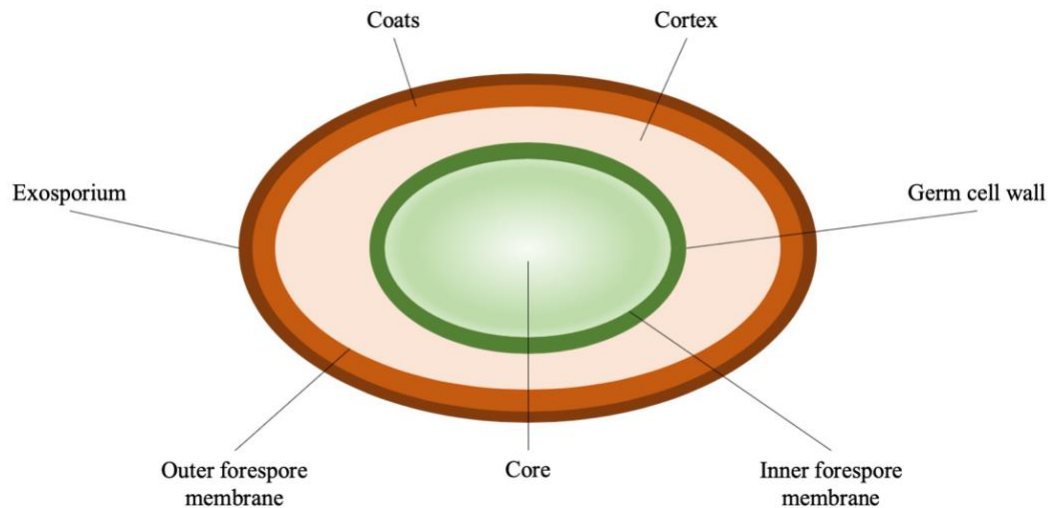


Figure 1.1 Dormant spore structure (all layers are not drawn to scale) (Matthews et al., 2017).

(Redrawn from Matthews KR, Kniel KE, and Montville TJ, p. 59, in *Food Microbiology: An Introduction*, 4th ed, ASM Press, Washington, DC, 2017.)

Additionally, the low water content of the core plays a major role in the spore dormancy and resistance. Compared to vegetative cells with approximately 4 grams of water per gram of dry weight of cells, the core of the spore has only a tenth of the water (0.4 to 1 gram of water per gram of dry weight of spores). The spore cortex aids in the dehydration of the core, but the mechanism(s) involved is currently unknown. The SASP within the core are responsible for protecting the DNA from chemical and enzymatic damage. In combination, the many layers and unique characteristics of a spore work

function to provide durability and impermeability when encountering environmental conditions encountered during processing and sanitation.

1.1.2 Spore Lifecycle

Bacterial spores are formed as a result of unfavorable environmental conditions and nutrient depletion. There are five stages to complete a full lifecycle transformation of a vegetative cell into a spore: sporulation, dormancy, activation, germination, and outgrowth (Figure 1.2).

Sporulation begins when the vegetative cell starts to divide and create a small spore compartment and mother cell compartment, each with complete genomes (Matthews et al., 2017). The mother cell engulfs the forespore and result in an endospore formation. Eventually developing into a spore, the spore cortex and coat will differentiate itself from the vegetative cells. Also unique to spores versus vegetative cells is the production of dipicolinic acid (DPA) and small acid-soluble proteins (SASP) (Matthews et al., 2017).

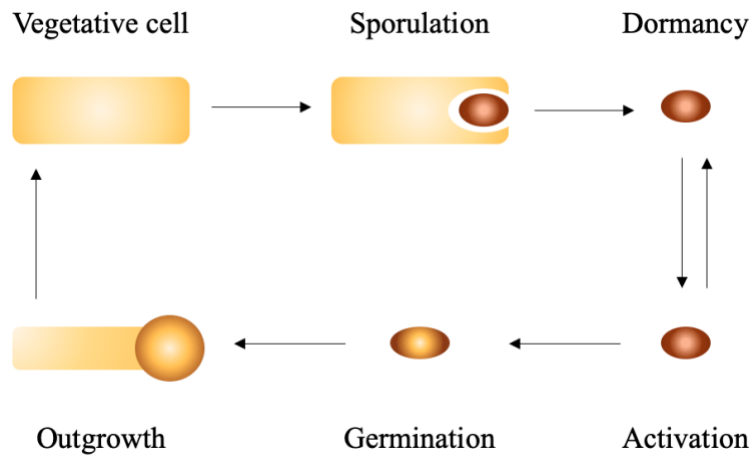


Figure 1.2 Spore lifecycle (not drawn to scale) (Matthews et al., 2017).

(Redrawn from Matthews KR, Kniel KE, and Montville TJ, p. 65, in *Food Microbiology: An Introduction*, 4th ed, ASM Press, Washington, DC, 2017.)

Spores will remain dormant and inactive until a sufficient amount of nutrients and favorable conditions trigger the activation of the spore. Spores transform back into vegetative cells through the process of activation, germination, and outgrowth (Matthews et al., 2017). Appropriate nutrients will induce metabolism and germination, and the initial activation of a spore speeds up germination, which can be imposed in many ways. Bacterial spores are activated with a heat shock treatment at certain temperatures for a short (~5 min) duration of time, and these heat shock conditions vary for mesophilic or thermophilic bacterial spores. Some strains, such as *Bacillus megaterium* are heat activated at 60°C, while other strains, such as *Bacillus stearothermophilus*, require up to 115 °C (Berg and Sandine, 1970). During the activation stage, the spore will no longer be dormant (biologically inactive) but retains heat resistance, non-sustainability, refractility, and DPA (Berg and Sandine, 1970).

After the activation stage, the spore will proceed in germination, active metabolism will start, DPA and SASP will excrete, the cortex will degrade, and large molecules will synthesize (Matthews et al., 2017). Once initiated, the germination process is irreversible and the cell will lose all its physiological spore characteristics (Berg and Sandine, 1970). During germination, the spore will lose up to 30% of its dry weight, and calibrate back to the water content level typical of vegetative cells. This spore life cycle event leads to the loss of its key stress resistance characteristics: SASP, low water activity in the core, and impermeability. Finally, during the outgrowth period, the spore will increase in size as DNA replication takes place, and the spore will synthesize new amino acids, nucleotides; along with generating the membrane and cell wall components of its new vegetative cell form.

1.1.3 General Characteristics of *Bacillus* Genus and Spores

Taxonomically belonging to the family *Bacillaceae*, the *Bacillus* genus is one of the oldest and most diverse group of bacteria (Gopal et al., 2015). *Bacillus* species are rod-shaped, nonmotile, and are capable of forming endospores (Soni et al., 2016). Although, given the expansive range of the physiological characteristics, it is impossible

to categorize and generalize the *Bacillus* spp. appropriately. In particular, the constant influx of novel species isolated from unusual environments, more sophisticated analytical methods, and comparisons on the genome level led to major reclassifications of species within *Bacillus* (Zeigler and Perkins, 2015). In general, these species are ubiquitous in soil sources but with the wide array of physiology are commonly isolated from all-natural and extreme environments, such as lake sediments or hot springs (Claus and Berkeley, 1872).

There are currently over 280 published *Bacillus* species, but most of these species are non-pathogenic and only a handful are associated with foodborne outbreaks. Some of these species capable of producing toxins and causing foodborne disease include *B. cereus*, *B. licheniformis*, *B. subtilis*, and *B. anthracis*.

1.1.4 *Bacillus* Spores in Food and Contamination

Bacillus spores can be isolated from a variety of foods such as milk, eggs, spices, and rice. Contamination of spore-forming bacteria in dairy products starts on the farm and the production of raw milk and continues to increase from abuses in temperature during transportation and distribution, and following pasteurization. The occurrence of spores inside of eggs may occur from cracked and contaminated eggs. It has also been reported that slow cooling and extended storage at room temperature of cooked rice will lead specifically to activation and toxin production of *B. cereus* spores, and is responsible for one of the most common causes of *B. cereus* food poisoning, known as the “fried rice syndrome” (Ross, 2019).

There are many factors that contribute to the contamination of spore-forming bacteria in food products from the environmental exposure of raw starting ingredients and during processing. For example in raw milk, pasteurization eliminates nutrient-competing vegetative cells, but spores survive this heat treatment that permits later outgrowth of *Bacillus* spores. Moreover, spore-forming bacteria have the ability to attach to stainless steel surfaces on processing equipment and develop into biofilms in areas hard to reach (i.e. dead ends, corners, valves, cracks, etc.); making it difficult to access and clean. Since

the use of stainless steel surfaces in dairy manufacturing plants is the standard for most equipment, it is difficult to prevent the contamination, aggregation, and adherence of microbial biofilms to stainless steel components.

Bacillus spore formation occurs in biofilms after maturation (Burgess et al., 2014). Although biofilms can contain vegetative cells, spores attach more readily since they are relatively hydrophobic, are chemical and heat resistant, and have greater capabilities for adherence to solid surfaces (Gopal et al., 2015). Microbes in biofilms are able to withstand the bactericidal effects of antimicrobials and disinfectants during sanitation; and consequently, can detach from the biofilm matrix and enter the food product during processing (Soni et al., 2016). The spores remain dormant in the food product until environmental conditions become favorable to activate germination of spores and the outgrowth for the production of vegetative cells. If the level of vegetative cells of *B. cereus* reaches 10^5 to 10^8 CFU/mL in foods, the bacteria may produce enough toxin to cause foodborne illnesses, such as intoxication and in some cases diarrheal food poisoning.

The most prevalent aerobic thermophilic/thermoduric spore-formers found in dairy processing plants come from the *Bacillus* and *Geobacillus* genera; including *Bacillus licheniformis*, *Bacillus cereus*, and *Geobacillus* spp. (Gopal et al., 2015). Mesophilic spore-formers, such as *B. cereus* and *B. licheniformis*, are found commonly in raw milk and throughout all stages of the dairy processing continuum (Miller et al., 2015). These mesophilic spores have the ability to survive pasteurization in milk processing and then germinate when conditions are suitable in milk products prior to the high-temperature treatment (Janstova et al., 2001). The predominance of some spore-forming bacterial species in the final milk product will lead to quality concerns such as odor and taste, and food safety concerns such as toxins causing foodborne intoxication and/or diarrheal disease.

1.1.5 *Bacillus cereus* spores

Bacillus cereus is an important foodborne pathogen that is less known, and foodborne disease highly underreported, but it is associated with a hospitalization rate of 0.4% in the United States, and between 2% to 33% across different European Countries (Matthews et al., 2017). *B. cereus* produces emetic (vomiting) toxin and protein an enterotoxin that causes fast-acting human diarrheal illness within 0.5 to 6 hours. In addition, foodborne disease caused by *B. cereus* diarrheal toxins production leads to symptoms in humans at 6 to 14 hours following contaminated food consumption since the toxins are produced by vegetative cells in the small intestine. The spore-forming microorganism, *B. cereus*, thrive as spores when less competition for nutrients by vegetative cells is eliminated through heat treatments. Foodborne outbreaks from *B. cereus* are commonly linked to fried rice, meat, pasta, sauces and gravies, puddings, and dairy products (Soni et al., 2016).

Ubiquitous in nature, *B. cereus* can be isolated from soil and plants, and can also be found in decaying matter. They can exist in spore form or as vegetative cells (Sankararama, 1999). Within the vegetative cell, spores can be found in the central to subterminal segment (Matthews et al., 2017). In spore form, *B. cereus* spores can germinate back into vegetative cells with the presence of germinants (nutrients like amino acids, glucose, or lysozyme), temperature, or heat-activation.

B. cereus contamination in dairy foods begins on the farm from the bacteria present in soil, bedding used for cows, and on the surface of the udders of cows, and in combination leads to the contamination of raw milk. *B. cereus* is a Gram-positive and rod-shaped bacteria that are able to survive in aerobic and facultative anaerobic environments (Janstova et al., 2001). *B. cereus* grows optimally at moderate temperatures, and the germination of *B. cereus* spores will take place between the range of 8 to 30°C. Some *B. cereus* strains are psychrotrophic; giving them the ability to survive in cold milk storage temperatures (typically 4 to 8°C) and influence shelf-life stability of the milk products (Janstova et al., 2001). Furthermore, *B. cereus* spores can withstand conditions such as high heat or strong chemical treatments. In combination

with the hydrophobic nature, heat, and chemical tolerance of *B. cereus*, spores can form biofilms on processing equipment. The appendages and/or pili found on the outside of *B. cereus* spores are involved in adhesion to processing surfaces. In terms of quality impacts, *B. cereus* is responsible for the sweet curdling of milk, and odor and taste changes in the milk. Overall, both food safety and quality-related issues due to *B. cereus* are a concern for the dairy industry.

1.1.6 *Bacillus licheniformis* spores

Bacillus licheniformis has been isolated from infant milk formula and whole milk powders from manufacturers in China and linked to foodborne outbreaks in the United Kingdom (Matthews et al., 2017; Yuan et al., 2012). *B. licheniformis* is the most prevalent spore-forming bacteria in raw milk and dry milk powders (Kent et al., 2016). *B. licheniformis* is characterized as a mesophile but some strains can grow at thermophilic temperatures. Similar to *B. cereus*, *B. licheniformis* is ubiquitous in the soil of farm environments, which leads to the contamination of raw milk once transferred from the udder of cows. *B. licheniformis* is not considered a foodborne pathogen, but may influence the spoilage of dairy products by producing enzymes as spores that can degrade compounds found in milk (Dhakal et al., 2014).

