Optimization of pulsed light treatment conditions for improving microbial destruction in liquid foods

By

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Abstract

Over the years, minimal or non-thermal techniques have been explored to address the limitations of traditional thermal processing, which can reduce the nutritional and sensory qualities of food. Pulsed light (PL) is one such technique, using intense white light pulses, for rapid microbial decontamination while preserving food quality. However, PL's effectiveness in liquids has shown varying results, with clearer liquids responding better due to greater light penetration. Larger liquid volumes pose challenges, as they limit the light penetration and lead to uneven PL distribution, highlighting the need for optimized mixing and treatment durations. This study was aimed at developing efficient PL treatments for microbial decontamination of water and apple juice.

In the first part, sterile water inoculated with *Escherichia coli* K-12 was treated under various PL conditions, including treatment duration (0, 6, 10, 14, 17, and 20 s), agitation mixing speed (500, 750, and 1,000 rpm), and sample volume (100, 200, 300 and 400 mL). A first-order kinetic model was used to calculate the associated D-values (time to destroy 90% of the microbial population). Increasing the sample volume at a constant agitation speed significantly increased the D-values, from 0.63 s for 100 mL to 4.40 s for 400 mL. Conversely, at a constant sample volume of 400 mL, increasing agitation speed significantly decreased D-values, from 6.06 s under static conditions (0 rpm) to 2.96 s at 1,000 rpm. Additionally, treating multiple containers simultaneously showed microbial inactivation comparable to single-container treatments, offering potential for scale up.

In the second part of the study, 400 mL of pasteurized apple juice, inoculated with *E. coli* K-12, were PL treated for varying duration (0-48s) and different agitation speeds (500-1,000 rpm). Agitation-assisted PL treatment successfully reduced *E. coli* counts to undetectable levels within 24, 30, and 42 s, depending on the agitation speed. In contrast, non-agitated samples only showed a modest reduction to around 6 log CFU/mL after 24 s, with no significant further decrease. The D-values decreased from 9.10 s to 4.70 s as the agitation speed increased. Apple juice quality attributes, including antioxidant activity, total soluble solids, pH, color, ascorbic acid content, and rheological properties, were not significantly impacted by the PL treatment. However, the total phenolic content increased slightly up to an optimal treatment duration before decreasing, eventually falling below the initial levels. The highest temperature increase during the longest treatment duration (48 s) was 3.13 ± 0.06 °C, remaining within the acceptable limits for non-thermal processing.

Overall, this study highlights the potential of mixing agitation-assisted PL as a promising non-thermal pasteurization technique for liquid foods, offering both effective microbial inactivation and quality retention. These findings contribute to the potential development of optimized PL treatment protocols and provide a foundation for scaling up PL applications for water treatment and liquid food processing.

Résumé

Au fil des années, des techniques minimales ou non thermiques ont été explorées pour remédier aux limites du traitement thermique traditionnel, qui peut réduire les qualités nutritionnelles et sensorielles des aliments. La lumière pulsée (PL) est l'une de ces techniques, utilisant des impulsions de lumière blanche intenses, pour une décontamination microbienne rapide tout en préservant la qualité des aliments. Cependant, l'efficacité du PL dans les liquides a montré des résultats variables, les liquides plus clairs répondant mieux en raison d'une plus grande pénétration de la lumière. Les volumes de liquide plus importants posent des défis, car ils limitent la pénétration de la lumière et conduisent à une distribution inégale du PL, soulignant la nécessité d'optimiser le mélange et la durée du traitement. Cette étude visait à développer des traitements PL efficaces pour la décontamination microbienne de l'eau et du jus de pomme.

Dans la première partie, l'eau stérile inoculée avec *Escherichia coli* K-12 a été traitée dans diverses conditions PL, y compris la durée du traitement (0, 6, 10, 14, 17 et 20 s), la vitesse de mélange par agitation (500, 750 et 1 000 tr/min) et le volume de l'échantillon (100, 200, 300 et 400 ml). Un modèle cinétique de premier ordre a été utilisé pour calculer les valeurs D associées (temps nécessaire pour détruire 90 % de la population microbienne). L'augmentation du volume d'échantillon à vitesse d'agitation constante a augmenté de manière significative les valeurs D, de 0,63 s pour 100 ml à 4,40 s pour 400 ml. Inversement, à un volume d'échantillon constant de 400 ml, l'augmentation de la vitesse d'agitation a diminué de manière significative les valeurs D, de 6,06 s dans des conditions statiques (0 tr/min) à 2,96 s à 1 000 tr/min. De plus, le traitement simultané de plusieurs conteneurs a montré une inactivation microbienne comparable aux traitements à conteneur unique, offrant un potentiel de mise à l'échelle.

Dans la deuxième partie de l'étude, 400 ml de jus de pomme pasteurisé, inoculé avec *E. coli* K-12, ont été traités par PL pendant différentes durées (0 à 48 s) et différentes vitesses d'agitation (500 à 1 000 tr/min). Le traitement par PL assisté par agitation a réussi à réduire le nombre d'*E. coli* à des niveaux indétectables en 24, 30 et 42 s, selon la vitesse d'agitation. En revanche, les échantillons non agités n'ont montré qu'une réduction modeste d'environ 6 log UFC/ml après 24 s, sans diminution supplémentaire significative. Les valeurs D ont diminué de 9,10 s à 4,70 s à mesure que la vitesse d'agitation augmentait. Les attributs de qualité du jus de pomme, notamment l'activité antioxydante, les solides solubles totaux, le pH, la couleur, la teneur en acide ascorbique et les propriétés rhéologiques, n'ont pas été significativement affectés par le traitement par PL. Cependant, la teneur phénolique totale a légèrement augmenté jusqu'à une durée de

traitement optimale avant de diminuer, tombant finalement en dessous des niveaux initiaux. L'augmentation de température la plus élevée pendant la durée de traitement la plus longue (48 s) était de 3,13 ± 0,06 °C, restant dans les limites acceptables pour un traitement non thermique.

Dans l'ensemble, cette étude met en évidence le potentiel du PL assisté par agitation comme technique prometteuse de pasteurisation non thermique pour les aliments liquides, offrant à la fois une inactivation microbienne efficace et une conservation de la qualité. Ces résultats contribuent au développement potentiel de protocoles de traitement PL optimisés et fournissent une base pour la mise à l'échelle des applications PL pour le traitement de l'eau et la transformation des aliments liquides.

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Contributions of Authors

Mr. Bosco Mategeko is an M.Sc. candidate at McGill University, where he is enrolled in the "Food Science and Agricultural Chemistry" program. Under the supervision of his supervisor, he conducted all experiments, collected and analyzed data, and presented results. He composed drafts of the thesis, the posters, and the manuscripts for scientific conferences and publications.

Prof. Hosahalli S. Ramaswamy is the supervisor under whose guidance the research was conducted. He assisted the M.Sc. applicant through the research process by providing special processing equipment, supervising the experiments, assessing the results, and final editing the thesis. He also assisted in the editing of posters and manuscripts for conferences and publications.

List of Presentations and Publications

Parts of this thesis have been presented as a poster at the following scientific conferences:

Mategeko, B., & Ramaswamy, H.S. (2024). "Optimization of pulsed light treatment of liquid foods for improving microbial inactivation". Poster presentation at the Northeast Agricultural and Biological Engineering Conference (NABEC) in State College, Pennsylvania, USA.

Mategeko, B., & Ramaswamy, H.S. (2025). "Enhanced inactivation of *Escherichia coli* in water using agitation-assisted pulsed light treatment". Poster presentation at the IFTPS annual meeting in Reno, Nevada, USA.

Parts of this thesis have been prepared as manuscripts for publication in a refereed scientific journal:

Mategeko, B., & Ramaswamy, H.S. (2025). Recent advancements in pulsed light processing of liquid foods: A review. Prepared for submission to *Food Research Reviews* (or an alternative journal).

Mategeko, B., & Ramaswamy, H.S. (2025). Enhanced inactivation of *Escherichia coli* in water using agitation-assisted pulsed light treatment. Prepared for publication in the *Journal of Food Science* (or an alternative journal).

Mategeko, B., & Ramaswamy, H.S. (2025). Impact of agitation-assisted pulsed light treatment on microbial inactivation and quality attributes in apple juice. Prepared for publication in the *Journal of Food Science* (or an alternative journal).

The research was carried out by the candidate under the supervision of Prof. Ramaswamy.

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List of Abbreviations

ANOVA	Analysis of Variance
CFU	Colony forming unit
FDA	Food and Drugs Administration
GAE	Gallic acid equivalent
J/cm^2	Joules/Square Centimeter
mL	Milliliters
PL	Pulsed Light
TPC	Total Phenolic Content
UV	Ultraviolet
UFC	Unité formant colonie
mJ/cm^2	Millijoules/Square Centimeter
FC	Folin-Ciocalteu
TSS	Total Soluble Solids
PPO	Polyphenol Oxidase
PME	Pectin Methyl Esterase
POD	Peroxidase
RMSE	Root Mean Square Error
TSA	Tryptic Soy Agar
AOA	Antioxidant Activity
AA	Ascorbic Acid
rpm	Revolutions Per Minute
kV	Kilovolt

Nomenclature

D	D-value
d	Thickness
DPPH	2,2-diphenyl-1-picrylhydrazyl
E. coli	*Escherichia coli*
Log	$\log_{10}$
V	Volume
%	Percent
^{o}C	Degree Celsius
s	Second
R^2	Coefficient of determination
N_0	Initial microbial population
N	Microbial population after treatment

Chapter 1

General Introduction

Traditional thermal processing techniques are commonly used to reduce or eliminate unwanted microorganisms from various food products. However, the associated heat is known to negatively impact the nutritional and sensory attributes of food (Basak et al., 2022; Mandal, 2022; Santamera et al., 2020). Although thermal processing can reduce microbial loads to safe levels, there is a growing consumer demand for fresh-like food with minimal alteration of its natural properties (Ragaert et al., 2004). This demand has driven the development of nonthermal food processing techniques such as pulsed light (PL), high hydrostatic pressure, ultrasound, irradiation, and plasma, as alternatives to heat processing for producing minimally processed foods (Khouryieh, 2021; Knorr et al., 2011; Stoica et al., 2013). These non-thermal technologies aim to ensure food safety while limiting negative impacts on nutritional and sensory qualities. Ensuring microbial safety is imperative regardless of the decontamination method used. Mandal et al. (2020) identified PL as a highly strategic nonthermal method due to its broad applicability.

PL is used to rapidly inactivate microorganisms on various surfaces within the food industry, including food itself, equipment (Oms-Oliu et al., 2010), and packaging materials (Kramer et al., 2017; Oms-Oliu et al., 2010). PL is generated by flash lamps that use inert gases (e.g., xenon) to emit electromagnetic radiation with wavelengths ranging from 100 to 1100 nm, covering the UV, visible, and infrared spectra (John & Ramaswamy, 2018; Koutchma, 2019). Pulsed UV light offers advantages over continuous UV light in terms of microbial inactivation effectiveness, penetration power, and lamp safety (Keklik et al., 2012). PL treatment uses instantaneous pulses of intense light to decontaminate food quickly with minimal changes to quality attributes (Wang et al., 2021). In addition to microbial inactivation, PL has been shown to destroy aflatoxins in food (Mandal et al., 2020). PL technology was initially developed in Japan and introduced to Western nations when Hiramoto (1984) patented PL as a sterilization method in the United States. Subsequently, a Californian firm patented a PL-based method for food preservation (Gómez-López, 2011). The adoption of PL technology in the food industry gained popularity after receiving FDA approval in 1996, though its widespread use in large-scale food applications is still limited (Birwal et al., 2019).

The worldwide consumption of fruit-based food products, particularly juices, has increased significantly, largely due to lifestyle changes. However, the mechanical processes, chemical

additives, and native microbes involved in juice production accelerate their degradation. These products are particularly prone to microbial growth and physicochemical changes, resulting in spoilage and a decline in nutritional and sensory quality. As a result, considerable research is being focused on minimal processing techniques and innovative methods like PL (Salazar-Zúñiga et al., 2022). Treating liquid samples with PL presents greater challenges due to microorganisms being distributed throughout the liquid. Additionally, studies have identified a significant barrier to the successful application of current PL systems known as the "shadow effect," where food particles near the lamp protect others from light exposure (Artíguez & Martínez de Marañón, 2015; Mandal et al., 2020). PL effectiveness depends on various factors, including the distance between the sample and the lamp, exposure level, turbidity, and the optical and physicochemical characteristics of the sample. The depth of the sample also affects PL efficacy (Mandal et al., 2020). Studies indicate that longer exposure durations and shorter distances between the light source and the sample increase microbial inactivation (John & Ramaswamy, 2020). Preetha et al. (2021) observed an inverse relationship between the absorption coefficient and microbial inactivation, suggesting that clear liquids are most susceptible to PL. Additionally, liquids tend to show better results when treated in thin profiles (Pollock et al., 2017).

There are generally two types of PL equipment used for treating liquids: batch systems and continuous flow systems. According to Gómez-López (2011), experiments utilizing flow-through systems better mimic real-world food processing procedures. This aligns with findings from several studies that suggest sample agitation improves microbial inactivation (Franco-Vega et al., 2021; Pataro et al., 2015; Sauer & Moraru, 2009). Despite its promising potential, PL as a method for liquid treatment remains significantly understudied, highlighting opportunities for further research.

Last year (Mittal, 2023), our lab conducted a study on the impact of PL on microbial inactivation kinetics of *E. coli* K-12 in liquid media using small plastic pouches. The results showed that PL was more effective with thinner profiles, and microbial inactivation was significantly influenced by sample volume and distance between the sample and the light source (Mittal, 2023). While the results demonstrated PL's effectiveness in decontaminating water, the small volumes and pouches used pose challenges for scaling up. This suggested the need for further research involving larger volumes and different PL treatment techniques. Therefore, the current research was aimed at:

1. Investigating the potential of mixing agitation assisted pulsed light treatment in enhancing the inactivation of *Escherichia coli* K-12 (EC1-5G) suspended in water, focusing on the effects of mixing speed, sample volume, and treatment time.
2. Studying the impact of mixing agitation-assisted pulsed light treatment on microbial inactivation and quality attributes in apple juice.

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Preface to Chapter 2

The preceding chapter introduced a knowledge gap in the application of pulsed light technology, particularly concerning its use in processing liquid food products. Generally, research has predominantly focused on PL applications for surface decontamination of solid matrices or the treatment of small liquid volumes. To broaden the scope of PL applications in liquid food processing, a comprehensive literature review is essential to assess the current understanding of PL principles and their industrial applications.

Chapter 2 dwells into the intricacies of PL technology, exploring its mechanisms and the advanced equipment utilized in its application. The chapter thoroughly examines the notable advantages and inherent limitations of PL, providing insights into its efficacy as a microbial control method and its impact on product quality. It aims to offer a comprehensive overview of PL's significance in both scientific and industrial contexts, tracing its evolution from inception to contemporary advancements, and highlighting the existing knowledge gap regarding its use in liquid food products.

This work was conducted under the supervision of Professor H. S. Ramaswamy.

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Chapter 2

Recent Advancements in Pulsed Light Processing of Liquid Foods: A Review

2.1. Abstract

The industrial treatment of liquid foods often relies on thermal techniques for microbial decontamination, but the associated heat can compromise nutritional and sensory qualities. In response to consumer preferences for minimally processed foods, nonthermal processing technologies like pulsed light (PL) have gained attention. PL technology uses intense light pulses for rapid decontamination with minimal quality changes. It employs high-intensity light emitted by flash lamps across UV, visible, and near-infrared spectral ranges, with the UV-C range being particularly crucial for microbial inactivation. The mechanism of action of PL includes photochemical, photothermal, and photophysical damage to microorganisms. The combination of these mechanisms, along with high emission power, enhances PL's penetration and decontamination effectiveness. A typical PL setup consists of a power supply, capacitor, and lamp. The flashlamp, filled with inert gases like xenon, is a critical component. PL systems can be batch or continuous, with batch being more common. Despite its FDA approval in 1996, the widespread use of PL in large-scale food applications is still evolving. This review presents the latest advancements in the application of PL for microbial decontamination in liquids and evaluates its effects on quality attributes of the treated liquid foods, aiming to scale up its use. Studies on PL's microbial inactivation and its effect on quality attributes in liquid foods showed varying results. The application of PL on transparent fluids exhibited higher efficiency than in opaque ones. Limitations, such as the shadow effect and sample heating, necessitate careful consideration while designing a PL system. Overcoming these challenges involves optimizing the process conditions and exploring comprehensive hurdle approaches. While PL offers promising opportunities for the food industry, addressing these challenges is essential for its widespread industrial applications. Ongoing developments focus on improved treatment chamber designs and optimization of PL treatment conditions.

Key words: Pulsed light, microbial decontamination, nonthermal technologies, liquids

2.2. Introduction

In the industrial treatment of liquid foods, thermal techniques are largely employed (Mahnot et al., 2022). While these techniques ensure microbial safety and contribute to product stability for adequate shelf life, it is recognized that heat can compromise the nutritional and sensory qualities of food (Santhirasegaram et al., 2015). In addition, there is an evolving consumer preference for food with a fresh-like quality and minimal alteration of its inherent properties (Ragaert et al., 2004). To address such customers' needs, nonthermal processing technologies, including pulsed light, high hydrostatic pressure, ultrasound, irradiation, and plasma, have been proposed as alternatives for heat processing to produce minimally processed foods (Khouryieh, 2021; Knorr et al., 2011). Irrespective of the decontamination method selected, it is imperative to ensure microbial safety. Mandal et al. (2020) reported pulsed light (PL) as the most strategic nonthermal method due to its tremendous applicability. PL treatment is a nonthermal processing technology that employs instantaneous pulses of intense light to decontaminate food within a short time accompanied by marginal changes in quality attributes (Wang et al., 2021). In addition to microbial inactivation, PL was revealed to exhibit further desired decontamination advantages, including the destruction of aflatoxin present in food (Mandal et al., 2020).

PL has been employed on an industrial scale for disinfecting packaging materials for several years (Kramer et al., 2017). In contrast, the adoption of PL technology in the food industry for processing gained popularity after receiving FDA approval in 1996 (FDA, 2024), though its widespread use in large-scale food applications is yet to be realized (Birwal et al., 2019). Despite PL being shown to achieve the desired microbial decontamination in a short time (John & Ramaswamy, 2020; Xu et al., 2019), there is still a need for more improvement in efficiency to scale up its use at the industry level (Mandal et al., 2020; Xu et al., 2019). Several investigations highlighted significant obstacles to the effective implementation of the existing PL systems, with notable challenges such as the occurrence of a "shadow effect". This phenomenon refers to a situation where particles adjacent to the lamp shield the remaining food particles from the light (Artíguez & Martínez de Marañón, 2015; Mandal et al., 2020). Therefore, this review provides a comprehensive understanding of the recent advancements in PL applications, as well as prospects, focused on improving microbial decontamination in liquid foods and expanding the utilization of PL technology on a large scale.

2.3. PL technology

PL also referred to as pulsed UV light, pulsed white light, high-intensity light, intense pulsed light, or broad-spectrum white light in scientific literature, utilizes high-intensity light emitted by gas discharge lamps (Baysal & Taştan, 2022; Pataro & Ferrari, 2022). These lamps generate radiation across the wavelength range of 200-1,100 nm, encompassing ultraviolet (UV): 200-400 nm, visible: 380-780 nm, and near-infrared (NIR): 700-1,100 nm spectral ranges (De Moraes & Moraru, 2018; John & Ramaswamy, 2018; Koutchma, 2019). About 25% of the wavelengths fall within the UV range, 45% in the visible range, and 30% in the infrared range (Chen et al., 2015). UV portion itself is subdivided into three spectral regions, UV-A: 400-315 nm, UV-B: 315-280 nm, and UV-C: 280-100 nm (Diffey, 2002). However, it is important to note that microbial inactivation is primarily driven by the UV-C component of the spectrum (Koutchma, 2019). The PL technique involves a wide spectrum of white light characterized by short duration (100–400 μs) and intense pulses (Baysal & Taştan, 2022). Under FDA regulation (21CFR179.41), the utilization of PL treatments in the food industry requires the employment of xenon flash lamps, usually operating at 1-20 pulses per second, with a pulse duration limited to 2 milliseconds (2ms). Also, the total energy doses applied during the process must not exceed 12J/cm^2.

2.3.1. Mechanisms of action of PL

The antimicrobial effect of PL has been documented to be associated with three main mechanisms: photochemical, photothermal, and photophysical.

***Photochemical damage*:** The photochemical effect has been underlined as the primary factor contributing to microbial inactivation in PL treatments. The UV component of PL can be absorbed by proteins, DNA, and RNA, causing photochemical damage that results in the death of microorganisms. Upon absorption of the PL light, the target molecules, such as thymine, undergo structural changes through a photochemical transformation which consequently prevents cell replication and finally leads to death (Baysal & Taştan, 2022; Birwal et al., 2019; Güneş & Turan, 2017; Mahnot et al., 2022; Pataro & Ferrari, 2022; Salazar-Zúñiga et al., 2023).

***Photothermal damage*:** The photothermal effect in PL treatment is associated with the absorption of PL from the infrared region which delivers heat to the surface of the processed substrate. This induces an immediate, localized temperature increase in a thin surface layer, typically just a few micrometers thick, without significantly raising the internal temperature of the treated materials. As a result, this localized overheating can swiftly vaporize the intracellular liquid in microbial

cells, creating a small steam flow that disrupts the cell membrane and wall(Baysal & Taştan, 2022; Birwal et al., 2019; Güneş & Turan, 2017; Mandal et al., 2020; Salazar-Zúñiga et al., 2023). Due to the relatively slow migration of heat from the cell's exterior to its interior, the germicidal impact of the photothermal effect in PL treatment is limited compared to the photochemical activity (Salazar-Zúñiga et al., 2023). As per the literature, elevated flux densities, particularly those above 0.5 J/cm^2, result in the rupture of microbial cells due to thermal stress (Birwal et al., 2019; Salazar-Zúñiga et al., 2023).

***Photophysical damage*:** Certain literature recognizes the photophysical activity of PL as the third mechanism responsible for microbial inactivation. Structural changes, distortion of cell walls, and modifications in cell form induced by intermittent, high-intensity pulses are associated with this photophysical damage. Studies have observed damage to cell walls, membrane rupture, cytoplasm damage, and more in bacterial cells exposed to PL, even when the temperature increase was minimal (Birwal et al., 2019; Salazar-Zúñiga et al., 2023).

Beyond the three widely acknowledged mechanisms of PL germicidal activity, Mahnot et al. (2022) introduced an additional mechanism, the *indirect chemical effect*. In this mechanism, germicidal chemicals induced by PL, such as hydroxyl radicals, ozone, or hydrogen peroxide, contribute to microbial reductions. However, due to a scarcity of extensive literature providing further clarification on this mechanism and distinguishing it from the other three mechanisms, the indirect chemical effect is not considered the main mechanism of PL germicidal activity in this review.

The combination of these various microbial inactivation mechanisms, whether acting simultaneously or sequentially, in conjunction with the high emission power that likely enhances PL's penetration of treated substrates, provides a plausible explanation for the superior decontamination effectiveness of PL compared to continuous UV light treatment (Pataro & Ferrari, 2022).

2.3.2. PL equipment

A typical PL system comprises three key components: a power supply, a capacitor, and a lamp as illustrated in Figure 2.1. According to Elmnasser et al. (2007), the energy is stored in a high-power capacitor for a relatively extended period (a fraction of a second), and it is subsequently released to a specially designed lamp unit within a much shorter time frame ranging from nanoseconds to milliseconds. The high energy is delivered to the lamp filled with inert gas in

which this gas is ionized to generate an intense pulse of light concentrated on the treatment area, typically lasting a few hundred microseconds (Chen et al., 2015). The emitted light encompasses a broad-spectrum wavelength, ranging from UV to infrared, with the distribution detailed in the mentioned wavelength ranges.

