Evaluation of Intense Pulsed Light to Inactivate Thermophilic Spore-Forming Bacteria *Anoxybacillus flavithermus* and *Geobacillus stearothermophilus* in Dairy Powders

A THESIS

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Abstract

The goal of this study was to evaluate the effects of Intense Pulsed Light (IPL) on the inactivation of *Anoxybacillus flavithermus and Geobacillus stearothermophilus* spores found in the dairy powders of nonfat dry milk (NFDM), milk protein concentrate (MPC70), and whey protein concentrate (WPC80). *Anoxybacillus flavithermus* and *Geobacillus stearothermophilus* are two types of thermophilic, spore-forming bacteria found to cause quality issues in the milk powder industry. Bacterial spore heat resistance permits survival in the processing conditions of an industrial environment. Traditional thermal processing of foods leads to undesirable off flavors in the final product and a decrease in consumer acceptance. Intense Pulsed Light (IPL) is a novel technology being evaluated for use in powdered foods and ingredients to deactivate spore forming bacteria. This study evaluates the effect of IPL on spores of *A. flavithermus* and *G. stearothermophilus*.

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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
LGLI	Lawn grown liquid inoculation
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	Titratable 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

Throughout the dairy industry, there is increasing concern regarding the levels of spore-forming bacteria present in milk and dairy powders. Some spore-forming bacteria are foodborne pathogens, while others can cause product spoilage and affect quality. Spore-forming bacteria are ubiquitous in the environment, and enter the milk continuum through direct coming into contact with the farm soil, cow bedding and udders, where it is then shipped to the processing plants (Ortuzar et al. 2018). Thermal processing is one processing technology that can inactivate bacterial spores, but leads to undesirable functional and organoleptic outcomes in the product. Therefore, new technologies for food processing to decrease levels of active spores and maintain flavor and quality of treated foods are warranted. Intense Pulsed Light (IPL) utilizes a Xenon-light bulb, which produces an intense burst of light in broad-spectrum wavelengths from UV to near-infrared at high energy and short durations. IPL shows promise for dairy powdered foods and ingredients for safety and quality by eliminating the use of high temperatures and long exposure time, intense pulsed light reduces levels of molds, yeasts, and bacteria, while potentially keeping the integrity of the product intact (Chen et al. 2018).

With increasing demand global demand for exportation of dry milk powders, spore forming bacteria increasingly pose an ongoing problem in milk powders for the dairy industry and downstream use of powders and ingredients by food companies. Traditional thermal processing of milk powder to deactivate spore forming bacteria leads to undesirable flavor and quality attributes, and

therefore are not acceptable control methods to reduce foodborne pathogens and spoilage organisms in many foods and ingredients (Morr, 1991). Therefore, incorporation of new technologies such as intense pulsed light are promising, and are currently being evaluated for non-thermal processing of foods. Use of IPL to treat powdered foods has been demonstrated to reduce numerous foodborne pathogens present in dry food matrices without heating the food above 60°C, but evaluation of the efficacy of IPL to deactivate bacterial spores has been limited to the mesophilic foodborne pathogen *Bacillus cereus*. Therefore, use of IPL technology to deactivate spore-forming bacteria is currently underway for processing dry powdered foods, to try to ensure no viable foodborne pathogenic bacteria or spoilage organisms remain in the final treated powdered foods.

Spore-forming bacteria in dairy powders are typically limited to *Bacillus*, *Geobacillus*, *Anoxybacillus* and *Clostridum* spp. Studies have shown that bacterial spores enter the dairy continuum starting on the farm through contact with soil, then cow bedding, and finally on the udders of cows leading to spores in liquid milk (Egopal, et al. 2015). During milk powder processing, bacterial spores are resistant to heat, chemical, and radiation treatments. All aerobic, thermophilic, spore-forming bacteria are included in the *Bacillales* order (Remize 2017). As such, *Anoxybacillus flavithermus and Geobacillus stearothermophilus* are problematic in milk processing plants in the United States (Burgess, et al. 2013). Albeit, the presence of *G. stearothermophilus* and *A. flavithermus* in dairy products can be hygiene indicators of the farm or processing plant environments.

All bacterial cells exist as vegetative cells and some can transform into dormant spores (survival, non-active form), in response to nutrient depletion or when in the presence of a stressful extrinsic environment. The anatomy of a spore lends itself the ability to survive extrinsic stressors in harsh environments. Gram-positive bacteria are resistant to penetration of extrinsic stressors due to their thick peptidoglycan layer – a polymer of amino acids and sugars, which easily absorbs foreign materials (Schaalje 2018). All bacterial cells exist as vegetative cells and some can transform into dormant spores (survival, non-active form), in response to nutrient depletion or when in the presence of a stressful extrinsic environment. For microbiology challenge studies involving spores in foods, a high inoculation level is required for milk powders initially in order to ensure a countable and noticeable reduction in viable spores following exposure to IPL treatment.

In the dairy industry, non-fat dry milk powder (NFDM), milk protein concentrate (MPC70), and whey protein concentrate (WPC80) are most problematic for contamination and spoilage from bacterial spores. Therefore, the work described here is focused on the use of IPL to deactivate thermophilic bacterial spore forming bacteria in NFDM, MPC and WPC, while maintaining the quality of the dry milk product by evaluating the impacts of IPL on the quality and functionality of NFDM.

1 Literature Review

1.1 Spore-forming Bacteria

During the bacterial life cycle, nutrient depletion and/or environmental stress can lead some bacteria to a period of dormancy through spore formation. In spore forming bacteria, the master transcriptional regulator gene Spo0A is activated as a response to environmental stress. It binds to DNA and influences the expression of over 500 genes to initiate the process of sporulation (McHugh 2017). There are more than 100 sporulation genes required for spore formation (Meeske 2016). In this process of sporulation, the cell's genomic DNA is condensed, forming a mother cell in which the DNA divides and a forespore is develops and is engulfed by the mother cell. The developing spore is surrounded by a peptidoglycan layer called the cortex, which is important for dehydration of the core, aiding in high temperature resistance (Figure 1.1). A protein coat forms around the spore as maturation progresses, which becomes the cell wall of the bacterium after the spore germination (Figure 1.2). Lytic enzymes destroy the mother cell in a process called cell death and the newly formed spore is released into the environment (Figure 1.1) (Cornell University 2019). Spores are dormant and in this physiological state are highly resistant to physical and chemical stress and cellular damage. Spores can be revived and transform back to the vegetative state when nutrients and conditions are favorable in the external environment. The outgrowth process of spore-forming bacteria is of concern to the food industry since spores may germinate and cells multiply after packaging, leading to a potential decrease of the quality and shelf

life of the product. Spores utilize DNA repair mechanisms as survival strategies to protect and repair genomic DNA damage that may occur from external stressors (Connon 2008). Thermophilic spore-forming bacteria can attribute heat-resistance to large amounts of calcium and dipicolinic acid, which bind together to mineralize and dehydrate the spore core. Higher amounts of calcium dipicolinate equates to a more dehydrated core (Jamroskovic 2016). Therefore, thermophiles have a lower core water content in the spore coat compared to mesophilic spores, conferring thermophilic spores more heat-resistance (Setlow 2005). The type of spore core, mineral ions, and essential stability of spore proteins also play a role in heat resistance. The impregnation of thermophilic spore DNA with alpha/beta-type small, acid-soluble spore proteins (SASP) protects DNA against damage from heat. Thermophiles contain enzymes with unique capabilities to survive at extreme temperatures by modifying the composition of their cytoplasmic membrane to keep their cellular components stable and active. Thermophilic bacteria do this due to adaptation of their amino acid sequence over time. Enzymes from thermophiles contain a higher amount of tightly packed, hydrophobic amino acids and less polar and charged amino acids when compared to mesophilic proteins, making thermophilic proteins highly heat stable (Sarmiento 2015).

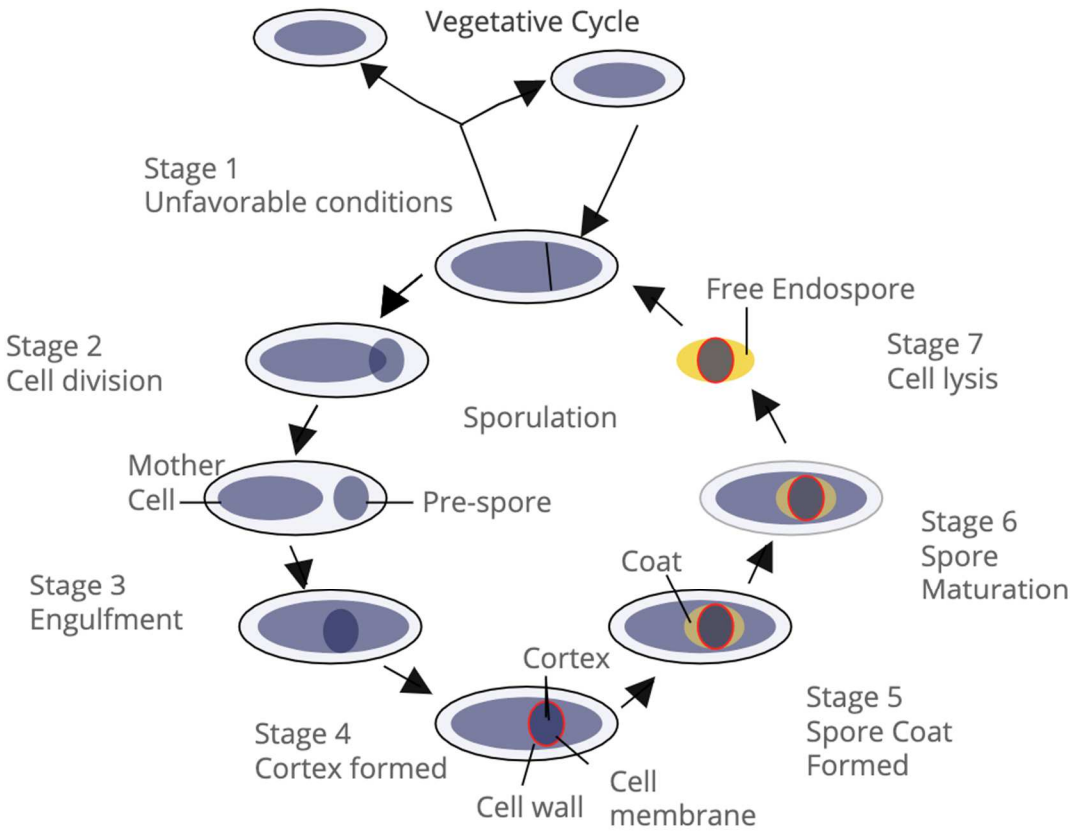

Figure 1.1 The lifecycle of a spore.

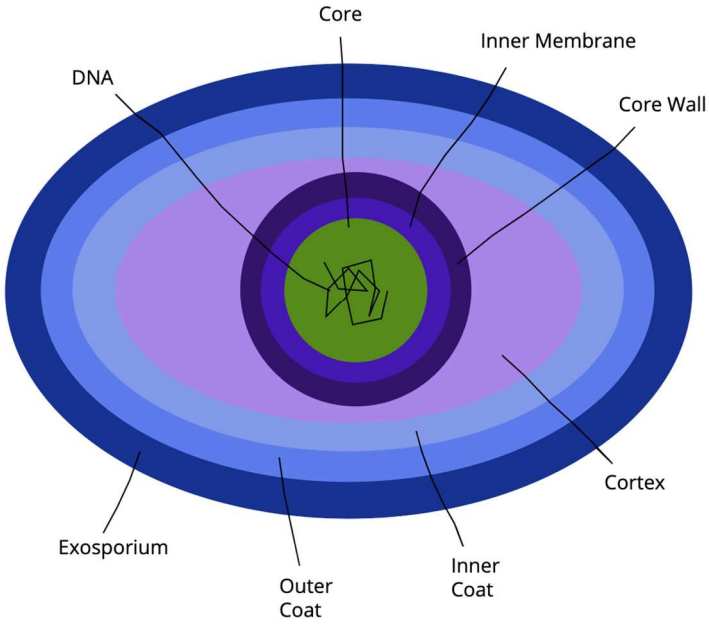

Figure 1.2 Anatomy of a spore.

1.2 Bacterial Spores of importance to the Food Industry

A study quantifying changes in spore-forming bacteria throughout milk production from the farm to packaged pasteurized milk reported that approximately one-third of the fluid milk produced in the United States is lost annually, due to contamination with spore-forming bacteria that survive the pasteurization process (Ortuzar, et al. 2018). Bacteria enter the dairy processing continuum initially at the milking machine on the farm. Various bacteria (psychrophic, mesophilic and thermophilic) are found in raw milk and continue to increase in number during transport, bulk tank, and silo storage. Mesophilic and thermophilic bacteria are found in pasteurized and packaged milk. *A. flavithermus* and *G. stearothermophilus*, are not commonly isolated from raw milk, rather in preheating equipment, evaporators and drying sections of the milk

powder process present favorable conditions for growth of these thermophiles in milk processing environments (Gauvry et al. 2017). It has been shown that bacterial spores increased from 0.58 to 1.72 log_{10}CFU/mL from the milking machine to packaged milk, showing an increase in spores along the process (Ortuzar, et al. 2018). This study identifies control points that could be effective along the milk processing continuum as the levels of spore-forming bacteria remained constant on the farm and transport, but increased during processing. The addition of control steps to reduce initial numbers at the farm, as well as during processing are necessary.