1.1.6 Methods for Eliminating *Bacillus* Spores

Microbial contamination of food products is commonly controlled through conventional heat applications greater than 75°C, such as pasteurization, sterilization, canning, or retort (Oomes et al., 2007). The goals of these heat-processing methods are to inactivate both pathogenic and non-pathogenic vegetative cells and prolong product shelf-life. Due to the heat resistance exhibited by spores, thermal processing coupled with the impact of high heat on food product degradation, alternatives to controlling spore-forming bacteria using non-thermal treatments (< 60°C) are being explored in the food industry.

Nonthermal or mild thermal technologies include ultraviolet (UV) radiation treatment, high-pressure processing (HPP), and pulsed electric field (PEF). UV radiation uses wavelengths between the range of 200 and 280 nm to inactivate vegetative bacterial cells, but spores are more resistant to this type of treatment and can repair the cellular damage caused from UV treatments (Setlow, 2014). HPP uses the energy high-pressure compression and mild heating to reduce vegetative spores. HPP shows promise to eliminate spores when the initial treatment is done to induce germination and the second treatment is done to maximize inactivation (Devatkal et al., 2015). However, HPP has its limitations on powdered ingredients due to the lower water activity and water content in the powdered food matrices that hinder the pressure to penetrate the food particles and efficiently eliminate spores. In PEF treatment of food, matrices uses short pulses of electricity to inactivate microorganisms, and is suitable for liquid, solid, and semisolid foods and preserves nutritional quality but little research has been done to show the effects of PEF on spores; or specifically, spores in powdered matrices (Gerlach et al., 2008).

Intense pulsed light (IPL) treatment is an emerging technology that uses intense, short-time pulses of light in broad-spectrum wavelengths (UV to near-infrared) on food surfaces. IPL is formed from the release of light pulses generated by electromagnetic energy accumulated in a capacitor within fractions of a second. This technology is approved by the US Food and Drug Administration for use on food and food contact surfaces using a light source such as a Xenon flashlamp (FDA, 1996; Miller et al., 2012). IPL emission leads to microbial inactivation through membrane disruption and by damaging DNA causing dimer formation, which stops DNA translation and replication processes (Rowan et al., 1999). It has been suggested that IPL has a photothermal and photophysical effect on microbial inactivation (Cheigh et al., 2012). IPL technology has the combined ability to induce germination in spores, followed by its inactivation in the food product. This novel non-thermal technology is a promising approach to eliminate heat and chemical resistant spores in powdered matrices during processing while maintaining the nutritional and sensory quality of the food or ingredient.

1.2 General Properties of Dairy Powders

The Codex Standard 207-1999 defines milk powders as “milk products that can be obtained by the partial removal of water from milk” (FAO/WHO Codex Aliment, 2011). Liquid milk concentrates are converted into solid powder form to stabilize and prolong the quality and food safety of the product during processing, transportation, and storage, and the stabilized milk constituents result from the dehydration of milk. There are a variety of dairy powders that are classified based on composition: whole milk powder (WMP), skim milk powder (SMP), whey powders, casein and caseinate powders, and milk ingredients (Singh and Singh, 2016). Additionally, the dairy industry has developed many value-added products from the by-products of dairy manufacturing such as milk protein concentrate (MPC) and isolate (MPI), whey protein concentrate (WPC) and isolate (WPI), micellar casein concentration (MCC) and isolates (MCI) (Schuck, 2013b).

In this range of dairy powder products, different compositions of milk proteins are extracted and purified using one or a combination of membrane separation and chromatography techniques. Following extraction and purification steps, dairy products are subjected to dehydration which is most frequently achieved by spray drying. The drying parameters will also influence the specific properties and nutritional quality of the final milk powder product.

Dairy powders differ by composition, and microbiological and physical properties (Table 1.1). Particularly, milk powders are categorized into functional, physical, sensory, biochemical, and microbiological properties (Caric and Milanovic, 2003). The interactions between each of the properties influence the final functional and physical quality of the product.

Table 1.1: Classification of dairy powder Characteristics (Schuck, 2013)

Dairy Powder Characteristics	Description
Composition	Proteins, carbohydrates, fats, minerals, water
Physical Properties	Bulk and particle density, instant characteristics, flowability, floodability, hygroscopicity, degree of caking, whey protein nitrogen index, thermostability, insolubility index, dispersity index, wettability index, sinkability index, free fat, occluded air, interstitial air, particle size
Microbiological Properties	Total bacterial count (TBC), somatic cell count (SCC), coliform count (CC)

(Adapted from Schuck P, p. 438, in *Handbook to food powders - Processes and properties*, 1st ed, Woodhead Publishing, Philadelphia, PA, 2013.)

According to regulations in the United States, microbiological standards test for the total bacterial count (TBC), somatic cell count (SCC), and coliform count (CC) in milk products. The acceptable upper limits for raw milk are TBC < 100,000 CFU/mL and SCC < 750,000 cells/mL and for pasteurized Grade A milk are TBC < 20,000 CFU/mL and CC < 10 CFU/mL (Schmidt, 2009). TBC gives an estimate of the overall quality of the milk, but it does not provide information on the effects of microbial contamination on shelf-life. SCC production is linked to the inflammation in the mammary gland of the cow, known as mastitis, and is an indicator of milk quality and poor milking hygiene, and other sources of contamination, such as unsanitary equipment (Murphy et al., 1999).

1.2.1 General Manufacturing Processes of Dairy Powders

Primarily, concentrated milk products are made into dry powders by spray drying; and membrane filtrations (ultrafiltration and diafiltration) are commonly used prior to spray drying to produce different compositions of powdered milk products (Figure 1.3). Spray drying will affect the water content, structural and physicochemical characteristics of the powder (Vignolles et al., 2007). Spray drying uses a hot air stream at 180-200°C to atomize the liquified milk, and will evaporate most of the moisture out of the product and result in dry milk ingredients (Chandan, 2016). Also due to evaporative cooling, the temperature of the individual particles do not reach above 60°C during drying (Schuck,

2013b). The size of the droplet, air temperature, and airflow are controlled to yield high-quality solubility, flavor, and color. Another process to produce dry milk products is roller drying, and during this process, hot rotating rollers evaporate the moisture off of the layer of concentrated milk that is in direct contact with the hot surface. Although the end product results in less moisture from roller drying than spray drying, the powder is more susceptible to irreversible heat damage from the intense heat treatment.

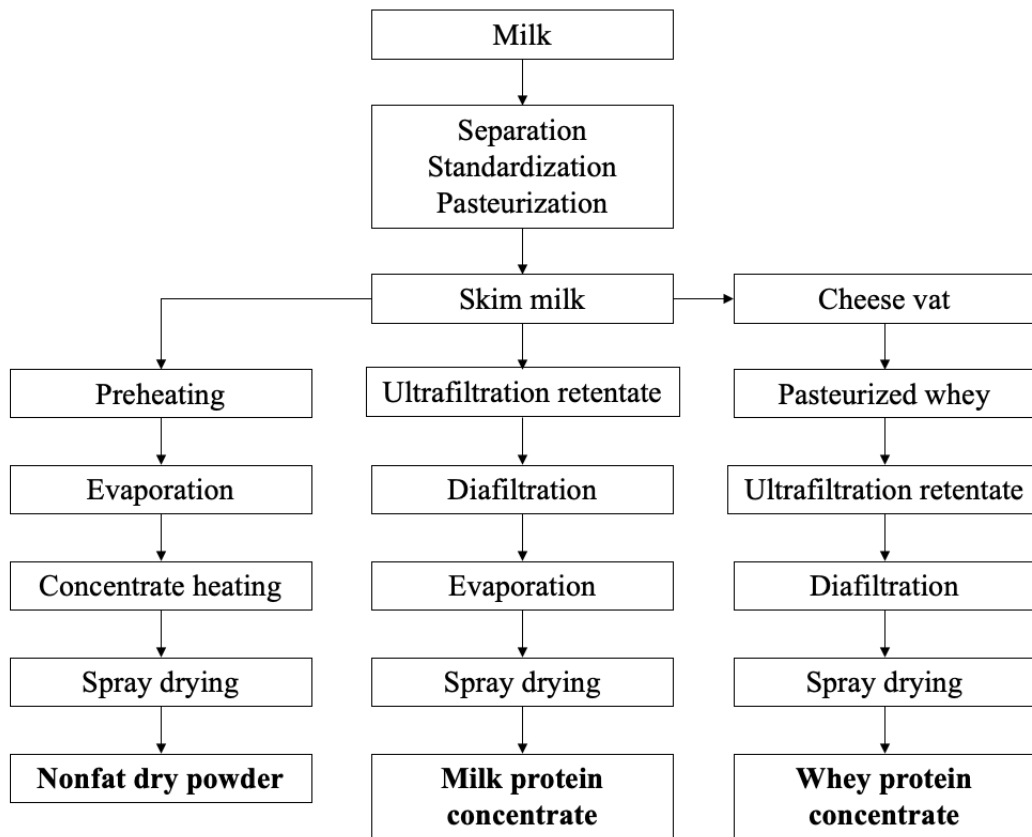


Figure 1.3 General manufacturing processes for SMP, MPC, and WPC powder.

(Adapted from Singh PK and Singh H, p. 335, and Kilara A, p. 355, in *Dairy Processing and Quality Assurance*, 2nd ed, John Wiley & Sons, Hoboken, NJ, 2016.; Burrington KJ, Schoenfuss T, and Patel S, p. 2, in *Technical Report: Coproducts of milk and whey processing*, Dairy Research Institute, 2014.)

1.2.2 Composition of Dairy Powders

The compositions of various types of dairy powders and the abbreviated version commonly used to identify the powder type followed by a number that denotes the approximate protein concentration is provided (Table 1.2).

Table 1.2: Typical composition of dairy powders (Chandan, 2016)

Products	Moisture (%)	Fat (%)	Protein (%)	Lactose (%)	Ash (%)
Nonfat Dry Milk	3.2	0.8	36.0	52.0	8.0
Milk protein concentrate (MPC 70)	5.0	2.0	68.5 – 72.5	18.2	10.0
Whey protein concentrate (WPC 80)	6.0	6.2	81.5	4.1	2.9

(Adapted from Chandan RC, p. 13, in *Dairy processing and quality assurance: An overview*, 1st ed, Global Technologies Inc., Coon Rapids, MN, 2016.)

Nonfat dry milk (NFDM): NFDM is made from condensed skim milk through the process of spray drying, and the removal of fat and water from whole milk results in a product containing lactose, milk proteins, and minerals (Chandan, 2016). NFDM contains less than 5% [wt/g] of moisture and less than 1.5% [wt/g] of fat. Although NFDM has low-fat content, lipid oxidation can still play a major role in off-flavor production and flavor stability. NFDM is used as a functional ingredient in a wide range of products, such as chocolate, bakery, beverages, and confectionery (Singh and Singh, 2016).

Milk protein concentrate (MPC): MPC is produced by ultrafiltration and diafiltration, followed by spray drying of skim milk, and the whey proteins remain in their native state since this processing method does not involve preheating after pasteurization. Through ultrafiltration, casein micelles and whey proteins are retained on the filter membrane, while water, lactose, and minerals are filtered out (Singh and Singh, 2016). Similar to NFDM, lipid oxidation of the low-fat content influences the flavor and flavor stability of MPC. MPC is beneficial for food applications since it contains the same ratio of casein and whey proteins as milk but with lower lactose content (Baldwin

and Pearce, 2005). Finally, MPC is used to improve yield or increase protein content of milk or cheese products, and enhance the textural characteristics of yogurts.

Whey protein concentrate (WPC): Whey protein can be retrieved from milk following the extraction of fat and casein or can be produced from the whey by-product of casein or cheese production (Kilara, 2016). WPC is produced through further processing: filtering and spray drying, and can contain 34%, 55%, or 75-80% protein on a moisture-free basis. Whey permeate that includes lactose, moisture, and ash decreases as more fat and protein solids are retained. WPC can contain a high amount of lactose which can become sticky while heating and cause powder caking during storage due to the hygroscopic nature of lactose (Sehrawat et al., 2018). WPC is also used for its functional properties such as foaming, emulsification, and gelation in a wide variety of products.

1.2.3 Functional Properties of Dairy Powders

As mentioned, the milk powder structure and function is affected by the drying technique. The milk powder properties are dependent on different factors including the raw material, drying method, and a combination of temperature, relative humidity, and various chemical reactions influenced by storage duration of the dairy powders (Pugliese et al., 2017). The type of product category testing is determined based on the sensory, nutritional, and functional needs of the end product. However, standard tests are commonly done to determine the characteristics of the particle structure, size distribution, flowability, density, and reconstitutability.

Particle structure and size distribution: The way in which the chemical components are scattered and linked determines the physical structure (Schuck, 2013b). The particle size of milk powders ranges depending on the manufacturing technique. The viscosity of liquid milk concentrate and atomization spray drying conditions impact the particle size of powders; for instance, high atomization pressure and low viscosity results in smaller particles (Caric and Milanovic, 2003). Typically, NFDM ranges from 85 to 230 microns. The particle size distribution will determine the appearance, reconstitution properties, surface reactivity, and the flow characteristics of the dairy powder (Pugliese et

al., 2017). Generally, larger-sized particles (greater than 200 microns) are free-flowing while finer powders are more resistant to flow and subject to cohesion (Kim et al., 2005). How closely the bulk of solid particles are packed depends on the shape, size, and uniformity of the particles (Sehrawat et al., 2018). Other considerations related to particle structure is the presence of lactose and fat. Typically, lactose is present in non-crystalline state and hygroscopic in nature, which aids in caking in humid environments. Fat in the powder can exist in free-state or surrounded by the protein membrane. Fat in free-state promotes oxidation but can be mitigated through milk homogenization.

Flowability: The ability of a powder to flow freely without aggregating determines its flowability. Factors such as particle size, shape, density, and the electrical charge will affect the flowability of milk powders.