The power supply unit: The PL generation necessitates direct current (DC), meaning that the alternating current (AC) obtained from the outlet undergoes conversion into DC through a transformer (De Moraes & Moraru, 2018; Mandal et al., 2020).

Capacitor unit: Consists of high voltage capacitors arranged in parallel to accumulate energy from the power supply unit during the charging cycle and discharge it during the discharge cycle, creating a high electrical current. This system is also connected to specialized high-power switches that execute on/off cycles of very short duration, converting continuous low electrical power into pulsed high-electrical power (Mandal et al., 2020).

Lamp unit: The lamp unit comprises a lamp housing containing one or more flash lamps enclosed in quartz envelopes. Each lamp is equipped with two electrodes and is typically filled with inert gases (such as Xenon or Krypton) or a mixture of noble gases due to their efficient conversion of electrical energy to optical energy (Franco-Vega et al., 2021). The configuration (linear, spiral, etc.) and dimensions of the lamp can be tailored for the particular application to achieve consistent irradiation of the target surface (Chen et al., 2015).

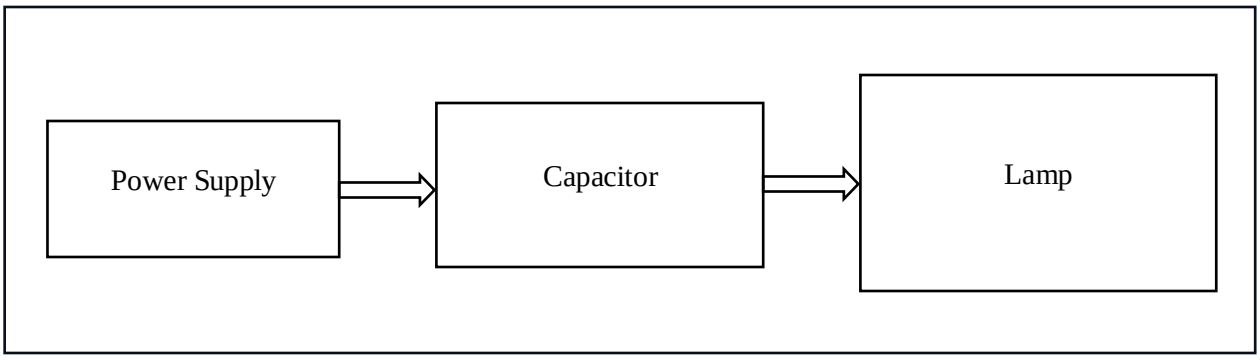

Figure 2.1. Main components of a typical PL equipment.

In addition to the core components mentioned earlier, the PL equipment may possess some auxiliary devices, such as a pump or conveyor belt for moving the product through the irradiation zone, cooling mechanisms, and instruments for monitoring temperature, fluence rate, or fluence (Pirozzi et al., 2021).

As described by De Moraes and Moraru (2018), the most crucial component of PL equipment is the flashlamp, which is filled with an inert gas like xenon or krypton. Xenon is the most

frequently used gas due to its superior conversion efficiency, and it is the preferred choice for most applications involving microbial inactivation. The primary structural elements of the flashlamp include the envelope, seals, and electrodes. The envelope serves as the casing that encloses the electrodes and holds the filling gas, and this envelope is typically crafted from clear fused quartz. The PL system is also equipped with a cooling fan to forestall overheating, a notable challenge encountered in the operation of flash lamps. To maintain reliable operation and extend the lamp's lifespan, additional cooling is required when operated at high power and flash rates. Forced air cooling, facilitated by a cooling fan integrated into the PL equipment, is employed to reduce heat accumulation in the treatment chamber. Reflectors are sometimes included in a treatment area to help redirect light towards the sample. In general, the effectiveness of PL technology for liquid decontamination depends on factors like total energy dose, emitted light spectrum, distance between sample and light source, liquid characteristics, flow conditions, inoculum size, processing unit geometry, and the number of lamps (Baysal & Taştan, 2022). Therefore, optimizing these factors is crucial for enhancing PL efficiency.

2.3.3. PL systems

The PL systems can be grouped into two main categories: *batch* and *continuous* systems. Both PL systems are available for PL treatments, although batch systems are more commonly used at present.

In batch setups, samples are placed inside a treatment chamber in which lamps are positioned along the chamber walls. Basic designs involve a single lamp located above the sample, often with an adjustable tray for holding the samples (De Moraes & Moraru, 2018; Pataro & Ferrari, 2022). More sophisticated configurations include multiple lamps in a chamber along with a quartz stand that holds the sample, enabling 360° exposure and treatment (De Moraes & Moraru, 2018). While undergoing liquid treatment in batch mode (Figure 2.2), the sample enclosed in a specific container is positioned within the treatment chamber on a customizable tray which enables the adjustment of the distance between the sample and the light source.

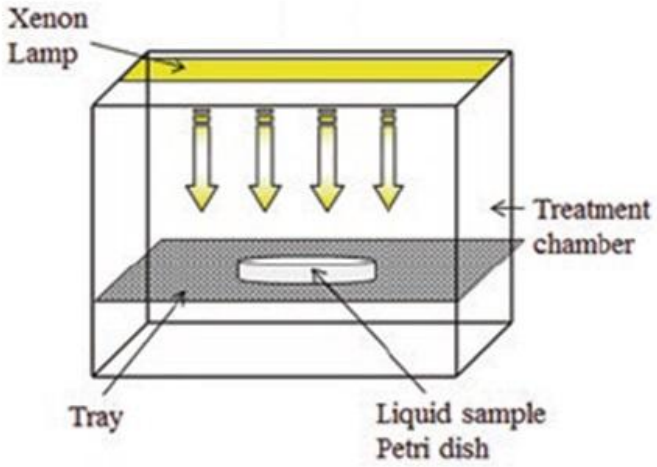

Figure 2.2. Schematic representation of batch PL system (Pataro & Ferrari, 2022).

Enhancements in the efficiency of pulsed light (PL) in batch operations have been realized by incorporating additional components into the PL system to generate multidirectional PL. This involves employing more than one lamp (Collazo et al., 2019) or facilitating manual or automated rotation or mixing of products to ensure uniform irradiation of all surfaces or volumes of treated material (Franco-Vega et al., 2021; Pataro et al., 2015).

Continuous setups can be designed to treat liquid products, either before or after packaging. Their use can provide better control over processing temperature, the ability to process a large volume of product, and increased efficiency in microbial inactivation due to enhanced light exposure, which improves treatment uniformity compared to discontinuous systems (Pataro et al., 2011). During the continuous treatments of fluids, the length of the exposure and, thus, the total number of flashes or fluence administered to the treated product are determined by the pulse repetition frequency and the residence time of the treated product in the irradiated zone. This is governed by the ratio between the volume of the treated product and its flow rate (Pataro & Ferrari, 2022).

In continuous treatments of liquid foods (Figure 2.3), the treatment chamber usually contains linear (Krishnamurthy et al., 2007; Muñoz et al., 2012; Pataro et al., 2011) or spiral quartz tubes (Wang et al., 2021; Xu et al., 2019). These tubes allow the liquid (such as water, fruit juices, and milk) to pass through while being exposed to the flash of light. Achieving optimal decontamination efficacy with minimal energy consumption requires careful optimization of the geometry, number of quartz tubes, and their relative position to the light source (Pataro & Ferrari, 2022). For instance, Ouyang et al. (2020b) positioned the quartz tube within a V-groove reflector,

which was subsequently placed on the shelf in the UV pulsed chamber (Figure 2.3. c). The smooth surface of the V-groove reflector, featuring angles of around 56 and 117 degrees, was employed to secure the quartz tube and improve energy absorption by redirecting the light energy towards the central regions of the quartz tubing.

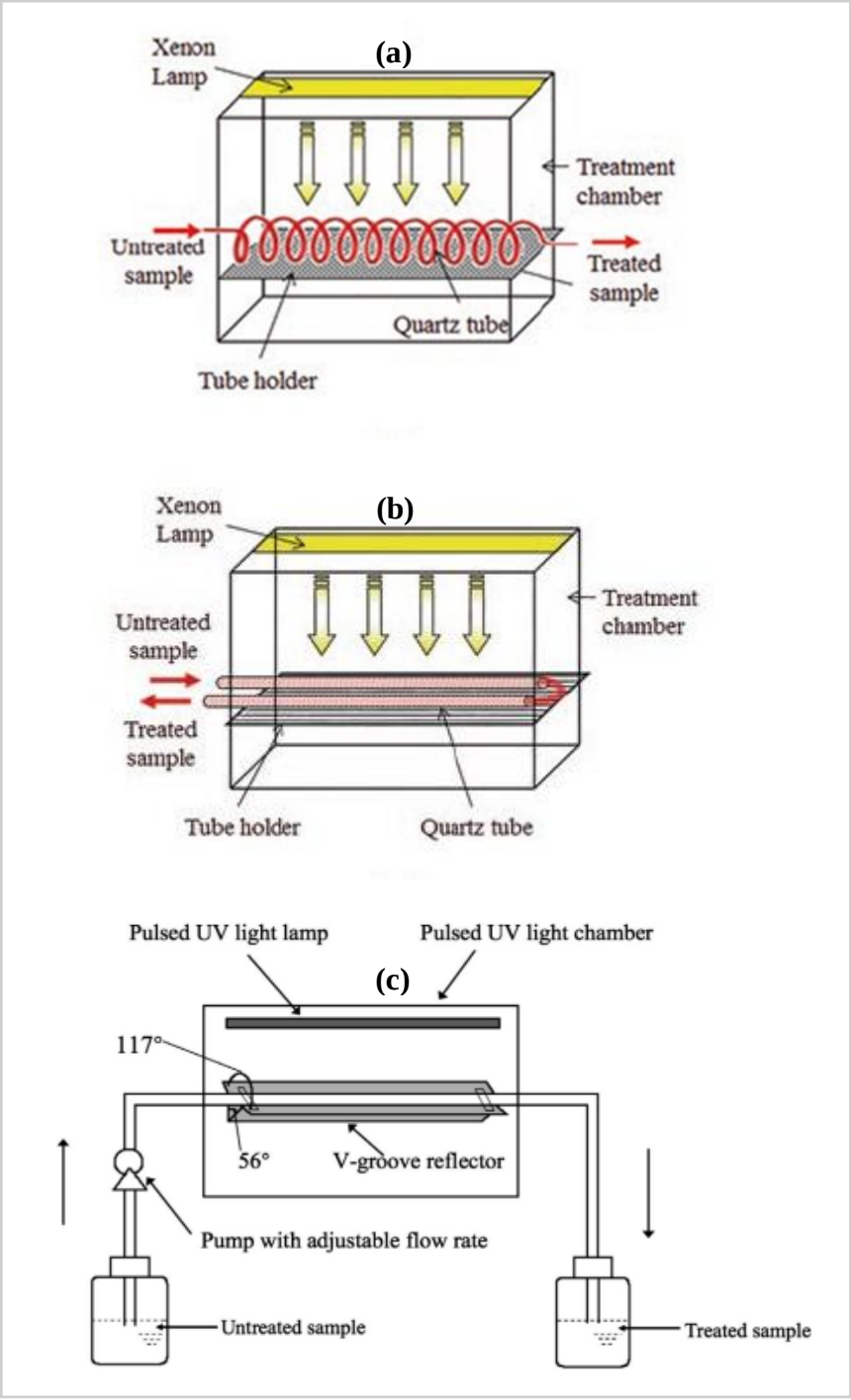

Figure 2.3. Schematic representation of continuous PL system. (a) continuous flow with linear quartz tubes. (b) continuous flow with spiral quartz tubes (Pataro & Ferrari, 2022), (c) continuous flow with a single linear quartz tube placed in a reflector (Ouyang et al., 2020b).

Pirozzi et al. (2021) highlighted the availability of various PL systems from different manufacturers, specifically tailored for laboratory-scale applications. Among the commonly used systems are the RS-3000C SteriPulse-XL system (Xenon Corp., Wilmington, Mass., USA), the XeMaticA-2L System (SteriBeam Systems GmbH, Germany) equipped with a single linear Xenon flash lamp, and the PL mobile decontamination unit (Claranor, Rouaine, France) equipped with 4 Xenon lamps (JA series, Verre et Quartz, Busy Saint Georges, France). These devices differ in wavelength distribution, pulse duration (50-360μs), pulse repetition rate (0-5.5Hz), input voltage (100-3800V), and the lamp cooling system (forced air or circulating water). While most operate in batch mode, only a few laboratory-scale PL systems are available for the continuous flow treatment of liquid and other products. For liquid treatments, the systems include in-house developed equipment (Ferrario & Guerrero, 2016; Xu et al., 2019) or commercial dynamic flow-through pilot units like the Maria PUD system (Claranor, Monosque, France) (Artíguez & Martínez de Marañón, 2015).

2.4. Microbial inactivation of PL in liquid foods

The utilization of PL as a nonthermal method for liquid decontamination represents a recent trend in liquid food processing research. While studies have documented a substantial decrease in the initial microbial count in liquid products (e.g., water, juice, milk) through PL application, the extent of this reduction varies considerably and is influenced by the transparency as well as turbidity of the fluids (Mahendran et al., 2019).

As summarized in Table 2.1, numerous researchers investigated the microbial decontamination efficiency of PL in various liquid products including water (Artíguez & Martínez de Marañón, 2015; Hwang et al., 2015; Yi et al., 2016), fruit juices (Bhagat & Chakraborty, 2022; Ferrario et al., 2013, 2015a, 2015b; Ferrario & Guerrero, 2018; Hwang et al., 2015; Karaoglan et al., 2017; Preetha et al., 2021; Preetha et al., 2023; Wang et al., 2021; Xu et al., 2019), milk and products thereof (Artíguez & Martínez de Marañón, 2015; Kasahara et al., 2015), tender coconut water (Basak et al., 2023; Preetha et al., 2021; Preetha et al., 2023), fresh liquid egg yolk (Ouyang et al., 2020a, 2020b), and wine (Harrouard et al., 2023).

Table 2.1. Microbial decontamination of liquid foods treated by PL

Liquid food	Process conditions	Flow Process	Microorganism	Reduction (log_{10} CFU/mL)	Reference
Apple juice	2.4-71.6 J/cm^2, <12°C, 10cm from lamp	Batch	*S. cerevisiae* cells *Alicyclobacillus* spores	4.4 3.0	Ferrario et al. (2015b)
Melon juice	2.4-71.6 J/cm^2, <20°C, 10cm from lamp	Batch	Cells of naturally occurring microorganisms	0.3-6.9	Ferrario et al. (2013)
Strawberry juice	2.4-71.6 J/cm^2, <20°C, 10cm from lamp	Batch	*E. coli* cells *L. innocua* cells *Salmonella* cells *S. cerevisiae* cells	<1 <1 <1 <1	Ferrario et al. (2013)
Grape juice	0.97-29.21 J/cm^2, 3.5cm from lamp	Batch	*Ps. aeruginosa* cells	1.9	Hwang et al. (2015)
Plum juice	0.97-29.21 J/cm^2, 3.5cm from lamp	Batch	*Ps. aeruginosa* cells	7.0	Hwang et al. (2015)
Skimmed whey	11 J/cm^2, 3000V, 5L/min, 22±2°C	Continuous	*L. innocua* cells	<0.5	Artíguez and Martínez de Marañón (2015)
Distilled water	11 J/cm^2, 3000V, 5L/min, 22±2°C	Continuous	*L. innocua* cells	6.0	Artíguez and Martínez de Marañón (2015)
Apple juice	0-71.6 J/cm^2, 60s, 3800V, 3 pulses/s, 10cm from lamp	Batch	*A. acidoterrestris* spores	3.0-3.5	Ferrario and Guerrero (2018)
Goat milk	10 J/cm^2, 8s		*E. coli*	6.0	Kasahara et al. (2015)
Water	14.02J/cm^2, 290s, 12L/min, 9cm from lamp	Continuous	*E. coli* C600	4.8	Yi et al. (2016)
Apple juice	71.6J/cm^2, 60s, 10cm from lamp	Batch	*S. cerevisiae* KE162 *L. innocua* ATCC33090 *E. coli* ATCC35218 *S. enteritidis* MA44	1.0 1.6 2.1 2.4	Ferrario et al. (2015a)
Tender coconut water	465J/cm^2, 8.5cm from lamp	Batch	*E. coli* *B. cereus* *L. monocytogenes*	5.1 3.0 3.4	Basak et al. (2023)
Pomegranate juice	761.4J/ cm^2, 90s	Batch	*E. coli* ATCC43888	5.0	Bhagat and Chakraborty (2022)
Red wine	22.8J/ cm^2,	Continuous	*Brettanomyces*	>6	Harrouard et

	9L/h, 15cm from lamp		*bruxellensis* cells		al. (2023)
Turnip juice	19.71J/ cm^2, 60s, 3,800V, 5cm from lamp	Batch	*Candida inconspicua* cells	2.8	Karaoglan et al. (2017)
Liquid egg white	45.6J/ cm^2, 40s, 5cm from lamp	Batch	*E. coli* K-12 *S. enteritidis*	1.3 2.0	Ouyang et al. (2020a)
Liquid egg white	0.37J/ cm^2, 40mL/min, 5cm from lamp	Continuous	*E. coli* K-12	1.6	Ouyang et al. (2020b)
Orange juice	95.2J/ cm^2, 100mL/min, 20cm from lamp	Continuous	*E. coli* MTCC433	4.0	Preetha et al. (2021)
Pineapple juice	95.2J/ cm^2, 100mL/min, 20cm from lamp	Continuous	*E. coli* MTCC433	4.5	
Tender coconut water	95.2J/ cm^2, 100mL/min, 20cm from lamp	Continuous	*E. coli* MTCC433	5.3	
Orange juice	756J/ cm^2, 45s, 100mL/min, 5cm from lamp	Continuous	Yeasts and Molds	4.4	Preetha et al. (2023)
	729J/ cm^2, 45s, 100mL/min, 5cm from lamp	Continuous	Aerobic plate counts	5.2	
Pineapple juice	756J/ cm^2, 45s, 100mL/min, 5cm from lamp	Continuous	Yeasts and Molds	4.5	
	729J/ cm^2, 45s, 100mL/min, 5cm from lamp	Continuous	Aerobic plate counts	5.4	
Tender coconut water	756J/ cm^2, 45s, 100mL/min, 5cm from lamp	Continuous	Yeasts and Molds	4.7	
	729J/ cm^2, 45s, 100mL/min, 5cm from lamp	Continuous	Aerobic plate counts	5.5	
Grape juice	66J/ cm^2, 30mL/min, 5cm from lamp	Spiral Continuous	*E. coli*	2.9	Wang et al. (2021)
Grape juice	52.8J/ cm^2, 40mL/min, 10cm from lamp	Spiral Continuous	*E. coli*	4.9	Xu et al. (2019)

PL treatments demonstrated a higher efficient microbial reduction in substantially transparent fluids compared to opaque liquids (Artíguez & Martínez de Marañón, 2015; Hwang et al., 2015). For example, the PL treatment distilled water reduced inoculated with *Listeria innocua* exhibited a maximum reduction of approximately 6 log cycles (Artíguez & Martínez de Marañón, 2015).

Conversely, when applied to opaque fluids, such as grape juice, orange juice, or skimmed whey, PL processing exhibited lower disinfection efficiencies, regardless of the characteristics of targeted microorganisms (Artíguez & Martínez de Marañón, 2015; Preetha et al., 2021; Preetha et al., 2023).

There is potential for improving the disinfection efficiency of the PL in opaque drinks under certain conditions. An effective design of continuous flow of PL reactors, operating with a thin layer of liquid (Wang et al., 2021; Xu et al., 2019), and employing flow conditions that generate high turbulence intensity (Franco-Vega et al., 2021), is essential to enhance treatment homogeneity as it ensures that all fluid particles receive the homogenous energy dose, thereby increasing the effectiveness of the PL process. Enhancing microbicidal effects, along with minimally processed foods with improved nutritional and nutritional sensory attributes and reduced energy consumption, can be achieved by employing a hurdle approach with PL in combination with other factors or decontamination techniques. Commonly and reliable combined approaches include the use of heat (Artíguez & Martínez de Marañón, 2015), ultrasound (Ferrario & Guerrero, 2016; Ferrario & Guerrero, 2017), thermosonication (Muñoz et al., 2012), and storage at low temperatures (<20°C) (Ferrario et al., 2013).

2.5. Effect of pulsed light on quality attributes in liquid foods

Conventional methods like thermal processing have a significant drawback due to their negative impact on various food quality attributes, such as color, taste, flavor, and the nutritional and bioactive components (Mandal, 2022). To meet the increasing global demand for safe, high-quality food products, researchers are increasingly exploring and evaluating innovative technologies such as pulsed light (PL). This nonthermal processing method is gaining attention as it is expected to preserve food quality without causing adverse effects (John & Ramaswamy, 2020).

2.5.1. Effect of pulsed light on total phenolic content

Total phenolic content (TPC) serves as a vital indicator of the nutritional and functional properties of liquid foods, particularly fruit juices, and plant-based beverages, due to its antioxidant capacity and role in promoting health. Numerous studies have investigated the effects of pulsed light (PL) treatment on TPC, with findings generally indicating that PL preserves or slightly enhances TPC under optimized conditions. For instance, Bhagat and Chakraborty (2022) reported a 97% retention of TPC in pomegranate juice following PL treatment at fluence levels

up to 2,988 J/cm^2. Similarly, Basak et al. (2023) observed no significant reduction in TPC in tender coconut water subjected to PL treatment at 2.5 kV (1,073 J/cm^2) for 2.5 min. Moreover, Jayachandran et al. (2024) found that fresh sugarcane juice, which initially contained 38.95 mg gallic acid equivalents (GAE)/100 mL, retained its TPC well after PL processing. The researchers noted that the impact of PL treatment on total phenols was minimal, with a maximum reduction of 7% observed only at a high voltage and pulse combination of 2.7 kV with 200 pulses. Mandal et al. (2020) reviewed that PL treatment for 30 s did not result in any statistically significant differences ($p > 0.05$) in blueberry wine samples compared to the control.

In some instances, PL treatment has led to increased TPC, which researchers attribute to the breakdown of phenolic precursors or improved extractability due to cell disruption induced by PL. Dhar and Chakraborty (2020) documented a 14% increase in TPC in a mixed fruit beverage at a fluence of 3,143 J/cm^2, attributing the enhancement to improved phenolic extraction. Moreover, Murugesan et al. (2012) suggested that the initial increase in TPC may be linked to the activation of phenylalanine ammonia-lyase (PAL), an enzyme in the phenylpropanoid pathway, stimulated by thermal stress generated during PL exposure up to a certain temperature threshold.

The stability of phenolic compounds during PL treatment is largely credited to the short exposure duration and limited heat generation, which help minimize thermal degradation. However, excessive PL exposure may trigger photochemical reactions that degrade phenolic compounds (Dhar et al., 2022). For example, Chakraborty et al. (2020) reported that TPC in Indian gooseberry (*amla*) juice increased by 10% following PL treatment at 2,727 J/cm^2 but decreased by 7% after exposure to 3012 J/cm^2, likely due to high-energy light pulses disrupting phenolic compounds. Similarly, Pihen et al. (2024) observed a 38.4% reduction in TPC in fresh apple juice treated at a maximum fluence of 176.4 J/cm^2 for 70 s, attributing the decline to a temperature increase to approximately 60°C, leading to bioactive compound degradation. Further studies have highlighted the negative impact of prolonged exposure or higher fluence levels on TPC. Chakraborty et al. (2022) reported a 10-17% reduction in TPC, with the highest loss observed at a fluence of 5,000 J/cm^2. Chakraborty and Parab (2023) reported a 6.3% reduction in the total phenolic content of table grape juice with a pH of 3.0 following PL treatment at a fluence of 2,772 J/cm^2. Although results vary, research consistently indicates that PL treatment can effectively maintain or enhance TPC in liquid foods when treatment parameters are carefully controlled to minimize photochemical degradation.