Some of the spore-forming bacteria of concern in the food industry include *Bacillus cereus, Bacillus licheniformis, Bacillus subtilis, Clostridium botulinum, G. stearothermophilus* and *A. flavithermus*. *B. cereus* have been linked to outbreaks in many types of foods, from beef to rice to vegetables and cheese products, and can cause diarrheal, ocular and respiratory tract disease in humans (McDowell, et al. 2019). *B. licheniformis* can cause gastrointestinal foodborne illness and are found in a variety of foods, such as cooked meat products and ice cream (Logan 2011). *B. subtilis* is associated with causing bread to become ropey and sweet on the bakery processing line and can be an opportunistic pathogen of human disease (Thompson 1993). *C. botulinum* are pathogenic, spore-forming bacteria found primarily in anaerobic conditions, such as those found in canned foods and can cause botulism, a severe form of food poisoning, which weakens muscles and respiratory system, and lethality in severe cases (Solomon 2001).

Thermophilic organisms, such as certain bacteria, grow at optimum temperatures between 50°C and 80°C. The optimal growth temperature and growth ranges are below 50°C for most mesophilic spore-forming bacteria, in comparison to those growth temperatures reported for thermophilic spore-forming bacteria, such as *A. flavithermus* and *G. stearothermophilus* (Table 1.1).

Table 1.1 Optimum Growth temperatures and ranges of spore-forming bacteria relevant to food industry

Bacteria	Optimum Growth Temperatures (Ranges) (°C)
Bacillus cereus	28 – 35 (5 – 50)[a]
Bacillus licheniformis	50 (30 – 55)[a]
Bacillus subtilis	25 – 35 (10 – 50)[a]
Geobacillus stearothermophilus	57 (55 – 65)[b]
Anoxybacillus flavithermus	60 (30 – 70)[c]
Clostridium botulinum	35 – 40 (10 – 48)[a]

[a](Yousef, A.E. 2003), [b](Mtimet 2015), [c](Goh, et al. 2014)

For all spore-forming bacteria, the level of heat inactivation varies, and decimal reduction times (D-value) are commonly used to determine the time it takes to reduce levels of an organism by one log (90%) at specified conditions (temperature).

Table 1.2 D-values and z-values of spore-forming bacteria relevant to food industry

	D-values (min)							z-values
Bacteria	85°C	95°C	100°C	110°C	115°C	121°C	143°C	°C
Bacillus cereus	32.1[b]	2[b]	3-200[a]	0.13[g]	0.07[g]	0.02[g]	0[g]	6.6[b] 8.5[b]
Bacillus licheniformis	29.9[c]	12.2[c]	13.5[a] 5.9[c]	0.68[g]	0.38[g]	0.18[g]	0[g]	14.2[2]
Bacillus subtilis	29.5[c]	15.8[c]	7-70[a] 5.7[c]	0.25[g]	0.14[g]	0.05[g]	0[g]	14[c]
Clostridium botulinum	-	-	15-25[a]	1.17[d] 4.4[e]	0.24[d] 1.3[e]	0.19[f]	-	9.9[e]
*Anoxybacillus flavithermus**	-	-	-	2[h]	-	-	0.07[i]	13[h]
*Geobacillus stearotherm-ophilus**	-	-	100-1600[a]	18-20[h]	-	0.12[i] 2.4[j]	-	11.1[j] 11[h]

[a](Yousef, et al. 2003), [b](Byrne 2006), [c](Rodriguez, et al. 1993), [d](Odlaug 1978), [e](Odlaug 1977), [f](Diao 2014), [g](Janštová 2001), [h](Zhao, et al. 2013), [i](Burgess, et al. 2010), [j](Wells-Bennik, et al. 2019)
*Thermophile

The comparison of D-values for spore-forming bacteria in table 1.2 demonstrates the ability of thermophilic spore forming bacteria to survive for much longer times at higher temperatures, based on higher D-values reported for 100°C to 143°C.

The two specific thermophilic spore-forming bacteria evaluated in these thesis projects were *A. flavithermus* and *G.stearothermophilus.* Neither bacteria cause foodborne disease, but can lead to decreased shelf life of foods, since both organisms produce enzymes that break down components of foods, which reduces the value of the food product (Soni, et al. 2016). *A. flavithermus* and *G. stearothermophilus* are both obligate thermophiles that have generation times/growth rates ~ 15 - 20 m at optimal growth temperatures and can form biofilms (Burgess 2010). *A. flavithermus* is found in dairy foods, especially during

the production of powdered products such as infant formula, confectionery, sports supplements and dietary health supplements (McHugh 2017). *A. flavithermus* thrives in dairy processing environments as the temperatures the bacteria encounter in the separation, pasteurization and evaporation processing steps are optimal for growth and subsequent sporulation (Wedel 2018). These processing steps reduce levels of vegetative microbes present in the milk, however thermophilic spore-forming bacteria are able to survive heat treatment due to their ability to form stress-resistant endospores (McHugh 2017).

According to Ziegler (2014), *G. stearothermophilus* are ubiquitous in the environment (Zeigler 2014), and have been isolated from industrial environments including paper mills, canning, juice pasteurization, sugar refining, gelatin production, dehydrated vegetable and dairy manufacturing facilities (Chen, et al. 2006),(Burgess, et al. 2010). *G. stearothermophilus* is most notoriously associated with the 'flat-sour' spoilage of low-acid canned food products, such as peas, corn and asparagus (Lin 1968). 'Flat-souring' results from the presence of *G. stearothermophilus* being transported on raw vegetables from the soil exposure, and then this bacteria encounters warm environments such as holding tanks and blanchers (Ito 1981). Growth of *G. stearothermophilus* does not produce any gas in canned foods, but is capable of lowering the pH of the product and producing off odors and cloudy liquids (Matthews 2017). Similarly, the warm processing environments used in the dairy industry encourage spore formation and although mesophilic spore-formers, such as *B. cereus* and *B. subtilis*, have been found at the highest levels in bulk raw tank milk (Miller 2015),

thermophiles are more prevalent in dairy powders (Watterson 2014). Various studies have steadily confirmed *B. licheniformis, A. flavithermus* and *G. stearothermophilus* as the three primary spore-forming bacteria present in dairy powders (Scott 2007)(Yuan, et al. 2012)(Watterson 2014). *B. licheniformis* is able to grow at both mesophilic and the lower range of thermophilic temperatures (Ivy, et al. 2012). Nazina et al. (2001), performed a taxonomic study, which compared the 16S rDNA sequences and fatty acid compositions of new isolates with established species of thermophilic *Bacillus* spp. The taxonomic analysis supported a proposed new nomenclature for the species within a new genus leading to *B. stearothermophilus* being reclassified as *G. stearothermophilus* (Nazina 2001). In a similar study by Pikuta et al. (2000), *A. pushchinoensis* was isolated from manure samples and taxonomically found to be closely related to *B. flavithermus* (thermophilic anaerobe with neutral pH), hence a new nomenclature was proposed as *A. flavithermus* (Pikuta, et al. 2000). Before 2001, *A. flavithermus* was not recognized as a valid taxonomic species and may have been misidentified as *B. stearothermophilus* (Burgess 2010).

1.3 History, Isolation and Identification of *Anoxybacillus flavithermus*

A. flavithermus has been isolated from geothermal springs, cow manure and milk processing plants and was first discovered inhabiting hot springs in Wairakei, New Zealand and are taxonomically classified based on 16S rDNA in the family of *Bacillaceae* (Saw, et al. 2008). Although *A. flavithermus* have smaller genomes, about 2.85 Mb (Belduz, et al. 2015), which contain genes that provide the ability to grow at high temperatures between 30°C and 70°C, a pH

range of 5.5 to 10.0 and are able to tolerate exposure to UV light (Goh, et al. 2014). *A. flavithermus* are a frequent contaminant in milk powders, and are resistant to milk processing control steps, such as preheating, evaporation and drying, due to the ability to grow at high temperatures, such as environments found in plate heat exchangers used for pasteurization and are a frequent contaminant in milk powders (Scott, et al. 2007),(Tasara, et al. 2017). Due to the high level of hydrophobicity of both vegetative cells and spores, these bacteria adhere to stainless steel used in food processing, and then form biofilms and posing a large problem in milk processing plant equipment (Zhao, et al. 2013). Intrinsic and extrinsic environmental factors such as humidity, water activity, residence time and temperature conditions affect the ability of control technologies to inactivate spores (Buehner, et al. 2014).

A. flavithermus strain WK1, was the first to be isolated, and one of the only complete genome sequenced for *Anoxybacillus* spp., along with *A. flavithermus* 52-1A, *A. flavithermus* TNO-09.006 and *A. flavithermus Kn10* (Tasara 2017),(Caspers 2013),(Matsutani 2013). *A. flavithermus* WK1 was isolated from a water drain near Wairakei geothermal power station in New Zealand and is 99.8% identical to that of the originally isolated strain DSM2641 (Saw, et al. 2008). When the genome is compared to sequenced genomes of other members of the family *Bacillaceae*, the genome of *A. flavithermus* strain WK1 is smaller, about 2.6 Mb, whereas most other members of the *Bacillaceae* family (such as *Geobacillus* spp. and *Bacillus* spp.) are larger than 3.0 Mb. Furthermore, it has been suggested that the strain underwent significant

gene loss over evolutionary time in comparison to *Bacillus* spp. genomes (Goh, et al. 2014). Growth in the lab occurs in sporulation agar and their yellow cells or colony color is a result of an accumulation of carotenoid pigment in the cell membrane (Burgess, et al. 2009). Along with their compact genome, characteristics such as color, silicification and biofilm proteins provide similarities to ancestral bacteria cells that are believed to have been involved in the biogeochemical processes that formed biominerals 4.4 billion years ago on Earth (Saw, et al. 2008).

1.4 General Characteristics of *Anoxybacillus flavithermus*

A. flavithermus are facultative anaerobes, Gram-positive, rod-shaped, cells (0.85 μm long, 2.3 – 7.10 μm wide) (Atanassova, et al. 2008), typically observed as pairs or short chains of cells and are spore-forming bacteria that grow between the temperature range of 30°C and 70°C, and optimally at 60°C. The pH range of growth is 5.5 to 10.0, and therefore are classified as either alkaliphilic or alkalitolerant and the optimal pH for growth is 7.0 to 7.5. (Atanassova, et al. 2008).

A. flavithermus can grow in waters super-saturated with amorphous silica (Saw, et al. 2008). This was observed in a location where geothermal fluids at 95°C from the Wairakei power station flush into a concrete channel and cool to 55°C where subaqueous silica deposits enter the channel, forming precipitates of amorphous silica, where *A. flavithermus* is able to grow. This occurrence increases the surface area of the fluid, slowing the precipitation and changing textural characteristics of silica (Saw, et al. 2008).

Studies of genomes of various strains of *A. flavithermus* show that adaptive genes and genetic exchange are important for their survival (Goh et al. 2014). Regardless of having a small genome (2.6 Mb), *A. flavithermus* has a complete set of enzymes for biosynthesis of all amino acids, nucleotides and cofactors (Saw, et al. 2008). 2,929 proteins make up the proteome for *A. flavithermus* (www.Uniprot.com), and of the protein-coding genes detected, 1,929 are predicted to have probable biological functions, 418 were conserved with only general predicted function and 516 had no predicted function (Saw, et al. 2008). A study by Swarge, et al. determined that spores contain a small amount of proteins sufficient for restoring metabolic activity when finished germinating (Swarge 2019). This study determined ~60% of the total proteins identified in vegetative cells of *B. subtilis* are found in spores.

1.5 Prevalence of *Anoxybacillus flavithermus* in Milk Powders

The ability of *A. flavithermus* to form spores and attach to stainless steel surfaces leading to the development of biofilms provides a challenge for the dairy industry in food processing facilities. Microbes enter raw milk through direct contact with the cow, air, feed, milk-handling equipment on the farm before being transported to the dairy processing facility (Ortuzar, et al. 2018). *A. flavithermus* multiply rapidly once exposed to warm environments found in niches in milk processing facilities such as plate heat exchangers, thus exemplifying the major role that thermal processing plays in the microflora of milk (Cortes 2016). *A. flavithermus* have been found ubiquitously distributed in milk powders regardless of the country of origin (Rueckert 2005).

A. flavithermus grow optimally in anaerobic environments at 65°C, which is higher than the conventional batch pasteurization temperature of 63°C, and spores and vegetative cells of *A. flavithermus* are able to survive this heat treatment (Khanal 2015). High-Temperature, Short-Time (HTST) pasteurization uses stainless steel heat exchange plates where product flows on one side while the heating media flows on the opposite side to raise milk temperatures to >161°F (72°C) for at least 15 seconds, followed by rapid cooling. HTST kills vegetative cells of *A. flavithermus,* but spores survive this thermal process, and may lead to outgrowth during storage, producing components that degrade lactose and proteins, resulting in lactic acid and souring the milk, thus decreasing quality and shelf-life (Reich, et al. 2017). Decimal reduction times (D-value) are commonly used to determine the time it takes to reduce an organism at specified conditions (temperature) by one log (90%). Zhao et al. reported the D-value of *A. flavithermus* spores as 2.0 minutes at 110°C (Zhao, et al. 2013).

1.6 Effect of *Anoxybacillus flavithermus* on Food Quality

A. flavithermus can form biofilms on stainless steel surfaces and survive thermal processing conditions as spores, and a study of *A. flavithermus* grown in a continuous flow laboratory reactor demonstrated that temperature has a significant impact on the formation of spores and biofilms, which can occur rapidly and simultaneously (Burgess, et al. 2009). Therefore, new food processing methods are needed for deactivation of spore-forming bacteria in milk powders. Intense Pulsed Light is a promising, non-thermal technology that has been demonstrated as effective for the deactivation of vegetative cells

(*Cronobacter sakazakaii, Enterococcus faecium* and spores of *B. cereus)* in powdered foods (Chen, et al. 2018).