Density: There are three interrelated classifications of milk powder densities: bulk (apparent), particle, and dry milk solids density (Table 1.3) (Schuck, 2013b). The densities are influenced by many processing factors along with the milk composition prior to drying.

Table 1.3: Milk powder density classifications (Schuck, 2013)

Density Classification	Definition	Unit	Influencing Factors
Bulk density	Weight per unit volume	Kg/m ³	Feed concentration, feed temperature, feed foamability, milk preheating, age thickening, feed composition, type of atomizer, particle temperature history, particle size distribution, particle density, occluded air, interstitial air
Particle density	Mass of particles with a total volume of 1 cm ³	g/ml	Amount of entrapped air, concentrate viscosity, incorporation of air prior to drying, type of atomizer
Occluded air	Difference between the volume of a given mass of particles and the volume of the same air-free solids	ml/100g	Incorporation of air into the feed, spray drying system, whipping action (before/during atomization), feed properties, stable foam formation from the feed (depends on fat and protein state/concentration)

Interstitial air	Difference between the volume of a given mass of particles and the volume of the same mass of powder tapped 100 times	ml/100g	Particle size distribution, degree of agglomeration
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(Adapted from Schuck P, p. 441-442, in *Handbook to food powders - Processes and properties*, 1st ed, Woodhead Publishing, Philadelphia, PA, 2013.)

Reconstitutability: Milk powder interactions with water are an important ingredient property for food applications since milk powders are dissolved with liquids before use. Rehydration and dissolution of milk powders are measured using various methods that determine the characteristics of the ingredient: wettability, swellability, sinkability, dispersibility, and solubility (Table 1.4). As a consequence of milk protein denaturation and aggregation after further processing of dried powder, the powder solubility is reduced (Thomas et al., 2004). Dairy powders high in protein and casein, such as WPC, are difficult to reconstitute due to surface casein micelles which inhibits the transfer of water to the powder particles (Schuck, 2013a).

Table 1.4: Milk powder rehydration characteristics (Schuck, 2013)

Rehydration characteristics	Definition	Unit	Influencing Factors
Wettability	Ability of a powder to hydrate and absorb water on the surface (expressed as time in seconds)	sec	Surface activity, surface area, surface charge, particle size, density, porosity, moisture-absorbing substances
Sinkability	Ability of a powder to overcome and penetrate the water surface tension and sink (expressed amount of powder that sinks per unit time per unit surface area)	%	Particle density (mass and amount of occluded air)
Dispersibility	Ability of a powder to become uniformly dispersed when hydrated with water	%	Temperature and duration of drying (heat treatment of casein)
Insolubility index	The fraction of sediments that do not dissolve under standardized conditions (temperature, concentration, time, intensity of stirring)	ml	Temperature and duration of drying, pre-concentration of milk

(Adapted from Schuck P, p. 443, in *Handbook to food powders - Processes and properties*, 1st ed, Woodhead Publishing, Philadelphia, PA, 2013.)

1.2.4 Dairy Powder Quality and Food Safety

Many factors impact the quality or changes in color, flavor, and solubility of dairy powders, and include milk composition, initial microbial load, good manufacturing practices (GMPs), packaging, or storage conditions (Sehrawat et al., 2018). The quality of raw milk has a substantial impact on milk powder flavor, and varies by animal feed, season, or microbiological quality (Park and Drake, 2014). Milk powders with high fat and moisture content are more prone to spoilage and changes in quality, therefore, control of heat treatment, moisture, and hygiene is important during and after processing. The moisture level needs to remain below 4% by weight of moisture and appropriate packaging materials need to be used to prevent moisture absorption and oxygen permeation. Proper GMPs and sanitation of equipment mitigates potential bacterial contamination, especially formation of heat resistant spore-formers on equipment. Storage conditions also have a major impact on product quality, and are influenced by exposure to sunlight, fat oxidation, and hydrolytic rancidity (Sehrawat et al., 2018). Lactose and protein interactions (caused by the Maillard reaction) impacts color, off-flavor, and insolubility, and protein denaturation is also responsible for reducing solubility.

The flavor of dairy powders will determine consumer acceptance of the dried dairy ingredient applications, and with significant off-flavors produced by lipid oxidation, consumers acceptance decreases (Caudle et al., 2005). Lipid oxidation is a primary contributor to shelf-life stability (Drake et al., 2006). It is generally recognized that flavor deterioration in dairy powders are a result of lipid oxidation and Maillard browning (Karagul-Yuceer et al., 2001). Many external factors can play a role in lipid oxidation, which include light exposure, anti-oxidant addition, preheating treatment, nitrogen flushing, moisture content, relative humidity, and storage temperature (Park and Drake, 2014). Intrinsic factors that influence lipid oxidation in dairy powders are the concentration of unsaturated fatty acids and the distribution of fat in the powders (Romeu-Nadal et al., 2007).

In the production of WPC, the processing steps will determine the consequences of lipid oxidation. Starter cultures can impact off-flavors due to the hydrolytic enzymatic activity, and bleach use to decolor Cheddar cheese results in an increase in off-flavors and lipid oxidation in whey powders produced from the by-products of cheese manufacturing (Campbell et al., 2011).

Surface free fat is defined as fat that is not entirely coated by amphiphilic molecules or shielded by the carbohydrate and protein matrix during drying, which impacts off-flavors, lipid oxidation, and the functional characteristics of dried dairy powders (Vignolles et al., 2007). Functional properties that are impacted by surface free fat in milk powders include oxidative stability, wettability, dispersibility, solubility, and flowability (Vignolles et al., 2007).

Water activity is an important measurement in dairy powders that will determine product safety and quality. The difference between water content and activity is that water content is related to the total amount of water present, whereas, water activity measures the “bound water” or energy status of the water in the system (Decagon Devices, 2006). Water activity determines the lower limit of water availability, and the water activity level dictates bacterial growth. To control bacterial growth in dairy powders the amount of available water needs to stay below a critical level. A water activity of 0.60 or less will not support the growth of pathogenic bacteria, however, spore-forming bacteria are still a concern (Beuchat et al., 2013). Chen et al. (2018) determined that the water activity level in NFDM can influence the level of log reduction of *Cronobacter sakazakii* using IPL technology. Pathogenic bacterial survival depends on water activity level, fat content, and solutes in the food matrix, and foods classified as low water activity foods, such as dairy powders, will have a water activity level < 0.7 (Farakos et al., 2014).

The water activity influences final quality of dairy powder products due to chemical reactions such as nutrient degradation, lipid oxidation, nonenzymatic browning, and protein denaturation. During processing, transportation, and storage, the stability of the dairy powders are affected by water activity level fluctuations. Improper handling and packaging of powdered products can lead to changes in texture, stability, density, and

rehydration properties. Caking, clumping, collapse, and stickiness are caused by the lack of water activity control during processing, transportation, and storage, and may lead to a “collapse phenomena” that is related to sticking, caking, and crystallization, which occurs when the matrix containing amorphous carbohydrates no longer maintains its own weight (Chuy and Labuza, 1994). Caking is the result of clumping of the powders and reduced flow of the particles, and leads to powders with poor rehydration and dispersibility properties. Saltmarch and Labuza (1980) studied whey powders, and determined that physical property changes occur from increased water activity and lead to diminished food quality and reduced shelf-life. Particularly, physical changes lead to dairy powders sticking to food processing equipment; thus reducing yield and efficiency, forming biofilms from insufficient cleaning, and diminishing the overall product food safety and quality.

Chapter 2

2. Evaluation of intense pulse light technology on the inactivation of mesophilic *Bacillus cereus* and *Bacillus licheniformis* spores in dairy powders

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2.1 Overview

Bacterial spores pose a quality and spoilage problem throughout the dairy powder industry. New nonthermal technologies to control and deactivate spore-forming bacteria to achieve acceptable levels in dairy powders is warranted. This study investigated the effects of intense pulsed light (IPL) on the inactivation of mesophilic *Bacillus cereus* and *Bacillus licheniformis* spores in nonfat dry milk (NFDM), milk protein concentrate (MPC 70), and whey protein concentrate (WPC 80). This study revealed that the extent of log reduction of spores in dairy powders by IPL treatment were significantly ($P < 0.05$) dependent on the bacterial spore type. The maximum inactivation achieved on *B. cereus* spores was 0.80 log₁₀ CFU/g and the maximum inactivation achieved on *B. licheniformis* was 0.20 log₁₀ CFU/g. This is the first report to evaluate use of a continuous IPL apparatus to decontaminate mesophilic spores in dairy powdered matrices.

Key words:

Bacillus cereus spores, *Bacillus licheniformis* spores, intense pulsed light, dairy powders

2.2 Introduction

The global dairy powder market was valued at 27.8 billion USD in 2017 and is projected to reach 38.0 billion USD by 2025, growing at a compound annual growth rate (CAGR) of 4.4% from 2018 to 2025 (Bhandalkar and Das, 2019). The nutritional advantages of milk powders in food products and increase in usage of milk powders in infant formula drive the global dairy powder market. The increased shelf-life and reduced transportation cost of dairy powders, as opposed to liquid dairy products, also aid in the growth of the market. The milk powder export market is growing nationwide due to changes in work and lifestyles, led by globalization and the penetration of products via the internet and social media. With the rise in international consumers of dairy powders, it is particularly important to meet growing market demands without compromising quality and food safety.

2.2.1 General Dairy Industry Standards for Milk Processing and Products

Liquid milk pasteurization temperatures are set to deactivate the most heat-sensitive of the non-spore-forming pathogenic organisms present in raw milk. Dairy manufacturers typically use the batch (holding) or high-temperature-short-time (HTST) pasteurization method. For batch holding, milk is heated to 63°C and for 30 minutes. Milk treated using the HTST method is heated to at least 72°C for a minimum of 15 seconds through the use of plate heat exchangers in a continuous process. Previous studies determined that pasteurization treatments at increased holding times or temperatures reduce the quality of milk during storage at low temperatures ($4 \pm 1^\circ\text{C}$) (Deeth and Lewis, 2017a). Both pasteurization conditions result in lower levels of vegetative bacterial cells, but bacterial spores may survive these thermal processes. Spores that survive heat treatment reduce shelf-life of milk products due to subsequent germination, and growth of vegetative cells from the spores.

The Codex Alimentarius (2003) states that pasteurization processes achieves a 5-log reduction of *Coxiella burnetii*, which ensures there is a greater inactivation of non-

spore-forming bacteria including *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* (Codex Alimentarius, 2003; Deeth and Lewis, 2017a). Pasteurization also serves to eliminate spoilage molds, yeast, and bacteria; that if viable, multiply over time during storage, particularly psychrotrophic bacteria. Adequate pasteurization is determined by monitoring the inactivation of an enzyme naturally found in milk, called alkaline phosphatase, which is inactivated at the same temperature as pathogenic bacteria found in milk; and this method is called the phosphatase test to ensure that pasteurized milk is not under-treated (Kay and Graham, 1935).

Pasteurization is a universally accepted method for milk heat treatments with continuous HTST used commonly, yet some countries prefer ultra-high temperature (UHT) processing of raw milk. UHT parameters are determined for effective deactivation of all non-spore-forming and most spore-forming bacteria, with the exception of heat resistant spore-forming *Bacillus* and *Geobacillus* spp. The conditions for UHT include heating the liquid milk between 138-145°C for 1-10 seconds (Deeth and Lewis, 2017a). The D-value and z-value are universal parameters used to characterize the extent of heat treatments in milk manufacturing. The D-value is the time in minutes it takes to eliminate 1-log₁₀ (90%) of the target microorganism population at a given temperature. The z-value is the change in temperature required to produce a tenfold change in the decimal reduction time (D-value). Brown (2000) reported a D-value of 2.35 minutes at 121.1°C for spores of *B. cereus*, and a D-value of 4-8 minutes at 100°C for *B. licheniformis* spores. Numerous studies report a range of D and z-values for spores of *B. cereus* or *B. licheniformis* in milk products (Table 2.1). Overall, *B. licheniformis* D-values are higher for each temperature than those for *B. cereus*, and while higher temperatures for pasteurization are more effective to deactivate bacterial spores, they may diminished the quality and consumer-acceptance of milk.

Table 2.1 D-values and z-values of *Bacillus* spp. spores in milk products

Bacteria	D-values (°C)							z-value
	94-95	99-100	103-105	110	115	120-121	135	
<i>Bacillus cereus</i>	107-166 ₁ 30-121 ₂ 81 ₃	58-138 162-186 36-1520 13-21 ₄	16-45 672 4-7	4-25 690	2-11	0-5 1.8-141	0-0 >240 0	6.7-13.8 8.5
<i>Bacillus licheniformis</i>	121-708 ₁	67-304 171-246 ₂	31-202	16-110	7-97	2-28	0-11	6.4-7.8

Within rows, values are reported from the same reference.