2.5.2. Effect of pulsed light on antioxidant activity

Antioxidant activity (AOA) in liquid foods is closely associated with phenolic compounds and other bioactive molecules. Research indicates that PL treatment can preserve, enhance, or minimally reduce AOA under specific conditions. For instance, Chakraborty et al. (2020) reported a 4% increase in AOA in *amla* juice after PL treatment at 2.8 kV for 5 min (2,727 J/cm^2), while observing a maximum 15% decrease at 2.9 kV for the same duration (3,012 J/cm^2). Muñoz et al. (2012) found no significant effect on antioxidant capacity in apple juice treated at a fluence of 5.1 J/cm^2. Similarly, Palgan et al. (2011) noted that the antioxidant activity of a reconstituted apple juice remained unchanged after 2 and 4 s of PL exposure (7 and 14 J/cm^2, respectively); however, an 8-second treatment (28 J/cm^2) led to a 5.5% decrease ($p < 0.05$). Vollmer et al. (2020) observed that fresh pineapple juice, with an initial antioxidant capacity of 16.4 ± 0.1 mg GAE/100 mL, was unaffected by 47 PL pulses (160-375 J/cm^2). A significant reduction ($p \leq 0.05$) in antioxidant capacity occurred with 94 and 187 pulses (325-1,479 J/cm^2), with the most intense treatment (2.4 kV/187 pulses) causing a 14% reduction. Chakraborty et al. (2022) reported that AOA retention varied between 87% and 98%, with higher losses at fixed fluence rates during longer exposures. Basak et al. (2022) found that a beverage blend of apple ber, carambola, and black table grapes retained 88.1% of its AOA after PL irradiation (3,000 J/cm^2). Jayachandran et al. (2024) observed minimal impact on AOA, with a maximum reduction of 16.7% at a high voltage and pulse combination (2.7 kV with 200 pulses). Conversely, (Pihen et al., 2024) documented a 14.5% decrease in DPPH inhibition in fresh apple juice subjected to a higher fluence of 176.4 J/cm^2 for 70 s, likely due to the significantly higher energy dose compared to other studies. Collectively, these findings suggest that antioxidant compounds are generally preserved under short PL exposure durations or low fluence levels. However, prolonged exposure or high fluences may lead to oxidative degradation, diminishing antioxidant activity (Cai et al., 2024).

2.5.3. Effect of pulsed light on total soluble solids

Total soluble solids (TSS), indicative of dissolved sugars and other solutes, are crucial quality indicators in liquid foods. Multiple studies have investigated PL's impact on TSS across various liquid foods. For example, Pihen et al. (2024) reported that the soluble solids (°Brix) in apple juice samples remained consistent with the control sample at 14.40°Brix, despite undergoing PL treatment. Similarly, Kasahara et al. (2004) observed no significant differences in the TSS of clarified apple juice when exposed to pulsed UV light at energy dosages ranging from 1,850 to

3,354 mJ/cm^2 compared to the control. Chakraborty et al. (2020) found that PL treatments had no significant effect ($p > 0.05$) on the TSS of Indian gooseberry juice subjected to different voltages (2.7, 2.8, and 2.9 kV) and treatment times (3, 4, and 5 min). Jayachandran et al. (2024) reported that the TSS of sugarcane juice remained unaffected across all samples processed under varying voltages (2.1-2.7 kV) and treatment durations of 100, 150, and 200 s, corresponding to 100, 150, and 200 pulses, respectively. Basak et al. (2023) found that the changes in total soluble solids were insignificant after PL pasteurization at 2.5 kV for 2.5 min (1073 J/cm^2). Moreover, Kamble et al. (2024) observed no significant influence ($p < 0.05$) on total soluble solids in dragon fruit juice processed under PL treatment at 1.4 J/cm^2. The stability of TSS is attributed to the non-destructive nature of PL treatment, which does not induce substantial chemical changes or solute degradation.

2.5.4. Effect of pulsed light on pH

The pH of liquid foods is a critical factor influencing both microbial stability and sensory properties. Studies indicate that PL treatment does not cause significant changes in the pH of most liquid foods. For example, Kamble et al. (2024) observed no significant influence ($p < 0.05$) on pH and titratable acidity in dragon fruit juice processed under PL treatment at 1.4 J/cm^2. Similarly, Basak et al. (2023) found that changes in pH and acidity were insignificant after PL pasteurization at 2.5 kV for 2.5 min (1,073 J/cm^2). Palgan et al. (2011) reported that the pH of apple juice samples remained steady at 3.66, matching the control, even after PL processing for 2, 4, and 8 s (corresponding to 7, 14, and 28 J/cm^2, respectively). Cai et al. (2024) also noted no significant changes in the pH of apple juice treated with pulsed light. In their study, 5.65 mL samples (solution thickness of 2 mm) were exposed to PL at fluences ranging from 6.75 to 54.00 J/cm^2, with total processing times varying accordingly. These findings align with those of Qi et al. (2023), who observed no impact of PL on the pH of apple juice. Similarly, Chakraborty et al. (2020) reported that PL treatments had no significant effect ($p > 0.05$) on the pH of Indian gooseberry juice (20 mL) subjected to varying voltages (2.7, 2.8, and 2.9 kV) and treatment durations of 3, 4, and 5 min. Likewise, Jayachandran et al. (2024) noted that the pH of sugarcane juice (100 mL samples) remained unaffected across all treatments involving different voltages (2.1-2.7 kV) and durations of 100, 150, and 200 s, equivalent to 100, 150, and 200 pulses, respectively. These consistent results suggest that PL treatment does not significantly alter the pH of various liquid foods.

2.5.5. Effect of pulsed light on color

Color is a vital quality attribute in liquid foods, significantly influencing consumer perception and acceptance. PL treatment has been shown to have a minimal impact on the color of various fruit juices when applied under moderate conditions. For example, Basak et al. (2023) observed that color changes (ΔE^* between 0.49 and 1.51) were insignificant after PL pasteurization at 2.5 kV for 2.5 min (1,073 J/cm^2). Intriguingly, Qi et al. (2023) reported a significant ($p < 0.05$) increase in ΔE^* with longer PL treatment durations in apple juice, reaching a maximum ΔE^* value of 5.9. In a study on table grape juice, Chakraborty and Parab (2023) noted that a PL fluence of 2,772 J/cm^2 caused a noticeable color change, while a fluence of 3186 J/cm^2 led to a more pronounced change. Comparable increases in ΔE^* were observed in PL-treated bael fruit juice (Dhar & Chakraborty, 2023) and Indian gooseberry juice (Chakraborty et al., 2020). Similarly, in the study by Pihen et al. (2024) on apple juice, the primary shifts in color were noted in the a^* and b^* parameters, representing changes in redness-greenness and yellowness-blueness, respectively. These values exhibited a notable decrease with higher treatment doses, such as at a fluence of 176.4 J/cm^2 for 70 s. Moreover, in the study conducted by Kamble et al. (2024) on dragon fruit juice, the redness value (a^*) decreased in the PL-treated juice sample under PL treatment at 1.4 J/cm^2.

The observed PL-induced color changes may result from enzymatic browning at lower fluences (when enzymes remain active) or non-enzymatic browning at higher fluences (when most enzymes are inactivated). Non-enzymatic browning could involve PL-induced Maillard reactions, changes in pigment content, or the formation of dark or brown substances (Dhar et al., 2022; Dhar & Chakraborty, 2020; Mahendran et al., 2019).

2.5.6. Effect of pulsed light on ascorbic acid

Ascorbic acid (vitamin C) is highly sensitive to thermal and oxidative degradation, making its retention during processing a critical factor. PL treatment has shown promise in preserving ascorbic acid content in liquid foods when applied under moderate conditions. Several studies have explored the effects of PL on ascorbic acid (AA) content. For example, Chakraborty et al. (2020) reported an 8% loss of AA content in *amla* juice pasteurized with PL at 1,504 J/cm^2. However, a higher fluence (3,012 J/cm^2) in enzymatically stable *amla* juice resulted in a 16% reduction in AA content. Vollmer et al. (2020) found that mild PL treatment at 2.1 kV with 47 pulses (253 J/cm^2) retained AA content entirely in pineapple juice, while treatment at 2.4 kV with

187 pulses (1,479 J/cm^2) led to a 29% reduction, indicating that AA degradation increases with higher pulse numbers and voltages.

In another study, Pihen et al. (2024) observed a 38.4% decrease in AA content (7 mg AA/100 mL) in fresh apple juice subjected to PL treatment at a maximum fluence of 176.4 J/cm^2 for 70 s. This higher loss may be attributed to their use of longer treatment durations (70 s) and higher total fluence compared to the milder conditions in other studies (maximum 48 s and approximately 5.04 J/cm^2 fluence). Chakraborty and Parab (2023) noted a 12% reduction in vitamin C in PL-treated pasteurized table grape juice (pH 3.5) when treated with fluences ranging from 1,152 to 3,186 J/cm^2. In addition, Basak et al. (2022) reported a 23% reduction in AA content in a beverage blend of apple ber, carambola, and black table grapes following PL irradiation at 3,000 J/cm^2. Kamble et al. (2024) observed that the AA content in dragon fruit juice remained largely unchanged (p> 0.05) under PL treatment at 1.4 J/cm^2. These findings highlight the potential of PL treatment to retain ascorbic acid in liquid foods under controlled processing conditions while also emphasizing the need to avoid excessive treatment intensities.

2.5.7. Effect of pulsed light on rheological properties

Rheological properties, such as viscosity and flow behavior, are crucial determinants of the texture and mouthfeel of liquid foods. Research on the effects of PL treatment on these properties is limited but generally indicates minimal impact.

For instance, Pihen et al. (2024) reported no significant alterations in the viscosity of apple juice after PL treatment. Similarly, Kasahara et al. (2004) found that clarified apple juice exposed to PL at fluences between 1,850 and 3,354 mJ/cm^2 exhibited no notable viscosity differences compared to untreated samples. In a subsequent study, Kasahara et al. (2015) observed that goat milk treated with fluences of 5 J/cm² and 10 J/cm² showed only a minor, non-significant increase in viscosity.

Conversely, Pok et al. (2023) demonstrated that PL treatments ranging from 3 to 60 s, corresponding to fluences between 3.6 and 71.6 J/cm², led to a slight but significant reduction in the apparent viscosity of melon juice. They also noted shear-thinning behavior, where viscosity decreased with increasing shear rates across all samples.

The rheological behavior of fruit juices is influenced by interactions between the dispersed phase (e.g., pulp) and the serum phase, which includes soluble polysaccharides, sugars, salts, and acids

(Diamante & Umemoto, 2015). According to Ibarz et al. (1996), juices containing pectin and pulp exhibit non-Newtonian behavior, while depectinised and clarified juices follow a Newtonian pattern. The minimal and non-significant changes in rheological properties observed in these studies may be attributed to the nature of the juices used, which lacked pectins and pulps that could absorb pulsed light and potentially trigger the depolymerization of pectins and polysaccharides. Therefore, while PL treatment generally has a minimal impact on the rheological properties of fruit juices, variations can occur depending on specific liquid compositions and treatment parameters.

2.5.8. Effect of pulsed light on enzymes

Enzyme activity plays a pivotal role in determining the quality and shelf life of liquid foods. PL treatment has emerged as a promising method to reduce enzymatic activity, thereby enhancing product stability.

For example, Chakraborty et al. (2020) achieved complete inactivation of polyphenol oxidase (PPO) and peroxidase (POD) in Indian gooseberry (*amla*) juice using PL treatment at 2.9 kV for 5 min, corresponding to a fluence of 3,012 J/cm^2. Similarly, Kamble et al. (2024) reported effective inactivation of PPO and POD in dragon fruit juice with a PL treatment of 1.44 J/cm^2. (Bhagat & Chakraborty, 2022) observed complete inactivation of PPO and POD in pomegranate juice treated with PL at 2,988 J/cm^2. Shaik and Chakraborty (2022) also reported complete inactivation of PPO, POD, and pectin methyl esterase (PME) in sweet lime (*Citrus limetta*) juice after PL treatment at 3.0 kJ/cm^2. In the case of table grape juice, Chakraborty and Parab (2023) investigated the inactivation of PPO, POD, and PME at fluence levels ranging from 1,152 to 3,186 J/cm^2. They found that enzyme inactivation exceeding 90% was achieved at 3,186 J/cm^2, rendering the juice both microbially safe and enzymatically stable. In pineapple juice, Vollmer et al. (2020) observed significant reductions in PPO and POD activities following PL treatments. The mildest treatment (1.8 kV/47 pulses, 160 J/cm^2) resulted in 10% and 25% reductions in POD and PPO activities, respectively, while the strongest treatment (2.4 kV/187 pulses, 1,479 J/cm^2) led to maximum reductions of 42% (POD) and 50% (PPO).

In dairy applications, Innocente et al. (2014) studied the effect of increasing PL fluence (0.26-26.25 J/cm^2) on alkaline phosphatase (ALP) in raw whole milk. They found that higher fluence levels resulted in approximately 94% ALP inactivation, accompanied by a temperature increase to 55 °C.

The reduction in enzymatic activity is attributed to the disruption of enzyme structures caused by high-intensity light pulses. However, the extent of inactivation varies depending on the enzyme type, food matrix, and treatment conditions.

2.6. Advantages of pulsed light

PL technology offers several advantages in food processing, particularly in enhancing microbial safety and extending shelf life. One of the primary benefits is its rapid disinfection capability. PL delivers intense, short-duration pulses of broad-spectrum light, effectively inactivating a wide range of microorganisms on food surfaces and packaging materials and this swift action makes it suitable for high-throughput processing environments (De Moraes & Moraru, 2018; John & Ramaswamy, 2018; Mandal et al., 2020). Another significant advantage of PL is its non-thermal nature. Unlike traditional heat-based methods, PL does not rely on elevated temperatures, thereby preserving the sensory and nutritional qualities of foods. This characteristic is particularly beneficial for heat-sensitive products, as it minimizes alterations in taste, texture, and nutritional content (Mahnot et al., 2022; Mandal, 2022; Pataro & Ferrari, 2022).

Environmental considerations also favor the use of PL technology. It eliminates the need for chemical sterilizing agents such as hydrogen peroxide, propylene oxide, or peracetic acid, reducing the risk of chemical residues on food products and lowering the environmental impact associated with chemical usage. Additionally, PL systems typically utilize xenon lamps, which are mercury-free and consume less energy compared to continuous-wave systems, further enhancing their environmental friendliness (De Moraes & Moraru, 2018).

From an operational perspective, PL equipment is compact and can be easily integrated into existing production lines. This ease of integration, coupled with the technology's high efficiency and relatively low operational costs, makes PL a cost-effective solution for food processors aiming to enhance safety and quality without significant infrastructure changes (Heinrich et al., 2016).

Furthermore, PL can be used to inactivate microorganisms on the surface of food packaging materials and potentially on the surface of products packaged in clear, ‘‘UV-transparent’’ packaging (De Moraes & Moraru, 2018).

2.7. PL application limitations

The main limitations documented in the literature that hinder the success of PL for effective microbial decontamination in liquid products include the shadow effect and sample heating (Pataro & Ferrari, 2022). Studies on fruit juice decontamination revealed that microbial inactivation was higher in clear juices than in cloudy ones, suggesting that turbid substances in juices caused a shadow effect on light penetration into the sample which ultimately reduced process efficiency because food particles adjacent to the lamp shield the rest from light (Pataro et al., 2011). In a study conducted by Gómez-López et al. (2005), lower inactivation in samples with higher microbial load was attributed to the shading effect, where microorganisms in high-density populations were layered, resulting in uneven inactivation. While PL is highly efficient in microbial inactivation, Massier et al. (2013) suggested that careful consideration of the shadow effect, linked to the liquid matrix or contamination density, is essential. Additionally, heat generation during extended processes can impact juice quality, a fact that necessitates the use of cooling systems (Pataro et al., 2011). For enhancing microbial inactivation, the combination of PL with other treatment techniques in a hurdle approach has been explored. Moreover, the renovated PL treatment requires an intelligent PL system that allows the optimal exposure of entire food to light thereby enhancing decontamination and enabling the widespread application of PL technology (Mahendran et al., 2019; Pirozzi et al., 2021).

2.8. Conclusions

The direct application of pulsed light (PL) to liquid food products offers promising opportunities for the food industry, aiming to enhance safety, shelf life, and functionality without compromising quality. Despite these advantages, achieving industrial-scale implementation requires further efforts. Challenges such as the shadow effect, limiting uniform light exposure, and reducing treatment efficiency, along with sample heating, remain significant concerns. While transparent drinks are efficiently treated, PL effectiveness diminishes with opaque or turbid liquids. Overcoming these challenges involves optimizing processing conditions and adopting technological solutions for improved uniformity and reduced heating effects. Ongoing developments include enhancements in treatment chamber design with integrated cooling systems for treating a broad range of liquid foods. Additionally, combining PL with other decontamination techniques is explored to enhance antimicrobial effects while minimizing energy consumption. Lastly, further comprehensive studies are essential to fully exploit PL's potential in industrial applications.

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Preface to Chapter 3

In the preceding chapter, a comprehensive literature review identified existing knowledge gaps concerning the application of pulsed light (PL) technology in processing liquid foods. Notably, there is limited research on treating liquid products, particularly in exploring the potential of agitation to enhance PL treatment efficacy for larger volumes and various liquids with differing transparency levels. Consequently, this research focuses on evaluating the effectiveness of agitation-assisted PL applications in liquid products.

This chapter (Chapter 3) investigated the efficacy of agitation-assisted PL treatment using water as a model liquid medium to establish process parameters and understand the influence of both process and product variables. The study aimed to determine the relationship between PL effectiveness, agitation speed, and water volume, employing *Escherichia coli* K-12 as a surrogate microorganism.

All experimental work, data analysis, and manuscript preparation were conducted by the candidate under the supervision of Prof. H. S. Ramaswamy.

Based on this study, the following manuscript has been prepared for publication in a peer reviewed journal:

Mategeko, B., & Ramaswamy, H.S. (2025). Enhanced inactivation of *Escherichia coli* in water using agitation-assisted pulsed light treatment. Prepared for publication in the *Journal of Food Science* (or an alternative journal).

Chapter 3

Enhanced Inactivation of *Escherichia coli* in Water Using Agitation-Assisted Pulsed Light Treatment

3.1. Abstract

Pulsed light (PL) treatment is a promising non-thermal technology for ensuring the safety and quality of food and water. Its effectiveness in liquids, however, varies due to challenges such as reduced light penetration and uneven exposure, especially in larger volumes. Effective mixing and optimized treatment durations are therefore critical for enhancing PL's efficiency. In this study, sterile water inoculated with *Escherichia coli* K-12 (EC1-5G) was subjected to various PL treatments to evaluate the impact of treatment duration, mixing agitation speed, and sample volume on microbial inactivation. A first-order kinetic model was applied to calculate D-values (time required for a 90% reduction in the microbial population). At a constant sample volume of 400 mL, increasing agitation mixing speed significantly decreased D-values, from 6.06 s under static conditions (0 rpm) to 2.96 s at 1,000 rpm. Conversely, increasing sample volume at a constant agitation speed of 500 rpm resulted in a significant rise in D-values, from 0.63 s for 100 mL to 4.40 s for 400 mL, emphasizing the need for longer treatment times in larger volumes. Interestingly, treating multiple containers simultaneously resulted in microbial inactivation levels comparable to those observed in single-container treatments. The findings underscore the importance of optimizing agitation mixing speed and appropriate sample volumes or simultaneously treating multiple containers to enhance PL decontamination efficiency and scale-up considerations. These strategies ensure effective microbial reduction while maintaining operational efficiency. This study highlights the potential of PL as a scalable and energy-efficient method for drinking water treatment, providing a reliable solution for microbial inactivation across varying volumes and possible continuous flow conditions.

Key words: Pulsed light, water, agitation, liquid volume, inactivation

3.2. Introduction

Water is an essential resource for all living organisms, including humans, who rely on it for survival. Comprising about 70% of the human body, water is indispensable to life. The 2021 United Nations World Water Development Report reveals that global freshwater usage has grown sixfold over the last century and has been rising at a rate of about 1% annually since the 1980s (United Nations, 2021). The increasing water demand emphasizes the need to protect drinking water from contamination. Water quality is increasingly at risk, with pollution primarily stemming from industrial processes, agricultural activities, natural environmental factors, and inadequate water supply and wastewater treatment infrastructure (Lin et al., 2022). Whether physical, chemical, biological, or radiological, water contamination can have detrimental effects on all forms of life (Kristanti et al., 2022). Biological contaminants typically refer to pathogenic microorganisms, including harmful bacteria, viruses, parasites, and protists (Behnam et al., 2013). Microbial contamination of water is frequently linked to fecal matter originating from human sources, domesticated animals, or wildlife (Hunter et al., 2002; Jung et al., 2014). In 2022, approximately 1.7 billion people worldwide were compelled to rely on drinking water contaminated with feces, while 2.2 billion lacked access to basic water services (World Health Organization, 2023). Unsafe drinking water, poor sanitation, and inadequate hand hygiene contributed to over 829,000 deaths from diarrhea, including nearly 300,000 children under the age of five, accounting for 5.3% of all deaths in this age group (United Nations, 2021).

Effective disinfection and rigorous sanitary quality monitoring are essential to safeguard drinking water and minimize the risk of waterborne diseases. *Escherichia coli* (*E. coli*), a widely recognized indicator of fecal contamination, has been a focal point for water quality assessment due to its direct association with gastrointestinal illnesses and the presence of pathogenic strains such as *E. coli* O157:H7 (Coleman et al., 2013; Health Canada, 2019, 2021). Health Canada, in alignment with other globally recognized regulatory bodies such as the World Health Organization (WHO), the European Union, the United States Environmental Protection Agency (U.S. EPA), and the Australian National Health and Medical Research Council, has set stringent standards for drinking water quality, requiring that *E. coli* be none detectable per 100 mL of water, emphasizing that meeting these standards is crucial for protecting public health (Health Canada, 2019). To meet these standards, various water disinfection technologies have been developed, including chemical disinfectants such as chlorine, chloramine, ozone, and chlorine dioxide, and physical methods like ultraviolet (UV) irradiation. Among these, chlorine remains one of the most used disinfectants due to its cost-effectiveness and the ability to provide residual

protection. However, the use of chlorine has been linked with the formation of harmful disinfection by-products (DBPs), such as trihalomethanes, which pose major long-term health risks, including carcinogenicity (Kalita et al., 2024).

As concerns over DBPs grow, there is a heightened emphasis on exploring alternative disinfection methods that are both effective and environmentally sustainable. Among these, continuous ultraviolet (UV) light has emerged as a viable option. Despite the need for relatively high doses to achieve effective microbial inactivation, UV light continues to be regarded as a promising disinfection strategy (Bohrerova et al., 2008). UV irradiation has emerged as an efficient method of disinfection due to its ability to inactivate a wide range of microorganisms without producing chemical by-products (Song et al., 2016). Traditional UV disinfection has relied on low-pressure and medium-pressure mercury lamps, but these have several drawbacks, including the use of toxic mercury, low energy efficiency, and relatively short operational lifespans of approximately 10,000 h (Autin et al., 2013; Chevremont et al., 2013). Additionally, traditional UV systems generate continuous UV light with power dissipation ranging from 100 to 1,000 W. Achieving the high-intensity energy density required for effective disinfection with continuous UV light can be expensive, necessitating designs that maximize the conversion and collection efficiency of UV radiation. To address these challenges, pulsed light systems have been developed, capable of dissipating megawatts of electrical power within the light source (Uslu-Senel & Demirci, 2024).