A recent study demonstrated a reduction of 3.18 $\log_{10}$ CFU/g of *Cronobacter sakazakii* in NFDM, and after 28 seconds of exposure to the IPL apparatus, there was very little change in the amino acid composition, particle physical appear and typical volatile compounds (Chen, et. al. 2018). After further investigation, they found that when combining IPL with a vibratory feeder and passing three to four times through the machine, a larger inactivation of *C. sakazakii* of 5.27 $\log_{10}$ CFU/g was achieved (Chen, et al. 2019). Other non-thermal methods being examined for reduction of bacteria and spores in powdered foods are UV light treatment or cold atmospheric plasma. A recent study determined that cold atmospheric plasma reduced levels of *Cronobacter sakazakii* in NFDM by ~1-3 $\log_{10}$ CFU/g (Chen, et al. 2019). Each of these non-thermal technologies have advantages and disadvantages, since they are not rapid, effective, or feasible throughput at high-scale for use in the dairy industry.

1.7 History, Isolation and Identification of *Geobacillus stearothermophilus*

G. stearothermophilus has been isolated from soil, hot springs, ocean sediment and milk processing plants (Zeigler 2014). Taxonomically based on 16S rRNA, they are the closest related genus to *A. flavithermus* (Zhao, et al. 2013). *Bacillus* spp. and *Geobacillus* spp. represent more than 99% of the total sequenced genomes for *Bacillaceae* (Goh, et al. 2014). In comparison to *A. flavithermus,* the *G. stearothermophilus* which have similar sized genomes (2.63 Mb), as well as temperature growth range from 37 to 70°C (Burgess, et al.

2017). The proteome of *G. stearothermophilus* consists of 2,552 proteins (Uniprot). *G. stearothermophilus* are a frequent contaminant in food industries that manufacture products at elevated temperatures (>40°C), such as canning, juice pasteurization, sugar refining, gelatin production, dehydrated vegetable production and dairy products (Wells-Bennik, et al. 2019). *G. stearothermophilus* are able to survive in foods with low moisture, high temperatures (up to 70°C), forms biofilms on processing equipment, and may remain dormant during storage. Spores of *G. stearothermophilus* are some of the most thermotolerant bacterial spores identified, and these qualities make them unique biological indicators for validation studies and sterilization tests of autoclaves.

In the early 1980's, 'Raptidase', an enzyme used in the food industry since the 1920's to replace the extreme acid hydrolysis process, began to be added to the system during the starch degradation process, where gelatinization happens at high temperatures (110°C), rather than after cooling allowing for a more rapid hydrolysis and lowered production costs (Matthews 2006). This new process was achieved using a more thermostable α-amylase from *B. licheniformis*. Subsequently, an even more stable enzyme from *B. stearothermophilus* was identified. Unfortunately, it is not used commercially as it produces maltodextrins from starch degradation that are undesirable for the glucoamylase treatment (Matthews 2006). Gray, et al. (1986), attempted to attach the NH_2-portion of this *B. stearothermophilus* enzyme with the COOH-portion of *B. licheniformis*, but the resulting hybrid enzyme was less stable than the parent enzymes (Gray 1986). Most commercial amylases used today are

engineered from enzyme sequences from multiple *Bacillus* spp. Through enzyme and protein engineering, stable variants of *Bacillus* spp. have been and will continue to be developed for use in the food industry (Matthews 2006).

G. stearothermophilus, along with most of the *Bacillus* spp., contains unique cellular structures that form in response to nutrient deprivation, increasing their resistance to environmental extremes, such as high-temperatures (Berendsen 2016). It is unknown what factors specifically contribute to these structures, but much evidence supports genes that encode genes carrying operon spoVA. Berendsen, et al. (2016) identified which genes influence higher heat resistance of spores of *B. subtilis*. These results suggest that more copies of spoVA loci in the genomes of spore-forming bacteria tend to provide higher heat resistance of the spore. For example, the sequences of $spoVA^1$ and $spoVA^2$ operons are present in genomes of thermophilic *Geobacillus* spp. and *A. flavithermus*, yet only the $spoVA^1$ operon is found in genomes of mesophilic spore formers such as *B. subtilis* and *B. licheniformis* (Berendsen 2016).

1.8 General Characteristics of *Geobacillus stearothermophilus*

G. stearothermophilus are rod-shaped, Gram-positive, aerobic bacteria that have an optimal growth temperature range of 55-65°C. Strains of *G. stearothermophilus* isolated from dairy foods have developed a niche adaptation of a unique physiological characteristic of utilizing lactose in specific environments, because these dairy-isolates contain a lac operon that is not found in other genomes of *G. stearothermophilus* (Burgess, et al. 2017). This finding suggests that some spore-forming contaminants, such as *G. stearothermophilus,*

may be favored among specific food ingredients and food processing technologies (Postollec, et al. 2012). *G. stearothermophilus* form spores in the heat-treating environment of processing plants, such as heat-exchangers, spray-drying and sterility control steps (Durand, et al. 2015). *G. stearothermophilus* form biofilms and slough off into the final product, where they remain dormant until exposed to elevated temperatures, which activates the cells, leading to germination and outgrowth, causing food spoilage (Eijlander, et al. 2019). *Geobacillus* spp. make up a significant population of bacteria worldwide as they are highly resistant, have heat-induced dormancy and are therefore transported long distances (Wells-Bennik, et al. 2019). A study showed that activation of spores of *G. stearothermophilus* began to occur at 100°C and maximized between 110 and 115°C (Finley, et al. 1962). *G. stearothermophilus* spores remain dormant for long periods of time until conditions become favorable for germination and outgrowth, resulting in large populations (Zeigler 2014).

G. stearothermophilus spores formed at 45°C were four times less heat-resistant than spores formed at 57°C, and growth, sporulation and recovery boundaries occur in similar conditions to those present when they initially formed, such has temperatures between 55 and 65°C and pH between 6.2 and 7.5 (Mtimet, et al. 2015). Kakagianni's research suggests that the risk of *G. stearothermophilus* contamination in food processing environments may be directly related to environmental temperature (Kakagianni 2018). 127 strains of *G. stearothermophilus* were examined to determine diversity and although their growth temperature, pH, NaCl tolerance and sporulation ability differed very little,

heat resistance varied between the *G. stearothermophilus* strains examined (Durand, et al. 2015). In this study, spores from each *G. stearothermophilus* strain were exposed to 120°C for half minute increments from 0 to 8 minutes, and then spread plated and incubated at 55°C for two days, and resulting colonies were enumerated and $\log_{10}$ reduction calculated. The D-value ranged from <0.5 minutes to 8.0 minutes for strains of *G. stearothermophilus* examined. The growth temperature, pH and salt tolerance of the most heat resistant strains were compared and the strains were able to grow at the lowest temperatures (40°C), pH (5) and 3% NaCl, showing resistance of some *G. stearothermophilus* strains to be related to extreme environments. *G. stearothermophilus* grew ideally from 40°C to 70°C. Therefore, the findings from these studies recommend that processing line temperatures be maintained above 70°C, to avoid allowing the most heat resistant strains to sporulate (Durand 2015).

Putri et al. (2017) evaluated the kinetics of *Geobacillus* growth, biofilm development and spore formation in milk processing (Putri 2017). The findings from this study were consistent with the previously reported values for growth temperature, and between the 16 strains evaluated, growth occurred between ~45°C and ~70°C, with optimal growth at ~60°C. One of the objectives of this study was to decrease the bacteria's ability to replicate, sporulate and form biofilms. Therefore, Putri et al. (2017) developed a mock flow system, with commercially-relevant conditions, and inoculated with the vegetative cells of *G. stearothermophilus*, and then monitored growth, spore-formation and biofilm development over time. The findings of this study revealed that around an

optimum growth temperature of ~60°C, viable cell and spore counts began to decrease, but rose after 3 - 6 hours, indicating attachment, germination and proliferation of cell and spore production of *G. stearothermophilus* occurred (Putri 2017). Although variable flow rate of milk did not show significant differences in levels of *G. stearothermophilus*, pulsed flow rate (1 h) versus continuous (~24 – 40 h) did exhibit differences. Pulsed flow rate resulted in a longer time before an increase in spore count was observed (>8 hours), while the continuous process resulted in higher counts after 4 hours (Putri 2017). A study performed by Knight et al. (2004), who evaluated the effects of temperature as the driving force influencing biofilm formation, suggests that fluctuation of temperature throughout the processing system would inhibit biofilm formation. By alternating cycles by between cool (35°C) and warm (72°C), by 5°C intervals over 8 passes through otherwise normal operating conditions (plate heat exchangers), with the idea that the bacteria will not have enough time in optimum growth temperature range to replicate, sporulate and form biofilms (Knight 2004). These parameters of temperature cycling proved to be effective at reducing attachment and outgrowth of thermophilic spore-forming bacteria.

1.9 Prevalence of *Geobacillus stearothermophilus* in Milk Powders

G. stearothermophilus have been isolated from low-acid foods, such as dairy products and are commonly known to cause flat-souring spoilage in milk (Egopal, et al. 2015). The sour flavor typically associated with *G. stearothermophilus* growth in foods occurs from the fermentation of carbohydrates with the production of short-chain fatty acids, with minimal gas

production, hence the 'flat' flavor description (Stevenson, et al. 2013). *G. stearothermophilus* produce enzymes like heat-stable lipases, which degrade food components, thus decreasing quality and leading to rapid spoilage of milk (Chopra, et al. 1984). Spores of *G. stearothermophilus* are exceptionally thermal resistant and food packages considered commercially sterile may still harbor spores of *G. stearothermophilus*. The thermotolerance of *G. stearothermophilus* spores is commercially used to validate moist heat sterilization such as sterilization conditions of autoclaves (Watanabe, et al. 2003). Wells-Bennik et al. reported the D-value of *G. stearothermophilus* spores to be 2.13 minutes at 125°C (Wells-Bennik, et al. 2019), thus its utility to test autoclave conditions at 121°C.

High-Temperature, Short-Time (HTST) pasteurization uses stainless steel heat exchange plates where fluid product flows on one side while the heating medium flows on the opposite side of the heat exchange plates to raise milk temperatures to at least 161°F (72°C) for at least 15 seconds, followed by rapid cooling. HTST kills vegetative cells of *G. stearothermophilus,* but spores survive this thermal process, and subsequently the spore forming bacteria end up in the final product. This may lead to outgrowth during storage, producing components that degrade lactose and proteins, resulting in lactic acid and souring the milk, thus decreasing quality and shelf-life (Reich, et al. 2017). Spores of *G. stearothermophilus* enter the dairy continuum similar to that described previously for *A. flavithermus*, through coming into contact with soil, cow bedding and

udders when it is then transported to processing milk facility and downstream powder processing facilities (Egopal, et al. 2015).

Spores in powdered milk products result from detachment from biofilms formed on the stainless-steel surfaces in the dairy processing plant. Spores of *G. stearothermophilus* have been found in dairy powders at numbers up to 10^6 CFU/g (Zhao, et al. 2013). *G. stearothermophilus* produce enzymes such as heat-stable lipase, which lead to spoilage of the milk product (Machado, et al. 2017). Therefore, non-thermal technologies are needed to deactivate spores of *G. stearothermophilus*, since this bacteria is able to grow and survive at higher temperatures during invasive heat-treatments that can reduce the quality of the milk. Reduction of levels of these spore-forming bacteria in dairy powders leads to safer, better quality foods, without decreasing freshness or nutritional value (Zhang, et al. 2018).

1.10 Effect of *Geobacillus stearothermophilus* on Food Quality

G. stearothermophilus do not cause human disease, but are problematic in the food industry. *G. stearothermophilus* spores can survive heat processing conditions, and lead to germination under favorable growth conditions leading to undesirable quality changes decreasing shelf-life and leading to spoilage of the food product. *G. stearothermophilus* possess a unique heat resistance and cellular repair/recovery mechanisms after heat treatment. The maximum heat resistance was reported with *G. stearothermophilus* spores that were generated at 57°C (D_{115}=12.27 minutes) (Mtimet, et al. 2014), whereas most other microbial cells are unable to survive for prolonged periods of time above 49°C (Angelotti, et

al. 1961). In comparison to other spore-forming bacteria, *G. stearothermophilus* are known for their high heat-resistant spores, which may explain why *G. stearothermophilus* spores are found in much higher concentrations in milk powders than in raw milk, since additional heat treatments are used in the powdered food and ingredient production processes (Eijlander, et al. 2019).

A study investigating the germination and outgrowth efficiency of spores from dairy products determined that *G. stearothermophilus* was one of nine types of spore-forming bacteria that was able to survive 20 minutes at 115°C (Eijlander, et al. 2019). When the dairy products evaluated were reconstituted, a decrease of pH of 5.57 was observed, indicating fermentation by *G. stearothermophilus* likely occurred, leading to spoilage (Eijlander, et al. 2019). The temperature tested was 106°C for a duration of 30 minutes, and it was determined that spores of *G. stearothermophilus* were not deactivated under these challenge conditions, likely leading to *G. stearothermophilus* outgrowth and spoilage in the final product. *G. stearothermophilus* was the only type of microorganism of 38 spore-forming bacteria examined to cause spoilage of recombined UHT-treated milk powders following these temperature and time conditions (Eijlander, et al. 2019). Thus, *G. stearothermophilus* poses a significant problem for thermal processing of foods as traditional thermal processing is not effective in inactivating spores.