References: 1. Janštová & Lukášová, 2001; 2. Milkolajcik, 1970; 3. Bergere & Cerf, 1992; 4. Wescott et al., 1995

Due to increased international trade in UHT milk, levels of spore-forming bacteria in milk and dairy powders are of concern to the dairy industry. Spore-forming bacteria are detected and associated with transport of milk products throughout tropical climate zones and storage at ambient temperatures (Deeth and Lewis, 2017b). Mesophilic and thermophilic spore-forming bacteria are a primary concern to dairy manufacturers. The optimal growth temperature range for *B. cereus* is 30 to 37°C and growth has been reported between the range 4 to 50°C (Brown, 2000; Deeth and Lewis, 2017b). The growth temperature range for *B. licheniformis* is between 30 to 60°C, with optimum growth between the range of 42 to 50°C (Brown, 2000; Deeth and Lewis, 2017b; Dong et al., 2017). Therefore, both *B. cereus* and *B. licheniformis* are classified as mesophilic. The US Dairy Export Council has set strict tolerance levels for spores in dairy powders. Specifically, the tolerance level for mesophilic spores in dairy foods is less than 1,000 CFU/g and less than 500 CFU/g for thermophilic spores (Watterson et al., 2014). *Bacillus* spore-forming bacteria, such as *B. cereus* and *B. licheniformis*, are a particular concern in dairy manufacturing plants as they survive industrial pasteurization. If bacterial spores survive pasteurization conditions, they attach and outgrowth to vegetative cells occurs, forming biofilms in pipes, dead ends, cracks, corners, crevices, valves, gaskets, and joints in stainless steel equipment in processing facilities (Gopal et al., 2015). The formation of endospores by these bacteria of concern imposes a formidable challenge to deactivate the dormant spores in dairy products after pasteurization.

2.2.2 Bacillus Bacterial Spores Defense Properties

The exosporium is the outermost external coat of the bacterial spores that provides the ability to attach to surfaces (Wood and Waites, 1988; Stewart, 2015). The spore coat is comprised of proteins and glycoproteins that plays a role in filtering molecules, such as enzymes and nutrients, and detoxifying chemicals that might otherwise cause damage to the spore (Driks, 2002; Setlow, 2006). Under the exosporium coat is the cortex composed of peptidoglycan, which plays the role of protecting the core from chemical damage by organic solvents. Beneath the cortex layer is the germ cell wall and the inner membrane. The inner membrane provides a chemical barrier for the core. Finally, the core contains its DNA that binds to small acid-soluble proteins (SASP) for protection. It has been reported that a mobile genetic element called *spoVA_{2mob}* is responsible for the heat resistance of *Bacillus* spores (Berendsen et al., 2016). It is believed that sporulation occurs through a phosphorelay (pathway involving multiple regulators and phosphotransferases) triggering a series of steps involving the secondary messenger (SpoA) and transcription of genes that translate into proteins/factors responsible for the process of sporulation (Olmedo et al., 1990; Trach et al., 1991; Hoch, 1993). Spores can lie dormant for millions of years with enhanced ability to survive environmental extreme conditions, such as acidic pH values of 1 to 5.2, elevated temperatures beyond pasteurization, antimicrobial agents, and UV and gamma radiation. The germination of spores into vegetative cells, a stage called outgrowth, can be induced by temperature, germinants, and heat-activation (generally 60 to 80°C). The germination of *B. cereus* spores and subsequently the replication of vegetative cells can result in emetic or diarrheal food poisoning if found between the range of 10⁵ to 10⁸ CFU/mL in food (Granum, 1993).

2.2.3 IPL Technology and on Bacterial Spores

Intense pulsed light (IPL) methodology uses the application of a broad wavelength output of light (190 to 1100 nm) in the form of high-intensity radiation

supplied by a flashlamp. The flashlamp is a Xenon gas-filled chamber that produces bursts of electrical currents generating pulses of light. The light pulses encompass a range of light from ultraviolet (UV-C, UV-B, UV-A), visible, and infrared light. IPL is typically 20,000 times more intense than the sunlight on the earth's sea level surface (Dunn et al., 1995).

Typically, the fluence (Joules/cm²) required to inactivate spores by 5 log₁₀ CFU/mL is 18-fold higher than vegetative cells (Levy et al., 2012). The lethal effects of this IPL technology are attributed to the broad light spectrum, short pulse duration, high peak power, and frequency output of the flashlamp (Dunn et al., 1995). The pulses of light emitted by IPL cause DNA mutations and damage, which interferes with the transcription and translation process of DNA replication. The broad wavelength output of light generates sufficient energy to cause a photochemical transformation of pyrimidine dimers in the DNA. Setlow and Li (2015) reported use of UV radiation alone is not effective in eliminating spores due to the spores' ability to repair cellular and DNA damage caused by UV radiation. The precise mechanism of IPL induced damage is due to the photolyase enzyme that binds to UV radiation-induced dimers and reverses the enzymatic reaction by using visible light energy (Nicholson, 1995). IPL technology provides a more thorough inactivation of spores as compared to UV radiation due to the application of high peak pulsed light energy. When bacterial spores are exposed to IPL, the first exposure to pulses of light will initiate germination, and continued durations of IPL inactivate the cells after germination. Through a time of continuous pulses for seconds ensures the spore will not repair the irreversible light-induced cellular damage leading to detrimental deactivation of spores (Dunn et al., 1995; McDonald et al., 2000).

2.2.4 IPL Technology and Treatment of Dairy Powders

In addition to bactericidal capabilities, the importance of evaluating a new nonthermal control technology is the preservation of sensory, nutritional, and functional properties of a food matrix. Milk powders impose a challenge of particle clumping during IPL treatments due to various inter-particle forces that cause powder agglomeration

(Peleg and Bagley, 1983). Agglomeration of powder is due to moisture absorption and elevated temperatures if extrinsic parameters are not controlled during IPL processing. To provide efficient decontamination of spores in dairy powders, the specification of light intensity, vibration frequency, food matrix water activity or temperature, and environmental relative humidity need to be monitored and/or controlled. If environmental processing factors are not monitored or strictly controlled, there may be variation in inactivation efficiency of microorganism, specifically spores, from IPL treatments.

One limitation of IPL is the 1-2 mm penetration depth onto food matrixes, which may not expose microorganisms in crevices, clumps, or irregularities creating a shadowing effect on food surfaces (Elmnasser et al., 2007). Pulsed-light treatments hold promise for use on outermost food surfaces (Wallen et al., 2001). Gomez-Lopez et al. (2005) reported undesirable product quality effects may occur to foods exposed to IPL treatment due to increasing temperatures with longer treatment times. Currently use of IPL technologies is rapidly expanding for decontamination of an array of foods and packaging materials, however, little work has been conducted for the application of IPL on dairy powder matrices. This study examines the application of intense pulsed light on dairy powders with respect to mesophilic bacterial spore decontamination.

2.3 Materials and Methods

2.3.1 Experimental Design of This Study

Nonfat dry milk (NFD), milk protein concentrate (MPC 70), and whey protein concentrate (WPC 80) were obtained from a commercial manufacturer within the United States (Land O'Lakes, Arden Hills, MN). Each powder type was inoculated separately with mesophilic bacterial spores, *Bacillus cereus* (ATCC 14579) and *Bacillus licheniformis* (ATCC 14580). The inoculated dairy powder samples were divided into 8 samples, to be treated with intense pulsed light with residence times 0, 30, 60, 120 seconds in duplicate. The log reduction of each sample of bacterial spores was calculated after the IPL treatment.

2.3.2 Dairy Powders and Bacterial Strains Used in This Study

Nonfat dry milk (NFDM), milk protein concentrate (MPC 70), and whey protein concentrate (WPC 80) were purchased from Land O'Lakes. NFDM was manufactured by Land O'Lakes (Arden Hills, MN), MPC 70 was manufactured by Grassland Dairy Products, Inc. (Greenwood, WI), and WPC 80 was manufactured by Bongards' Creameries (Perham, MN). The compositional analysis of each powder was provided by the manufacturers (Table 2.2).

Table 2.2 Dairy powder composition

Powder Type	Moisture (%)	Fat (%)	Protein (%)	Lactose (%)	Ash (%)
NFDM	3.2	0.8	36.0	52.0	8.0
MPC 70	5.0	2.0	68.5 – 72.5	18.2	10.0
WPC 80	6.0	6.2	81.5	4.1	2.9

The bacterial strains used in this study (*Bacillus cereus* ATCC 14579 and *Bacillus licheniformis* ATCC 14580) were obtained from the American Type Culture Collection (ATCC). Each bacterial strain (1.0 mL) was propagated into a single tube (6.0 mL) of sporulation broth (HiMedia) and incubated at 37°C for 24 hours. The sporulation broth consisted of: peptone (6.0 g/l), casein enzymic hydrolysate (4.0 g/l), yeast extract (3.0 g/l), meat extract B# (1.5 g/l), dextrose (1.0 g/l), manganous sulphate (0.30 g/l). Cultures were stored at 4°C.

2.3.3 Bacterial Spore Inoculum Preparation and Inoculation

The spore preparation method used in this study was adapted from Daelman et al. (2019). Each *Bacillus* strain was inoculated onto Petri plates (150 mm x 15 mm) containing a mixture of sporulation broth (HiMedia) and High Gel Strength Agar (plantMedia). Cultures of *B. cereus* and *B. licheniformis* were grown at 37°C for at least 7 days to allow for sporulation. Spores were harvested from the agar surface using an 18 cm Corning cell lifter (Corning Life Sciences, Corning, NY) and then placed into a sterile

50 mL conical tubes containing 10 mL sterile deionized (DI) water. Using a 40 µm cell strainer (Corning Life Sciences), the inoculum was filtered to remove any residual agar from the filtrate. Next, each conical tube was centrifuged in a Sorvall Legend X1R Centrifuge (Thermo Scientific) at 6000 rpm for 10 minutes to pellet cellular suspensions. The supernatant was decanted and the pellet was washed with sterile DI water, resuspended, and centrifuged three additional times. The final cell pellet was resuspended in 10 mL of a 50% (v/v) ethanol solution and stored at 4°C for 24 hours to deactivate remaining vegetative bacterial cells (Koransky et al., 1977). Finally, the suspension was centrifuged at 6000 rpm for 10 minutes and resuspended in 4 mL sterile DI water per 100 g inoculated; then stored at 4°C. Presence of spores was confirmed by Schaeffer-Fulton staining and examination under a light microscope after the samples were heated 10 minutes at 80°C to eliminate vegetative cells (Hamouda et al., 2002; Daelman et al., 2012; Tanaka et al., 2012). Each vial of spore suspension contained levels of approximately 7 log₁₀ CFU/mL.

2.3.4 Bacterial Spores on Filter Paper Preparation

Bacterial spore inoculum of approximately 7 log₁₀ CFU/mL of either *B. cereus* and *B. licheniformis* were dispersed onto filter discs. The filter discs had a 0.2 µm pore size, Nylon 66, and 47 mm diameter (Agilent). The filter discs were air-dried once placed inside a Buchner funnel (Sigma Aldrich) attached to a flask, and connected to a vacuum pump. About a 1 mL solution of the bacterial spore inoculum (7 log₁₀ CFU/mL) was carefully poured onto the filter in the funnel and the spores collected onto the filter paper and filtrate discarded. Triplicate samples of individual *B. cereus* and *B. licheniformis* discs were exposed to residence times of 30 and 60 seconds of IPL treatment.

2.3.5 Dairy Powder Inoculation

The water activity (a_w) of each dairy powder was determined using a water activity meter (Aqualab P_{aw}kit, Decagon Devices, Inc., Pullman, WA). The inoculation

procedure followed methods previously reported by Wiertzema et al. (2019). In brief, 100 g of each dairy powder sample (NFDM, MPC 70, WPC 80) was spread out in a thin layer (1 cm) in separate sterile stainless-steel mixing bowls (30 cm in diameter). A 10.0 mL pipette was used to transfer 4 mL of the inoculum for each bacterial spore one drop at a time onto 110 grams of each individual dairy powder. Dairy powders were covered with sterilized Avant Gauze non-woven sponges (Caring) to absorb excess moisture and then covered with aluminum foil to prevent contaminants. The inoculated sample was placed into a desiccator ($a_w = 0.30 \pm 0.05$) at 37°C for 24 hours to dehydrate the inoculated powder for the subsequent blending step. Following 24 hours, each sample was blended in a Waring commercial spice grinder (Grainger, Lake Forest, IL) to evenly distribute the dried spore inoculum throughout the powder sample. Samples were immediately treated with the intense pulsed light (IPL) after blending.

2.3.6 Water Activity Equilibrium of Dairy Powders

Dairy powders were adjusted to the target a_w value in vacuum desiccators by absorption at 37°C. The target a_w level was 0.25-0.30 (Potassium Fluoride, Acros Organics). Water activity was determined using a handheld meter (Aqualab Pawkit, Decagon Devices, Inc., Pullman, WA) with ± 0.02 precision.

2.3.7 IPL Apparatus and Parameters

The IPL apparatus and parameters were designed by Chen et al. (unpublished data). Intense pulsed light (IPL) treatments were conducted using a Xenon X-1100 steripulse- XL system (Xenon Corporation, Woburn, MA) equipped with a 76 cm linear Xenon flash lamp. The IPL apparatus (Figure 2.1) employed the following parts: Model-66°C vibratory feeder (Eriez Manufacturing Co., Erie, PA), two 6-inch 390 CFM inline duct mixed flow fans connected with a thermostatic circulating water bath (LabX, Midland, ON, Canada), Model-105 volumetric feeder (Tecnetics Industries, Inc., St. Paul,

MN), ultrasonic humidifier/dehumidifier, nitrogen tank, infrared heater, and X-1100 power/control module.

The X-1100 steripulse- XL system generates polychromatic radiation in the wavelength range of 190-1100nm. Flow fans were connected to the lamp housing to generate cool air and prevent lamp overheating during processing. The system's processing parameters can be manually adjusted, which includes the pulse rate (0.3-14.0 Hz), pulsed duration (50-7000 μ s), voltage (1000-3000 V), and energy up to 2433 J/pulse. For the IPL apparatus, the feed rate of the samples are dependent on the auger, paddle, and vibratory feeder speed.