Pulsed light (PL) disinfection is an emerging technology that has been accepted by both the European Union (EU) and the U.S. Food & Drug Administration (FDA) as an effective microbial disinfection method for the food industry (Elmnasser et al., 2007). PL technology involves the application of intense, short bursts of broad-spectrum white light with wavelengths ranging from 100 nm to 1,100 nm, generated by inert-gas flash lamps, commonly xenon lamps. Each light pulse lasts only a fraction of a second and has an intensity approximately 20,000 times stronger than sunlight at sea level (John & Ramaswamy, 2018; Pollock et al., 2017). The germicidal action of PL is primarily attributed to the UV component, which induces the formation of thymine dimers in microbial DNA, thereby disrupting replication and transcription and leading to cell death (Elmnasser et al., 2007; Kramer & Muranyi, 2014; Woodling & Moraru, 2007). Additionally, the localized temperature increase caused by the absorption of UV and IR radiation enhances microbial reductions, accelerating bacterial disruption (Mandal et al., 2020; Takeshita et al., 2003; Wekhof, 2000).

PL treatment has demonstrated superior effectiveness compared to conventional UV light treatment, primarily due to its higher and instantaneous energy impact. In a comparative study on pulsed light (PL) and continuous wave UV irradiation for water decontamination, the results demonstrated that *E. coli* inactivation occurred significantly more rapidly with PL than with continuous wave low- or medium-pressure UV systems at equivalent doses (Bohrerova et al., 2008). Furthermore, pulsed UV has shown potential in reducing the organic load in wastewater, highlighting its dual capability for disinfection and organic matter degradation (Uslu et al., 2016). Despite its advantages, PL technology faces certain challenges. The high doses of energy required to achieve effective microbial inactivation and the potential for thermal damage to the treated medium are concerns that limit its application. Additionally, the effectiveness of PL treatment can be influenced by factors such as water turbidity, UV transmission, and flow dynamics within the treatment system (Uslu et al., 2016). Enhancing the efficacy of PL while mitigating these limitations requires innovative approaches, such as incorporating agitation to improve uniform exposure and penetration.

Agitation is a mechanical process that facilitates the mixing of a liquid medium, ensuring that microorganisms within the water are uniformly exposed to the light source. By preventing the settling of suspended solids and reducing shadowing effects, agitation can enhance the distribution of PL energy, thereby improving the overall disinfection efficiency. Studies have demonstrated the effectiveness of agitation-assisted processes in various water treatment applications (Lehr et al., 2005; Nie et al., 2021), but its integration with PL technology for microbial inactivation remains underexplored. The concept of agitation-assisted PL treatment is particularly relevant for achieving effective disinfection in larger sample volumes or in systems where uniform energy distribution is critical. Agitation ensures that all microbial cells are exposed to high-intensity pulses of light, thereby addressing the limitations associated with penetration depth and uneven exposure in static systems. Additionally, agitation can potentially reduce the total energy requirement by enhancing the efficacy of each PL pulse, making the process more energy-efficient and cost-effective.

Therefore, this study aims to investigate the enhanced inactivation of *E. coli* K-12 (EC1-5G), a surrogate for the pathogenic strain *E. coli* O157:H7, in water using agitation-assisted pulsed light treatment. The main objectives are to assess the impact of agitation on the microbial inactivation kinetics of pulsed light and evaluate inactivation across different sample volumes. Additionally, the study will model and validate the inactivation kinetics to determine the decimal reduction

time (D-value) under various treatment conditions, offering insights into optimizing PL systems for large-scale water disinfection applications.

3.3. Materials and methods

3.3.1. Preparation of *Escherichia coli* K-12 inoculum culture

3.3.1.1. Reactivation of lyophilized cell stock

Lyophilized *Escherichia coli* K-12 (EC1-5G) cells were obtained from MilliporeSigma (a division of Merck KGaA, Darmstadt, Germany, operating in the USA and Canada). To reactivate the cell stock, 10 mL of Tryptic Soy Broth (TSB, Sigma-Aldrich, Difco, MO, USA) was added to the vial containing the lyophilized bacteria. The bacterial powder was then thoroughly mixed with the broth to ensure dissolution. The resulting suspension was streaked onto Tryptic Soy Agar (TSA, Difco) plates to isolate single colonies. These plates were incubated at 37°C for 24 h to promote bacterial growth. Uniform single colonies were selected using a sterile loop and transferred individually into 1.7 mL microcentrifuge tubes containing 1 mL of 20% glycerol for long-term storage at -40°C.

3.3.1.2. Preparation of stock solution

The preparation of the stock solution followed the methodology described by John and Ramaswamy (2020), with minor modifications. The culture of *Escherichia coli* K-12 (EC1-5G), stored in 20% v/v glycerol solution at -40°C for long storage purposes, was first thawed on ice. Thawed cells were further streaked onto TSA plates with a sterile loop and incubated at 37°C for 24 h. A single colony from the TSA plate was then transferred into 50 mL of tryptic soy broth (TSB, Sigma-Aldrich, Difco, MO, USA) and incubated under shaking conditions at 37°C for 24 h. After incubation, the dense culture solution was centrifuged at 4,000 RPM for 20 min using a benchtop centrifuge system, and the supernatant was discarded. The resulting pellet was washed by resuspending it in sterile distilled water, followed by centrifugation at 4,000 RPM for 20 min. This washing process was repeated three times, and the final pellet was resuspended in 50 mL of sterile distilled water, yielding a stock solution with an approximate bacterial population of 10^8 CFU/mL. The prepared stock solution was stored at 4°C until it was inoculated into samples for PL treatment.

3.3.2. Sample preparation and inoculation

The water sample was prepared by autoclaving distilled water at 121°C for 20min to get sterile distilled water. Inoculation was carried out by adding 50 mL of *E. coli* K-12 (EC1-5G) stock solution into 450 mL of sterile distilled water, followed by thorough mixing to ensure uniform distribution. Various volumes (100 mL to 400 mL) of the inoculated samples were then transferred into 600 mL glass jars (Figure 3.1) of dimensions 9.4x10.8cm (diameter x height) covered with quartz lids, which were subsequently subjected to different PL treatments.

3.3.2.1. Pulsed light treatment

The SteriPulse®-XL RS-3000 C pulsed light system (Xenon Corp., Wilmington, Massachusetts, USA) was utilized for the PL treatment of the samples. This system comprises a power/control module, a linear Xenon flash lamp, and a lamp enclosure module. According to the manufacturer's specifications, it generates three high-intensity pulses per second (pulse width: 360 μs) of polychromatic light with wavelengths ranging from 100 to 1,100 nm and an energy output of approximately 1.27 J/cm^2 per pulse at a distance of 1.9 cm from the quartz window surface (John & Ramaswamy, 2020; Pataro et al., 2011). The system includes a treatment chamber measuring approximately 16 × 40 × 16 cm (W × L × H) with a tray rack featuring 11 positions at distances ranging from 1.9 cm (Tray 0) to 14.6 cm (Tray 10) from the light source (Figure 3.1.a). The proximity of the sample to the light source plays a critical role, as closer distances result in higher PL intensity and faster bacterial inactivation (John & Ramaswamy, 2020). The treatment chamber is equipped with a forced air evacuation system to prevent heat accumulation from the lamp. This system included filters at both ends that effectively remove germs and are ozone-resistant (Prusty, 2020).

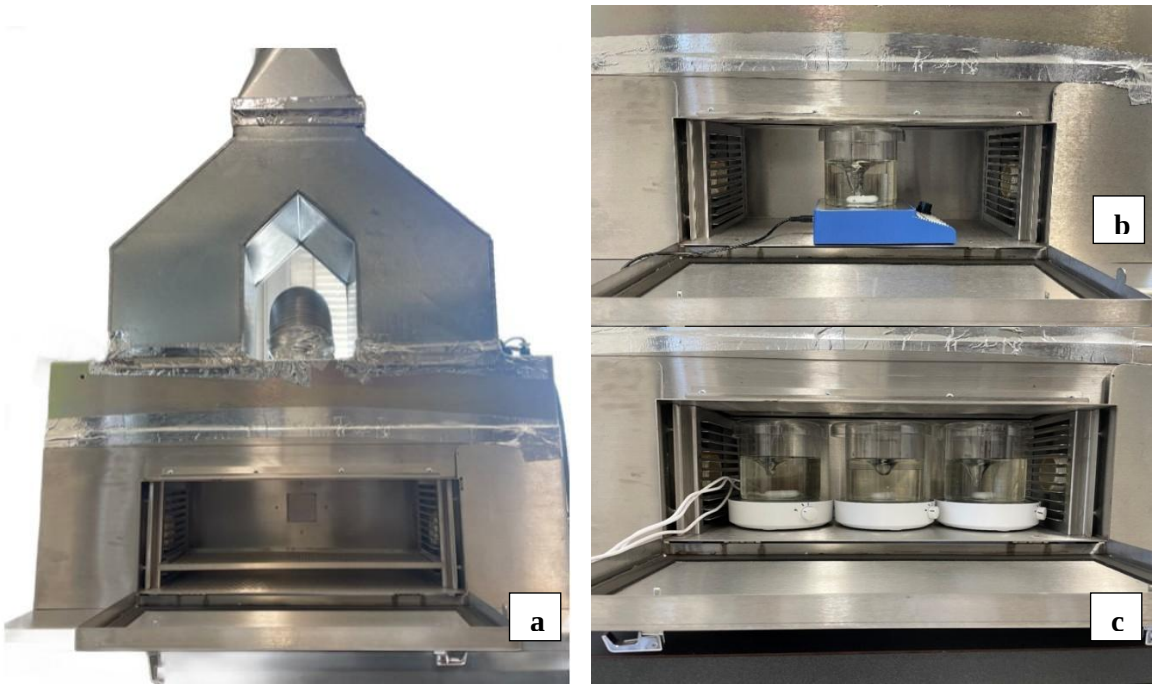


Figure 3.1. Pulsed light treatment system: (a) SteriPulse-XL RS-3000C, agitation setup with (b) single container, and (c) triple containers.

A magnetic stirrer (approximately 4.8 cm in height) with a maximum agitation speed of 3,000 rpm was used to create an agitation mechanism (Figure 3.1.b). The water samples, contained in glass jars with magnetic bars, were placed on the stirrer, which was set to operate at three different agitation speeds: 500 rpm, 750 rpm, and 1,000 rpm. This setup was used to investigate the effect of agitation on enhancing microbial inactivation by PL. Since the combined height of the magnetic stirrer and the glass jar (15.6 cm) matched the treatment chamber's height (16 cm), all treatments were conducted at a consistent distance of approximately 11.2 cm from the light source. The intensity of light diminishes proportionally to the square of the distance from the light source, as described by the inverse square law (Bolton & Linden, 2003; Gómez-López & Bolton, 2016). The formula to determine the fluence at a different distance is given in Equation 3.1:

$$F_2 = F_1 \, x \, \left(\frac{d_1}{d_2}\right)^2 \qquad (3.1)$$

Where F_1 is the fluence at distance d_1 and F_2 is the fluence at distance d_2.

Agitation speed was limited to 1,000 rpm because higher speeds caused increased splashing onto the jar lid, which could potentially obstruct light penetration. The samples were subjected to PL treatment durations ranging from 1 s (3 pulses) to 20 s (60 pulses), depending on the sample

volume. Additionally, PL treatments were performed using multiple containers (Figure 3.1.c) to compare microbial inactivation across concurrent setups (single, double, or triple containers).

3.3.3. Microbial enumeration and log-linear kinetic model

After undergoing PL treatment, the samples were aseptically mixed and serially diluted. A volume of 100 µL from serial dilutions was then plated in duplicate onto Tryptic Soy Agar (TSA) (DIFCO). The plates were incubated at 37°C for 24 h, after which colony enumeration was performed. Only plates containing 30-300 colonies were considered for analysis. All experiments were carried out in triplicates. The microbial concentration (CFU/mL) was calculated using Equation 3.2:

$$CFU/mL = \left(\frac{\text{Number of colonies}}{\text{Volume of sample plated}}\right) x\ Dilution\ factor \tag{3.2}$$

When necessary, microbial counts were initially normalized to a uniform starting load (control) of 7.0 log CFU/mL using Equation 3.3 to enable better comparison of microbial survival across PL treatments.

$$log_{10}\ N = log_{10}(\frac{N}{N_0} x\ 10^7) \tag{3.3}$$

Where N_0 (CFU/mL) denotes the initial population of *E. coli* before treatment, while N (CFU/mL) represents the number of bacteria after the PL treatment at time t (s).

To analyze the data using a semi-logarithmic model (representing first-order kinetic behavior), it was considered that an increase in PL treatment time would result in a logarithmic decrease in bacterial survival. For the log-linear model, also referred to as the D-value model, the following equation was utilized:

$$log_{10}(N/N_0) = -\ t/D\ ;\ (t \geq 0);\ Slope = -\ 1/D \tag{3.4}$$

In Equation 3.4, N_0 (CFU/mL) refers to the initial population of *E. coli*, while N (CFU/mL) represents the number of bacteria that survived after PL treatment at a specific time t (s). The D-value indicates the time required to achieve a 90% reduction in the microbial population. The D-value was calculated based on the slope of the plot of $\log_{10}$ (N/N_0) versus treatment time (s) as detailed by John and Ramaswamy (2020).

3.3.4. Statistical analysis

The experiments were carried out in triplicates, and the results are presented as mean ± standard deviation. Data analysis was performed using one-way ANOVA (α = 0.05) with SPSS Statistics for Windows, version 29.0 (IBM Corp., Armonk, USA). Statistically significant differences were further evaluated by comparing mean values using Tukey's test at a 95% confidence level.

3.3.5. Model evaluation

The goodness of fit for the inactivation model (log-linear model) in this study was evaluated using the root mean square error (RMSE), with lower values closer to 0 indicating a better fit. Additionally, a higher regression coefficient (R^2), approaching 1, was used to determine the most accurate fit among different relationships among the data, including linear, exponential, logarithmic, and polynomial.

3.4. Results and discussions

3.4.1. Pulsed light microbial inactivation as influenced by agitation speed

Water was selected for this study to assess the impact of agitation on enhancing the microbial inactivation efficiency of pulsed light due to its high light transmittance, which makes it more responsive to PL treatment compared to other non-transparent beverages. Also, *E. coli* was chosen as the model organism because of its common use as an indicator of water quality and its relevance in evaluating the effectiveness of disinfection methods. The non-pathogenic *E. coli* K-12 (EC1-5G) strain was used as a surrogate for the pathogenic *E. coli* O157:H7, ensuring safety while preserving the validity of the experimental results.

The 3-D bar graph in Figure 3.2 illustrates the residual survivor counts in nominal logarithmic units, starting from an initial microbial population of approximately 7.0 log CFU/mL. The data clearly demonstrates that *E. coli* K-12 inactivation in 400 mL of water was influenced by both treatment time and agitation speed. For any given agitation speed, the level of inactivation increased with longer treatment times. Similarly, for any given treatment time, a higher degree of agitation speeds led to greater inactivation efficiency. This graph (Figure 3.2) provides a spatial mapping of the effect of pulsed light intensity on microbial reductions across varying treatment times and agitation speeds. The findings align with expectations, showing that the extent of

inactivation increased with longer exposure times, as greater treatment duration resulted in a higher degree of microbial reduction.

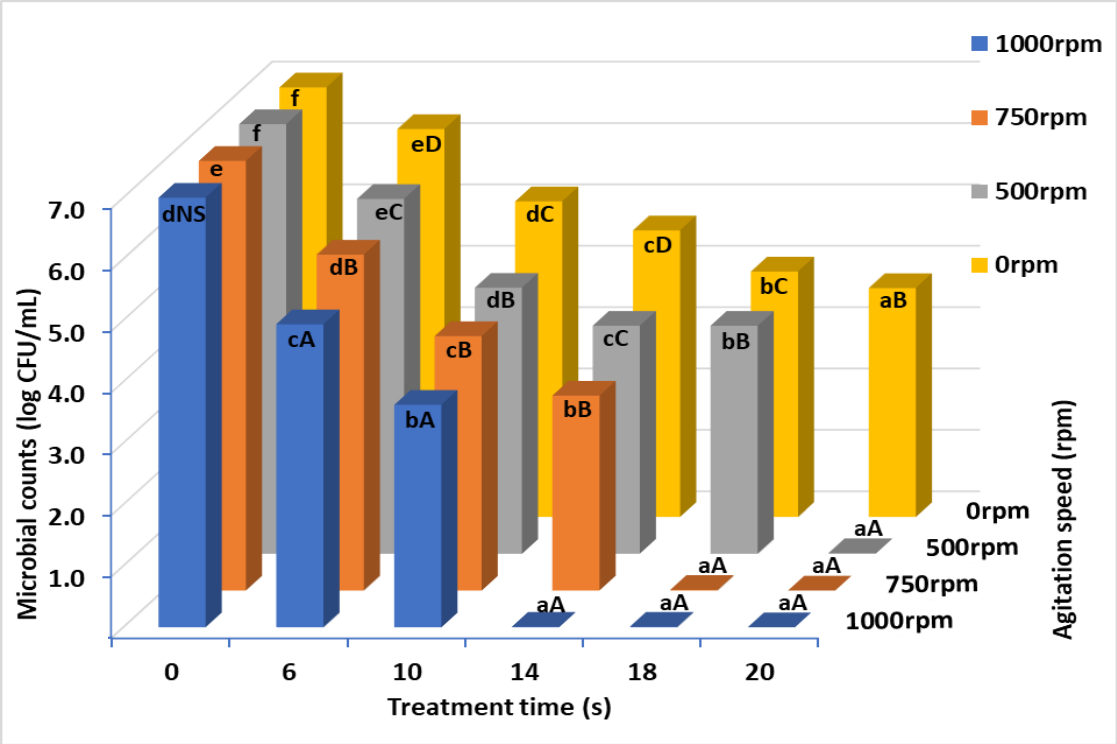


Figure 3.2. PL destruction of *Escherichia coli* K-12 in water as influenced by treatment time and agitation speed using the same volume of 400mL.

Note: NS stands for non-significant difference (p <0.05) amongst agitation speeds for treatment time.

The inactivation patterns at different agitation speeds appeared similar, suggesting they followed a comparable inactivation model. At an agitation speed of 1,000 rpm, no residual microbial activity was detected after a 14-s treatment, while similar results required 18 s and 20 s of PL treatment at 750 rpm and 500 rpm, respectively. The effect of agitation speed on inactivation followed the expected trend, with higher speeds enabling faster and greater exposure of liquid particles to pulsed light. In contrast, non-mixed water experienced minimal PL intensity, requiring more time for the light to effectively penetrate the entire liquid volume. The influence of agitation on the inactivation of *E. coli* was two folds. It provided an effective mixing of the contents and a gradual and uniform inactivation of the bacteria. Secondly, it provided new and continuous exposure of bacterial cells to PL to make sure the PL distribution is good even with lower penetration situations (limiting the absorption to the top layers). This is the most serious limitation in static volume treatments because the inner volumes will not receive adequate PL exposure.

As shown in Table 3.1, the microbial inactivation efficiency of PL treatment for 400 mL of water was significantly improved by mixing water inoculated with *E. coli* K-12. This is evident from the results, where a 10-second PL treatment reduced the initial microbial load from 7.0 log to 5.1 log, 4.3 log, 4.1 log, and 3.6 log at agitation speeds of 0 rpm, 500 rpm, 750 rpm, and 1,000 rpm, respectively. Furthermore, increasing the agitation speed by 500 rpm enhanced the microbial reduction rate by approximately 1 log for the same PL treatment duration.

The use of agitation during PL treatment of water has demonstrated that higher agitation speeds can achieve the desired level of microbial decontamination more quickly. This approach can save both time and energy, provided the energy required to create the mixing mechanism is lower than that consumed by the PL treatment. As shown in Table 3.1, a 20-second PL treatment reduced microbial counts to non-detectable levels at 1,000 rpm, whereas still water under the same conditions retained approximately 3.7 log of microbial load.

Table 3.1. Counts of *E. coli* K-12 after treating 400mL of water at various agitation speeds

Treatment time (s)	**Microbial counts (log CFU/mL)**			
	1,000rpm	**750 rpm**	**500 rpm**	**0 rpm**
0	7.00 ± 0.00^{dNS}	7.00 ± 0.00^{e}	7.00 ± 0.00^{f}	7.00 ± 0.00^{f}
6	4.9 ± 0.03^{cA}	5.47 ± 0.03^{dB}	5.78 ± 0.07^{eC}	6.32 ± 0.10^{eD}
10	3.62 ± 0.17^{bA}	4.14 ± 0.02^{cB}	4.33 ± 0.03^{dB}	5.14 ± 0.03^{dC}
14	$<1^{aA}$	3.18 ± 0.08^{bB}	3.72 ± 0.04^{cC}	4.67 ± 0.15^{cD}
18	$<1^{aA}$	$<1^{aA}$	3.22 ± 0.04^{bB}	4.00 ± 0.10^{bC}
20	$<1^{aA}$	$<1^{aA}$	$<1^{aA}$	3.73 ± 0.09^{aB}

Note: Values represent the means (n=3) ± SD of microbial counts. Different lowercase letters indicate, for each evaluated agitation speed, significant differences ($p < 0.05$) among treatment times. Different uppercase letters indicate, for each treatment time, significant differences ($p < 0.05$) among the evaluated agitation speeds. NS: non-significant difference amongst agitation speeds for a given treatment time.

The first-order plot of log reduction versus time (s) shown in Figure 3.3 demonstrates a strong fit to the data (R^2 close to 1), allowing for the calculation of the corresponding D-values. The time effect observed in Figure 3.2 was incorporated into the kinetic model, making the 2-D plot sufficient to assess the impact of agitation. The curves exhibited steeper slopes at higher agitation speeds, with the slopes gradually decreasing as the mixing speed was reduced to 0 rpm (static condition). This clearly illustrates that microbial destruction was significantly greater at higher agitation speeds compared to non-agitated water, aligning with the reduced exposure of water particles to pulsed light in the absence of mixing.

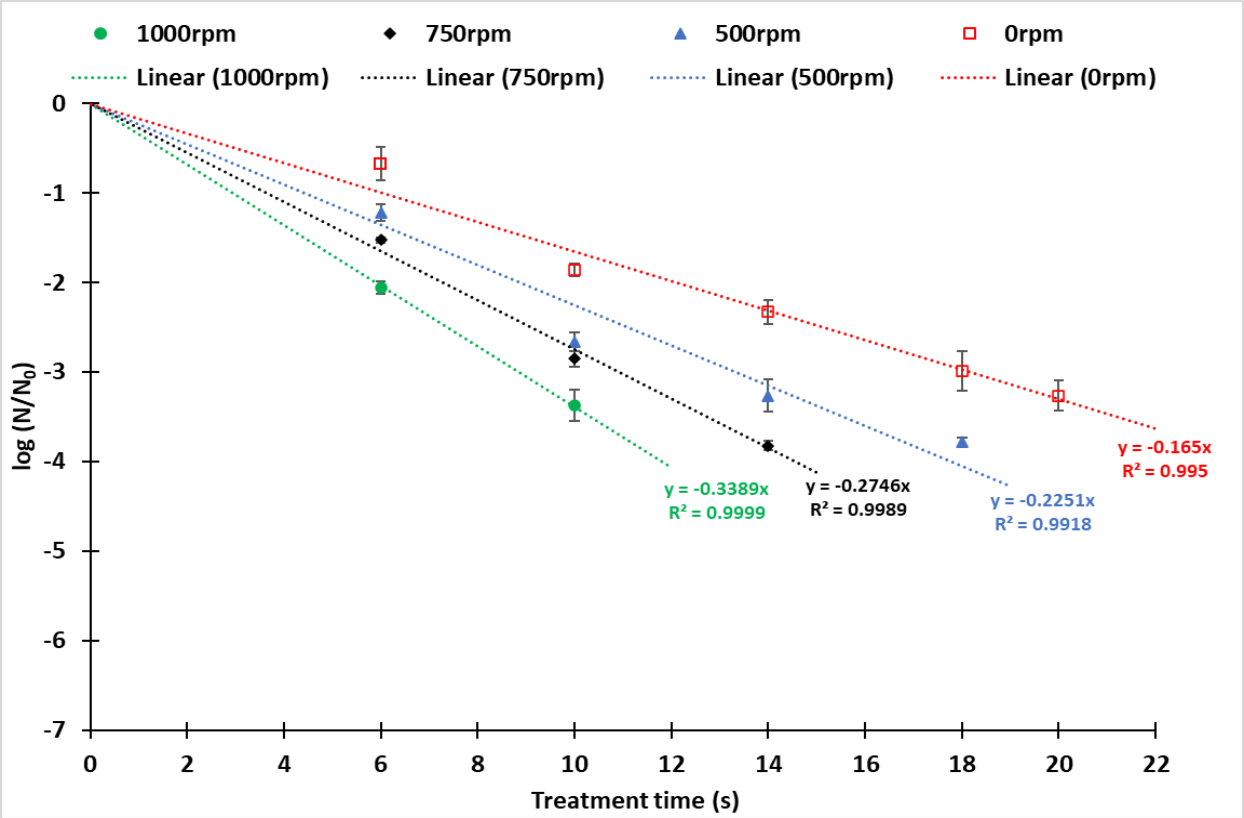


Figure 3.3. Log-linear model of *E. coli* K-12 inactivation kinetics in water as agitation speed increases (0-1,000rpm) at a constant water volume (400mL).