1.11 Composition of Milk Powders

Water activity (a_w) is defined as the escaping tendency of the water in a sample, and a_w is the measure of how secure water is within the product (Chirife,

et al. 1996). a_w varies significantly with temperature and is dependent on product components, such as those found in low-moisture foods. There may be an influence of a_w on the ability to eradicate spore-forming bacteria that contaminate milk powders. Foods with small a_w ranges are associated with changes in color, odor, flavor, texture, and stability (Decagon Devices 2006). Some processing technologies that inactivate bacterial spores may change the a_w of the food, therefore affecting functional properties for downstream usage. Implementation of preventative controls to keep low-moisture foods from caking during processing may be necessary. Subsequently, additional processing methods such as IPL treatment may alter the levels of microbiological contaminants in dairy powders. Milk powder composition is different from other non-dairy powdered foods, since dairy powders clump more easily due to the highly hydroscopic nature due to the high levels of lactose present in the powder (Decagon Devices 2006). This suggests that IPL treatment of milk powders with low a_w may lead to less powder clumping and hold promise as an ideal non-thermal control treatment that may be suitable for decreasing microbial spore levels in dairy powders.

Milk protein concentrate (MPC70), whey protein concentrate (WPC80) and nonfat dry milk (NFDM) are the three dairy powders examined in this study because they are some of the most produced and exported dairy powders (Figure 1.3) (US Dairy Export Council). The analytical composition of dairy powders may play a role in how the powder particles react to different control treatment such as IPL, and may lead to variation of efficacy for deactivation of

spores. The composition of the batch of MPC70 received from Grassland Dairy Products, Inc. (Greenwood, Wisconsin) used for research in this dissertation has been determined (Table 1.3). MPC is produced by ultrafiltration of milk, which does not damage the proteins, so it is highly soluble and behaves similarly to milk (Dairy Proteins).

Table 1.3 Composition of NFDM, WPC80 and MPC70

Type	Results (%)		
	WPC80	NFDM	MPC70
Fat	6.44	0.80	2.00
Moisture	6.10	3.20	5.00
Protein	81.07	36.00	68.50 - 72.50
Lactose	4.10	52.00	18.20
Ash	2.90	8.00	10.00

The WPC80 used for our studies is from Bongard's Creameries in Perham, Minnesota and was produced utilizing a proprietary ultrafiltration process, which concentrates native whey protein. WPC80 is produced by the ultrafiltration of whey with protein content ranging from 37% to 90% and the higher the protein composition, the less lactose is present (University of Wisconsin Center for Dairy Research). WPC80 is highly dispersible and soluble in water, and used in beverages and foods such as sport drinks, nutritional supplement, bakery products, dressings and fat replacer, and the composition of WPC80 has been determined (Table 1.3). WPC80 contains 4% moisture without casein, and once ingested, WPC80's proteins are released into the stomach quickly, in comparison to MPC70 which is composed of ~80% casein and 20% why and protein is more slowly released during digestion (Idaho Milk Products).

Furthermore, WPC80 is much less heat-stable than MPC70 as WPC80's proteins denature and unfold more easily with heat (Burrington 2015).

NFDM was produced from the Land O' Lakes facilities in Arden Hills, MN and the composition has been determined (Table 1.3). NFDM is similar in composition to dehydrated skim milk (Figure 1.3). It is composed of 3.2% moisture, 0.8% fat, 36% protein, 52% lactose and 8% ash and significant mineral content (University Wisconsin). NFDM can be heat treated before drying, which denatures whey proteins and alters NFDM's functional properties (High heat treated NFDM is less soluble than low heat powder), and the amount of whey protein denaturation is given by a measurement called the Whey Protein Nitrogen Index (WPNI), which determines whether the NFDM is low, medium or high heat-treated powder.

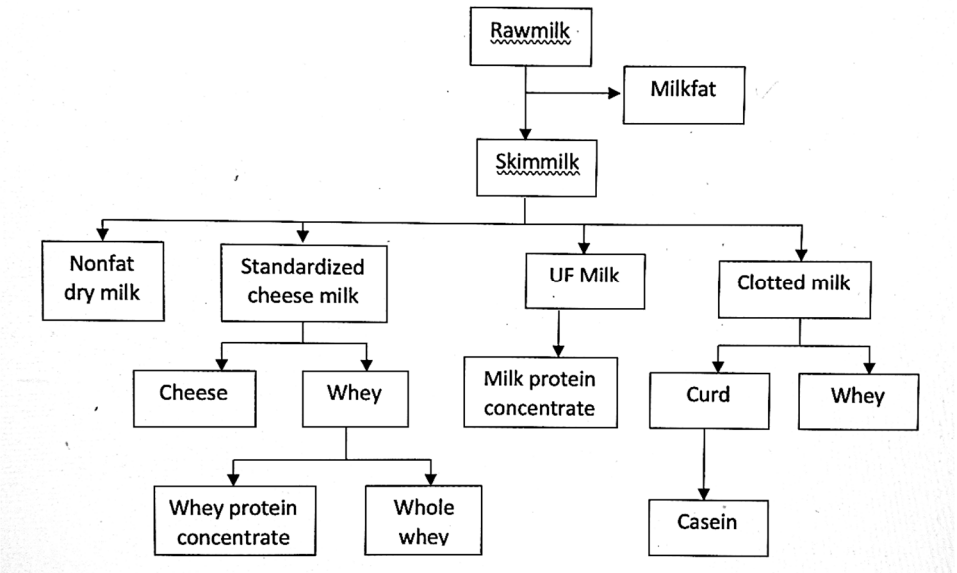

Figure 1.3 Flow diagram of products produced from raw milk

1.12 Functionality of Control and IPL Treated Powder (NFDM)

An important aspect for new non-thermal control methods is that the treatment does not significantly change functional properties of treated powders or foods. Therefore, in this project, we studied the effects of the Intense Pulsed Light apparatus on milk powders overtime by conducting a shelf-life study. For this study, IPL-treated NFDM was chosen for the shelf-life study to determine physical composition and functional changes following IPL exposure. For this analysis, necessary composition tests include determination of levels of protein, lactose, fat, ash, moisture and scorched particle before and after exposure to the IPL apparatus. Functionality testing such as titratable acidity, solubility index, color, flavor, wettability, dispersibility, water activity, flowability, whey protein nitrogen index (WPNI), pH and heat stability are also recommended tests to determine IPL induced changes of NFDM.

To conduct these analyses on IPL-treated NFDM, protein, lactose, fat, ash, moisture, scorched particle, titratable acidity and WPNI were determined for this study and the results are presented and discussed in chapter 3. The International Dairy Federation (IDF) methods used in this study for solubility index, wettability, heat stability and dispersibility were conducted following recommendations from the food processing technology supplier GEA (a brand-name abbreviated from “Gesellschaft für Enststaubungsanlagen,” German for deducing plants). To determine if color changes occur to IPL-treated NFDM, a Minolta chromameter measured the color of the powders before and after exposure to IPL treatment. To assess flavor changes of IPL-treated NFDM, a small group tasting panel was used to taste the products and discuss the flavor

changes that occurred post IPL treatment. Water activity of IPL treated NFDM was measured using a Pa_wkit standard water activity meter from decagon. Flowability of IPL-treated NFDM was examined using an avalanche spectrum from Mercury Scientific and by using a FloDex method. Finally, pH measurements determined if IPL treatment changed the pH of NFDM. Overall, a thorough evaluation of the physical composition and functional properties of NFDM following IPL treatment will provide new insight to reveal if this non-thermal treatment method is suitable for use to reduce levels of spores in dairy powdered foods and ingredients.

1.13 IPL induced damage of bacteria and bacterial spores

Over the past century, food industry researchers have gained increasing understanding of microbial transmission and pathogenicity affecting foodborne disease. Contaminated foods and food-borne diseases continue to pose problems across the food industry and throughout the healthcare system (Anderson 2000). Bacterial spores are resistant to many of the conventional treatments commonly used in the food industry (Liao 2019). Recent and current research of Intense Pulsed Light (IPL) usage on foods continues to provide a broader understanding of the effectiveness, in eradication of pathogenic bacteria or bacterial spores in both processed and unprocessed foods. Approved by the Food and Drug Administration (FDA) in 1996, and heralded as a promising method for inactivating microorganisms in foods without decreasing food quality, IPL is still being vetted for broad use as a non-thermal food disinfection (Barba, et al. 2015).

IPL is generated by accumulation of electromagnetic energy in a capacitor and is released in a broad spectrum of wavelengths. A capacitor, also known as a condenser, is a device with a two-terminal passive electronic component that stores electrical energy in an electric field. Invented by Ewald George von Kleist, a capacitor contains two electrical conductors, often in the form of metallic plates, foil or thin filters separated by a dielectric medium, usually glass, plastic film, ceramic, paper, mica, air and oxide layers. This work was predicated in 1748 by Benjamin Franklin, who connected a series of water-filled glass jars (leyden jars) that stored electric charge, creating the first electric battery. At present, capacitors are widely used in electronic circuits for blocking direct current while allowing alternating current to pass. In this way, they stabilize voltage and power flow (Bird 2010).

IPL is generated from inert gasses housed in a flashlamp. The flashlamp uses electric current to start flash powder burning in order to provide a brief sudden bust of bright light. It was originally used in flash photography in the early 20th century. Such flashlamps ignited an explosive powder that provided the bright light used in early photography. Invented by Joseph Cohen in 1899, it is used as a mine-detonator fuse by the US navy. Flashlamps remain useful in the generation of pulsed light as described here. In its current form, the flashlamp uses inert gasses – most typically xenon (Dunn, et al. 1995) (Gómez-López et al. 2007) (Sanchez-Maldonado, et al. 2018).

The advent of the use of IPL in food related research started with the work of Dunn, et al (1995), who suggested that, through a broad light spectrum, with

short pulse duration, and high peak power and frequency, that the output of a flashlamp used for IPL produced lethal results on bacterial spores (Dunn 1995). Initially used for the decontamination of food and food packaging since the 1990's, Farrell, et al. (2010) confirmed that IPL eradicates bacterial spores through the use of ultrashort duration pulses of intense broadband emission spectrum (190-1100 nm band) (Farrell, et al. 2010).

A recent study aimed at using IPL to inactivate clinically relevant Gram-positive and Gram-negative bacterial pathogens in healthcare settings and food-preparation settings, Farrell et al. (2010) found that all clinically relevant bacteria exposed to IPL were reduced by up to 7 $\log_{10}$ CFU/mL. The bactericidal effects of IPL in this study were due to rich broad-spectrum ultra-violet wavelengths between 200 – 280 nm, high peak power, short duration and ability to regulate the pulse duration and frequency output of the flashlamps (Farrell, et al. 2010). Anderson et al., (1999) focused on the use of IPL for the decontamination of bacteria present on food and food preparation surfaces. IPL treated bacteria were reduced (up to 8 $\log_{10}$ CFU/mL) suggesting that in specific medium, IPL shows some effectiveness for bacterial decontamination. Fungal spores showed greater resistance to IPL by comparison to vegetative bacterial cells (Anderson, et al. 2000).

While vegetative cells are susceptible to inactivation from IPL treatment, the fluence required to reduce bacterial spores by 5 $\log_{10}$ CFU/mL is 18 times higher than the Joules per unit (J/cm^2) used for vegetative cells (Levy, et al. 2012). Researchers have postulated that IPL breaks up the

transcription/translation process of DNA replication through a photochemical transformation of pyrimidine dimers in DNA. Continuous IPL treatment stresses spores, preventing them from utilizing DNA repair mechanisms from mutations caused from light damage (McDonald, et al. 2000).

Bacterial spore-formers are present in many foods. They are a common cause of food spoilage, causing economic loss across the industry. They fall into two taxonomic groups – *Bacillales* order and the *Clostridium* genus. *Clostridium* spp. are anaerobic bacteria and some species are capable of causing human disease through food poisoning and neurotoxicity (Egopal, et al. 2015). Spore resistance to extrinsic stressors is conferred through the internal membrane, cortex external membrane, coat and exosporium that each have specific structural, biochemical and permeability properties that contribute to their heat resistance (Remize 2017). Due to their thick peptidoglycan layer, Gram-positive bacteria are more resistant to penetration of IPL and UV than Gram-negative bacteria, as the outer LPS membrane is susceptible to IPL induced damage (Williams, et al. 2007), (Schottroff, et al. 2018).

The central focus of the research described in the subsequent research chapters of this thesis is on evaluating IPL deactivation of spore-forming bacteria in dry milk powders as dry milk, milk proteins and whey powder exhibit a high levels of spore formers (Burgess 2010), (Watterson 2014). More specifically, thermophiles are a group of heat-loving microbes that survive high temperature (40-120°C) decontamination techniques. While high temperatures generally destroy cells through denaturing vital proteins and damaging DNA, some

microorganisms have innate protective mechanisms against heat (Johnson 2014). DNA structural changes and protein cross-linking that protect them from the destructive effects of high heat allow thermophilic bacteria to have much higher heat resistance than mesophiles (Doyle 2013). Through a process of storing electricity and releasing it in a short, pulsed technique emitting high peak power, IPL continues to demonstrate some usefulness in effective decontamination in research settings.

Chapter 2

Evaluation of Intense Pulsed Light technology on the inactivation of thermophilic *Geobacillus stearothermophilus* and *Anoxybacillus flavithermus* spores in dairy powders

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2.1 Abstract

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. Intense Pulsed Light (IPL) is one technology being evaluated as an alternative non-thermal process to kill spore-forming bacteria. This study investigated decontamination of thermophilic *Geobacillus stearothermophilus* and *Anoxybacillus flavithermus* spores in dairy powders. The maximum inactivation of spores achieved on filter paper, without food powders, was 0.81 CFU/g for *A. flavithermus* and 0.93 CFU/g for *G. stearothermophilus*. Dairy powder samples were inoculated separately with each bacterial spore type at a_w values of (0.3 or 0.4) and treated with IPL under different residence times between 0-120 seconds using a fluence of 1.27 J/cm^2/pulse . Of the three food matrices exposed to IPL treatment, maximum reductions for *A. flavithermus* on NFDM, MPC 70 and WPC 80 were 0.50, 0.84, and 0.33 CFU/g, respectively. The maximum reduction achieved for *G. stearothermophilus* on NFDM, MPC 70 and WPC 80 were 0.47, 0.48, and 0.48 CFU/g, respectively. This is the first report to evaluate use of a continuous IPL apparatus to decontaminate thermophilic spores in powdered dairy matrices, and although some level of inactivation of *A. flavithermus* and *G. stearothermophilus* spores by IPL treatment, further work is necessary on the food safety parameters and efficiency of the continuous IPL apparatus design to allow for commercial success.