Optimal IPL processing and bacterial spore inactivation parameters for dairy powders were determined for spores of *B. cereus* in a prior affiliated study by Chen et al. (unpublished data). IPL apparatus parameters for all samples used a thermostatic circulating water bath at 55-60°C, two flow fans at 54 m³/h of cooling air, a subsurface cooling air at 40.8 m³/h, and a nitrogen tank (flow rate 6 L/min) used to purge the IPL system 5 minutes prior to sample runs. High intensity pulses at a rate of 1 pulse per second and a pulse width of 360 μ s were generated and applied to each dairy powder sample. Each pulse delivered 1.27 J/cm² at an input of 3000 voltage, and distance from the quartz window of 8 cm. Dairy powder samples (NFD, MPC 70, WPC 80) were exposed to four different residence times of 0 seconds, 30 seconds, 60 seconds, and 120 seconds. The vibratory frequency with a feed rate of ~100 g/min was used to maintain approximately a layer of 1-2 mm of each dairy powder on the feeder bed. Powders were preheated at a temperature of about 57°C prior to IPL processing. All samples were inoculated with approximately 7 log₁₀ CFU/mL of either *B. cereus* or *B. licheniformis* spores prior to IPL treatment. The IPL apparatus was cleaned and sterilized using a vacuum and an electric air duster following each experimental run, followed by manually wiping all food-contact surfaces with Clorox Bleach Germicidal Wipes, and allowed to cool before the next sample run. The temperature and water activity of the powders were recorded before and after each IPL treatment.

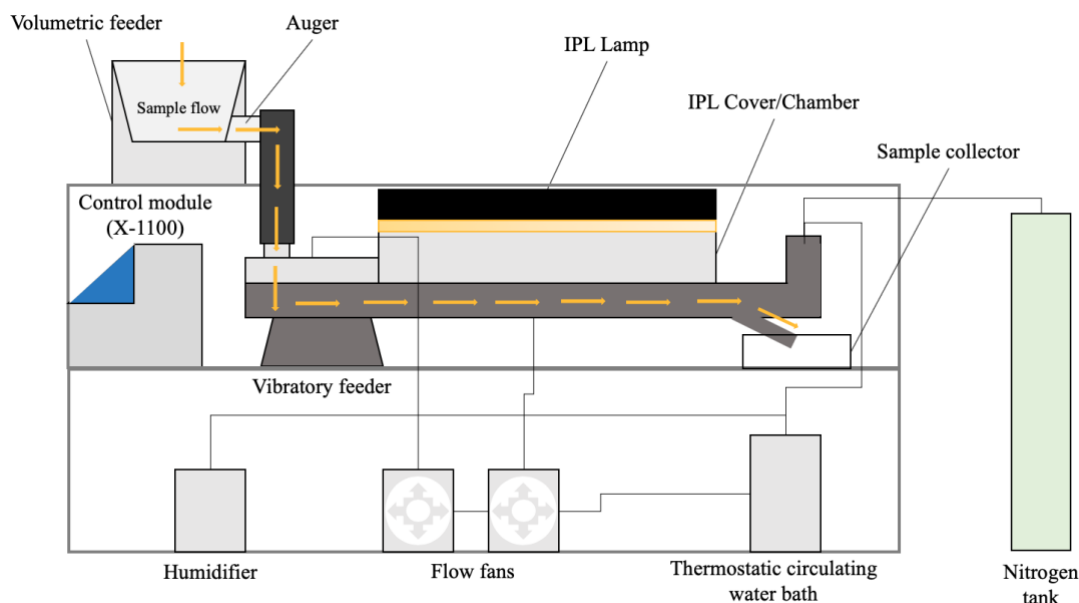


Figure 2.1 A schematic diagram of the Intense Pulsed Light Apparatus prototype.

2.3.8 Enumeration Procedure and Statistical Analysis

IPL-treated samples of NFDM, MPC 70, and WPC 80 were enumerated by diluting 1:10 in 0.1% (w/v) sterile peptone broth. Specifically, 11 g of the powdered samples were diluted in 99 ml of peptone water, then homogenized using the Brinkmann Seward Stomacher 400 Circulator for 30 seconds at 110V. To eliminate vegetative cells, the first dilution of IPL-treated dairy powder samples were placed into 9 ml test tubes and heated in the water bath at 65°C for 10-15 minutes (Berg and Sandine, 1970; Hanson et al., 2005). The heat treatment activates spores and enhances initial germination, which creates a more uniform degree of spore germination and also kills any remaining vegetative cells (Pandey et al., 2013). The homogenized samples were serially diluted to 10^7 and surface plated onto tryptic soy agar (TSA) media plates with dilutions levels ranging from 10^4 to 10^8 . The TSA medium consisted of enzymatic digest of casein (15 g/l), enzymatic digest of soybean meal (5 g/l), sodium chloride (5 g/l), and agar (15 g/l) (Acumedia, Neogen). All plates were incubated at 37°C for 18 to 24 hours. Bacterial counts of CFU/mL from germinated spores were converted to \log_{10} CFU/g. Finally, the log reduction of active spores was determined by equation 2.1, where the difference in

the initial bacterial population in CFU/g (A) was taken from the bacterial population in CFU/g after the IPL treatment (B).

$$\text{Log reduction} = \log_{10}\left(\frac{A}{B}\right) = \log_{10}(A) - \log_{10}(B) \quad (\text{Equation 2.1})$$

For each IPL experiment, two trials were conducted in duplicate under four different residence times (0s, 30s, 60s, 120s) and the values converted to average \log_{10} CFU/g. The mean values and standard deviation of the log values from the individual replicate experiments ($n = 4$) were determined across each powder. The analyses of variance (ANOVA) procedure was used to determine significant differences among the treatments ($p < 0.05$), and correlation analysis was conducted to verify the correlation between the different independent variables.

2.4 Results and Discussion

2.4.1 Microflora in Untreated Dairy Powders

As per the certificate of analysis provided by Eurofins, the bacterial background population of each dairy powder used in this study is shown in Table 2.3. The levels of spore-forming mesophilic microorganisms present in uninoculated dairy powders was 100, 120, and less than 10 CFU/g for NFDM, MPC 70, and WPC 80, respectively. The results of this test ensured that the initial bacterial population was very low and should not impact the bacterial spore count for IPL experiments of spore-inoculated powders. The high heat-shock step prior to enumeration on TSA deactivated germinated spores and vegetative cells, without effecting dormant spores (Wuytack et al., 2000).

Table 2.3 Bacterial population background testing performed on various dairy powders

Powder Type	Test	Method Reference	Results (CFU/g)
Nonfat Dry Milk	Yeast (Mold)	FDA BAM Chapter 18	< 10 (< 10)
	Total Coliforms (<i>E. Coli</i>)	AOAC 991.14	< 10 (< 10)
	Aerobic Spore-forming Mesophilic Count	SMEDP 8.090	100 est
	Aerobic Spore-forming Thermophilic Count	CMMEF Chapter 26.5	< 10
	Standard Plate Count	FDA BAM Chapter 3	130 est
Milk Protein Concentrate (MPC 70)	Yeast (Mold)	FDA BAM Chapter 18	< 10 (< 10)
	Total Coliforms (<i>E. Coli</i>)	AOAC 991.14	< 10 (< 10)
	Aerobic Spore-forming Mesophilic Count	SMEDP 8.090	120 est
	Aerobic Spore-forming Thermophilic Count	CMMEF Chapter 26.5	180 est
	Standard Plate Count	FDA BAM Chapter 3	80 est
Whey Protein Concentrate (WPC 80)	Yeast (Mold)	FDA BAM Chapter 18	< 10 (10 est)
	Total Coliforms (<i>E. Coli</i>)	AOAC 991.14	< 10 (< 10)
	Aerobic Spore-forming Mesophilic Count	SMEDP 8.090	< 10
	Aerobic Spore-forming Thermophilic Count	CMMEF Chapter 26.5	< 10
	Standard Plate Count	FDA BAM Chapter 3	1,600

The objective of this study was to evaluate IPL technology on the inactivation of *B. cereus* and *B. licheniformis* spores in dairy powders. Preliminary control testing was conducted with *B. cereus* and *B. licheniformis* spores on filter paper to ensure that the IPL treatment without any powdered foods would reduce viable bacterial spores, and the results are presented (Figure 2.2). The maximum inactivation achieved on *B. cereus* and *B. licheniformis* spores at 30 seconds (1.27 J/cm² /pulse) was a log reduction of 1.81 (± 0.28) and 1.73 (± 0.45) log₁₀ CFU/g, respectively (Figure 2.2). The IPL treatment of *B. cereus* and *B. licheniformis* spores at 60 seconds showed a log reduction of 2.07 (± 0.33) and 2.08 (± 0.52) log₁₀ CFU/g, respectively (Figure 2.2). For IPL treatments used in this study, a fluence dosage of 1.25 J/cm², which is similar to that used in IPL studies by Levy et al. (2012), who reported a 5 log₁₀ CFU/mL reduction on spores of *B. subtilis* strain 168 (DSM 402) on LB agar plates. Planchon et al. (2011) achieved a similar log reduction with IPL treatment of 5 log₁₀ CFU/mL using a fluence of 1.8 J/cm² on spores of *B. cereus* strain KBAB4 (isolated from soil, Versailles, France) on Petri dishes of LB agar. Aguirre et al. (2015) demonstrated a maximum inactivation of spores of *B. cereus* strain CECT 131 (ATCC 10876) of 6 log₁₀ CFU/cm² with IPL treatment at a fluence of 2.1 J/cm² on the surface of TSA plates. The inactivation results of from this study were lower than

previous studies, however, these differences may be attributed to strain to strain variation and the IPL treatment was used on bacterial spores inoculated onto dry filter paper rather than agar plates. Levy et al. (2012) reported that microbial reductions from IPL treatment are dependent on the type of surface used.

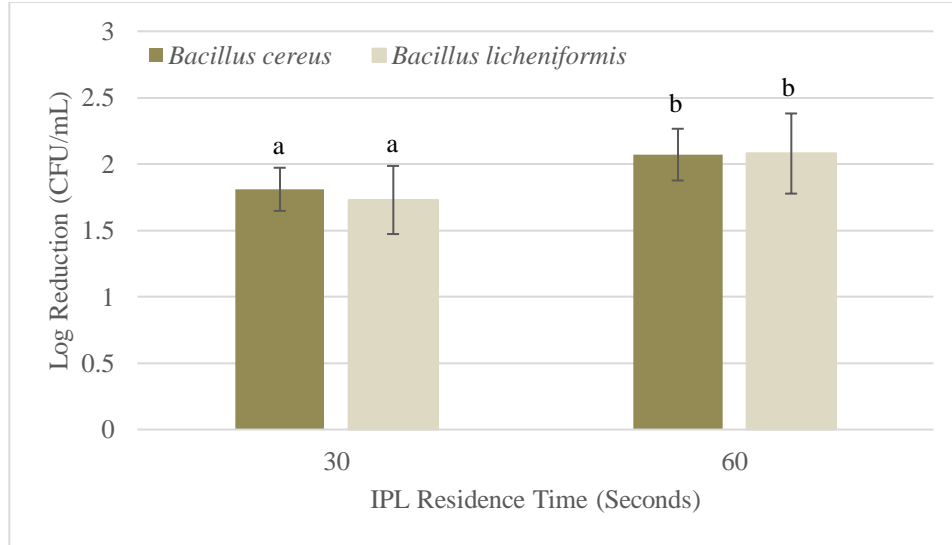


Figure 2.2 Average log reduction of mesophilic spores on filter paper treated with IPL.
^{a-b} Mean values for different IPL residence times with a different superscript differ ($P < 0.05$).

2.4.2 IPL Treatment of Spore Inoculated Dairy Powders

The effects of IPL to inactivate *B. cereus* and *B. licheniformis* spores were evaluated for three dairy powder types (NFDM, MPC 70, and WPC 80) and treatment times (0, 30, 60 and 120s) using a fluence of 1.27 J/cm²/pulse. The log reduction of *B. cereus* and *B. licheniformis* spores after IPL treatment at different residence times was determined (Table 2.4). Our results confirmed that the effect of IPL on inactivation is dependent on the *Bacillus* spore type, since differences were observed between *B. cereus* and *B. licheniformis*. The inactivation of *B. cereus* spores in the three dairy powders was more effective than IPL treatment on *B. licheniformis* spores in each dairy powder (Table 2.4). The log reduction of *B. cereus* spores in NFDM was significantly ($P < 0.05$) larger than *B. licheniformis* in NFDM, at 0.60-0.80 log₁₀ CFU/g as compared to 0.11-0.20 log₁₀ CFU/g, respectively. Moreover, log reduction of *B. cereus* spores in MPC 70 was

significantly ($P < 0.05$) larger than *B. licheniformis* in MPC 70, at 0.26-0.81 log₁₀ CFU/g as compared to 0.05-0.17 log₁₀ CFU/g, respectively. The results from this study also revealed that the log reduction of *B. cereus* in dairy powders is not dependent on the type of dairy powder ($P > 0.05$). Gomez-Lopez et al. (2005) compared the IPL sensitivity of *B. cereus* with other spore-forming bacteria and was able to determine that it was less resistant than *Alicyclobacillus acidoterrestris* and *Bacillus circulans*. Accordingly, the sensitivity of *B. cereus* to IPL treatment was not influenced by the dairy powder type.