The curves exhibited steeper slopes at higher agitation speeds, with the slopes gradually decreasing as the mixing speed was reduced to 0 rpm (static condition). This clearly illustrates that microbial destruction was significantly greater at higher agitation speeds compared to non-agitated water, aligning with the reduced exposure of water particles to pulsed light in the absence of mixing.

Table 3.2 summarizes the computed D-values obtained from the first-order model curves shown in Figure 3.3 for PL treatments of a 400 mL liquid sample at different agitation speeds. These values were calculated with the stirrer's top surface located approximately 11.2 cm from the light source, corresponding to a fluence of approximately 0.035 J/cm^2/pulse, determined using the inverse square law (Equation 3.1) based on an initial fluence of 1.27 J/cm^2/pulse at 1.9 cm from the light source. The table also includes the variability associated with the D-values. At the highest agitation speed of 1,000 rpm, the D-value was calculated to be 2.96 s, indicating that a five-log reduction could be achieved with approximately 15 s of treatment, corresponding to a total fluence of 1.58 J/cm^2. In contrast, under static conditions (0 rpm), achieving the same five-log reduction would require about 30 s, which is roughly double the treatment time at 1,000 rpm. This demonstrates that appropriate treatment conditions can facilitate a five-log reduction while

remaining within the FDA limit of 12 J/cm^2. In a different approach using plastic pouches to fill the inoculated water samples (limiting the depth to a minimum level), Mittal (2023) observed that a PL treatment of 150 mL for 15 s resulted in only a 2.6-log reduction. Similarly, John and Ramaswamy (2020) treated 5 mL samples inoculated with *E. coli* in a 50 mm petri dish, achieving a five-log reduction within just 5 s at a distance of 10.8 cm from the light source. The differences in outcomes between these studies and the present research are likely influenced by multiple factors, with the most significant being variations in sample volume and the distance from the light source.

Table 3.2. D-values for 400mL water treated with PL at different agitation speeds

Agitation speed (rpm)	D-value (s)	5D (s)	7D (s)
0	6.06 ± 0.15^{d}	30.3	42.4
500	4.40 ± 0.09^{c}	22.0	30.8
750	3.64 ± 0.06^{b}	18.2	25.5
1,000	2.96 ± 0.13^{a}	14.8	20.7

Note: The data represents the mean of three replicates ± SD. Different superscripts within a column indicate a significant difference at $p \leq 0.05$.

A significant decrease in D-value ($p < 0.05$) was observed with increasing agitation speed. This is because greater mixing allows the liquid particles to be exposed to the light more quickly and effectively, resulting in higher received light intensity and shorter treatment times. This underscores the critical role of agitation in improving the efficiency of PL microbial inactivation, thereby reducing the treatment time needed to meet specific microbial reduction targets. For instance, achieving a 7-log reduction of *E. coli* K-12 in 400 mL of water in this study required approximately 21 s of PL treatment, corresponding to a total fluence of about 2.17 J/cm^2. In comparison, Hwang et al. (2015) achieved a 7-log reduction of *Pseudomonas aeruginosa* in 10 mL of mineral and isotonic water using a 90 mm petri dish with a total fluence of 0.97 J/cm^2. The differences in results can be attributed to several factors, including variations in the equipment used. This study utilized the SteriPulse® XL-RS 3000C, which features a longer pulse width of 360 μs and higher energy, whereas Hwang et al. (2015) employed the Heraeus Noblelight XAP Series with a pulse width of 200 μs. Additionally, differences in sample volumes and the type of microorganisms studied may also contribute to the observed variations in microbial inactivation.

3.4.2. Influence of sample volume on microbial inactivation by pulsed light

This study also aimed to evaluate the effect of sample volume on the effectiveness of PL microbial decontamination in liquids and determine its optimal applications. Figure 3.4 presents a 3-D graph illustrating the logarithmic residual survivor counts after various treatment times, with sample volumes ranging from 100 mL to 400 mL. All samples were placed in the same glass jar, covered with a quartz lid, and agitated at a constant speed of 500 rpm. Minor variations in the initial microbial counts (approximately 7.0 log CFU/mL across all samples) were normalized using Equation 3. The normalization involved dividing the actual counts (N) by the initial microbial load (N_0) and multiplying by 10^7, ensuring all samples started at a value of 7.0 log. This adjustment would not affect the relative reduction trends observed in microbial inactivation.

As shown in Figure 3.4, the survivor population steadily decreased with increasing treatment time, following trends like those observed earlier with the 400 mL samples at a given agitation speed. The microbial destruction patterns were consistent across different sample volumes; however, larger volumes required longer treatment times to achieve the same level of inactivation. For instance, the microbial reduction achieved in 8 s with a 400 mL sample was drastically lower than that attained in 4 s with a 100 mL sample during PL treatment.

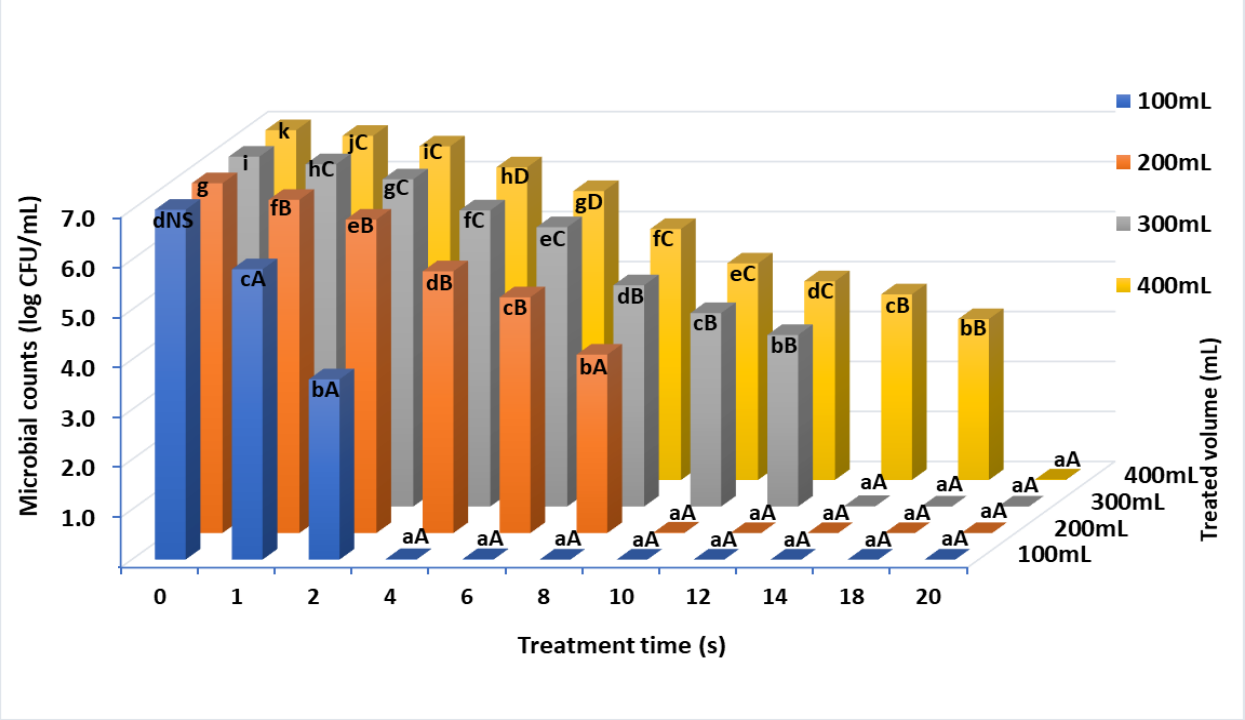


Figure 3.4. PL destruction of *Escherichia coli* K-12 in water as influenced by volume and treatment time at a constant agitation speed of 500 rpm.

Note: NS stands for a non-significant difference ($p < 0.05$) amongst agitation speeds for treatment time.

Table 3.3 illustrates the impact of liquid volume on the microbial inactivation efficiency of PL treatment at a constant agitation speed of 500 rpm. The results show significant differences in microbial reduction across various treatment times. For instance, after a 6-s PL treatment, the initial microbial load decreased from 7.0 log to 5.8 log, 5.6 log, 4.7 log, and became non-detectable for treated volumes of 400 mL, 300 mL, 200 mL, and 100 mL, respectively. Additionally, smaller sample volumes exhibited more rapid inactivation rates. For example, reducing *E. coli* K-12 from 7.0 log to a non-detectable level required just 4 s for a 100 mL sample, while the same reduction took 10 s, 14 s, and 20 s for 200 mL, 300 mL, and 400 mL samples, respectively.

A study by Mittal (2023), which utilized plastic pouches for treating water samples inoculated with *E. coli* K-12, reported a similar influence of sample volume on microbial inactivation. For instance, a 2-second PL treatment of a 10 mL water sample resulted in a 5-log reduction, whereas a 15-second treatment of 150 mL achieved only a 2.6-log reduction.

Table 3.3. Counts of *E. coli* K-12 after PL treatment of various volumes at 500rpm agitation

Treatment time (s)	Microbial counts (log CFU/mL)			
	100mL	**200mL**	**300mL**	**400mL**
0	7.00 ± 0.00^{dNS}	7.00 ± 0.00^{g}	7.00 ± 0.00^{i}	7.00 ± 0.00^{k}
1	5.81 ± 0.05^{cA}	6.67 ± 0.10^{fB}	6.85 ± 0.04^{hC}	6.89 ± 0.07^{jC}
2	3.60 ± 0.10^{bA}	6.27 ± 0.07^{eB}	6.55 ± 0.08^{gC}	6.67 ± 0.08^{iC}
4	$< 1^{aA}$	5.24 ± 0.02^{dB}	5.92 ± 0.06^{fC}	6.25 ± 0.04^{hD}
6	$< 1^{aA}$	4.72 ± 0.13^{cB}	5.59 ± 0.07^{eC}	5.78 ± 0.06^{gD}
8	$< 1^{aA}$	3.57 ± 0.11^{bA}	4.43 ± 0.05^{dB}	5.02 ± 0.06^{fC}
10	$< 1^{aA}$	$< 1^{aA}$	3.87 ± 0.06^{cB}	4.33 ± 0.03^{eC}
12	$< 1^{aA}$	$< 1^{aA}$	3.43 ± 0.02^{bB}	3.98 ± 0.08^{dC}
14	$< 1^{aA}$	$< 1^{aA}$	$< 1^{aA}$	3.72 ± 0.04^{cB}
18	$< 1^{aA}$	$< 1^{aA}$	$< 1^{aA}$	3.22 ± 0.03^{bB}
20	$< 1^{aA}$	$< 1^{aA}$	$< 1^{aA}$	$< 1^{aA}$

Note: Values represent the mean (n=3) ± SD of microbial counts. Different lowercase letters indicate, for each evaluated volume, significant differences ($p < 0.05$) among treatment times. Different uppercase letters indicate, for each treatment time, significant differences ($p < 0.05$) among the evaluated volumes. NS: non-significant difference amongst volumes for a given treatment time.

Similarly, Gómez-López (2011) observed the impact of sample volume on microbial inactivation, suggesting that in multilayered microbial systems, only surface microorganisms are effectively

inactivated, while those deeper within the layers remain protected due to shielding by the uppermost layers.

These findings underscore the importance of agitation in improving PL treatment efficiency. As demonstrated in earlier data, increasing the agitation speed enhances the inactivation rate by allowing faster and more uniform exposure of liquid particles to the light. Thus, optimizing the agitation speed according to the volume being treated is crucial to achieving efficient microbial inactivation while minimizing treatment time and conserving energy.

Figure 3.5 represents a first-order plot of log reduction versus time (s), derived from the 3-D data shown in Figure 3.4. This plot offers important insights into the impact of sample volume on the pulsed light inactivation of *E. coli*. The slope of the curve represents the inactivation rate, with steeper slopes indicating higher efficiency. The negative reciprocal of the slope corresponds to the D-value, where smaller D-values reflect greater inactivation rates.

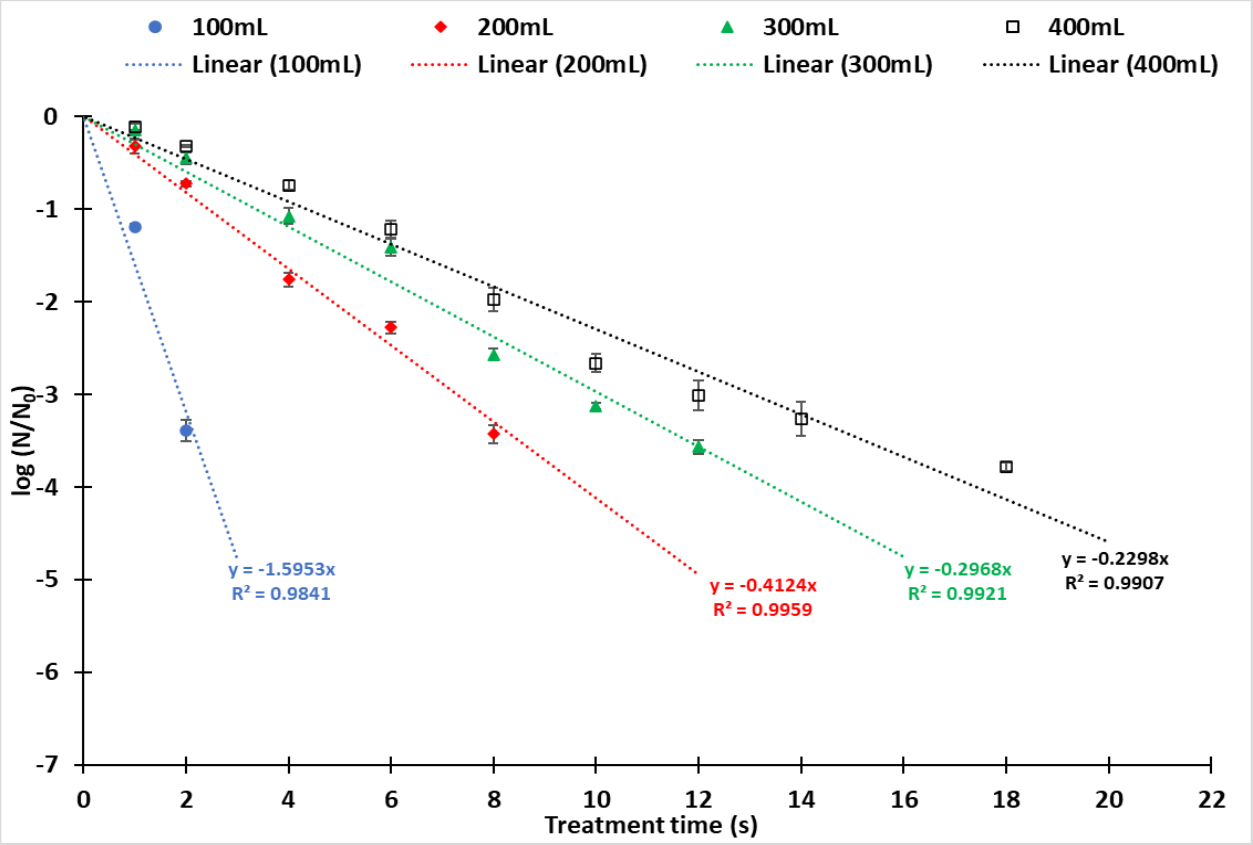


Figure 3.5. Log-linear model of *E. coli* K-12 inactivation kinetics in water with increase in volume (100-400mL) at a constant agitation speed (500rpm).

As anticipated, the 100 mL sample exhibited the steepest slope of -1.595 per s, demonstrating the highest inactivation rate, while the 400 mL sample had the gentlest slope at -0.2298 per s,

reflecting the lowest inactivation rate. This trend highlights that as the sample volume increases, the slope becomes less steep, indicating a reduced PL treatment efficiency with larger volumes.

Table 3.4 summarizes the D-value data for samples of varying volumes treated at a constant agitation speed of 500 rpm. As expected, the D-value increases significantly ($p < 0.05$) with sample volume. To gain deeper insight into the microbial inactivation behaviors, two additional parameters were calculated: sample thickness (volume divided by the surface area of the glass jar lid used for PL treatment) and the D-value per unit volume (D/V). These parameters provide a clearer perspective on the most efficient treatment conditions within the experimental range.

The results show that sample thickness increases proportionally with volume, as the surface area of the jar lid remained constant across all tests. This increase in thickness directly corresponds with a rise in the D-value**.** Larger volumes introduce greater liquid depth, which limits pulsed light penetration and reduces overall inactivation efficiency.

Table 3.4. Thickness, D-values and D/V ratio at different volumes at agitation of 500 rpm

Volume, V (mL)	Thickness, d [V/area] (mm)	D-value (s)	D/V (s/ml)
100	14.42	0.63 ± 0.02^{a}	0.0063
200	28.83	2.43 ± 0.04^{b}	0.0122
300	43.25	3.37 ± 0.05^{c}	0.0112
400	57.67	4.40 ± 0.09^{d}	0.0110

Note: The data represents the mean of three replicates ± SD. Different superscripts within a column indicate a significant difference at $p \leq 0.05$.

The D/V ratio highlights the efficiency of PL treatment per milliliter of liquid. The D-value for any sample can be estimated by multiplying the D/V ratio by the sample volume. For example, achieving a 5-log reduction required approximately 3 s for a 100 mL sample with a D-value of 0.63s. By contrast, 200 mL, 300 mL, and 400 mL samples required longer treatment times of 12 s, 17 s, and 22 s, respectively. These results align with findings from Mittal (2023), who demonstrated that achieving a 5-log reduction in water treated with pulsed light required a total fluence of 7 J/cm^2 for a 10 mL sample, remaining within the FDA dose limit of 12 J/cm^2. However, for larger sample sizes (40 mL or more), longer exposure times were necessary, which resulted in exceeding the FDA dose limit.

To overcome this challenge, we implemented an agitation system that ensures effective mixing, preventing the same surface of the liquid from being repeatedly exposed to PL. With this approach, a 400 mL sample treated for 22 s at 11.2 cm (from the light source) achieved a 5-log reduction using a fluence of approximately 0.035 J/cm^2 per pulse, corresponding to a total fluence of 2.31 J/cm^2. These results highlight the critical role of agitation in improving microbial inactivation during PL treatment. Agitation induces turbulence, which enhances the exposure of deeper liquid layers to PL, thereby boosting the inactivation rate. This effect was similarly observed by Mandal et al. (2020) and reported by Sauer and Moraru (2009), who demonstrated that turbulence significantly enhances PL effectiveness in liquid samples. The importance of agitation becomes even more pronounced when considering the protective nature of microbial multilayers. As suggested by Gómez-López (2011), microorganisms on the surface are readily inactivated, while those deeper within remain shielded from PL exposure. Agitation mitigates this effect by ensuring uniform mixing, thereby exposing all microorganisms to the light and maximizing inactivation efficiency.

The following figures (Figures 3.6, 3.7, and 3.8) provide additional insights by rearranging the data presented in Table 3.4. Figure 3.6 depicts the relationship between sample volume, sample thickness, and their corresponding D-values. It demonstrates that variations in D-values are closely aligned with changes in both volume and thickness. This correlation exists because the sample volume is calculated as the product of surface area (consistent across all samples) and thickness. The figure clearly indicates that the relationship between D-values and sample volume or thickness follows a logarithmic pattern rather than a linear one. To better understand this behavior, Figure 3.7 shows a plot of the logarithm of the D-value against the sample volume and thickness. The resulting polynomial relationship demonstrates a non-linear trend, further confirming that the D-value increases in a non-linear fashion as sample volume or thickness increases.

A more detailed observation of Table 3.4 suggests a reciprocal relationship between D-values and sample volume or thickness. This is further visualized in Figure 3.8.a, which shows the exponential relationship between the reciprocal of sample volume or thickness and the D-value. In contrast, Figure 3.8.b plots the logarithm of D-values against the logarithm of the reciprocal sample volume and the logarithm of reciprocal sample thickness, revealing a polynomial trend.

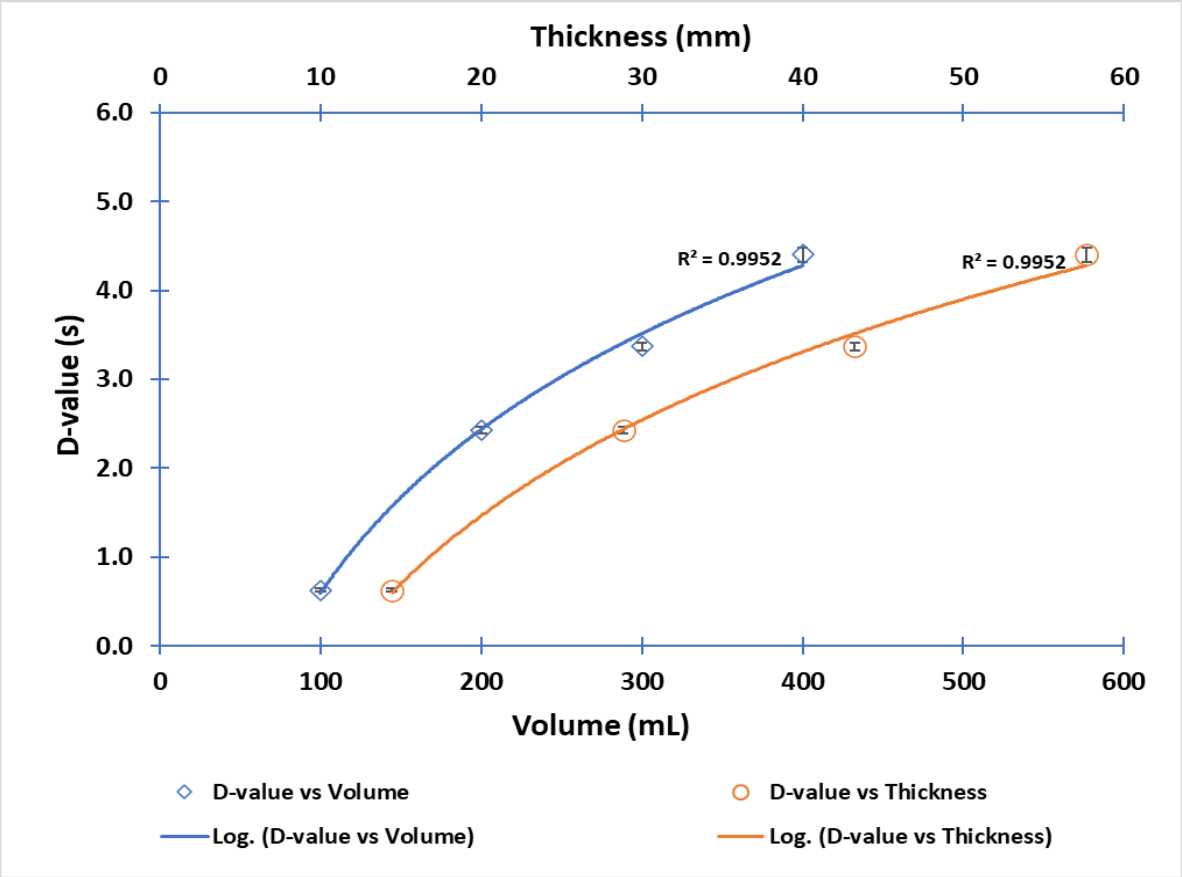


Figure 3.6. Variation in D-value as a function of changes in sample volume or sample thickness.