Key words:

Anoxybacillus flavithermus spores, *Geobacillus stearothermophilus* spores, intense pulsed light, dairy powders

2.2 Introduction

Approximately one-third of the fluid milk produced in the United States is lost annually due to contamination with spore-forming bacteria that survive the pasteurization process. These bacterial spores are able to withstand pasteurization and continue through the milk supply chain to packaged powders causing spoilage (Ortuzar, et al. 2018). According to the US Dairy Export Council, customers have low tolerances for acceptable limits of thermophilic and mesophilic spores present in dairy powders (<500 to <1000 spores/g) (Watterson, et al. 2014). Importing countries have set trade standards of low allowable levels of spores per gram, which vary per country. These stringent specifications present an important challenge to the dairy industry worldwide as studies indicate a high prevalence of thermophilic spore-formers on preheaters and evaporators in dairy processing powder environments, making them the primary organisms of concern for the trade market to meet customer requirements for spore-former counts (Watterson, et al. 2014). The global dairy powder market was valued at 27.8 billion USD in 2017 and is projected to continue growing at a compound annual growth rate (CAGR) of 4.4% from 2018 to 2025 to reach 38.0 billion USD by 2025 (Bhandalkar and Das 2019). The

increased usage and nutritional advantages of milk powders in food products such as infant formula drive the global dairy powder market. The prolonged shelf-life and reduced transportation cost of dairy powders, in contrast to liquid dairy products, also aid in the growth of the market. With the rise in international consumption of dairy powders, it is particularly important to meet growing market demands without compromising quality and food safety. The objective of this study was to evaluate the use of an Intense Pulsed Light (IPL) apparatus to determine its ability to reduce microbial spores in dairy powders. A major advantage of IPL over alternative non-thermal technologies such as UV treatment is that IPL is delivered over a very short period of time, resulting in lower costs and waste from off flavors produced (Chaine et al., 2012). This study evaluated the effect of IPL treatments on deactivation of thermophilic, spore-forming bacteria in three dairy powders: nonfat dry milk (NFDM), milk protein concentrate (MPC70) and whey protein concentrate (WPC80). The two specific spore-forming bacteria evaluated in the project were *A. flavithermus* and *G. stearothermophilus.* Various studies have consistently confirmed *Bacillus licheniformis, A. flavithermus* and *G. stearothermophilus* as the three primary spore-forming bacteria present in dairy powders (Scott 2007), (Yuan, et al. 2012), (Watterson 2014). IPL treatment showed promise in reducing the levels of thermophilic spores in each of the three dairy powders tested.

2.2.1 Increasing Demands for Process Improvements of Dairy Powders

Globally, there is increasing concern regarding the acceptable levels of spore-forming bacteria present in milk and dairy powders (Ortuzar et al. 2018).

Some spore-forming bacteria, such as *B. cereus* and *Clostridium botulinum* are foodborne pathogens, while other microbial spores cause product spoilage, reduce shelf life, and affect quality. While there is increasing evidence that thermal processing, such as HTST and UHT pasteurization, can inactivate bacterial spores, it could potentially lead to undesirable outcomes in the product (Reich et al. 2017). Continuous HTST pasteurization is a commonly accepted method for heat treatment of milk in the United States, yet some countries prefer ultra-high temperature (UHT) processing of raw milk. UHT parameters are optimal for deactivation of all vegetative and most spore-forming bacteria, with the exception of heat resistant spore-forming *Bacillus* spp. and *Geobacillus* spp. The conditions for UHT include heating the liquid milk between 138-145°C for 1-10 seconds (Deeth and Lewis 2017). 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-$\log_{10}$ (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). Numerous studies report a range of D-values for spores of *A. flavithermus* and *G. stearothermophilus* in milk products.

Table 2.1 D-values and z-values of spore-forming bacteria relevant to food industry

	D-values (min)							z-values
Bacteria	85°C	95°C	100°C	110°C	115°C	121°C	143°C	°C
Bacillus cereus	32.1[b]	2[b]	3-200[a]	0.13[g]	0.07[g]	0.02[g]	0[g]	6.6[b] 8.5[b]
Bacillus licheniformis	29.9[c]	12.2[c]	13.5[a] 5.9[c]	0.68[g]	0.38[g]	0.18[g]	0[g]	14.2[2]

Bacillus subtilis	29.5[c]	15.8[c]	7-70[a] 5.7[c]	0.25[g]	0.14[g]	0.05[g]	0[g]	14[c]
Clostridium botulinum	-	-	15-25[a]	1.17[d] 4.4[e]	0.24[d] 1.3[e]	0.19[f]	-	9.9[e]
Anoxybacillus flavithermus	-	-	-	2[h]	-	-	0.07[i]	13[h]
Geobacillus stearotherm-ophilus	-	-	100-1600[a]	18-20[h]	-	0.12[i] 2.4[j]	-	11.1[j] 11[h]

[a](Yousef, et al. 2003), [b](Byrne 2006), [c](Rodriguez, et al. 1993), [d](Odlaug 1978), [e](Odlaug 1977), [f](Diao 2014), [g](Janštová 2001), [h](Zhao, et al. 2013), [i](Burgess, et al. 2010), [j](Wells-Bennik, et al. 2019)

IPL technology utilizes a Xenon-light bulb, which produces an intense burst of light in broad-spectrum wavelengths from UV to near-infrared at high energy and short durations (190 – 1100 nm). IPL is a promising technology for dairy powdered foods and ingredients for safety and quality as it does not utilize high temperatures (<60°C) and has been demonstrated to reduce levels of molds, yeasts, and bacteria in NFDM, while causing minimal color or particle size change of powders from IPL treatment (Chen, et al. 2018). A similar IPL apparatus is used in this study to evaluate the efficacy of deactivation of thermophilic spore-forming thermophilic bacteria in dry powdered dairy foods.

2.2.2 Specific Spore-forming Bacteria Evaluated in this Study

A. flavithermus and *G. stearothermophilus* are a frequent contaminant in milk powders due to resistance to milk processing control steps, such as preheating, evaporation and drying, due to the ability of these microorganisms to grow and sporulate at high temperatures, such as those found in plate heat exchangers used for pasteurization (Scott 2007), (Tasara 2017). Due to the high level of hydrophobicity of both vegetative cells and spores, these bacteria adhere

to stainless steel used in food processing, form biofilms and are problematic for sterilization of milk processing plant equipment (Zhao, et al. 2013). Intrinsic and extrinsic environmental factors such as humidity, water activity, residence time and temperature conditions affect the ability of control technologies to inactivate bacterial spores (Buehner 2014). The US Dairy Export Council has set strict tolerance levels for spores in dairy powders between <500 CFU/g to <1000 CFU/g for mesophilic and thermophilic bacterial spores (Watterson et al., 2014). If bacterial spores survive pasteurization, they often form biofilms downstream in pipes, dead ends, cracks, corners, crevices, valves, gaskets, and joints in stainless steel equipment in processing facilities and outgrowth to vegetative cells may occur (Egopal et al., 2015). The formation of bacterial endospores by these bacteria of concern presents a challenge to deactivate dormant spores in dairy products after pasteurization.

2.2.3 Characteristics of Thermophilic Spore-forming Bacteria

G. stearothermophilus are rod-shaped, Gram-positive, aerobic bacteria that have an optimal growth temperature range of 55-60°C. *G. stearothermophilus* can catabolize lactose and are also commonly known to cause flat souring spoilage of low-acid foods. The sour flavor is produced from fermentation of carbohydrates resulting in production of short-chain fatty acids, with minimal gas production, hence the 'flat' description (Stevenson 2013). Since spores of *G. stearothermophilus* have exceptionally high thermal resistance, packages considered commercially sterile may still harbor dormant cells of this microorganism. Thermal deactivation conditions examined previously of *A.*

flavithermus and *G. stearothermophilus* vary (Table 2.1). Additional factors, such as sporulation conditions and strain-to-strain variability, impact spore heat resistance (Wells-Bennik 2019). Moreover, as *G. stearothermophilus* has been isolated nearly everywhere people have looked for a century (1920) (Zeigler 2014), its biofilm-forming ability leads to spores with very high heat resistance, these attributes make it a good model organism to which a comparison of more recently researched thermophiles is used as a research tool (Zhao, et al. 2013). Prior to 2001, *A. flavithermus* was not recognized as a valid taxonomic species and may have been mistaken for *B. stearothermophilus* (Burgess 2010).

2.2.4 Commercial Relevance of IPL Technologies on Bacterial Spores

Intense pulsed light (IPL) methodology uses a broad wavelength output of light (100 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, which generates pulses of light. The light pulses encompass a wide 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 surface at sea level (Dunn et al., 1995).

Typically, the fluence (Joules/cm^2) required to inactivate microbial spores by 5 $\log_{10}$ CFU/mL is 18-fold higher than used to inactivate vegetative cells (Levy et al., 2012). The bactericidal effects of 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. More specifically, the broad wavelength output of light generates sufficient energy to cause a photochemical transformation of pyrimidine dimers in the DNA (McDonald 2000).

In addition to bactericidal capabilities, the importance of evaluating a new nonthermal control technology is the impact of sensory, nutritional, and functional properties of a processed 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 results from moisture absorption and elevated temperatures if extrinsic parameters are not controlled during 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 closely monitored and/or controlled.

One limitation of IPL is the 1-2 mm penetration depth of light onto food matrixes, which may not expose microorganisms in crevices, clumps, or irregularities creating a shadowing effect on food surfaces (Elmnasser et al. 2007). However, pulsed-light treatments are suitable for use on outermost food surfaces (Wallen et al. 2001). Gomez-Lopez et al. (2005) reported that undesirable product quality effects may occur in foods exposed to IPL treatment due to increasing temperatures that may occur with longer treatment times. Currently, use of IPL technologies is expanding for microbial decontamination of an array of foods and packaging materials, however, the use of IPL on dairy powder foods and ingredients has not been extensively studied to date. This

study examines the application of IPL to reduce levels of thermophilic bacterial spores on three types of dairy powders.

2.3 Materials and Methods

2.3.1 Experimental Design of this Study

Each powder type (NFDM, WPC80 and MPC70) was inoculated separately with thermophilic bacterial spores, *Geobacillus stearothermophilus* (ATCC12980) was obtained from the American Type Culture Collection and *Anoxybacillus flavithermus* (WB1294) used in this study, was isolated from milk powders and obtained from Cornell University (Dr. Nicole Martin). Environment, humidity and temperature were controlled inside the IPL apparatus to limit the caking of milk powders. Oxidation was also controlled through the circulation of nitrogen.

2.3.2 Dairy Powders Used in this Study

Nonfat dry milk (NFDM), milk protein concentrate (MPC70), and whey protein concentrate (WPC80) were supplied from Land O'Lakes. NFDM was manufactured by Land O'Lakes (Arden Hills, MN), MPC70 was manufactured by Grassland Dairy Products, Inc. (Greenwood, WI), and WPC80 was manufactured by Bongards' Creameries (Perham, MN). The compositional analysis of each powder was provided by the manufacturers (Table 2.2).

Table 2.2 Composition of NFDM, MPC70 and WPC80.

Type	Results (%)		
	NFDM	MPC70	WPC80
Fat	0.80	2.00	6.44
Moisture	3.20	5.00	6.10
Protein	36.00	68.50 - 72.50	81.07

Lactose	52.00	18.20	4.10
Ash	8.00	10.00	2.90

2.3.3 Bacterial Spore Inoculum Preparation and Inoculation

The lawn-grown liquid inoculation (LGLI) spore preparation method used in this study was adapted from Daelman et al. (2019) and Lee, et al. (2011). For spore preparation of *G. stearothermophilus* and *A. flavithermus* an aliquot (1 mL per plate) of overnight grown cultures was spread plated on large Tryptic Soy agar (TSA) Petri plates (150 mm x 150 mm), since prior studies have determined that TSA is the most suitable for spore generation and enumeration of these cultures (Zhao et al. 2013). Cultures plates were incubated for 48 hours at 55°C and further incubated at 37°C for seven days to allow for sporulation. After seven days, cell sporulation was verified under the microscope using the Schaeffer-Fulton staining method (Oktari, A. et al. 2017). 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 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 1978). 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 examined under a light microscope after the samples were heated at 95°C for 20 minutes to eliminate vegetative cells (Hamouda 2002), (Burgess 2009), (Rueckert 2005). Each vial of spore suspension contained approximately 7 $\log_{10}$ CFU/mL. This baseline was determined by initially taking 1 mL of the inoculum to perform serial dilutions to obtain the concentration (Lee et al., 2011).