Table 2.4 Log reduction of various dairy powders with *B. cereus* and *B. licheniformis* based on the intense pulsed light (IPL) residence time

Powder Type	IPL residence time (sec)	<i>B. cereus</i> (log ₁₀ CFU/g)	<i>B. licheniformis</i> (log ₁₀ CFU/g)
Nonfat Dry Milk	30	0.74 (0.32) ^{a,x}	0.14 (0.31) ^{a,y}
	60	0.60 (0.33) ^{a,x}	0.20 (0.41) ^{a,y}
	120	0.80 (0.35) ^{a,x}	0.11 (0.41) ^{a,y}
Milk Protein Concentrate (MPC 70)	30	0.39 (0.51) ^{a,x}	0.17 (0.36) ^{b,y}
	60	0.81 (0.54) ^{a,x}	0.15 (0.04) ^{b,y}
	120	0.26 (0.11) ^{a,x}	0.05 (0.10) ^{b,y}
Whey Protein Concentrate (WPC 80)	30	0.03 (0.26) ^{a,x}	0.28 (0.31) ^{c,y}
	60	0.28 (0.17) ^{a,x}	0.15 (0.19) ^{c,y}
	120	0.08 (0.67) ^{a,x}	0.01 (0.31) ^{c,y}

^{a-c} Mean values for different powder types within a column with a different superscript differ ($P < 0.05$).
^{x, y} Mean values for the bacterial spore type between rows with a different superscript differ ($P < 0.05$).
 Results are reported as the mean values (standard deviation).

Additionally, since *B. licheniformis* are facultative thermophiles, they are able to grow at both thermophilic and mesophilic temperatures. Studies have reported that thermophilic bacterial strains can produce highly heat-resistant spores (Scheldman et al., 2006). In particular, Scheldman et al. (2006) determined that *B. licheniformis* spores had significantly higher heat resistance than *B. cereus* spores in raw milk at a D_{100°C}-value of approximately 100 min as compared to only 6-8 min for *B. cereus* spores in milk, and a similar trend is observed for IPL treatment suggesting that spores of *B. licheniformis* are more resistant than *B. cereus*.

However, the log reduction of *B. licheniformis* in dairy powders varied dependent on the dairy powder evaluated ($P < 0.05$). Chen et al. (2018) reported that undesirable agglomeration significantly reduces the efficacy of microbial inactivation of IPL treatment on NFD. A similar agglomeration may occur post-IPL treatment based on the

dairy powder compositions, since MPC 70 and WPC 80 both have higher fat, protein, and moisture content as compared to NFDM. Fitzpatrick et al. (2007) determined that fat, high protein, and moisture content all have a significant effect on dairy powder cohesion. Additionally, the emission of energy (produced by the IPL) can be absorbed by proteins and reduce the effective dose available for microbial inactivation (Elmnasser et al., 2007a). Some additional factors that influence the surface stickiness of powder particles are surface temperature and water content (Sharma et al., 2012). The work of Gomez-Lopez et al. (2005) determined that carbohydrate and water content have a variable effect on IPL efficiency on the inactivation of bacteria. Furthermore, the limitations of the IPL penetration depth will be less effective for microbial deactivation with increased particle thickness (Uesugi and Moraru, 2009). Therefore, if caking of powders occurs due to extrinsic factors or higher a_w values, this results in a build-up of bulk powder thickness throughout the IPL process, reducing IPL exposure and inactivation of spores.

No significant correlation ($P > 0.05$) was observed between the IPL treatment and increasing residence times between 30-120s. Therefore, our results conclude that residence time of IPL-treated dairy powders did not influence ($P > 0.05$) the inactivation of the *B. cereus* and *B. licheniformis* spores in dairy powders. This study determined the inactivation of *B. licheniformis* by IPL treatment was dependent on dairy powder types and not IPL residence times. Chen et al. (unpublished data) also found that extending the IPL treatment time might not be effective in increasing microbial inactivation. Microorganisms on the surface of food powders are likely eliminated by IPL treatment than microbes shielded by other cells on the bottom layer, or hidden in open pores of the powdered matrices (Elmnasser et al., 2007b).

2.4.3 Comparison of IPL Treatment on Spore Inoculated Dairy Powders with Adjusted Water Activity

The average log reduction of *B. cereus* and *B. licheniformis* spores on dairy powders with two different water activities values (average of $a_w = 0.40$ and 0.31) was determined (Figure 2.3). Significant differences ($P < 0.05$) in log reduction of both *B.*

cereus and *B. licheniformis* spores based on the water activity level were observed. Our results revealed that at water activity levels of 0.40, a larger log reduction of approximately 0.26 to 0.31 log₁₀ CFU/g was observed, in comparison to the reduced log reduction of approximately 0.06 to 0.14 log₁₀ CFU/g observed at average water activity levels of 0.31. The results were not consistent with a previous study by Chen et al. (2018) on the inactivation of vegetative cells of *Cronobacter sakazakii* in NFDM. Specifically, Chen et al. (2018) found that inactivation decreased with increased water activity levels from 0.25 to 0.35, where the greatest inactivation of 3.18 log₁₀ CFU/g occurred at a water activity of 0.25. A study by Hsieh et al. (1976) on thermal inactivation of both *Salmonellae anatum* NF3 and *Staphylococcus aureus* 196E vegetative cells increased with increasing water activity levels above 0.80, and were most heat resistant in the range of a_w 0.75-0.80. Although this has not been tested for bacterial spores, it supports this finding trend for bacterial spores, but future studies are needed to conclude this. Higher water activity levels of dairy powders will result in agglomeration of powders, which will cause a shielding effect for the inner bacteria against the IPL treatment (Uesugi and Moraru, 2009). Therefore, increased water activity levels correspond to undesirable agglomeration of dairy powders, which has shown to reduce the inactivation of IPL (Chen et al., 2018). This study revealed that *B. cereus* and *B. licheniformis* spore deactivation from IPL treatment in dairy powders is more effective at higher a_w levels, but as a_w increases, dairy powder agglomeration increases. In commercial dairy powder processing facilities, the environmental relative humidity level likely varies from 30% to 65% based on seasonal changes in some geographic locations (US Dairy Export Council, 2019), and these extrinsic factors need to be considered for implementation of IPL.

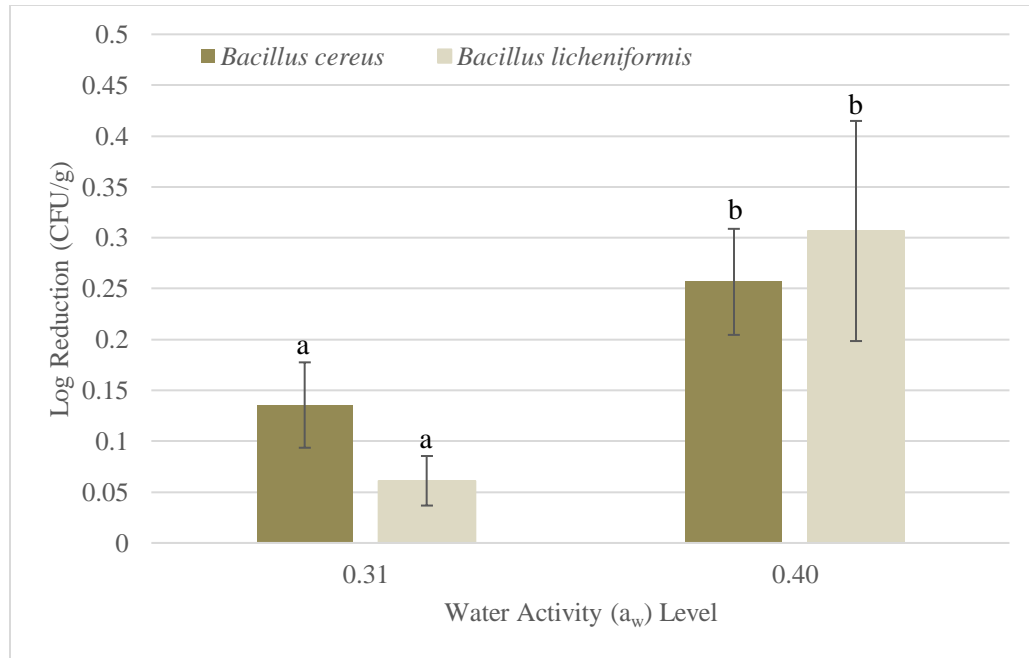


Figure 2.3 Average log reduction on dairy powders treated with IPL at different water activity.

^{a-b} Mean values for different water activity levels with different superscript differ ($P < 0.05$).

2.5 Summary

Although some level of inactivation of *B. cereus* and *B. licheniformis* spores by IPL treatment was achieved in dairy powders, IPL technology is a potential alternative or complement to conventional thermal or chemical decontamination processes for use in the food industry. However, further work is necessary on the food safety parameters and efficiency of the continuous IPL apparatus design to allow for commercial success.

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Chapter 3

3. Evaluation of intense pulsed light technology on the quality and functionality of nonfat dry milk powder

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3.1 Overview

Currently, new nonthermal technologies are being examined to decontaminate dairy powders from foodborne pathogens and spore-forming bacteria. The ideal treatment method should inactivate microorganisms, yet not cause detrimental effects on sensory, quality, or functionality to dairy powders. The objective of this study was to investigate the impacts of intense pulsed light (IPL) on the quality and functionality of nonfat dry milk (NFDM). IPL-treated samples were compared to untreated NFDM samples. Milk powder composition (protein lactose, fat, ash, moisture), quality changes (flavor, odor, appearance, color), and functional and physical characteristics were evaluated for IPL-treated NFDM. The hypothesis is that IPL treatment will cause minimal changes in the quality and functionality of NFDM. Our results indicate that IPL will not significantly affect the functionality of the nonfat dry milk, however, minimizing changes to the quality and sensory-related changes of IPL-treated NFDM need further evaluation.

Keywords: Intense pulsed light; nonfat dry milk; milk powder quality; milk powder functionality

3.2 Introduction

In contrast to liquid milk products, milk powders provide advantages to extended storage, processing, handling, transportation, and use for the formulation of food products. However, dried dairy powders are more susceptible to flavor, quality, and functionality changes such as uptake in moisture, browning, collapse or caking (Al Madhi et al., 2006). Raw milk sources, drying methods, or storage conditions can have an impact on milk powder quality and properties. The physical properties and chemical composition are indicators of milk powder quality. The flowability of milk powders is an important consideration in the dairy industry since it may affect transportation, packaging, or downstream mixing into food products. Rehydration properties impact milk powder reconstitution with water, which is an important criterion for end users. Understanding the general functional properties of milk powders provide food developers with the knowledge to optimize the usage of the powder in processing or food development environments. One of the most produced dairy products manufactured in the U.S. is nonfat dry milk (NFDM) (Abdalla et al., 2017). NFDM provides beneficial texture and flavor to its food applications. The objective of this study is to evaluate the impacts of intense pulsed light on the basic physical properties (flowability, solubility, wettability, heat stability, etc.) of IPL-treated NFDM. Our hypothesis is that IPL treatment on NFDM will cause minimal changes to the functionality and quality of the dairy powder.

3.2.1 NFDM Composition

The typical composition of NFDM has been determined in Table 3.1 (US Dairy Export Council, 2019). The composition of the powder may affect its downstream use and product shelf-life. In the production of NFDM, fat is removed before processing and the protein composition is generally casein or whey proteins. Casein comprises ~80% of the proteins in milk powder and precipitates with rennet or acid treatment. The remaining protein is typically whey, which is soluble unless denatured at high temperatures and

precipitate. The lactose in NFDM can interact with the proteins when exposed to high temperatures, causing browning. Fat content also influences the level of oxidation in milk powders (Wisconsin Center for Dairy Research).

Table 3.1 Composition of nonfat dry milk (US Dairy Export Council, 2019)

Type	Results (%)
Protein	34.0 - 37.0
Lactose	49.5 - 52.0
Fat	0.6 - 1.25
Ash	8.2 - 8.6
Moisture (non-instant)	3.0 - 4.0
Moisture (instant)	3.5 - 4.5

3.2.2 NFDM Sensory and Appearance

Flavor is one of the most important components of consumer acceptance for foods and ingredients. NFDM has a flavor similar to fluid skim milk, and off-flavors are a major concern for food companies. Factors such as lipid oxidation, enzymatic reactions, microbial growth, or environmental conditions impact the formation of off-flavors (Karagul-Yuceer et al., 2002). Ultimately, the development of off-flavors will reduce product shelf-life, sensory quality, and economic value. The odor is paramount for consumer acceptance, and NFDM should be pleasant and clean to the palate, but other flavors can be influenced by processing environments. Off-smells such as barn, cardboard, sour and chemical can result in rejection of the product (Abdalla et al., 2017).

In terms of appearance for USDA Standard Grade, NFDM should be absent of lumps or grains, free from dark particles and have uniform white to light cream color (USDA, 2001), and can be altered from the Maillard reaction occurring during heat processing and spray drying. The color of the product is important to the consumer as an off-color is not familiar and visually deters them from purchasing or using the product. The color of milk powders varies on a scale of whites to light cream. When exposed to high heats, the Maillard reaction may occur, causing browning of the powder particles.

3.2.3 NFDM Physical and Functional Properties

Standard physical testing is typically done on NFDM powders to understand the impacts of processing on the powder characteristics. These may include titratable acidity (TA), scorched particles, and whey protein nitrogen index (WPNI). Functional testing is important to determine the reconstitutability of the powder in food applications, which include wettability, dispersibility, insolubility, flowability, heat stability, and coffee stability.

Titratable acidity (TA) is the measure of acid in a solution using a titrant, reported as % g/L. The titrant, which is typically sodium hydroxide, is added at a known amount to determine the concentration of the second chemical species by association. TA should not exceed 0.15% in NFDM and is a good indicator of bacterial presence in the milk since decreased pH indicates fermentation may have occurred from culture growth within the product (Chandan et al., 2008).

Discolored specks that result from spray-drying is termed scorched particles, where the heat influences the Maillard reaction to occur (Sharma et al., 2012). NFDM generally contains 7.5-15 mg (Chandan et al., 2008), and is determined by comparison to the standard of four discs (7.5 mg, 15.0 mg, 22.5 mg, and 32.5 mg) established by the American Dry Milk Institute.