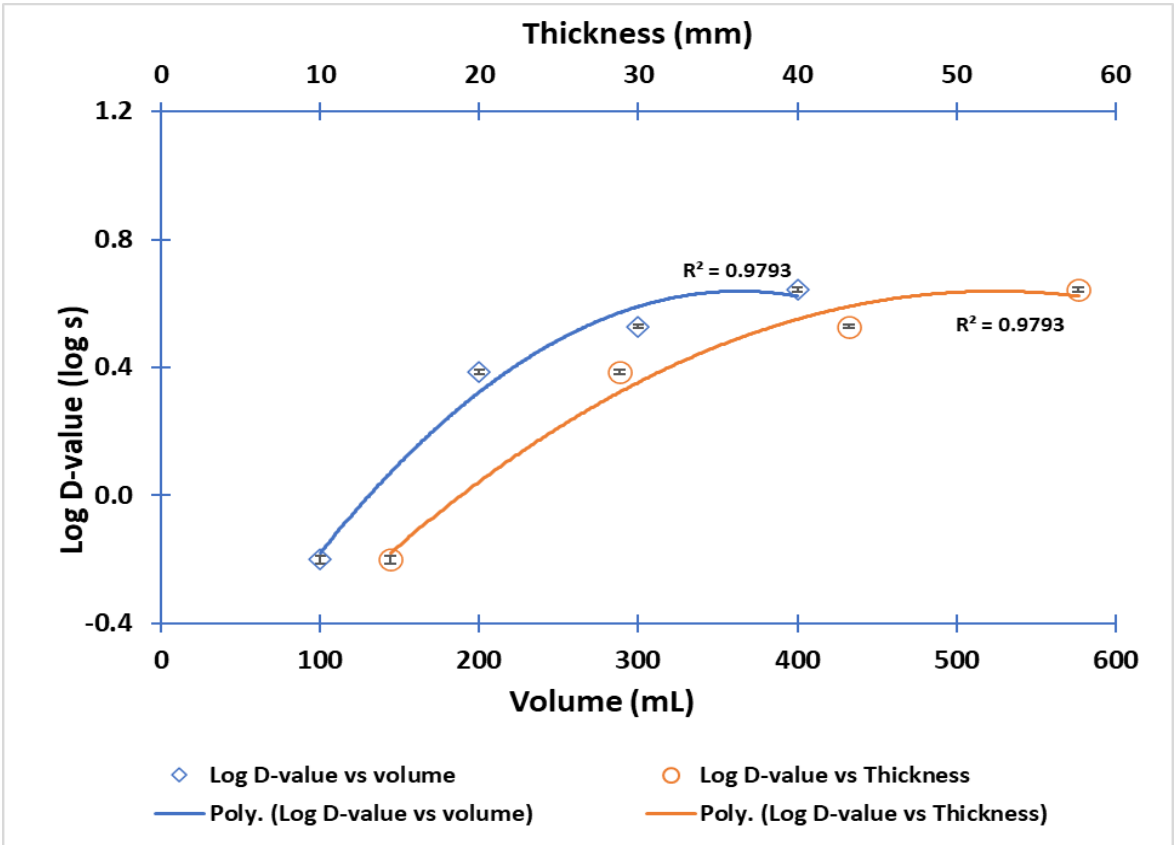
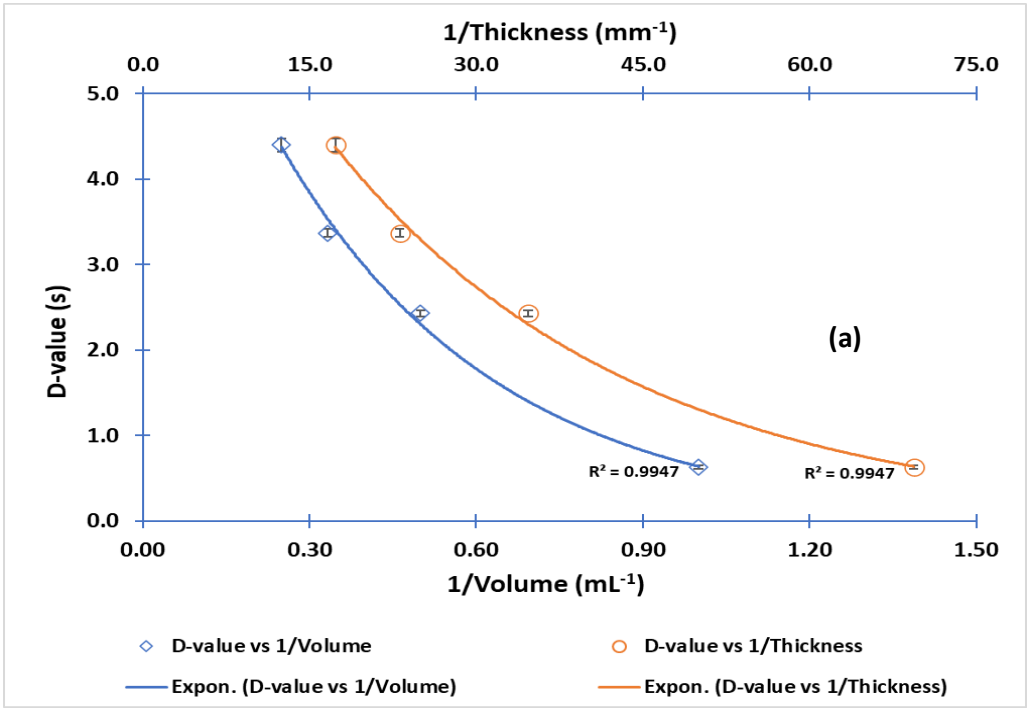


Figure 3.7. Sample volume (mL) and sample thickness (mm) vs Log D-value.

These findings emphasize that the D-value is influenced not only by the sample volume and thickness but also by their reciprocal relationships. Such analyses are useful for predicting microbial inactivation efficiency under varying treatment conditions and optimizing PL processes for different sample sizes. By understanding these trends, it becomes possible to better tailor PL treatment parameters, such as exposure time and energy requirements, to achieve desired microbial reductions efficiently across a range of sample volumes.



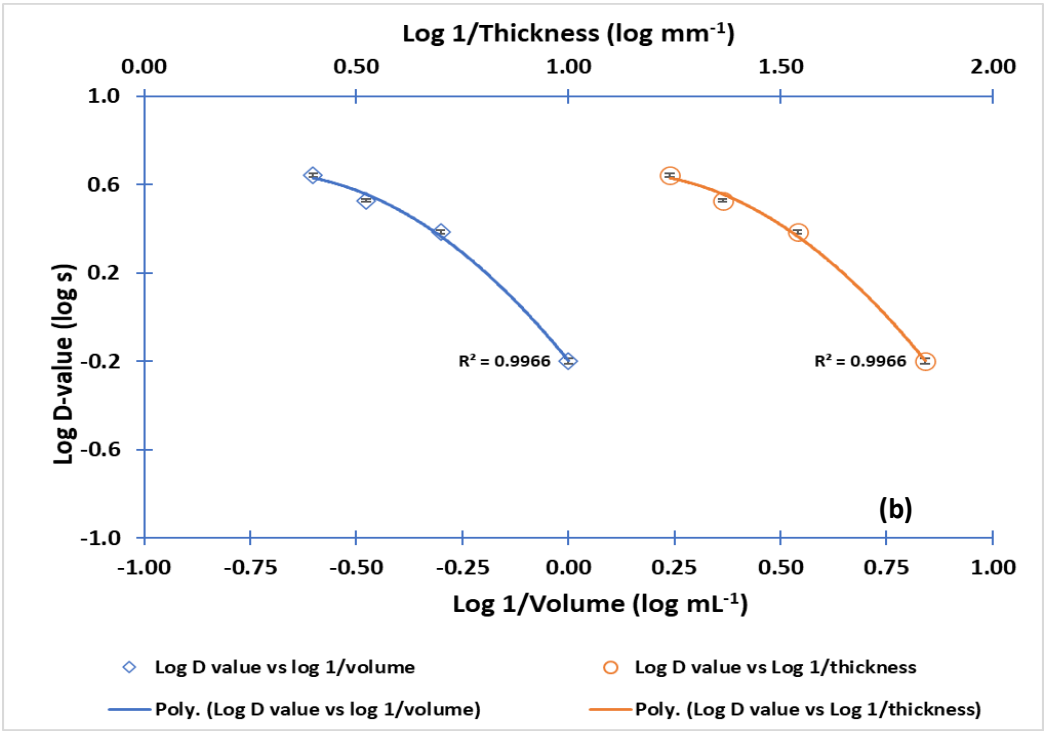


Figure 3.8. (a) Reciprocal sample volume (1/v*100) and reciprocal sample thickness (1/d*1,000) vs D-value and (b) log-log correlation of reciprocal volume and reciprocal thickness with log D-value.

3.4.3. Treatment of multiple containers

Table 3.5 compares the inactivation kinetics of *E. coli* K-12 in 400 mL of sterile water when treated using single, double, or triple containers positioned along the lamp length. The results show that the D-values for all three configurations were very similar, averaging around 4.5 s. Statistical analysis revealed no significant differences in D-values between single and double container setups, or between double and triple container setups.

Table 3.5. Comparison of D-values for various containers simultaneously exposed to PL

Container (s) exposed to PL	D-value (s)
Single container	4.44 ± 0.09^{a}
Left double container	4.73 ± 0.12^{b}
Right double container	4.60 ± 0.12^{ab}
Left triple container	4.52 ± 0.08^{ab}
Middle triple container	4.47 ± 0.09^{ab}
Right triple container	4.60 ± 0.11^{ab}

Note: The data represents the mean of three replicates ± SD. Different superscripts within a column indicate a significant difference at $p \leq 0.05$.

These findings demonstrate that pulsed light treatment can deliver consistent microbial inactivation efficiencies regardless of the number of containers treated simultaneously. This suggests that processing multiple containers at once is a viable and energy-efficient approach, enabling the decontamination of larger liquid volumes without compromising treatment effectiveness.

3.4.4. Pulsed light inactivation optimization with different sample volume

An analysis of D-value per unit volume (D/V) plotted against sample volume, as shown in Figure 3.9, reveals several critical insights. At a sample volume of 100 mL, the D-value was relatively low, and the D/V value reached its minimum at 0.006 s/mL, indicating the fastest microbial decontamination and, thus the highest energy efficiency for microbial inactivation. As the sample volume increased to 200 mL, the D/V value peaked at approximately 0.012 s/mL before slightly decreasing to 0.011 s/mL for 300 mL and 400 mL volumes.

These findings suggest that microbial inactivation was most efficient at the smallest sample volume (100 mL), where the minimal D/V value reflects optimal energy utilization for microbial destruction. The linear relationship between energy absorption and inactivation rate further

emphasizes this efficiency. Treating three separate containers, each holding 100 mL, simultaneously would allow for decontamination of a combined volume of 300 mL in less time and with greater effectiveness compared to treating the same total volume in a single container. For larger sample volumes, such as 200 mL to 400 mL, the D/V value nearly doubles compared to the 100 mL sample, reaching approximately 0.012 s/mL. This indicates that larger sample volumes require longer exposure to PL to achieve similar levels of inactivation, which could lead to energy inefficiency or overexposure effects.

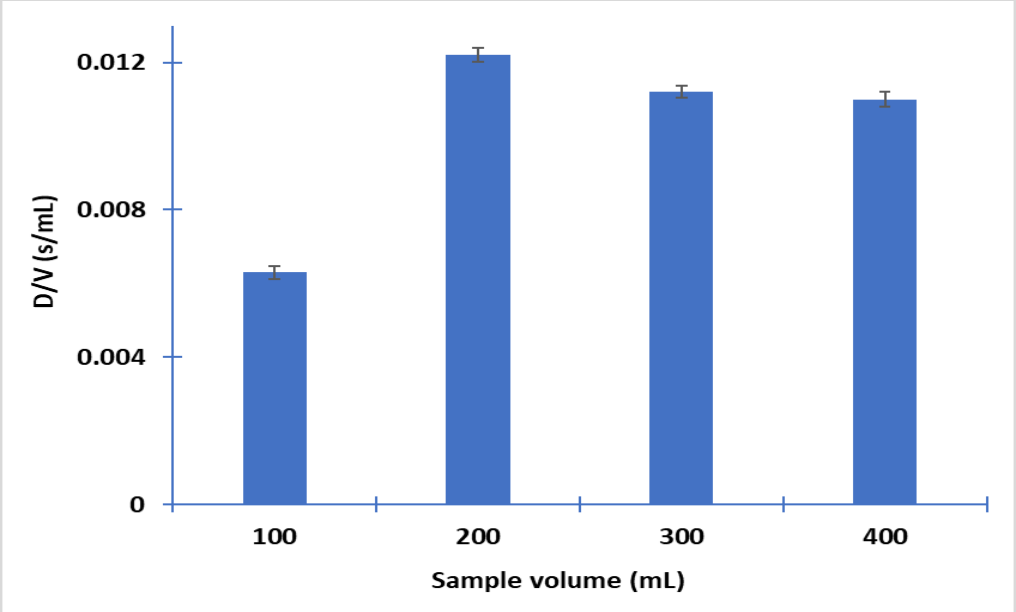


Figure 3.9. Decimal reduction time to sample volume ratio as influenced by sample volume.

A practical approach to maximize efficiency involves processing smaller volumes, such as 100 mL, across multiple containers simultaneously. Additionally, as demonstrated in prior results, supplementing PL treatment with liquid agitation can enhance inactivation efficiency for larger volumes. Increasing agitation speeds ensures better circulation and faster exposure of liquid particles to the pulsed light, ultimately accelerating microbial inactivation and improving overall treatment effectiveness.

3.4.5. Validation

Pulsed light treatment is an emerging non-thermal alternative to traditional thermal methods for producing safe and high-quality food products. This study utilized *E. coli* K-12 as a surrogate microorganism to represent pathogenic *E. coli* in assessing the decontamination efficiency of PL for ensuring the safety and quality of treated drinking water. To evaluate whether PL treatments could achieve specific target microbial reductions (equivalent to 2D, 3D, or 4D levels),

inactivation kinetics were calculated for the four sample volumes studied. These calculations provided a predictive model that was validated by comparing predicted microbial reductions with actual experimental results.

Table 3.6 exhibits the comparison between predicted and observed reductions for treatment times corresponding to 2D, 3D, and 4D. For instance, the processing time required to achieve a 2D reduction equals twice the D-value, as determined by the first-order model. Predicted values were derived using previously determined D-values from the log-linear model. The equipment operated at a fixed rate of 3 pulses per second, so treatment durations were converted into the equivalent number of pulses (Np) by multiplying the time in seconds by three and rounding to the nearest whole number. This rounding ensured consistency between predicted and experimental treatment durations, facilitating a direct comparison.

Table 3.6. Comparison of predicted and observed microbial reductions by PL for various treatment durations corresponding to 2D, 3D and 4D

Volume (mL)	**Reductions by 2D (log CFU/mL)**			**Reductions by 3D (log CFU/mL)**			**Reductions by 4D (log CFU/mL)**			**RMSE**
	Predicted	Actual	Np	Predicted	Actual	Np	Predicted	Actual	Np	
100	1.60±0.04	1.25±0.04	3	3.19±0.09	3.37±0.05	6	4.79±0.13	4.73±0.36	9	0.23
200	1.86±0.32	2.11±0.04	15	2.61±0.45	2.95±0.06	21	3.73±0.65	4.25±0.05	30	0.38
300	2.08±0.03	2.20±0.10	21	2.97±0.04	3.08±0.09	30	4.16±0.06	4.34±0.18	42	0.14
400	2.07±0.04	2.21±0.07	27	3.09±0.05	2.99±0.05	39	3.91±0.07	3.91±0.15	51	0.10
RMSE	0.23			0.21			0.28			

The results revealed a strong agreement between predicted and observed microbial reductions. Model validation was done by employing the root mean square error (RMSE), calculated across all sample volumes for each processing time (2D, 3D, and 4D) and across all processing times for each sample volume (100 mL, 200 mL, 300 mL, and 400 mL). Among processing times, the 3D treatment exhibited the highest accuracy, with the smallest RMSE of 0.23. For sample volumes, the 400 mL treatment showed the closest alignment between predictions and experimental results, with an RMSE of 0.10. These findings align with the principle, described by John and Ramaswamy (2020), which states that smaller RMSE values, closer to zero, indicate a better model fit. Overall, the developed predictive model demonstrates reliability and can serve as a valuable tool for designing efficient PL processes to ensure water safety and achieve target microbial reductions.

3.5. Conclusions

This study demonstrated that pulsed light treatment is a highly effective alternative for water decontamination. The surrogate microorganism used, *E. coli* K-12, which is often employed to assess the microbial quality of water due to its association with waterborne contamination, exhibited high susceptibility to PL. The results revealed that sample volume significantly impacts the efficiency of PL treatment, with thinner liquid profiles leading to better microbial inactivation. While larger volumes pose a challenge, the study highlighted that effective agitation during PL treatment can overcome this limitation by promoting uniform exposure of the liquid to PL, thereby enhancing microbial reduction while maintaining compliance with FDA fluence limits.

Moreover, treating multiple containers simultaneously was found to improve PL decontamination efficiency and reduce energy consumption. The methodologies and insights gained from these experiments will be applied in subsequent research to examine the effects of PL on microbial inactivation and quality attributes in apple juice. These findings also offer valuable guidance for developing treatment protocols, designing scalable systems, and conducting pilot-scale tests.

The experiments carried out in batch treatment chambers equipped with mixing systems to induce turbulence showed that agitation significantly enhances microbial inactivation. Looking ahead, continuous PL treatment systems that ensure uniform exposure of liquid samples could be explored. Such systems would allow for higher PL intensities to be used without the surface damage typically seen in static systems, paving the way for large-scale application of this technology.

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Preface to Chapter 4

In the preceding chapter, we investigated the kinetics of pulsed light (PL) inactivating the non-pathogenic strain *Escherichia coli* K-12 under varying power levels and sample volumes, using water as the suspension medium. This study aimed to elucidate the effects of water agitation on PL penetration and the resulting *E. coli* K-12 inactivation patterns. The integration of agitation with PL treatment demonstrated enhanced and more rapid *E. coli* destruction in water, attributed to improved exposure of liquid particles to light pulses, which also facilitated microbial decontamination in larger sample volumes.

Building upon these findings, the current chapter extends the research to apple juice, a turbid liquid. The objective is to assess the efficacy of agitation-assisted PL in inactivating *E. coli* suspended in apple juice and to examine its impact on quality parameters such as total phenolic content, antioxidant activity, total soluble solids, pH, color, ascorbic acid content, and rheological properties. This chapter focuses on exploring the relationship between increased microbial inactivation and treatment duration, in conjunction with varying agitation speeds. Subsequently, the effects of agitation-assisted PL treatment on the aforementioned quality attributes of apple juice are evaluated.

All experimental work and data analysis were performed by the candidate under the supervision of Prof. H. S. Ramaswamy.

Part of this chapter of this chapter will be prepared as a manuscript for submission to a peer-reviewed journal:

Mategeko, B., & Ramaswamy, H.S. (2025). Impact of agitation-assisted pulsed light treatment on microbial inactivation and quality attributes in apple juice. Prepared for publication in the *Journal of Food Science* (or an alternative journal).

Chapter 4

Impact of Agitation-Assisted PL Treatment on Microbial Inactivation and Quality Attributes in Apple Juice

4.1. Abstract

The current study explored the potential of agitation-assisted pulsed light (PL) treatment for microbial inactivation and the quality preservation of apple juice by subjecting 400 mL pasteurized samples (inoculated with *Escherichia coli* K-12) to PL treatments under various treatment durations (0-48 s) and agitation speeds (500 rpm, 750 rpm, and 1,000 rpm). Due to the limited penetration of PL in non-clear liquids, agitation was introduced to ensure better exposure of juice particles to PL, enhancing microbial destruction and enabling efficient processing. To create turbulence, the juice sample was transferred to a glass jar covered with a quartz lid and placed on a magnetic stirrer. The agitation-assisted PL treatment effectively inactivated *E. coli* K-12 suspended in apple juice. For example, *E. coli* counts (initially 7 log CFU/mL) were reduced to undetectable levels at treatment durations of 24, 30, and 42 s when agitation speeds of 1,000 rpm, 750 rpm, and 500 rpm were applied, respectively. In contrast, similar microbial reductions were not achieved in non-agitated samples, as *E. coli* counts plateaued at approximately 6 log CFU/mL after 24 s with no further noticeable reduction. Quality analyses indicated that PL treatment did not cause significant changes in total phenolic content, antioxidant activity, total soluble solids, pH, color, ascorbic acid content, or rheological properties. The maximum temperature rise (3.13 ± 0.06 °C) during PL treatment remained well below the threshold for nonthermal processing, reinforcing its suitability as a nonthermal pasteurization technique. These findings highlight the feasibility of agitation-assisted PL as a promising nonthermal technology for apple juice pasteurization, meeting the FDA-recommended five-log microbial reduction and fluence while maintaining essential quality attributes. The study provides insights for optimizing treatment protocols and scaling up for pilot-scale testing.

Key words: pulsed light, apple juice, quality attributes, microbial inactivation, agitation

4.2. Introduction

Fruits are an excellent source of nutrients with low-fat content, playing a vital role in maintaining a healthy lifestyle by reducing cholesterol and blood pressure (Pem & Jeewon, 2015; Slavin & Lloyd, 2012). However, their seasonal availability makes fruit juices a popular alternative, providing year-round nutritional benefits. This advantage, combined with their health-promoting properties, has led fruit juices to replace carbonated drinks, becoming the preferred choice among health-conscious consumers (Jain & De, 2016). Apple is one of the most widely available fruits globally, valued for its exceptional nutritional profile and numerous health benefits. Rich in antioxidants, dietary fiber, vitamins, minerals, flavonoids, and polyphenols, apple juice is a natural source of bioactive compounds that help prevent diseases, such as heart disease, cancer, and diabetes. These nutrients, along with its year-round availability, make apple juice one of the most demanded fruit juices worldwide (Jain & De, 2019). Apple juice comprises sugars (fructose, glucose, sucrose), organic acids (malic, quinic, and citromalic acids), tannins, pectin, vitamin C, minerals, and volatile esters that give its characteristic aroma. The composition varies depending on factors such as apple variety, growing conditions, maturity at harvest, and processing methods. However, like other fruit juices, apple juice is susceptible to contamination by acid-resistant pathogens, such as *Escherichia coli* O157:H7, which has been linked to outbreaks in the U.S. between 1995 and 2005. This necessitated the U.S. Food and Drug Administration (FDA) mandate for pasteurization processes to achieve a minimum 5-log reduction in pathogen levels for juices (USFDA, 2001).