2.3.4 Bacterial Spore Inoculation Procedure on Filter Paper

Bacterial spore inoculum of approximately 7 $\log_{10}$ CFU/mL of either *G. stearothermophilus* or *A. flavithermus* were independently dispersed onto filter discs. The filter discs had a 0.2 µm pore size, Nylon 66, and 47 mm diameter (Agilent) and were air-dried using a Buchner funnel (Sigma Aldrich) attached to a flask, and connected to a vacuum pump. One mL of the bacterial spore inoculum (7 $\log_{10}$ CFU/mL) was carefully dispersed onto the filter inside the funnel and the spores were subsequently collected onto the filter paper and the filtrate was discarded. Triplicate samples of individual *G. stearothermophilus* and *A. flavithermus* 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 all three dairy powders was determined using a water activity meter (Aqualab Pa$_w$kit, Decagon Devices, Inc., Pullman, WA). The inoculation procedure used for dairy powders followed methods previously

reported by Wiertzema et al. (2019). In brief, under a biological safety cabinet, 100 grams of each dairy powder sample (NFDM, MPC, WPC) 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 (*A. flavithermus or G. stearothermophilus*) 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 ± 0.05) at 37°C for 24 hours to dehydrate the inoculated power for the subsequent blending step. After 24 hours, each sample was blended in a Waring commercial spice grinder (Grainger Lake Forest, IL) to homogenize and evenly distribute the dry spore inoculum throughout the powder sample. After blending, samples were immediately treated with the IPL apparatus.

The spore preparation procedure for each strain in this study was inoculated in nutrient broth for 24 to 48 hours at 55°C as described in section 2.3.3 (Sella 2014). Inoculated dairy powder samples were divided into eight samples, to be treated with intense pulsed light with residence times of 0, 30, 60, 120 seconds in duplicate. The log reduction of each sample of bacterial spores was determined after IPL treatment. NFDM, WPC80 and MPC70 were separately inoculated with the thermophilic bacterial spores, which were exposed to IPL at 30, 60 and 120 seconds, respectively. Log reduction of spores in each

sample was determined in duplicate and compared to a control sample for each condition after a heat-shock, dilution, plating and incubation for 18 h at 55°C.

2.3.6 Water Activity Equilibrium of Dairy Powders

Dairy powders were spread in a thin layer on sterile petri dishes and were placed into Nalgene vacuum desiccators where the powders adjusted to the target a_w value, which was maintained by absorption with a saturated salt solution at 37ºC (250 mL Potassium Fluoride, Acros Organics). The target equilibrated a_w level was 0.25-0.30. Water activity was determined using a handheld meter (Aqualab Pa$_w$kit, Decagon Devices, Inc., Pullman, WA) with ± 0.02 precision. The a_w of uninoculated powders was found to range from 0.32 to 0.41. This variable was dependent on the composition of the powder and the season as a_w increases with temperature due to changes in the property of the water (Chirife et al., 1996).

2.3.7 IPL Instrument and Parameters

The IPL apparatus and parameters were similar to those previously reported, with minor modifications for internal environmental control (Chen et al. 2018). All samples were inoculated with approximately 7 $\log_{10}$ CFU/mL of either *A. flavithermus or G. stearothermophilus* spores prior to IPL treatment. 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 components: a Model-66C 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), a Model-105 volumetric feeder (Tecnetics Industries, Inc., St. Paul, MN), an ultrasonic humidifier/dehumidifier, a nitrogen tank, infrared heater, and an X-1100 power/control module.

The X-1100 steripulse- XL system generates polychromatic radiation in the wavelength range of 190-1100nm. Flow fans are connected to the lamp housing to generate cool air and prevent the lamp from 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 μs), voltage (1000-3000 V), and energy up to 2433 J/pulse. For the IPL apparatus, the feed rate of the samples were 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 m^3/h of cooling air, and subsurface cooling air at 40.8 m^3/h. A nitrogen tank (flow rate 6 L/min) was used to purge the IPL system 5 minutes prior to sample runs. High intensity pulses at a rate of 1/s with a width of 360 μs were generated and applied to each dairy powder sample. Each pulse delivered 1.27 J/cm^2 at an input of 3000 voltage, and a distance of eight centimeters from the quartz window. Dairy powder samples (NFDM, 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 a layer of approximately 1-2 mm of each dairy powder on the feeder bed. Powders were preheated at a temperature of 56ºC prior to IPL processing. The inoculated powders were added to an environment controlled volumetric feeder with paddles where they maintained their heat of 56 ºC and the IPL lamp, vibratory feeder and auger were turned on in that order. 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 determined before and after each IPL treatment.

Table 2.3 IPL parameters used for NFDM, WPC and MPC (Chen, et al. 2019).

Parameter	Value
Vibration	30%
Voltage	3000 volts
Energy	700 J
Period (ms)	1000 Hz
Run mode	Continuous

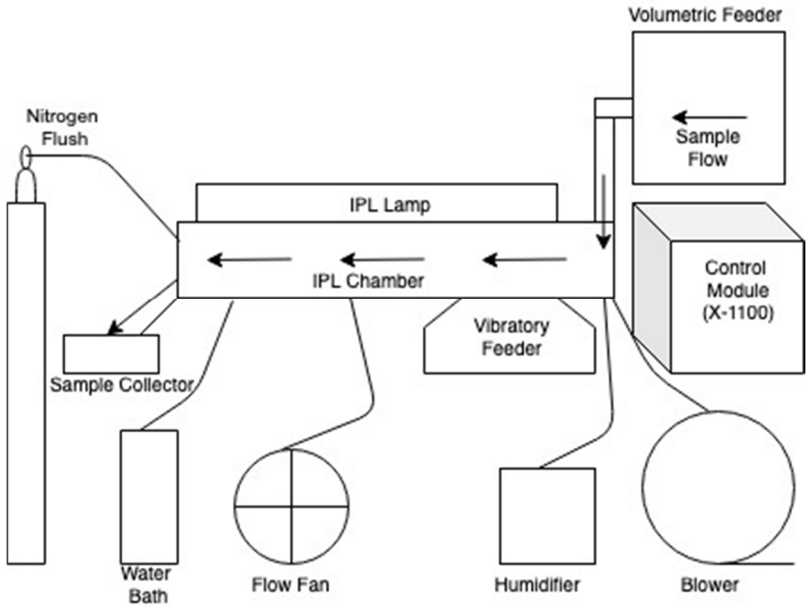

Figure 2.1 A Schematic Diagram of IPL Apparatus used in this study.

2.3.8. Determining Background Bacteria in Untreated Milk Powders

Prior to inoculation studies, all three dairy powders used in this study were analyzed for verification of background levels of any yeasts, molds, coliforms and spore-forming bacteria (Eurofins Microbiology Laboratories Inc. in Mounds View, MN). The purpose of this test was to confirm a very small population of initial bacterial, which should not impact the bacterial spore count for IPL experiments of inoculated powders. The certificate of analysis provided by Eurofins listed the bacterial background population of each dairy powder (Table 2.4).

2.3.9 Enumeration Procedure

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, 11g of each of the powdered samples were diluted in 99 ml of peptone water, then homogenized using a Brinkmann Seward Stomacher 400 Circulator for 30 s. To

eliminate vegetative cells, the first dilution of IPL-treated dairy powder samples were heated in the water bath at 95°C for 20 m as previously described (Hamouda 2002)(Burgess 2009)(Rueckert 2005). This heat treatment procedure has been shown to activate spores and enhances initial germination, thereby creating a more uniform degree of spore germination and subsequently killing any remaining vegetative cells in microbial challenge studies (Pandey 2013). The homogenized samples were serially diluted to 10^{-7} and surface plated onto Tryptic Soy Agar (Acumedia, Neogen) media plates with dilutions levels ranging from 10^{-4} to 10^{-8}. 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).

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

2.3.10 Statistical Analysis

For each IPL experiment, two trials were conducted in duplicate at residence times of 0, 30, 60 and 120 seconds, and the values were 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. Analysis of variance (ANOVA) was performed to determine significant differences among treatments and powders, ($p<0.05$), and correlation analysis was conducted to verify the correlation between the different independent variables. Student's t-tests were performed to compare treated versus untreated samples.

2.4 Results and Discussion

2.4.1 Microflora in Untreated Dairy Powders

The bacterial background population of each dairy powder was determined (Table 2.4). The levels of spore-forming thermophilic microorganisms present in uninoculated dairy powders was <10, 180, and <10 CFU/g for NFDM, MPC 70, and WPC 80, respectively. These results confirm that the initial bacterial population was very low and would not impact the bacterial spore count for IPL experiments of spore-inoculated powders.

Table 2.4 Background contaminants test results

Powder	Test	Method	Results (CFU/g)
NFDM	Yeast	FDA BAM Chapter 18	<10
	Mold	FDA BAM Chapter 18	<10
	Total Coliforms	AOAC 991.14	<10
	E. coli	AOAC 991.14	<10
	Aerobic Spore-forming Mesophilic Count	SMEDP 8.090	~100
	Aerobic Spore-forming Thermophilic Count	CMMEF Chapter 26.5	<10
	Standard Plate Count	FDA BAM Chapter 3	~130
	Yeast	FDA BAM Chapter 18	<10
	Mold	FDA BAM Chapter 18	<10
	Total Coliforms	AOAC 991.14	<10
	E. coli	AOAC 991.14	<10

MPC70	Aerobic Spore-forming Mesophilic Count	SMEDP 8.090	~120
	Aerobic Spore-forming Thermophilic Count	CMMEF Chapter 26.5	~180
	Standard Plate Count	FDA BAM Chapter 3	~80
WPC80	Yeast	FDA BAM Chapter 18	<10
	Mold	FDA BAM Chapter 18	<10
	Total Coliforms	AOAC 991.14	<10
	E. coli	AOAC 991.14	<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

2.4.2 IPL Deactivation of Spores on Filter Papers

The objective of this study was to evaluate IPL technology on the inactivation of *A. flavithermus* and *G. stearothermophilus* spores in dairy powders. Preliminary control testing was conducted with *A. flavithermus* and *G. stearothermophilus* spores on filter paper to ensure that the IPL treatment without any powdered foods present, would reduce viable bacterial spores. The maximum reduction from IPL treatment on filter paper varied at each timepoint, and maximum reduction of 0.81 (±0.02) $\log_{10}$CFU/g was achieved at 30 seconds for *A. flavithermus* and a reduction of 0.93 (±0.05) $\log_{10}$ CFU/g at 60 seconds for *G. stearothermophilus* (Figure 2.2). Levy et al. (2012), reported use of IPL (1.27 J/cm^2/pulse) for 3 pulses at 1 pulse/s achieved a 5 $\log_{10}$ CFU/mL reduction on spores of *B. subtilis* strain 168 (DSM 402) on LB agar plates. *G. stearothermophilus* CIP 66.23 T was also reported to have achieve a 5 $\log_{10}$ CFU/mL reduction on LB agar plates in this study. Planchon et al. (2011) achieved a 5 $\log_{10}$CFU/mL reduction with IPL treatment (fluence of 1.8 J/cm^2) 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 $\log_{10}$ CFU/cm^2 with IPL treatment at a fluence of 2.1 J/cm^2 on the surface of TSA plates. The inactivation results for thermophilic spores from this study were lower than previous studies with *B. cereus*, however, these differences may indicate that thermophilic spores are less susceptible than mesophilic spores to IPL treatment, or that bacterial spores inoculated onto dry filter paper, rather than agar plates, may be less susceptible to IPL treatment. Levy et al. (2012) reported that microbial reductions from IPL treatment are dependent on the type of surface used and that resistance to pulsed light between *B. cereus* strain KBAB4 and *G. stearothermophilus* was similar (Fluence of ~1 J/cm^2 to achieve a 3-log reduction). While resistance to heat was different between strains with 5.5 minutes required to achieve a 3 log reduction for *B. cereus* KBAB4 and >60 minutes for a similar reduction of *G. stearothermophilus* (Levy et al. 2012). An additional study performed by Chaine et al. (2012), of IPL usage on sugar syrups (5.5ml and 20ml volumes, corresponding to 3 mm and 10 mm thickness, respectively), which were poured into 5 cm diameter petri dishes and exposed to an IPL system using a xenon flash lamp. The sugar syrups were artificially contaminated with spores of *B. subtilis, G. stearothermophilus or Alicyclobacillus acidoterrestris.* At least a 3-log reduction of *B. subtilis, G. stearothermophilus* or *A. acidoterrestris* was reported after IPL treatment at 1.5 J/cm^2. (Chaine et al. 2012).