Whey Protein Nitrogen Index (WPNI) determines undenatured whey proteins resulting from heat treatments in NFDM as developed by the American Dairy Products Institute (ADPI, 2009). WPNI is based on the moisture-adjusted weight (mg/g) of NFDM and may change due to processing history, and affects reconstitution functional properties based on heat treatment (Zhao et al., 2019; Sharma et al., 2012). A study published in Dairy Science & Technology shows that WPNI is associated with protein content for both low (WPNI >6) and medium (WPNI >1.59<5.99) heat NFDM, and are therefore reported as standardized WPNI calculations to be independent of protein content, which will represent the actual heat treatment of the powder (Sikand et al., 2008).

The ability of the powder to absorb water on the surface is due to adhesive intermolecular interactions, which determines its wettability (Moldoceanu et al., 2017).

Particle size is correlated with wettability; the larger the particle size, the increase in wettability (Abdalla et al., 2017). Wettability is dependent on the contact angle between the surface of the liquid and the outline of the contact surface, and the smaller the angle leads to improved wettability. Given the hygroscopic nature of NFDM, the contact angle with a liquid is small, resulting in good wetting amounts. The liquid fills voids areas in food particles, therefore porous surfaces wet more easily (Sharma, et al., 2012).

Passibility through a sieve (typically 150 μm) due to its ability to break down describes dispersibility (Lee et al., 2014). This property of NFDM shows how well the particles separate in water, bettering its wettability. Dispersibility decreases as particle size decreases and as the fat content increases in milk powders (Sharma et al., 2012).

Dissolvability is important in the milk powder industry because it shows the ability of the powder to form a solution. A decrease in solubility may show a deterioration of the milk powder over time, potentially due to how it is stored and absorbed moisture from the atmosphere (Supplee and Bellis, 1925). Other than high heat NFDM, which should have insolubility levels less than 2.0 mL, the solubility index of NFDM should be less than 1.2 mL and is often an important determinant of reconstitution quality in the powder dissolution process (Selomulya and Fang, 2013).

Flowability ensures good fraction or granulation of powders. The importance of flow is for plant efficiency and movement in processing, as well as uniformity in the product. In a study done in the Flodex laboratories in Milan, Italy, a good range of disk-hole diameter was determined to be 10-24 mm (Gioia, 1980). Flowability is dependent on dispersion and agglomeration (Sharma et al., 2012).

Heat stability is important for powders that are intended to be reconstituted and is a representation of its protein stability (Sharma et al., 2012). Heat stability is dependent on its ability to withstand high temperatures and is determined by heat coagulation time at 140°C (Sikand et al., 2010). The stability of NFDM during evaporation while drying in high-heat treatments prompts a κ -casein and β -lactoglobulin complex formation (Sharma et al., 2012), which allows the powder to withstand the conditions of high-heat sterilization. The coffee stability test is a standard method for analyzing the heat stability of a powder. Milk powder properties, when mixed in coffee, is an important function to

analyze since coffee can be considered an acidic solution. The NFDM stability in the coffee is affected by undesirable floaters or sinkers. Presence of these during the test indicate the chemical make-up of the powder, and high surface fat content results in poor coffee stability (Teehan et al., 2007).

3.2.4 NFDM Intrinsic Properties

Intrinsic properties of NFDM, such as water activity and pH, are important properties to understand for its use as an ingredient during processing or food formulations. Water activity (a_w) is an indicator of how tightly the water is held by the molecules in the food product, and values typically range from 0 to 1 (Smith, 2018). Water activity increases with hygroscopicity of milk powders (Smith, 2018). Since NFDM is composed of a large amount of lactose, it is extremely hygroscopic. This means that the powder can absorb atmospheric moisture, which can lead to caking, although the proteins in the powder limit the hardness and clumping that occur over time. The pH values of milk powders are measurements of the number of free hydrogen molecules in the system, which contribute to protein function and may promote sour flavors. Milk powders can range from 6.6 to 6.8 in pH value (Kajal et al., 2012).

3.3 Materials and Methods

3.3.1 Experimental Design on Untreated and IPL-Treated NFDM

Nonfat Dry Milk (NFDM) was obtained from a commercial manufacturer, Lone Star Dairy Products (Canyon, TX). Following IPL treatment, NFDM powders were sealed in Uline matte (OPP/ VMPET/ PE) stand-up barrier pouches (Hudson, WI). Each sample was packaged in 10-gram aliquots and stored at ambient temperature (23°C) in a dry and dark area away from sunlight. Preliminary powder functionality testing was completed on the untreated (control) and IPL-treated samples (0-month testing), followed by monthly surveillance (1 month - 6 months) on the powder appearance, flavor, and odor for significant changes in quality (Figure 3.1). The IPL apparatus was ran using

optimal parameters as described in Chapter 2 with a continuous feed rate of approximately 1000 g/h (2 minutes residence time). This study evaluates month 0 through month 4 testing results.

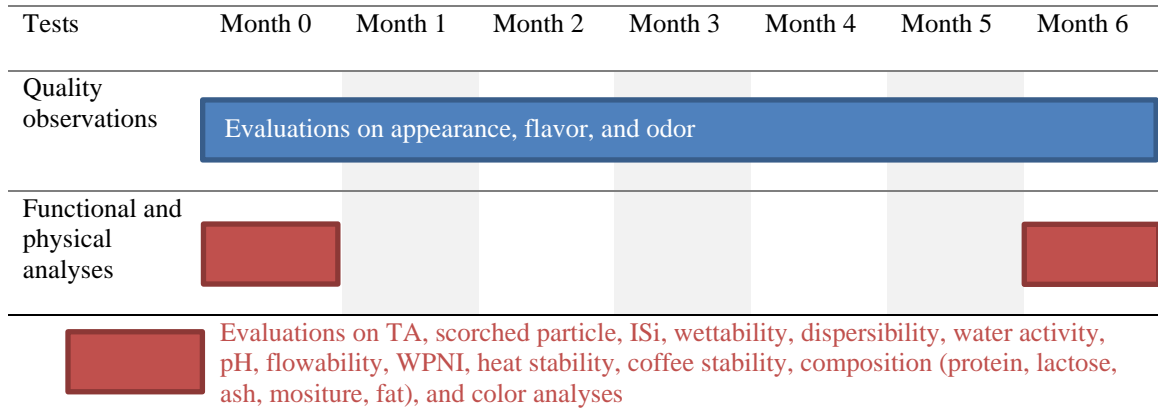


Figure 3.1 Timeline of shelf-life testing for untreated and IPL-treated NFDM.

3.3.2 Composition (Lactose, Fat, Ash, Moisture), Scorched Particle, and Protein and WPNI Analyses on Untreated and IPL-Treated NFDM

NFDM composition, scorched particle, and TA measurements of the initial NFDM untreated and IPL-treated samples were determined by the South Dakota State University Dairy Science Department (Midwest Dairy Foods Research Center, Brookings, SD). Protein and WPNI analyses were determined by Eurofins DQCI (Mounds View, MN). For each NFDM untreated and IPL-treated sample, the measurements were carried out in triplicate, and the mean values were obtained.

3.3.3 Flavor, Odor, and Appearance Analyses on Untreated and IPL-Treated NFDM

One small teaspoon of untreated and IPL-treated dried powder was placed directly into the mouth, separately, and flavor notes were recorded. The powders were also wetted and tasted for comparison. Odor descriptors were determined for dry and wetted powders.

Visual assessment of the appearance of the untreated and IPL-treated samples was observed with the naked eye.

Color comparison of the untreated and IPL-treated NFDM were determined using the Konica Minolta Chroma Meter CR-200 series (Konica Minolta Sensing, Inc., USA) to obtain the CIE L* a* b* values. L* represents lightness from black (0) to white (100), a* represents red (+) or green (-), and b* indicated yellow (+) or blue (-). A standard white tile (Y: 92.89, x: 0.3151, y: 0.3215) was used to calibrate the instrument before measurements. To measure color, the untreated NFDM sample and IPL-treated NFDM samples were placed on separate 100 x 15 mm plastic Petri dishes (Fisher Scientific) with a depth of approximately 2 mm. Each sample was measured in duplicate.

3.3.4 Wettability, Dispersibility, Insolubility Index (ISi), Flowability Index, Heat Stability, and Coffee Stability Testing on Untreated and IPL-Treated NFDM

Wettability: The wettability of NFDM was measured as the amount of time (seconds) required for the milk powder to become wetted when placed on the surface of the water (GEA Niro Method No. A5b, IDF Standard 87:1979). This method is commonly used to determine the wetting time of instant dried dairy products in water.

Dispersibility: A 200 mm, 150 µm woven metal wire cloth test sieve was used to evaluate the dispersibility of NFDM. Dispersibility is defined as the ability of the powder to break down into particles passing through a 150 µm sieve. In this test, the powder is initially wetted and manually stirred in water, followed by filtering through a sieve to collect the total solids content in the liquid (GEA Niro Method No. A6a, IDF Standard 87:1979). Equation 3.1 was used to calculate the dispersibility (D) of the NFDM samples.

$$D = \frac{(Total\ solids\ in\ \%\ of\ the\ liquid) \times 962}{100 - [(Moisture\ in\ \%) + (Total\ solids\ in\ \%\ of\ the\ liquid)]} \quad (\text{Equation 3.1})$$

Insolubility index (ISi): The NFDM powders were reconstituted at 10% (w/w) powder concentration in distilled water and the procedure was carried out following the GEA Niro Method No. A3a (GEA Niro Method No. A3a, IDF Method 129A:1988). The

measurement for the powder's ability to dissolve in water is the insolubility index (ml sediment) of that powder. A Sorvall Legend X1R Centrifuge (Thermo Scientific) was used to accommodate the 50 mL vials used in this method.

Flowability index: To estimate the flowability index of the NFDM powders, the Flodex (Hanson Research, Chatsworth, CA) tool was used to determine specifications using an arbitrary scale of 4 to 40. The Flodex determination of the intrinsic flowability is based on the ability of the powder to fall freely through different sized holes in a plate placed below a funnel. The ability for the powder to fall successfully three times through the smallest diameter hole is recorded as the flowability index. The flowability index provides information on the property of a powder to flow evenly under forces like gravity, compaction, and dosage (Gioia, 1980 - Intrinsic Flowability).

Heat stability: Coagulation of untreated and IPL-treated NFDM powders was evaluated by a heat stability test. Powder samples were reconstituted in 1 ml of water within a glass vial and agitated in a silicon oil bath at 143 °C for 5 minutes (Fisher Chemicals, Fair Lawn, NJ). The oil bath used in this test was the Yamato Oil Bath BO400 (Tokyo, Japan). No visualization of coagulation indicates heat-stable powder after 5 minutes of testing.

Coffee stability: The protein stability of dairy powders is determined by adding milk powder to hot coffee. This simple test is often used to test coffee creamers and milk powders. As outlined by GEA Niro Method No. A16a, 5 grams of powder is wetted and stirred in 150 ml coffee cooled to 80°C between the pH of 4.9-5.4. No observations of flocculated particles on the surface of the solution after 30 seconds as determined by this test indicates a thermally stable powder.

Water activity: The water activity of the NFDM was determined using a handheld meter (Aqualab Pawkit, Decagon Devices, Inc., Pullman, WA) that is reported to have a ± 0.02 precision. Water activity is defined as how tightly water is bound to the food system. More specifically, water activity is the ratio of water vapor pressure in a material (p) to the pure water vapor pressure (p_o) at the same temperature. The water activity of a product equals the relative humidity when the vapor and temperature equilibrium are

determined. A water activity level of 1.0 equates to pure water. Water activity is determined with equation 3.2.

$$a_w = \frac{p}{p_o} = \% \text{ equilibrium relative humidity} \times 100 \quad (\text{Equation 3.2})$$

pH: An Oakton pH 700 benchtop meter was used to determine the pH of the NFDM powders. NFDM powder samples were reconstituted at 10% (w/w) powder concentration in distilled water prior to quantification.

3.4 Results and Discussion

3.4.1 Composition of Untreated and IPL-Treated NFDM

The objective of this study was to evaluate the influence IPL treatment had on the quality and functional properties of NFDM in comparison to the untreated NFDM (control). The composition of the untreated and IPL-treated NFDM used in this study are shown in Table 3.2. Unlike skimmed milk powder (SMP) that is defined by CODEX Alimentarius, NFDM is defined by U.S. Food and Drug Administration, which has no standard for protein level (U.S. Dairy Export Council). The U.S. Dairy Export Council has provided the typical composition for NFDM for protein, 34.0 - 37.0%; lactose, 49.5 - 52.0%; fat, 0.6 - 1.25%; ash, 8.5 - 8.6%; and moisture, 3.0 - 4.0%. Protein, lactose, fat, ash, and moisture content for the IPL-treated NFDM were 35.81%, 41.05%, 1.23%, 7.86%, and 3.65%, respectively (Table 3.2). With the exception of lactose and ash, the results from this study are within the typical composition for NFDM. Sikand et al. (2008) reported that lower ash values are attributed to lower mineral content in NFDM. Furthermore, the United States Department of Agriculture (USDA) has outlined the basis for U.S. Standard Grade NFDM powder on the following: flavor, physical appearance, bacterial estimate (TPC), milk fat content, moisture content, scorched particle content, solubility, and titratable acidity (21 CFR part 131; 58.2527). The final U.S. Standard Grade is based on the lowest rating of the quality factors, and per these specifications, the

moisture shall not be more than 4.0% and the milkfat shall not be more than 1.25%. Our results of the IPL-treated NFDM are within the values to comply with this USDA standard.