Thermal pasteurization has traditionally been the most widely used technique in fruit juice processing due to its effectiveness in inactivating microorganisms and extending shelf life. However, thermal treatments can negatively impact the sensory, nutritional, and physicochemical qualities of juices, such as reducing antioxidant levels and altering taste, texture, and appearance (Kwaw et al., 2018; Zia et al., 2024). The growing consumer demand for minimally processed, fresh-like fruit juices with high nutritional quality has led to the development of nonthermal processing technologies as alternatives or complements to conventional thermal methods (Elmnasser et al., 2008; Finger et al., 2023).

Among non-thermal technologies, pulsed light treatment has gained significant attention. PL involves the use of intense, short pulses of broad-spectrum light (200-1,100 nm) to inactivate microorganisms through three primary photomechanisms: DNA damage via thymine dimer formation (referred to as the photochemical effect), localized heating of microbial cells (the

photothermal effect), and structural disruption caused by the pulsing action (the photophysical effect) (Dhar et al., 2022; John & Ramaswamy, 2018). These mechanisms typically occur simultaneously, with their relative importance varying based on the fluence applied to the food and the specific microorganism being targeted. Approved by the FDA for food processing in 1996 (FDA, 2024), PL has demonstrated high efficacy in microbial inactivation for various liquid foods, including apple, melon, orange and strawberry juices (Ferrario et al., 2013), tender coconut water (Basak et al., 2023; Preetha et al., 2021), pomegranate juice (Bhagat & Chakraborty, 2022), pineapple juice (Vollmer et al., 2020), milk (Smith et al., 2002), and bael fruit juice (Dhar & Chakraborty, 2023). However, the effectiveness of PL can be limited by factors such as sample turbidity, color, and volume, which hinder light penetration and reduce treatment uniformity (Dhar & Chakraborty, 2023; Miller et al., 2012).

The impact of sample turbidity on reducing the efficiency of PL decontamination was highlighted in a study by Mittal (2023), where diluting apple drink led to a decreased D-value, indicating an improved inactivation rate of *E. coli* K-12 suspended in the diluted sample. Similarly, in a study on the pasteurization of bael fruit juice, Dhar and Chakraborty (2023) observed challenges with PL penetration in colored and turbid bael fruit juice, particularly as the sample volume increased. To overcome these limitations, they conducted experiments at higher fluence levels exceeding the FDA-recommended limits of 12 J/cm^2 stipulated in Title 21 of the Code of Federal Regulations (FDA, 2024) and incorporated the use of a magnetic stirrer to agitate the juice during treatment. This approach improved microbial and enzyme inactivation, achieving a ≥5-log microbial reduction and enzyme inactivation of nearly 90%. However, the study did not evaluate the potential for microbial inactivation in larger volumes (exceeding 20 mL) or assess the effects on the juice's quality attributes. Furthermore, it required surpassing the FDA-recommended fluence limits.

Building on these findings, previous studies (Chapter 3) demonstrated that agitation-assisted PL enhanced decontamination efficiency in clear water volumes of 100-400 mL, with higher agitation speeds further improving microbial inactivation without exceeding the FDA fluence limit. Recognizing the dependence of PL penetration on liquid clarity, this study aimed to evaluate the impact of agitation-assisted PL on microbial inactivation and quality in colored liquids, specifically apple juice. The focus was on assessing the effects of agitation-assisted PL on inactivation of *E. coli* K-12 (EC1-5G) (a surrogate for *E. coli* O157:H7), temperature rise, and quality attributes viz. total phenolic content, antioxidant activity, total soluble solids, pH, color, ascorbic acid content, and rheological properties.

4.3. Materials and methods

4.3.1. Pulsed light treatment

The juice samples underwent PL treatment using the SteriPulse®-XL RS-3000 C pulsed light system (Xenon Corp., Wilmington, Massachusetts, USA). This system consists of a power/control unit, a linear Xenon flash lamp, and a lamp enclosure module. As per the manufacturer's specifications, it produces three high-intensity pulses per second (pulse duration: 360 μs) of polychromatic light, covering wavelengths from 100 to 1,100 nm, with an energy output of approximately 1.27 J/cm^2 per pulse at a distance of 1.9 cm from the quartz window surface (John & Ramaswamy, 2020; Pataro et al., 2011). The treatment chamber measures approximately 16 × 40 × 16 cm (W × L × H) and includes a tray rack with 11 adjustable positions, ranging from 1.9 cm (Tray 0) to 14.6 cm (Tray 10) from the light source (Figure 4.1.a). The distance between the sample and the light source is crucial, as closer proximity results in greater PL intensity and more rapid bacterial inactivation (John & Ramaswamy, 2020). To prevent heat buildup from the lamp, the treatment chamber features a forced air evacuation system equipped with filters at both ends that are effective at removing germs and are resistant to ozone (Prusty, 2020).

Figure 4.1. Pulsed light treatment system: (a) SteriPulse-XL RS-3000C and mixing setup (b).

A magnetic stirrer, approximately 4.8 cm in height, with a maximum agitation speed of 3,000 rpm, was utilized to establish an agitation mechanism (Figure 4.1.b). Apple juice samples, placed in glass jars containing magnetic bars, were agitated at three different speeds: 500 rpm, 750 rpm, and 1,000 rpm. This setup was designed to evaluate the impact of agitation-assisted PL on improving microbial inactivation as well as its effects on the quality attributes of apple juice. As the combined height of the magnetic stirrer and glass jar was 15.6 cm, nearly matching the treatment chamber's height of 16 cm, all treatments were carried out at a uniform distance of approximately 11.2 cm from the light source. According to the inverse square law, the intensity of light decreases proportionally to the square of the distance from the source (Bolton & Linden, 2003; Gómez-López & Bolton, 2016). The fluence at varying distances can be calculated using the formula provided in Equation 4.1:

$$F_2 = F_1 \, x \, \left(\frac{d_1}{d_2}\right)^2 \tag{4.1}$$

Where F_1 is the fluence at distance d_1 and F_2 is the fluence at distance d_2.

The agitation speed was capped at 1,000 rpm, as higher speeds led to excessive splashing onto the jar lid, which could hinder light penetration. The PL treatment durations ranged from 6 s (18 pulses) to 48 s (144 pulses).

4.3.2. Microbial inactivation in apple juice

4.3.2.1. Reactivation of lyophilized cell stock

Lyophilized *Escherichia coli* K-12 (EC1-5G) cells, obtained from MilliporeSigma (a division of Merck KGaA, Darmstadt, Germany, operating in the USA and Canada), were reactivated by adding 10 mL of Tryptic Soy Broth (TSB, Sigma-Aldrich, Difco, MO, USA) to the vial containing the lyophilized cells and thoroughly mixing to dissolve the bacterial powder. The suspension was then streaked onto Tryptic Soy Agar (TSA, Sigma-Aldrich, Difco, MO, USA) plates to isolate single colonies. After incubating the plates at 37°C for 24 h to promote bacterial growth, individual colonies were aseptically picked using a sterile loop and transferred into 1.7 mL microcentrifuge tubes containing 1 mL of 20% glycerol, which acted as a cryoprotectant to maintain cell viability for long-term storage at -40°C.

4.3.2.2. Preparation of stock solution

The preparation of the stock solution was carried out following the method outlined by John and Ramaswamy (2020), with slight modifications. The *Escherichia coli* K-12 (EC1-5G) culture, stored in a 20% v/v glycerol solution at -40°C for long-term preservation, was thawed on ice. Once thawed, the cells were streaked onto TSA plates using a sterile loop and incubated at 37°C for 24 h. A single colony from the TSA plate was then inoculated into 50 mL of TSB broth and incubated at 37°C for 24 h under shaking conditions. After incubation, the culture was centrifuged at 4,000 rpm for 20 min in a benchtop centrifuge, and the supernatant was discarded. The resulting pellet was washed by resuspending it in sterile distilled water, followed by centrifugation at 4,000 rpm for 20 min. This washing step was repeated three times, and the final pellet was resuspended in 50 mL of pasteurized apple juice, producing a stock solution with an approximate bacterial concentration of 10^8 CFU/mL. The stock solution was stored at 4°C until it was used to inoculate samples for PL treatment.

4.3.2.3. Sample inoculation

The apple juice used in this study was a commercially available product obtained from a grocery store in Quebec, Canada. All samples were of the same brand and manufacturer, ensuring uniformity throughout the study. Due to its acidic nature (pH < 4.6), pasteurization during production, and airtight packaging, the apple juice is free from vegetative bacteria and can be stored at room temperature for extended durations, exceeding six months, under appropriate storage conditions (Nehmé et al., 2024).

The inoculation of apple juice samples was performed aseptically by adding 50 mL of *E. coli* K-12 (EC1-5G) stock solution to 450 mL of pasteurized apple juice, followed by thorough mixing to achieve uniform distribution, resulting in a 1:10 dilution and an initial microbial concentration of approximately 10^7 CFU/mL prior to PL treatments. Inoculated samples of varying volumes (100-400 mL) were then transferred into 600 mL glass jars (Figure 4.1) with dimensions of 9.4 cm (diameter) × 10.8 cm (height), covered with quartz lids, and subjected to PL treatments under various combinations of agitation speeds and treatment durations.

4.3.2.4. Microbial enumeration and log-linear kinetic model

After PL treatment, the samples were aseptically mixed and serially diluted. From each dilution, 100 µL was plated in duplicate onto TSA plates and incubated at 37°C for 24 h. Colony

enumeration was performed on plates with 30-300 colonies, which were selected for analysis. All experiments were conducted in triplicate, and the microbial concentration (CFU/mL) was determined using Equation 4.2:

$$CFU/mL = \left(\frac{\text{Number of colonies}}{\text{Volume of sample plated}}\right) x\ Dilution\ factor \quad (4.2)$$

When required, microbial counts were normalized to an initial uniform load (control) of 7.0 log CFU/mL using Equation 4.3 to enable better comparison of microbial survival across PL treatments.

$$log_{10}\ N = log_{10}(\frac{N}{N_0} x\ 10^7) \quad (4.3)$$

Where N_0 (CFU/mL) denotes the initial population of *E. coli* K-12 before treatment, while N (CFU/mL) represents the number of bacteria after the PL treatment at time t (s).

To analyze the data using a semi-logarithmic model, which reflects first-order kinetic behavior, it was assumed that an increase in PL treatment time would lead to a logarithmic decrease in bacterial survival. The log-linear model, also known as the D-value model, was applied using Equation 4.4:

$$log_{10}(N/N_0) = -\ t/D\ ;\ (t \geq 0);\ Slope = -\ 1/D \quad (4.4)$$

In this equation, N_0 (CFU/mL) refers to the initial population of *E. coli* K-12, while N (CFU/mL) denotes the surviving bacterial count after a specific PL treatment time t (s). The D-value indicates the time required to achieve a 90% reduction in the microbial population. The D-value was determined by calculating the slope of the plot of $\log_{10}(N/N_0)$ against treatment time (s) as detailed by John and Ramaswamy (2020).

4.3.3. Temperature

The temperature increase in apple juice during PL treatments was monitored using a digital thermometer (Traceable Kangaroo Thermometer, Control Company, Texas, USA). Temperature measurements were taken immediately before and after each PL treatment. The temperature rise (ΔT, °C) was calculated as the difference between the post-treatment and pre-treatment temperature readings. All measurements were performed in triplicate, and the results are presented as the mean ± standard deviation.

4.3.4. Total phenolic contents

The total phenolic content (TPC) was determined using a method adapted from Derradji-Benmeziane et al. (2014). In this procedure, 5 mL of apple juice was mixed with 10 mL of 75% (v/v) ethanol and centrifuged at 3,000 rpm for 15 min. A 200 µL aliquot of the supernatant was then combined with 1 mL of 10% (v/v) Folin-Ciocalteu (FC) reagent and 800 µL of 7.5% (w/v) Na_2CO_3 in a test tube. Immediately after mixing, the tubes were covered with foil and incubated in the dark for 30 min. Following incubation, the absorbance of the samples was measured at 765 nm using a UV/VIS spectrophotometer (UV-3100PC, VWR International, LLC, USA). A standard curve was generated by measuring the absorbances of gallic acid solutions ranging from 0.02 to 0.5 mg/mL, and the resulting regression equation was used to calculate the TPC of the samples expressed in milligrams gallic acid equivalents per milliliter of apple juice (mg GAE/mL). All experiments were performed in triplicates and the results are presented as the mean ± standard deviation.

4.3.5. Antioxidant activity

The antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, as outlined by Mandal (2022), with slight modifications. DPPH is a stable free radical with a dark purple color, which, when exposed to antioxidants, undergoes reduction and results in decolorization. This change in color allows for the measurement of antioxidant activity using a spectrophotometer (Singh et al., 2015). In this procedure, 5 mL of the sample was mixed with 10 mL of 75% (v/v) ethanol and centrifuged at 3,000 rpm for 15 min. From the resulting supernatant, 400 µL was transferred to a test tube, and 1.6 mL of DPPH solution (1 mM in 75% (v/v) ethanol) was added. The test tubes were immediately covered with foil and placed in the dark at room temperature for about 15 min. After the incubation period, the absorbance of each sample was measured at 517 nm using a UV/VIS spectrophotometer (UV-3100PC, VWR International, LLC, USA), alongside a control consisting of 1.6 mL of DPPH solution and 400 µL of 75% (v/v) ethanol. The absorbance values of the sample (A_{sample}) and the control ($A_{control}$) were recorded, and the DPPH radical scavenging activity was calculated as a percentage using the following formula (Equation 4.5):

$$\% \text{ DPPH radical scavenging activity} = [1 - (A_{sample} / A_{control})] * 100 \quad (4.5)$$

All experiments were performed in triplicates and the results are presented as the mean ± standard deviation.

4.3.6. Total soluble solids

The total soluble solids (TSS) of the apple juices were measured using a digital pocket refractometer (Atago PAL-α, Tokyo, Japan) and expressed as °Brix, following the method described by Yang et al. (2019). All experiments were performed in triplicates and the results are presented as the mean ± standard deviation.

4.3.7. pH

The pH during PL treatments was monitored using a digital benchtop pH meter (PH550 Benchtop pH Meter, Oakton Instruments, IL, USA). The device was calibrated before use and employed to measure the pH of 60 mL of apple juice sample placed in a 100 mL beaker at 25°C. The pH meter provided stable and accurate readings. All measurements were conducted in triplicates, and the results are reported as the mean ± standard deviation.

4.3.8. Color

The color of the apple juice was evaluated during PL treatments by measuring the color parameters of untreated and PL-treated samples using a Minolta Tristimulus Colorimeter (Minolta Corp., Ramsey, NJ, USA), as employed in recent studies (Dhar & Chakraborty, 2023; Singh et al., 2015). The instrument utilized a pulsed xenon arc lamp with D65 illumination (day light source), and the observation angle was set to 10°, matching human visual perception and adhering to CIE recommendations. The color parameters L^*(lightness), a^*("+" red to "-" green), and b^* ("+" yellow to "-" blue) were recorded using SpectraMagic software (Minolta Corp., Ramsey, NJ, USA). Beyond the CIE L^*, a^*, and b^* values obtained from the software, the total color change (ΔE^*) was calculated using Equation 4.6 (Pathare et al., 2013) to further characterize the color changes in the PL-treated apple juice.

$$\Delta E* = \sqrt{(L^*_{untreated} - L^*_{treated})^2 + (a^*_{untreated} - a^*_{treated})^2 + (b^*_{untreated} - b^*_{treated})^2} \quad (4.6)$$

All experiments were performed in triplicates and the results are presented as the mean ± standard deviation.

4.3.9. Ascorbic acid

Vitamin C (ascorbic acid) content was analyzed using the indophenol method, as outlined in Nielsen (2024). The procedure involved the preparation of three key reagents: the standard solution, indophenol solution, and metaphosphoric acid-acetic acid solution.

The standard solution was prepared by dissolving 50 mg of ascorbic acid in 50 mL of metaphosphoric acid-acetic acid solution. To prepare the indophenol solution, 42 mg of sodium bicarbonate and 50 mg of 2,6-dichloroindophenol sodium salt were dissolved in 50 mL of deionized distilled water (dd water), and the volume was adjusted to 200 mL with dd water. This solution was stored in the dark under refrigeration until use. The metaphosphoric acid-acetic acid solution was prepared by adding 20 mL of acetic acid to 100 mL of dd water, followed by dissolving 7.5 g of metaphosphoric acid into the mixture while stirring. The solution was then diluted with dd water to a final volume of 250 mL, filtered through fluted filter paper, and stored refrigerated.

For standard solution titration, a mixture of 5 mL metaphosphoric acid-acetic acid solution and 2 mL of the standard solution was titrated against the indophenol solution. For the sample, a mixture of 5 mL of metaphosphoric acid-acetic acid solution and 2 mL of apple juice was titrated against the indophenol solution. The blank titration was performed using 7 mL of metaphosphoric acid-acetic acid solution. The endpoint of the reaction was noted when a persistent rose-pink color appeared and lasted for at least 5 s. The volume of indophenol solution required to reach the endpoint was recorded for each titration. The ascorbic acid content (mg AA/mL) of the samples was calculated using the formula below (Equation 4.7):

$$Ascorbic\ acid\ content\ (mg\ AA/mL) = (X - B) \times (F/E) \times (V/Y) \qquad (4.7)$$

$$F = \frac{mg\ ascorbic\ acid\ in\ volume\ of\ standard\ solution\ titrated\ **}{average\ mL\ used\ to\ titrate\ standards\ -\ average\ mL\ used\ to\ titrate\ blank}$$

** mg ascorbic acid in volume of standard solution titrated = (mg of ascorbic acid/50mL) x 2mL

Where:

X = average mL of indophenol solution used for sample titration,

B = average mL of indophenol solution used for blank titration,

F = titer of dye (= mg ascorbic acid equivalent to 1.0 mL indophenol standard solution),

E = mL sample assayed (= 2 mL),

V = volume of total assay solution (= 7 mL),

Y = volume of sample aliquot titrated (= 7 mL).

All experiments were conducted in triplicate, and the results are reported as the mean ± standard deviation.

4.3.10. Rheological properties

The effect of PL treatment on the rheological properties of apple juice was assessed by performing flow rheological tests on both untreated and treated samples. These tests included the determination of flow behavior index (n), consistency coefficient (m), and apparent viscosity (shear viscosity, η) as a function of shear rate ($\dot{\gamma}$) over a range of $0.1s^{-1}$ to $100s^{-1}$ for 3 min at 22 °C. The measurements were conducted using an AR2000 rheometer equipped with a 60-mm cone and a 2° angle (TA Instruments, New Castle, DE, USA), following the methodology outlined in recent studies (Pok et al., 2023; Taherian et al., 2019).

The apparent viscosity of the apple juice was analyzed as a function of shear rate using the Power-law model (also termed as Ostwald-de waele model), expressed by the following equation (Equation 4.8), which is widely applied to characterize shear-thinning and Newtonian fluids:

$$\eta = m\dot{\gamma}^{n-1} \tag{4.8}$$

Where η denotes the apparent viscosity (shear viscosity), m is the consistency coefficient, $\dot{\gamma}$ represents the shear rate, and n is the flow behavior index. A value of $n < 1$ indicates shear-thinning behavior, whereas $n = 1$ characterizes Newtonian behavior (Taherian et al., 2006).

For each treatment condition, samples were tested in duplicates, with two measurements taken per replicate, resulting in a total of four measurements per condition. The results were reported as mean values ± standard deviation.

4.3.11. Model evaluation

Kinetic modelling was conducted using Excel 365 (Microsoft Office System, USA). The first order (log-linear) kinetic model was evaluated using the straight-line equation. The regression coefficient (R^2) was applied to assess the model's goodness of fit, with values close to 1 indicating a more accurate representation of the data.

4.3.12. Statistical analysis

All experiments were conducted in triplicate or more, and the results are expressed as mean ± standard deviation. Data analysis was carried out using one-way ANOVA ($\alpha = 0.05$) with SPSS Statistics for Windows, version 29.0 (IBM Corp., Armonk, USA). Statistically significant differences were further assessed by comparing mean values using Tukey's test at a 95% confidence level.

4.4. Results and discussions

4.4.1. Microbial inactivation

Apple juice was tested to evaluate the potential of agitation in enhancing the efficiency of pulsed light for microbial inactivation in non-clear liquid foods. This potential had already been demonstrated for clear water in previous studies (Chapter 3). The apple juice used in this study was a pasteurized commercial product, naturally colored but without pulp and less clear than water. It was selected to assess the effectiveness of agitation-assisted PL in destroying *E. coli* suspended in 400 mL samples of apple juice. The goal was to determine if agitation could improve microbial inactivation compared to the findings of Mittal (2023), which reported that PL was ineffective in inactivating *E. coli* in 5 mL of apple juice spread evenly in a sterile polyethylene Whirl-Pak bag and treated without agitation.

Figure 4.2 presents a 3D visualization of the residual survivor counts (in logarithmic units), starting from an initial concentration of approximately 10^7 CFU/mL. The figure shows that microbial counts significantly decreased with increasing treatment durations during PL treatment combined with liquid mixing. In contrast, non-agitated samples (0 rpm) exhibited minimal microbial reduction (approximately 1 log) after 24 s of treatment, with no further significant reduction observed up to 48 s.

The success of liquid agitation in enhancing PL inactivation was further highlighted in Table 4.1. The table shows that *E. coli* counts were reduced to undetectable levels at treatment durations of 24, 30s, and 42 s when agitation speeds of 1,000 rpm, 750 rpm, and 500 rpm were applied, respectively. In contrast, achieving similar microbial reductions was not possible in non-agitated samples, as *E. coli* counts plateaued at approximately 6 log CFU/mL (from an initial count of 7 log CFU/mL) after 24 s of treatment, with no further noticeable reduction observed.

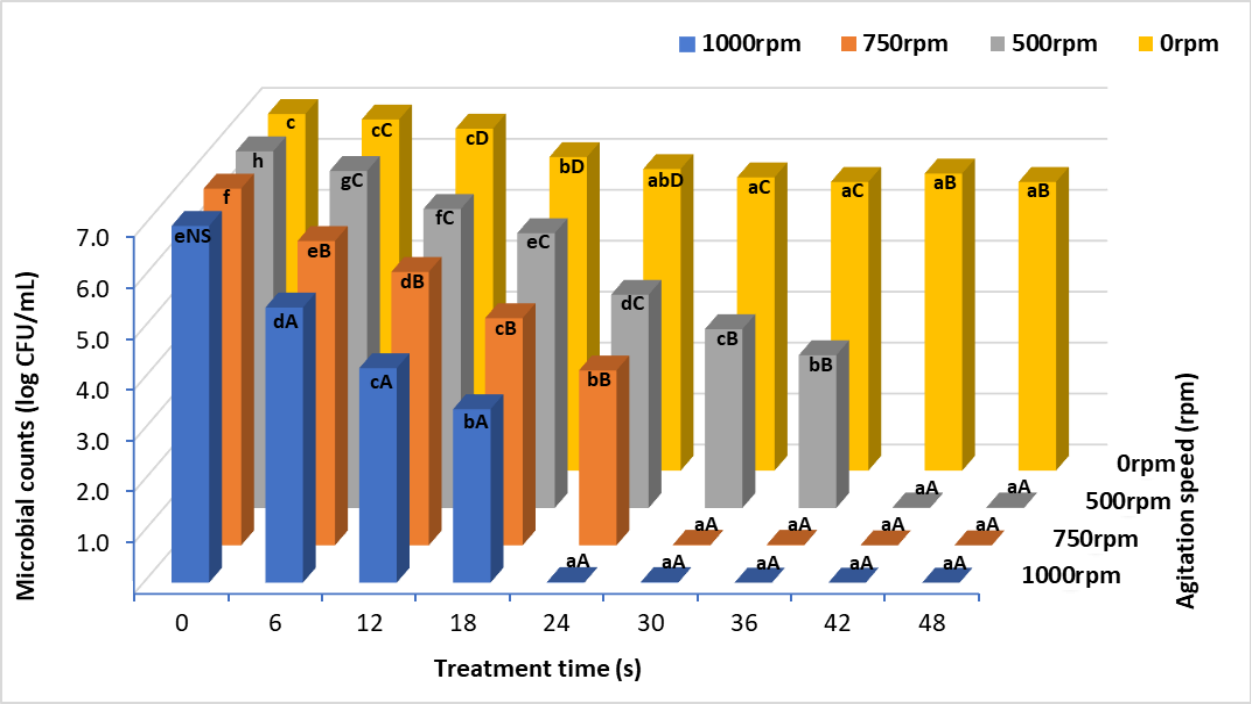

Figure 4.2. 3D plot showing the PL inactivation of *E. coli* K-12 in apple juice as influenced by agitation speed and treatment duration.