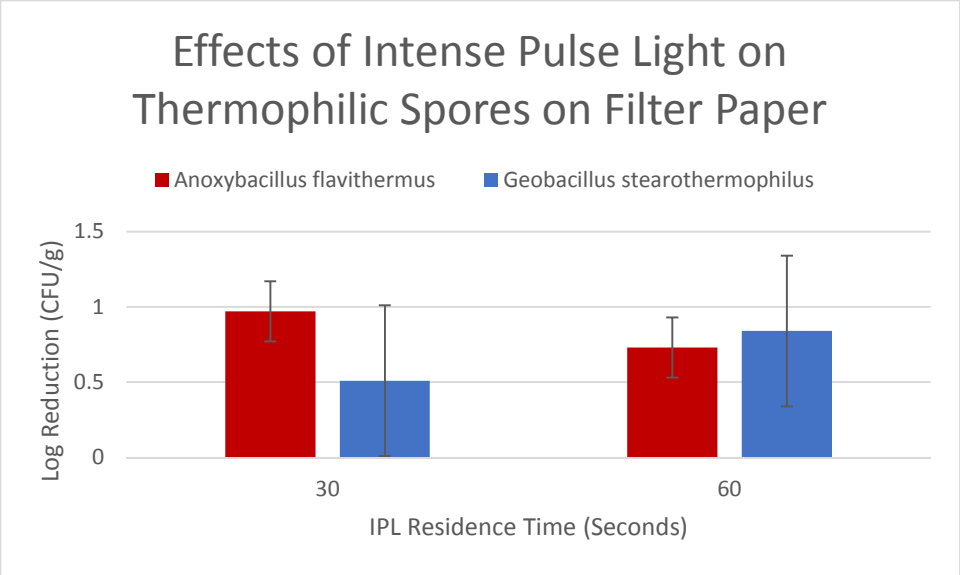

Figure 2.2 Average log reduction of *G. stearothermophilus* and *A. flavithermus* spores on Filter Paper treated with IPL. (n=4)

2.4.3 IPL Treatment of Spore Inoculated Dairy Powders

The effects of IPL to inactivate *G. stearothermophilus* and *A. flavithermus* 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/cm^2/pulse. The log reduction of *G. stearothermophilus* and *A. flavithermus* spores after IPL treatment at different residence times was determined (Table 2.5). Our results confirmed that the effect of IPL on inactivation is not dependent on the microbial spore type, since insignificant ($P > 0.05$) differences were observed between *G. stearothermophilus* and *A. flavithermus*. The inactivation of *A. flavithermus* spores in NFDM and MPC70 was more effective than IPL treatment on *G. stearothermophilus* spores and somewhat less effective in WPC80, showing IPL inactivation is dependent on spore type (Table 2.5). The log reduction of *A. flavithermus* spores in NFDM was slightly larger ($P < 0.05$) than *G.*

stearothermophilus in NFDM, at 0.10 – 0.50 $\log_{10}$ CFU/g as compared to 0.15 – 0.47 $\log_{10}$ CFU/g, respectively. The log reduction of *A. flavithermus* spores in MPC70 was larger (P > 0.05) than *G. stearothermophilus* in MPC 70, at 0.04 – 0.84 $\log_{10}$ CFU/g as compared to 0.03 – 0.48 $\log_{10}$ CFU/g, respectively. Finally, the log reduction of *G. stearothermophilus* spores in WPC80 was larger (P > 0.05) than *A. flavithermus* in WPC80, at 0.02 – 0.48 $\log_{10}$ CFU/g as compared to 0.08 – 0.33 $\log_{10}$ CFU/g, respectively. The results from this study also revealed that the log reduction of *G. stearothermophilus* in dairy powders was not dependent on the type of dairy powder (P > 0.05) for the three dairy powders examined in this study (Table 2.5). A study by Gomez-Lopez et al. (2005) compared the IPL sensitivity of *B. cereus* with other spore-forming bacteria and was able to determine that *B. cereus* was less resistant than *Alicyclobacillus acidoterretris* and *B. circulans*. The results from this study support the notion that thermophilic spores may be more resistant to IPL inactivation than mesophilic spores. Future studies should examine a wider range of sporeforming bacteria to determine IPL inactivation efficacy, and if similar mechanisms responsible for thermal tolerance in bacterial spores are used in response to IPL.

Table 2.5 Log reduction of treated *A. flavithermus* and *G. stearothermophilus* on each dairy powder at various IPL residence times.

			Reduction (log_{10} CFU/g)	
Powder	Time (sec)	CFU/g	*A. flavithermus*	*G. stearothermophilus*
NFDM	0	$2.58*10^9$ ($7.05*10^8$)	-	-
	30	$1.52*10^9$ ($4.90*10^8$)	0.20 (0.83) ^{a,x}	0.15 (0.03) ^{a,y}
	60	$1.89*10^9$ ($2.39*10^8$)	0.10 (0.98) ^{a,x}	0.47 (0.38) ^{a,y}
	120	$8.70*10^8$ ($3.07*10^8$)	0.50 (0.39) ^{a,x}	0.36 (0.30) ^{a,y}
MPC70	0	$3.70*10^8$ ($1.71*10^9$)	-	-
	30	$3.40*10^8$ ($1.82*10^9$)	0.04 (0.02) ^{b,x}	0.03 (0.31) ^{a,y}
	60	$1.58*10^8$ ($6.80*10^8$)	0.37 (0.11) ^{b,x}	0.40 (0.01) ^{a,y}
	120	$5.40*10^7$ ($5.70*10^8$)	0.84 (0.03) ^{b,x}	0.48 (0.02) ^{a,y}
WPC80	0	$5.10*10^8$ ($1.64*10^9$)	-	-
	30	$4.20*10^8$ ($1.58*10^9$)	0.08 (0.04) ^{c,x}	0.02 (0.35) ^{a,y}
	60	$2.52*10^8$ ($5.70*10^8$)	0.31 (0.29) ^{c,x}	0.46 (0.02) ^{a,y}
	120	$2.37*10^8$ ($5.40*10^8$)	0.33 (0.38) ^{c,x}	0.48 (0.04) ^{a,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). (n=4)

Studies have reported that thermophilic bacterial strains can produce highly heat-resistant spores (Scheldeman et al. 2006). In particular, it was determined that *G. stearothermophilus* spores had significantly higher heat resistance than *A. flavithermus* spores in milk at a D_{110^oC}-value of approximately 18 - 20 min as compared to only 2 min for *A. flavithermus* spores. A similar finding is observed for IPL treatment suggesting that spores of *G.*

stearothermophilus are more resistant to IPL deactivation than spores of *A. flavithermus*.

The results from this study also revealed that the log reduction of *A. flavithermus* in dairy powders is dependent on the type of dairy powder evaluated ($P < 0.05$) (Table 2.5). Chen et al. (2018) reported that undesirable agglomeration significantly reduced the efficacy of microbial inactivation of IPL treatment in NFDM. Ultimately, the composition of the milk powder seemed to play a role on the interaction and penetration of the intense pulsed light on the spores inoculated into the powder. NFDM, MPC and WPC vary in protein, fat and lactose content, which may explain various reductions in each powder (Berry 2015). 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 IPL, can be absorbed by proteins and thus reduce the effective dose available for microbial inactivation (Elmnasser et al. 2007). 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 is less effective for microbial deactivation as particle thickness increases (Uesugi and Moraru, 2009). Therefore, if caking of powders occurs due to extrinsic

factors or higher a_w values, a build-up of bulk powder thickness throughout the IPL process may occur, thus 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 *A. flavithermus* and *G. stearothermophilus* spores in dairy powders. This study determined that the inactivation of *A. flavithermus* 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 may not be effective in increasing microbial inactivation. Microorganisms on the surface of food powders are likely eliminated by IPL treatment as opposed to microbes shielded by other cells on the bottom layer, or hidden in open pores of the powdered matrices (Elmnasser et al. 2007).

The student's t-test on the dairy powder data determined if the treated powder log reductions are statistically significant when compared to untreated samples. The greatest log reduction of *G. stearothermophilus* on NFDM was significant ($P < 0.05$) at 30 seconds of exposure to IPL compared to the control. Every other greatest log reduction for both *G. stearothermophilus* and *A. flavithermus* for each powder was determined to not be significant ($P > 0.05$).

Table 2.6 Results compiled to show highest log reduction achieved for each powder

	Results (log CFU/g ± SD)	
Powder	*A. flavithermus*	*G. stearothermophilus*
NFDM	0.50 (0.39)	0.47 (0.38)*
MPC70	0.84 (0.03)	0.48 (0.02)
WPC80	0.33 (0.34)	0.48 (0.03)

*Results are considered significant ($P < 0.05$)

2.4.4 Comparison of IPL treatment on Spore Inoculated Dairy Powders with Adjusted Water Activity

The average log reduction of *A. flavithermus* and *G. stearothermophilus* 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 *A. flavithermus* and *G. stearothermophilus* spores based on the water activity level were observed. Results from this study revealed that at water activity levels of 0.40, a larger log reduction of approximately 0.30 to 0.32 $\log_{10}$ CFU/g was observed, in comparison to the reduced log reduction of approximately 0.08 to 0.26 $\log_{10}$ CFU/g observed at average water activity levels of 0.31. These results were not consistent with the research previously conducted by Chen et al. (2018) on the inactivation of vegetative cells of *Cronobacter sakazakii* in NFDM. Specifically, Chen et al. (2018) found that inactivation of vegetative cells of *C. sakazakii* decreased with increased water activity levels from 0.25 to 0.35, where the greatest inactivation of 3.18 $\log_{10}$ 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 study was performed with vegetative cells, it supports a trend general trend for bacterial spores and further studies may conclude this. Furthermore, higher water activity levels of dairy powders may result in agglomeration of powders,

which causes 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 *A. flavithermus* and *G. stearothermophilus* 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 for commercial use.

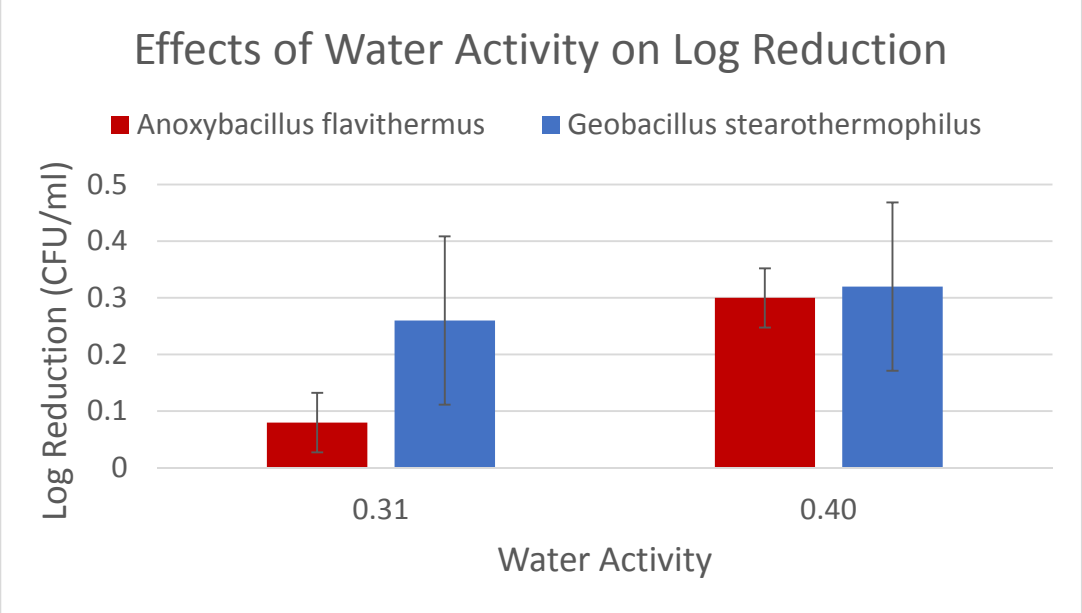

Figure 2.3 Average log reduction on dairy powders treated with IPL at different water activity. (n=4)

2.4.5. IPL deactivation of *A. flavithermus* compared to *G. stearothermophilus*

The D-values reported for spores of *G. stearothermophilus* have a higher heat resistance than those for spores of *A. flavithermus* (Table 2.1). The maximum log reduction results from this study support that on NFDM and MPC70, *A. flavithermus* spores were more susceptible to IPL inactivation than those of *G. stearothermophilus*. Alternatively, spores of *G. stearothermophilus* were more susceptible to IPL inactivation on WPC80 compared to *A. flavithermus.* Furthermore, the preliminary test of spores on filter paper resulted in a higher reduction of *G. stearothermophilus* spores from IPL treatment than *A. flavithermus*. A higher magnitude of IPL inactivation of *G. stearothermophilus* spores has been reported in previous studies (3 to 5 $\log_{10}$ CFU/g), which may be attributed to the differences in the food matrix studied since sugar syrup is a transparent liquid, which may allow for greater IPL penetration depth than an opaque dairy powder (Chaine et al. 2012). The results from this study are consistent with IPL studies performed by Chen et al. (unpublished data) in that inactivation of spores of either bacterial type was not dependent on residence time.

IPL provides a more thorough inactivation of spores as compared to UV radiation given the exposure of high peak pulsed light energy. Introduction to pulses of light initiates germination and after repeated applications of IPL, the cells are inactivated. Continuous pulses ensure that the spore cannot repair the light-induced cellular damage, which leads to deactivation of spores (Dunn et al., 1995), (McDonald et al., 2000). The mechanism of IPL induced damage is

accredited to the photolyase enzyme that binds to UV radiation-induced dimers and uses visible light energy to reverse the enzymatic reaction (Nicholson, 1995).

2.4.6. Future Direction

Thermophilic bacterial spores pose a problem in the dry powder dairy ingredient industry. This raises particular concern in the growing US dairy export market, as importing countries have strict allowable levels of spores/g for acceptable milk powders and ingredients. As such, there is increasing need for novel, non-thermal processing technologies to reduce levels of spores in dairy powders, while preserving sensory and quality attributes of the powders themselves and ensuring safety for consumption.

Implementation of IPL technology will effectively improve the safety of powdered foods, thereby reducing health hazards and enhancing the US food industry's competitiveness, which will increase the utilization of US agricultural products. Currently, more research is needed on use of IPL to better understand the effects of the decontamination process on nutritional value and quality of milk powders.

Although some level of inactivation of *A. flavithermus* and *G. stearothermophilus* 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.

2.5 Acknowledgements

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

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3.1 Abstract

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 physical characteristics (titratable acidity, scorched particle, solubility index, wettability, dispersibility, water activity, flowability, whey protein nitrogen index, pH, heat stability, and coffee stability) 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 NFDM, 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 (Madhi 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 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 (Non-fat Dry Milk and Skim Milk Powder 2018).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
(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 (Karagül-Yüceer 2001). 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 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 2009).

Discolored specks that result from spray-drying is termed scorched particles, where the heat influences the Maillard reaction to occur (Sharma 2012). NFDM generally contains 7.5-15 mg (Chandan 2009),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 (Zhao et al.

2019), and affects reconstitution functional properties based on heat treatment (Sharma 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 to 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 2008).

The ability of the powder to absorb water on the surface is due to adhesive intermolecular interactions, which determines its wettability (Moldoveanu 2016). Particle size is correlated with wettability; the larger the particle size, the increase in wettability (Abdalla 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 2012).