Table 3.2 Composition of untreated and IPL-treated nonfat dry milk

Type	Standard Test	Untreated (%)	IPL-Treated (%)
Protein	AOAC 926.15	35.95 (0.06)	35.81 (0.03)
Lactose	AOAC 984.22	40.78 ^a	41.05 ^a
Fat	AOAC 932.06	1.20 (0.02)	1.23 (0.04)
Ash	AOAC 932.30	7.60 (0.10)	7.68 (0.01)
Moisture	AOAC 932.06	3.42 (0.02)	3.65 (0.03)

^aResults obtained from Alfred Laboratory (South Dakota State University), no standard deviations were given from the report.

Results are reported as the mean values (standard deviation).

3.4.2 Functional, Physical, and Intrinsic Properties of Untreated and IPL-Treated NFDM

The functional properties of the untreated NFDM and IPL-treated NFDM were determined and the results are summarized (Table 3.3). U.S. Standard Grade NFDM requires specifications for scorched particle content that shall not contain more than 22.5 mg, insolubility index of < 2.0 mL, and titratable acidity < 0.17%. Our results determined TA values of 0.14% for untreated NFDM and 0.12% for IPL-treated NFDM, which aligned with the requirements for USDA Standard Grade.

IPL-treated NFDM had a 2.4 mg increase in scorched particles in comparison to the control sample. It is expected that this value will increase through IPL treatment since milk powders typically darken through the Maillard reaction, which is attributed to the low water activity of the powder and exposure to hot air (Sharma et al., 2012). Additionally, Gonzales et al. (2009) reported that NFDM is sensitive to the Maillard reaction due to high concentrations of lactose and lysine-rich proteins.

The WPNI of the IPL-treated NFDM was 7.99 mg/g, which was 9.20% lower than the WPNI of the untreated sample. WPNI values greater than or equal to 6.0 mg/g are classified as low heat to reflect the degree of heat treatment, and the samples from this study can be defined low heat-treated and are within the requirements for USDA Standard Grade NFDM (ADPI, 2009). The decreased WNPI of the treated sample suggests that protein denaturation increased (Pelegriane and Gasparetto, 2005). Pelegriane and Gasparetto (2005) reported that at neutrality (pH = 6.8), the solubility of the protein was observed to decrease with increasing temperatures, even below 60°C. Temperature can cause the noncovalent bonds that stabilize the secondary and tertiary protein structure to unfold and subsequently trigger hydrophobic groups to interact and reduce water-binding abilities. These reactions influence the WPNI determination, and the results from this current study suggest that IPL treatment of NFDM induces slight protein denaturation at a feed rate of approximately 1000 g/h.

A related functional property to WPNI is the coffee stability of a dairy powder. Specifically, the denaturation of whey protein impacts the coffee stability of skim milk powders (Sharma et al., 2012). Sharma et al. (2012) reported that powders with $WPNI \leq 3$ have the best coffee stability. Although the untreated and IPL-treated samples had WPNI values > 3 , no visible precipitation was observed during testing. Therefore, the IPL-treated NFDM can provide good stability in a coffee solution.

Heat stability of untreated and IPL-treated NFDM showed no visible coagulation after 5 minutes of heating in the oil bath. These results are expected since heat stability is related to the pH value and protein content of the powder, which were relatively the same for each sample analyzed in this study (Sikand et al., 2010). The pH values obtained from the samples in this study were 6.60 and 6.57 for untreated and IPL-treated NFDM, respectively. This value is within the pH of reconstituted milk samples from a previous study with values between the pH range of 6.5 to 6.7 (Jenness and Patton, 1959).

The rehydration process for powders includes three steps, wetting, dispersing, and solubilization (Ji et al., 2016). During this test, powder wetted within 60 seconds is classified as easy to wet (GEA Niro, 2005). The rehydration properties of the untreated and the IPL-treated NFDM demonstrated similar characteristics. According to the results

of this study of the immersion wetting procedure, both untreated and IPL-treated samples were difficult to wet with water (> 120 seconds). Wettability of powders is dependent on the surface composition of the powder (Kim et al., 2002), and NFDM contains lactose which provides good wetting properties due to its hygroscopic nature. Although both samples did not completely sink below the surface even after 120 seconds during testing, the IPL-treated powders achieved faster penetration of the still water surface. The untreated powder wetted more slowly due to the hydrophobic free fat on the powder surface that has the ability to reduce wettability (Selomulya and Fang, 2013). A possible explanation for why the IPL-treated NFDM achieved quicker wetting may be due to a small amount of IPL-induced lipid oxidation, which would have minimized the influence of surface free fat. Kim et al. (2002) observed that removing fat from NFDM causes milk powders to become wetted quickly. However, the quick wetting of the powder formed into lumps and hindered the wetting of the powder inside the lumps. Accordingly, both NFDM samples tested in this study are considered non-wettable.

The dispersibility of untreated NFDM values determined in this study were 34.27% and 34.81% for IPL-treated NFDM. These values are within the expected range since ordinary spray-dried powders are about 37% dispersible (Moats, Feinstein, and Golumbic, 1959).

The insolubility index (ISi) was used as an indicator of powder solubility in this study, which determined that the untreated and IPL-treated NFDM have an ISi value of > 0.1 mL. The IPL-treated NFDM may have resulted in slightly better dissolution due to the partial denaturation of proteins in dry form that would improve its functionality (Mine, 1997). Since the IPL treatment of powders was conducted below the temperature of 60°C, it has been reported that protein solubility increases with temperatures between 40°C to 50°C (Pelegri and Gasparetto, 2005). Similarly, Pugliese et al. (2017) reported the ISi values of skim milk powders were ≤ 0.1 mL. The results determined in this study for IPL-treated and untreated NFDM for the ISi values agree with the classical differentiation of milk powders based on WPNI (Bylund, 1995), and these ISi values conform with U.S. Standard Grade NFDM classification.

The results obtained from this study suggest that untreated and IPL-treated NFDM have good flowability. Kim et al. (2015) reported that skim milk powders flow more easily than other food powders due to its surface composition containing lactose, protein, and little fat content. No differences between samples were observed for the flowability of IPL-treated and untreated NFDM.

The water activity values of untreated and IPL-treated NFDM analyzed in this study were 0.32 and 0.39 for untreated and IPL-treated NFDM, respectively. Multiple studies have reported that the water activity of skim milk powders can range from 0.100 to 0.380 (Hogan et al., 2010; Morgan et al., 2005). These water activity values reported in prior studies are consistent with the results obtained from this study. The overall quality of dairy powders is often influenced by the water activity, which impacts the nutritional, sensory, and physical properties if water migration is not controlled (Pugliese et al., 2016), and our results indicate a minor increase in water activity occurred through IPL treatment of NFDM; likely due to humidity introduced from the IPL apparatus testing environment.

Additionally, the color of the IPL-treated NFDM was monitored to determine if reactions such as Maillard browning were initiated. The IPL-treated NFDM had a slightly lower b^* value (less yellow) compared to the untreated NFDM. However, our results determined there was no significant difference ($P > 0.05$) between the colors of the untreated and the IPL-treated NFDM. In general, increased b^* values reflect a change towards yellow or brown, which is associated with Maillard browning (Morales and van Boekl, 1998). Overall, our results indicate that no changes in the powder color of NFDM developed through the IPL treatment.

Table 3.3 Standard milk powder functional, physical, and intrinsic tests performed on untreated and IPL-treated nonfat dry milk

Type	Standard Test	Results	
		Untreated	IPL-Treated
Titrateable acidity	AOAC 947.05	0.14 (0.00) %	0.12 (0.01) %
Scorched particle	ADPI BUL. 916	3.1 mg ^a	5.5 mg ^a
Solubility index	IDF 129A:1988	< 1.00 (0.00) mL	< 1.00 (0.00) mL
Wettability	IDF 87:1979	> 120 (0) seconds	> 120 (0) seconds
Dispersibility	IDF 87:1979	34.37 (7.76) %	34.81(3.53) %
Water activity	Aqualab Pawkit	0.32 (0.03)	0.39 (0.02)
Flowability	Flowdex (Hanson Research)	Good flow, Smallest size disk: 9 mm	Good flow, Smallest size disk: 9 mm
Whey Protein Nitrogen Index	Eurofins internal method	8.80 mg/g ^b	7.99 mg/g ^b
pH	OakTon pH700	6.60 (0.02)	6.57 (0.02)
Heat stability	Yamato Oil Bath	No visible coagulation at 5 minutes (143°C)	No visible coagulation at 5 minutes (143°C)
Coffee stability	GEA A16a	No visible precipitation, or flocculated particles	No visible precipitation, or flocculated particles
Color	Minolta Chroma Meter	L* 92.94 (0.15) a* -3.47 (0.13) B* 13.23 (0.02)	L* 92.65 (0.30) a* -3.16 (0.09) B* 13.20 (0.01)

^aResults obtained from Alfred Laboratory (South Dakota State University), no standard deviations were given from the report.

^bResults obtained from Eurofins DQCI (Mounds View), no standard deviations were given from the report. Results are reported as the mean values (standard deviation).

3.4.3 Quality Observations (Appearance, Flavor, and Odor)

For untreated and IPL-treated NFDM flavor, odor, and physical appearance were determined from month 0 to month 4 (Table 3.4). In order for NFDM to conform with the physical appearance specifications with the U.S. Standard Grade, the physical appearance of the powder may possess a slight unnatural color, it shall be free from lumps, and be reasonably free from visible dark particles. No major changes were observed in the IPL-treated NFDM as compared to the untreated NFDM. IPL-treated NFDM resulted in a physical appearance that contained a uniform white to cream color, it was free from particles, and there were no visible dark particles.

The USDA specifies the flavor of U.S. Standard Grade NFDM shall possess a fairly pleasing flavor with bitter, oxidized, scorched, storage, or utensil flavors to a slight degree, and chalky, cooked, feed, or flat to a definite degree. Our results identified the flavor of the untreated NFDM to contain sweet, clean, pleasing, and desirable dairy flavors for month 0. Months 1 to 4 maintained the pleasing and desirable dairy flavors. In contrast, the IPL-treated powder acquired flavor characteristics of metallic, bitter, oxidized, cooked, and scorched throughout months 0 to 4. Our results also revealed undesirable odor changes to the IPL-treated NFDM. However, these preliminary results were only determined through observations and not with trained sensory panelists.

Visible and UV light wavelengths have been shown to contribute to the development of aroma compounds in milk (Rosenthal, 1992; Scheidegger et al., 2010). Photochemical reactions will cause singlet oxygen to react with lipids, proteins and vitamins compounds resulting in unpleasant off-flavors of the oxidation products (Hansen and Skibsted, 2000; Davies, 2003). These odors can be profiled as fishy, hay-like, and oxidized. Karagul-Yuceer et al. (2001) reported that lipid oxidation of milk will lead to these types of flavors (oxidized, metallic, fishy). Specifically, the aroma-active compound associated with a metallic, green note in NFDM identified in their study was (E)-4,5-Epoxy-(E)-2-decenal (Karagul-Yuceer et al., 2001). Fatty, hay-like, stale odor notes were also detected by lipid oxidation products, which include (E)-2-nonenal, (E,E)-2,4-nonadienal, and (E,Z)-2,6-nonadienal. Another aroma-active compound was

identified by Forss (1979), 1-Octen-3-one, which was described as a metallic, mushroom-like odor, and was identified as a primary odorant of milk products (Schieberle et al., 1993). The changes in flavor and odor from the IPL treatment needs to be studied further to understand the true mechanisms (light source) and compounds that influence these characteristics. Additionally, trained sensory panelists should be used to further evaluate and determine the sensory descriptors. To identify the compounds that directly contribute to the characteristic flavors produced by the IPL, fractionation should be carried out by gas chromatography (Shiratsuchi et al. 1995).

Table 3.4 Quality observations performed on untreated and IPL-treated NFDM

Month	Untreated			IPL-Treated		
	Flavor	Odor	Appearance	Flavor	Odor	Appearance
0	Sweet, clean, pleasing, desirable dairy flavors	Fresh, no off odors	Uniform white to light cream, free from lumps, no visible dark particles	Metallic, cooked, scorched	Fishy, slight oxidized smell	Uniform white to light cream, free from lumps, no visible dark particles
1	Pleasing, desirable dairy flavors	No off odors	Uniform white to light cream, free from lumps, no visible dark particles	Metallic, cooked, scorched, bitter, oxidized	Fishy, slight oxidized smell, hay-like	Uniform white to light cream, free from lumps, no visible dark particles
2	Pleasing, desirable dairy flavors	No off odors	Uniform white to light cream, free from lumps, no visible dark particles	Metallic, cooked, scorched, bitter, oxidized	Fishy, slight oxidized smell, hay-like	Uniform white to light cream, free from lumps, no visible dark particles
3	Pleasing, desirable dairy flavors	No off odors	Uniform white to light cream, free from lumps, no visible dark particles	Metallic, cooked, scorched, bitter, oxidized	Fishy, slight oxidized smell, hay-like	Uniform white to light cream, free from lumps, no visible dark particles
4	Pleasing, desirable dairy flavors	No off odors	Uniform white to light cream, free from lumps, no visible dark particles	Metallic, cooked, scorched, bitter, oxidized	Fishy, slight oxidized smell, hay-like	Uniform white to light cream, free from lumps, no visible dark particles

3.5 Summary

In this study, we confirmed that IPL treatment on NFDM will cause minimal changes to powder composition and functionality. However, the organoleptic properties related to flavor and odor were not desirable. This study provided precursory sensory descriptors but the determination of flavor compounds and descriptive sensory analysis should be considered for future research. This ongoing shelf-life study will continue to evaluate sensory and functional changes to the IPL-treated NFDM to determine necessary improvements on this novel technology for use in the dairy industry.

3.6 Acknowledgements

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