Passibility through a sieve (typically 150 μm) due to its ability to break down describes dispersibility (Lee 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 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 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 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 2012).

Heat stability is important for powders that are intended to be reconstituted and is a representation of its protein stability (Sharma 2012). Heat stability is dependent on its ability to withstand high temperatures and is determined by heat coagulation time at 140°C (Sikand 2010). The stability of NFDM during evaporation while drying in high-heat treatments prompts a κ-casein and β-lactoglobulin complex formation (Sharma 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 1997).

Water soluble vitamins, such as riboflavin (vitamin B2), aid in the body's ability to utilize carbohydrates, proteins and fats. Riboflavin is stable in the presence of heat, most oxidizing agents and acidic conditions. It is sensitive to basic conditions and light (Choe 2005). High amounts of riboflavin in the body have been associated with improved health and greater protection from diseases (Szczuko 2019). The vitamin is found in baby foods, breakfast cereals, sauces, processed cheese, fruit drinks and vitamin-enriched milk products and yeast (Choe 2005). Many studies on the content of riboflavin in milk and milk powders have been documented since 1937 with widely varying the results. NFDM has been shown to contain riboflavin levels ranging from 13.05 to 29.4 μg/g; methods of measuring the value, location and season of milk received can affect this value (Daniel 1943). According to the USDA SELF Nutrition data, 120 g of nonfat dry milk powder contains 1.9 mg of riboflavin.

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 is it 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 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). Prior to 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 (23ºC) in a dry and dark area away from sunlight. Preliminary powder functionality testing was completed on the untreated (control) and treated (IPL) samples (0-month testing), followed by monthly surveillance (1 month – 5 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 5 testing results.

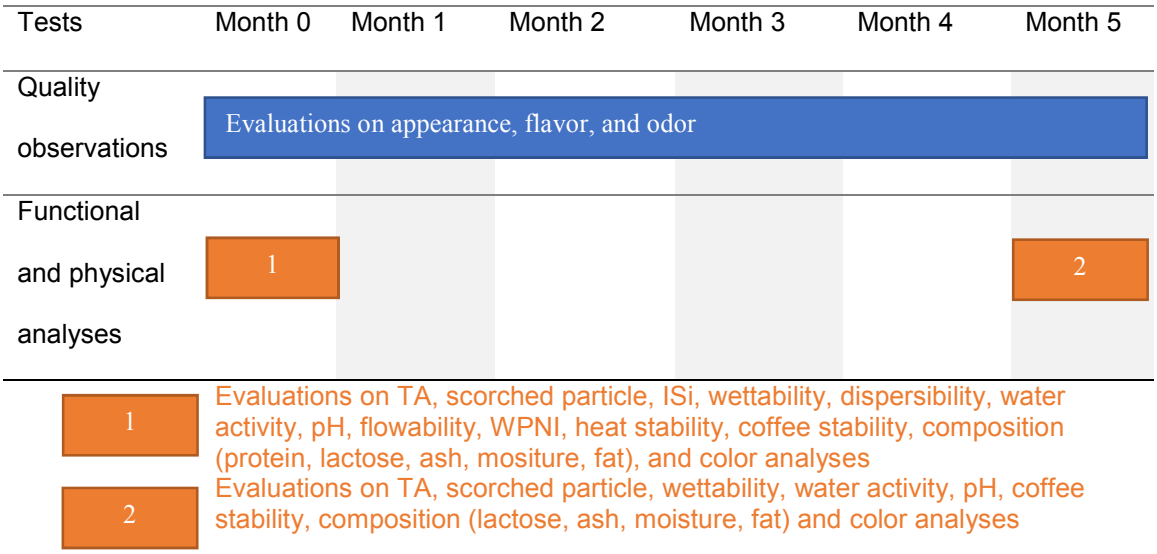

Figure 3.1 Timeline of shelf-life testing for untreated and IPL-treated NFDM.

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

NFDM composition, scorched particle, and TA measurements of the NFDM untreated and 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 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 treated and untreated 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 Konia Minolta Chroma Meter CR-200 series (Konia 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.

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

***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 (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 ºC 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 80ºC 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 Pa_wkit, 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.

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

***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 NFDM in comparison to the untreated NFDM (control). The composition of the untreated and IPL-treated NDFM 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

		Month 0		Month 5	
Type	Standard Test	Untreated (%)	IPL-Treated (%)	Untreated (%)	IPL-Treated (%)
Protein	AOAC 926.15	35.95	35.81	-	-
Lactose	AOAC 984.22	40.78	41.05	40.20	40.60
Fat	AOAC 932.06	1.20	1.23	1.25	1.26
Ash	AOAC 932.30	7.60	7.68	7.52	7.44
Moisture	AOAC 932.06	3.42	3.65	3.63	3.86

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 WPNI of the treated sample suggests that protein denaturation increased (Pelegrine 2005). Pelegrine and Gasparetto (2005) reported that at neutrality (pH = 6.8), the solubility of the protein was observed to decrease with increasing temperatures, even below 60ºC. 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 ≤ 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 6.6 ± 0.009 for untreated and IPL-treated NFDM. 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 1959).

The rehydration process for powders includes three steps, wetting, dispersing, and solubilization (Ji 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 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 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 60ºC, it has been reported that protein solubility increases with temperatures between 40ºC to 50ºC (Pelegrine 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 powers 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 ± 0.018 and 0.39 ± 0.009 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 2010) (Morgan 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.

Additionality, 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 1998). Overall, our results indicate that no changes in the physical appearance of NFDM developed through the IPL treatment.

The previous tests were run again after 5 months of storage. Similar results were reported when compared to 0 months (Table 3.3). Qualities without statistical differences ($P > 0.05$) between 0 and 5 months include: Wettability, Water activity, pH, Coffee stability, TA, Scorched particle, Composition (Moisture, Ash, Fat, Lactose) and Color. These findings indicate that NFDM was not altered at ambient storage in sealed in Uline matte (OPP/ VMPET/ PE) stand-up barrier pouches (Hudson, WI) between 0 and 5 months. The small amount of change in these attributes over a 5 month period is consistent with the typical characteristics of NFDM according to the Center of Dairy Research, who report that NFDM stored at <27ºC and <65% rh, should maintain quality characteristics for 12 – 18 months (Wisconsin Center for Dairy Research). Lloyd et al. (2004), reported that a group of panelists considered all 20 samples stored at ambient conditions from 0 to 29 years were acceptable for use in an emergency situation. Furthermore, they determined amounts of available lysine (1.4 to 2.6 mg/100g, thiamin (2.7 to 4.0 µg/g) and riboflavin (12.7 to 20.2 µg/g) were not significantly ($P > 0.05$) correlated with sample age (Lloyd, et al. 2004).

In addition, the riboflavin value in NFDM was determined for the first time after 5 months of storage to evaluate any changes in photodegradation. It was found to have a lower concentration after exposed to the IPL apparatus (Figure 3.3). The IPL-treated sample was 1.25 mg/100g, which is 0.17 mg/100g less than the control powder at 1.42 mg/100g. Riboflavin's sensitivity to light directly correlates with changing milk flavor forming singlet oxygen species, and

accelerating oxidative changes (Dalsgaard 2010). The decrease in riboflavin in the treated sample indicates degradation, thereby resulting in an off-flavor.

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

		Results			
		0 Months		5 Months	
Type	Standard Test	Untreated	IPL-Treated	Untreated	IPL-Treated
Titratable acidity	AOAC 947.05	0.14%	0.12%	0.15%	0.15%
Scorched particle	ADPI BUL. 916	3.10 mg	5.50 mg	4.90 mg	6.00 mg
Solubility index	IDF 129A:1988	< 1.00 mL	< 1.00 mL	-	-
Wettability	IDF 87:1979	> 120 seconds	> 120 seconds	> 120 seconds	> 120 seconds
Dispersibility	IDF 87:1979	34.37%	34.81%	-	-
Water activity	Aqualab Pa_wkit	0.32 ± 0.018	0.39 ± 0.008	0.30 ± 0.014	0.38 ± 0.007
Flowability	Flowdex (Hanson Research)	Good flow, Smallest size disk: 9mm	Good flow, Smallest size disk: 9mm	-	-
Whey Protein Nitrogen Index	Eurofins internal method	8.80 mg/g	7.99 mg/g	-	-
pH	OakTon pH700	6.596 ± 0.009	6.573 ± 0.009	6.579 ± 0.012	6.603 ± 0.021
Heat stability	Yamato oil bath	No visible coagulation at 5 minutes (143℃)	No visible coagulation at 5 minutes (143℃)	-	-
Coffee stability	GEA A16a	No visible precipitation	No visible precipitation	No visible precipitation	No visible precipitation

Color	Minolta Chroma Meter	L* 92.83 ± 0.105 a* -3.37 ± 0.095 B* 13.24 ± 0.015	L* 92.43 ± 0.215 a* -3.22 ± 0.065 B* 13.21 ± 0.010	L* 92.72 ± 0.078 a* -3.40 ± 0.021 B* 13.33 ± 0.064	L* 92.66 ± 0.163 a* -3.20 ± 0.014 B* 13.27 ± 0.042
Riboflavin	AOAC 970.65	-	-	1.42 mg/100g	1.25 mg/100g

Note: Month 5 tests were determined on changes in values between untreated and IPL-treated in month 0, cost factor analysis and fat content were below the limit of detection for some indicator tests.

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 5 (Table 3.3). 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 reasonability 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 1-5 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 0-5. Our results also revealed undesirable odor changes to the IPL-treated NFDM. 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 1993). The changes in flavor and odor from the IPL treatment need to be studied further to understand the true compounds that influence these characteristics. As detailed analysis of fat, protein and carbohydrate was not performed in this study, we can only conclude that our primary indicator of flavor changed after exposure to IPL and is not acceptable for the consumer.

Table 3.4 Quality observations performed on untreated and IPL-treated nonfat dry milk

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

5	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

3.4.4 Further Testing Exposure to IPL Apparatus

After examining the effects of IPL to sensory and functionality on NFDM, an additional test to determine which stage of the apparatus was causing the change in odor and flavor of the powder due to passage through the IPL apparatus. We passed four samples (100 g each) through the machine with different combinations of components used in the IPL apparatus (Table 3.5). The first sample (treatment #1) was taken straight from the bulk bag of low-heat NFDM with no treatments from the IPL apparatus. The second sample (treatment #2) was passed through the feeder and bed of the IPL apparatus without use of the infrared pre-heating element or IPL bulb on. The third sample (treatment #3) was passed through only the initial feeder under the preheat infrared treatment (56ºC). Next, for treatment #4 (Table 3.5) a sample inserted into the IPL apparatus after the pre-heat feeder and was placed on the vibratory conveyor bed for IPL flashlamp exposure using conditions previously described. Finally, for treatment #5 (Table 3.5), the last sample was exposed to all components of the IPL apparatus, under the infrared preheat bulb in the feeder and under the IPL flashlamp 1 pulse/s. Each sample was exposed to each specific condition for 120 seconds, using parameters as previously described in

Chapter 2. Each of the powders were then observed in an informal blind sensory tasting for odor, appearance and flavor changes in a 10% solution of each sample of NFDM rehydrated into water (Table 3.5).

Table 3.5 Quality observations performed on various conditions in the IPL apparatus

Treatment Type	NFDM treatment-Samples	Pre-heat temperature	IPL temperature	Sensory
1	Control (no treatment)	NA	NA	Cardboard, chalky odor. Powdery, flat, lacking flavor. Uniform white to light cream, free from lumps.
2	Pass through entire apparatus, only vibratory feeder on	23ºC	23ºC	Grain, egg odor. Sweet, chalky, cooked flavor. Uniform white to light cream, free from lumps.
3	Pre-heat in water bath and maintain under infrared heater in feeder only	56ºC	NA	Old, sulfur, oxidized odor. Sour, stale, burnt flavor. Uniform white to light cream, free from lumps.
4	Pass under IPL flashlamp only	NA	26ºC	Rancid, hay-like odor. Old, metallic, scorched flavor. Uniform white to light cream, free from lumps.
5	Pre-heat in water bath, maintain under infrared heater and pass through feeder and IPL flashlamp	56ºC	27ºC	Animal feed, barnyard odor. Scorched, stale flavor. Uniform white to light cream, free from lumps.

As previously mentioned, off-smells such as those described as barn, cardboard, sour and chemical can result in rejection of the product (Abdalla 2017). Therefore, it is important to analyze which part of the IPL apparatus may be contributing to the off-flavor development. It was determined that the flavors

and odors that were most consistently undesirable were similar between treatment #3, treatment #4 and treatment #5. This is consistent with flavor and odor profiles determined at time points from months zero through five with untreated and IPL-treated samples. As previously reported, no major functionality changes occurred in the IPL-treated NFDM as compared to the untreated NFDM through the five-month shelf study (Table 3.3). 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. Particle size and clumping can greatly affect the milk powder quality by influencing the physical properties of the milk powder (Abdalla 2017). Since the milk powder appearance remained consistent throughout the examined conditions, it is anticipated that NFDM may respond similarly to the conditions of the IPL process when exposed to preheat, IPL and both, since the sample preheat was set at 56ºC. Although the temperatures of the powders when leaving the IPL bed were at temperatures near 30ºC, Chen, et al. (2018) reported that the surface temperatures of the NFDM were monitored and never exceeded 58ºC (Chen et al. 2018). The changes in flavor and odor in NFDM from IPL treatment needs to be evaluated further to understand the true mechanisms of the degradation of compounds in dairy powders that influence these characteristics. Therefore, further testing is necessary to determine the temperature and conditions throughout the IPL process to evaluate what is specifically causing the off odor and flavor results.

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 fundamental 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.

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