Note: Values represent the mean (n=3) ± SD of D-value. Different letters indicate significant differences (p <0.05).

Additionally, comparing the same treatment duration across the three agitation speeds reveals that higher agitation speeds significantly improved *E. coli* reductions in apple juice. For instance, after 18 s of PL treatment, the microbial counts decreased by approximately 1.6 log, 2.5 log, and 3.6 log when agitation speeds of 500 rpm, 750 rpm, and 1,000 rpm were used, respectively.

Table 4.1. Counts of *E. coli* K-12 following PL treatment of apple juice at different treatment durations and agitation speeds

Treatment time (s)	Microbial counts (log CFU/mL)			
	1,000 rpm	750 rpm	500 rpm	0 rpm
0	7.00 ± 0.00^{eNS}	7.00 ± 0.00^{f}	7.00 ± 0.00^{h}	7.00 ± 0.00^{c}
6	5.40 ± 0.12^{dA}	5.97 ± 0.15^{eB}	6.62 ± 0.13^{gC}	6.89 ± 0.11^{cC}
12	4.21 ± 0.07^{cA}	5.36 ± 0.12^{dB}	5.87 ± 0.06^{fC}	6.71 ± 0.10^{cD}
18	3.41 ± 0.06^{bA}	4.46 ± 0.06^{cB}	5.39 ± 0.06^{eC}	6.15 ± 0.11^{bD}
24	$<1^{aA}$	3.43 ± 0.10^{bB}	4.18 ± 0.06^{dC}	5.92 ± 0.05^{abD}
30	$<1^{aA}$	$<1^{aA}$	3.51 ± 0.11^{cB}	5.75 ± 0.10^{aC}
36	$<1^{aA}$	$<1^{aA}$	3.00 ± 0.06^{bB}	5.66 ± 0.16^{aC}
42	$<1^{aA}$	$<1^{aA}$	$<1^{aA}$	5.83 ± 0.09^{aB}
48	$<1^{aA}$	$<1^{aA}$	$<1^{aA}$	5.67 ± 0.12^{aB}

Note: Values represent the mean (n=3) ± SD of microbial counts. Different lowercase letters indicate, for each evaluated agitation speed, significant differences (p < 0.05) among treatment times. Different uppercase letters indicate, for each treatment time, significant differences (p < 0.05) among the evaluated agitation speeds. NS: non-significant.

The first-order plot of log reduction versus time (s) in Figure 4.3 reveals a strong correlation with the data (R^2 approaching 1), enabling the calculation of D-values. The effect of treatment duration, as depicted in Figure 4.2, was incorporated into the kinetic model, making the 2D plot sufficient to evaluate the impact of agitation. The calculated D-values derived from the first-order model curves (Figure 4.3) depicting the PL treatment of a 400 mL apple juice sample under varying agitation speeds are summarized in Table 4.2. These treatments were performed using the stirrer positioned approximately 11.2 cm from the light source, corresponding to a fluence of around 0.035 J/cm^2/pulse. This fluence was determined using the inverse square law (Equation 4.1) based on an initial fluence of 1.27 J/cm^2/pulse measured at 1.9 cm from the light source.

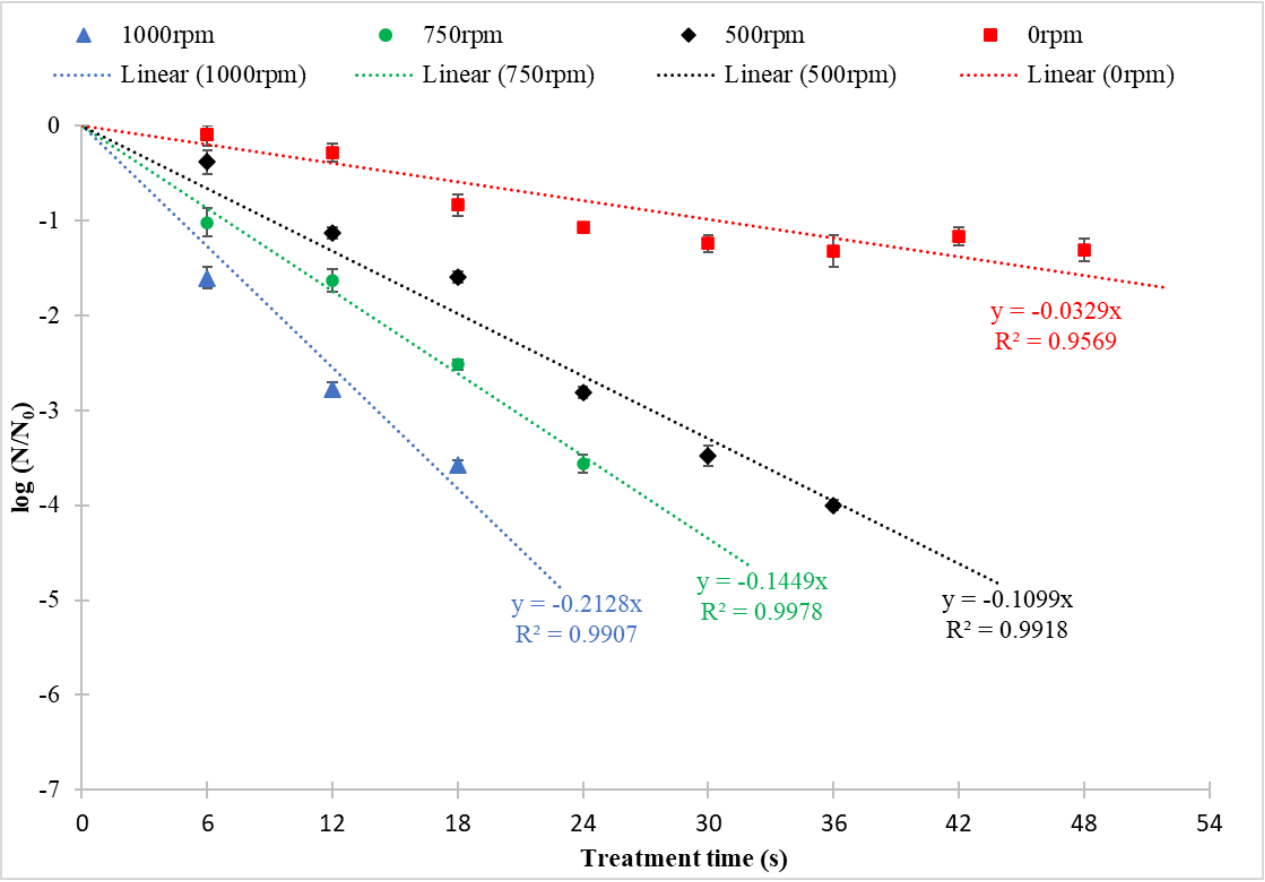

Figure 4.3. 2D plot illustrating the log-linear model of *E. coli* K-12 inactivation kinetics in a constant volume of apple juice as agitation speed increases.

At the highest agitation speed of 1,000 rpm, the D-value was determined to be 4.7 s, indicating that a five-log reduction could be achieved within approximately 24 s of treatment. In contrast, under static conditions (0 rpm), achieving the same reduction was not possible, as microbial counts plateaued at around 6 log CFU/mL after 24 s of PL treatment. This result highlights how optimized treatment conditions can significantly enhance microbial inactivation, enabling the recommended five-log reduction while staying within the FDA fluence limit of 12 J/cm^2.

A prior study by John and Ramaswamy (2020), reported achieving a five-log reduction using a 5 mL suspension (*E. coli* in 0.1% peptone water) subjected to PL in a 50 mm petri dish within 5 s at a distance of 10.8 cm from the light source. Differences between that study and the current research are likely due to factors such as sample type, sample volume, and the distance from the light source.

Table 4.2. D-values at different agitation speeds with an apple juice volume of 400mL

Agitation speed (rpm)	D-value (s)
0	30.48 ± 1.87^{c}
500	9.10 ± 0.30^{b}
750	6.90 ± 0.24^{ab}
1,000	4.70 ± 0.09^{a}

Note: Values represent the mean (n=3) ± SD of D-value. Different letters indicate significant differences ($p < 0.05$).

The present study observed a significant reduction in D-value ($p < 0.05$) when agitation was applied during PL treatment of apple juice. This reduction is attributed to improved mixing, which allows the liquid particles to be more evenly and effectively exposed to the light, resulting in greater light intensity received and shorter treatment times. The importance of agitation in enhancing PL efficiency is underscored by these findings, as it significantly reduces the time required to achieve specific microbial reduction targets. For example, in this study, a 7-log reduction of *E. coli* K-12 in 400 mL of apple juice at 1,000 rpm required approximately 33 s of treatment, corresponding to a total fluence of around 3.45 J/cm^2. In comparison, Hwang et al. (2015) achieved a 7-log reduction of *Pseudomonas aeruginosa* in 10 mL of mineral and isotonic water within a 90 mm petri dish with a total fluence of 0.97 J/cm^2. The variations in results between the two studies can be attributed to differences in equipment, with the current study using the SteriPulse® XL-RS 3000C, characterized by a longer pulse width (360 μs) and higher energy, whereas Hwang et al. (2015) used the Heraeus Noblelight XAP Series with a shorter pulse width (200 μs). Other contributing factors include differences in sample types, sample volumes, and the bacteria studied.

A similar trend to that observed in the current study was reported by Sauer and Moraru (2009), who demonstrated an increase in *E. coli* inactivation in apple juice subjected to 1-12 light pulses while inducing turbulence in a 1 mL liquid sample using an orbital shaker. The shaker operated at 500 rpm (low turbulence) and 3,000 rpm (high turbulence). Their study achieved a maximum reduction of 7.29 ± 0.73 log CFU/mL under high turbulence compared to 4.46 ± 0.39 log CFU/mL under low turbulence, with a fluence of 8.8 J/cm^2. These findings indicate a faster

inactivation rate than that reported in the present study. This difference could be attributed to several factors, including the smaller sample volume (1 mL), a shorter distance (5.59 cm) from the light source, and the application of a higher PL power dose. Furthermore, Dhar and Chakraborty (2023) proposed that enhancing PL treatment with a magnetic stirrer to generate turbulence in the liquid sample improved microbial and enzyme inactivation in 20 mL of bael juice exposed to PL treatments (0-1.64 kJ/cm^2) at 2.8 cm from the light source.

4.4.2. Temperature

The temperature change was continuously monitored during the PL treatments. Figure 4.4 presents a 3D visualization of the temperature increase in apple juice subjected to PL treatment at varying treatment durations and agitation speeds. As illustrated in the figure, the temperature rose significantly with prolonged treatment durations. Notably, the graph also indicates that there was no significant difference in temperature increase across different agitation speeds at the same treatment duration.

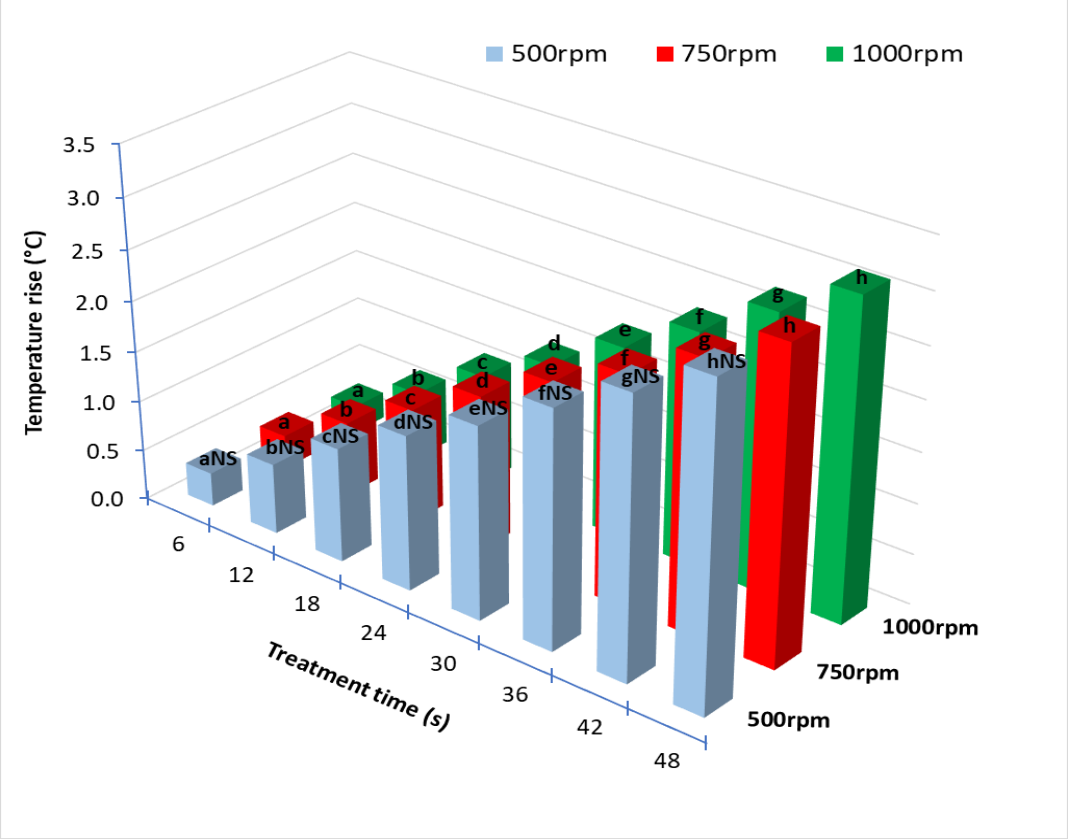

Figure 4.4. Temperature rise in apple juice subjected to PL treatment at varying durations and agitation speeds.

Note: NS: non-significant difference in ΔT among the evaluated agitation speeds for each treatment time.

A closer examination of Table 4.3 reveals that the temperature rise during PL treatment ranged from 0.27 ± 0.06 to 3.13 ± 0.06 °C. This aligns with previous studies that identified the infrared (IR) region of the PL spectrum as the source of heat in PL-treated samples (Mandal et al., 2020). This finding underscores the importance of the cooling blower in PL equipment, which serves to cool both the system and the sample during treatment (Pataro et al., 2011).

Table 4.3. Temperature rise in apple juice subjected to PL treatment at varying durations and agitation speeds

Treatment time (s)	Temperature rise (ΔT, °C)		
	500rpm	750 rpm	1,000 rpm
6	0.33 ± 0.06^{aNS}	0.33 ± 0.06^{a}	0.27 ± 0.06^{a}
12	0.70 ± 0.10^{bNS}	0.73 ± 0.06^{b}	0.67 ± 0.06^{b}
18	1.13 ± 0.06^{cNS}	1.13 ± 0.06^{c}	1.10 ± 0.10^{c}
24	1.53 ± 0.06^{dNS}	1.53 ± 0.06^{d}	1.47 ± 0.06^{d}
30	1.90 ± 0.10^{eNS}	1.93 ± 0.06^{e}	1.90 ± 0.10^{e}
36	2.33 ± 0.12^{fNS}	2.27 ± 0.06^{f}	2.30 ± 0.10^{f}
42	2.73 ± 0.06^{gNS}	2.67 ± 0.06^{g}	2.73 ± 0.06^{g}
48	3.13 ± 0.06^{hNS}	3.07 ± 0.06^{h}	3.13 ± 0.06^{h}

Note: Values represent the mean (n=3) ± SD temperature increase. Different lowercase letters indicate, for each evaluated agitation speed, significant differences ($p < 0.05$) among treatment times. NS: non-significant differences among the evaluated agitation speeds for each treatment time.

The results on temperature rise in this study further confirmed that PL treatment satisfies the requirements to be classified as a nonthermal technology. A processing method is considered nonthermal if the temperature increase in the treated product does not exceed 30°C (Chiozzi et al., 2022). In this study, the observed temperature changes (maximum of 3.13 ± 0.06°C) were well below this threshold, reaffirming the nonthermal nature of the applied PL treatment.

4.4.3. Total phenolic content

Phenolic compounds are essential for supporting a healthier human diet, making their retention in food crucial from a nutritional standpoint. This study evaluated the impact of PL processing on the phenolic compounds in apple juice. Like other non-thermal technologies, the main goal of PL treatment is to ensure microbial safety, enzymatic stability, and the preservation of nutritional quality. To investigate the effect of agitation-assisted PL on total phenolic content (TPC), apple juice samples were treated for varying durations under different agitation speeds.

Figure 4.5 provides a 3D visualization of the changes in TPC in apple juice samples treated with agitation-assisted PL for different durations (6-48 s) and compares them to untreated samples. The figure shows a notable increase in TPC with higher agitation speeds (500 rpm, 750 rpm, and

1,000 rpm). However, this effect of agitation may need further confirmation, as it could be influenced by variations in the initial TPC of untreated samples used for each agitation speed.

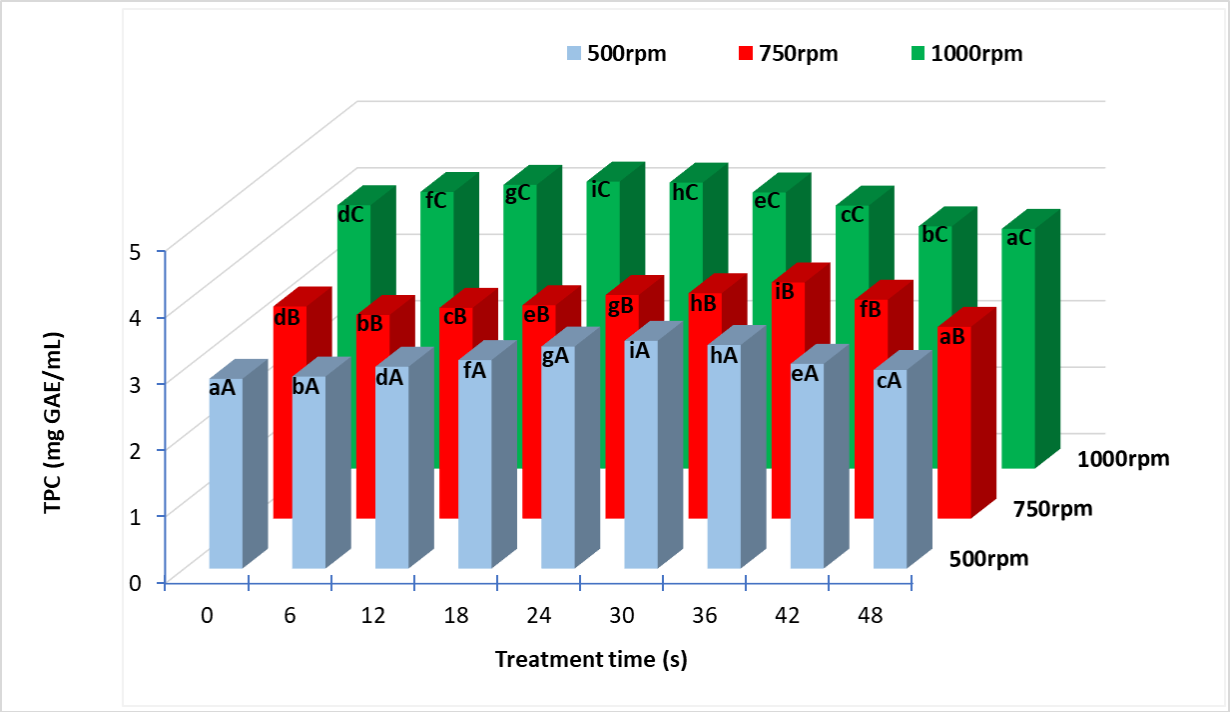

Figure 4.5. 3D visualization of TPC in apple juice under different PL treatment conditions.

A consistent trend observed was that TPC significantly increased up to a specific treatment duration (18 s at 1,000 rpm, 24 s at 750 rpm, and 30 s at 500 rpm). Beyond these points, TPC progressively declined with longer treatment times, eventually falling below the initial TPC levels during extended treatment durations.

A detailed examination of Table 4.4 reveals significant variation in the initial TPC of untreated apple juice across samples subjected to different agitation speeds, with values of 2.856 ± 0.001 mg GAE/mL, 3.193 ± 0.001 mg GAE/mL, and 3.964 ± 0.001 mg GAE/mL at 500 rpm, 750 rpm, and 1,000 rpm, respectively. Additionally, the peak TPC levels achieved during PL treatments at varying agitation speeds were 4.319 ± 0.002 mg GAE/mL for 1,000 rpm, 3.554 ± 0.001 mg GAE/mL for 750 rpm, and 3.428 ± 0.001 mg GAE/mL for 500 rpm. These findings highlight the importance of selecting optimal treatment conditions to maximize TPC retention in treated apple juice, as extended treatment durations and higher PL treatment fluences can lead to a significant decline in TPC levels.

Several studies investigating the effect of PL on TPC have reported findings consistent with the current study. For instance, Pihen et al. (2024) observed a 38.4% reduction in total phenols in

fresh apple juice treated at a maximum fluence of 176.4 J/cm^2 for a short duration of 70 s. This decline in TPC was attributed to the increase in temperature of the PL-treated samples, which rose to approximately 60°C, leading to a significant loss of bioactive compounds.

Table 4.4. Total phenolic content in apple juice under various PL treatment conditions

Treatment time (s)	Total phenolic content, TPC (mg GAE/mL)		
	500rpm	**750rpm**	**1,000 rpm**
0	2.856 ± 0.001^{aA}	3.193 ± 0.001^{dB}	3.964 ± 0.001^{dC}
6	2.890 ± 0.001^{bA}	3.064 ± 0.002^{bB}	4.163 ± 0.003^{fC}
12	3.037 ± 0.001^{dA}	3.171 ± 0.001^{cB}	4.270 ± 0.003^{gC}
18	3.138 ± 0.002^{fA}	3.211 ± 0.001^{eB}	4.319 ± 0.002^{iC}
24	3.342 ± 0.002^{gA}	3.364 ± 0.001^{gB}	4.304 ± 0.001^{hC}
30	3.428 ± 0.001^{iA}	3.391 ± 0.003^{hB}	4.155 ± 0.002^{eC}
36	3.364 ± 0.003^{hA}	3.554 ± 0.001^{iB}	3.958 ± 0.001^{cC}
42	3.081 ± 0.002^{eA}	3.293 ± 0.002^{fB}	3.645 ± 0.001^{bC}
48	2.990 ± 0.001^{cA}	2.885 ± 0.001^{aB}	3.608 ± 0.001^{aC}

Note: Values represent the means (n=3) ± SD of TPC. Different lowercase letters indicate, for each evaluated agitation speed, significant differences ($p < 0.05$) among treatment times. Different uppercase letters indicate, for each treatment time, significant differences ($p < 0.05$) among the evaluated agitation speeds.

Interestingly, Palgan et al. (2011) reported no significant difference in total phenol content between untreated control samples and apple juice subjected to an 8-second PL treatment at a fluence of 1.17 J/cm² per pulse. Their findings emphasized the importance of applying mild treatment conditions, such as shorter durations and lower fluence, to better preserve the quality of apple juice.

Murugesan et al. (2012) observed an increase in TPC when elderberry was treated with pulsed PL. A maximum increase of approximately 50% in total phenolics was achieved at an energy dose of 11,000 J/m²/pulse during a 10-s treatment, while a nearly 40% increase was recorded at the same energy dose after 5 s of exposure. However, prolonged treatment led to a decrease in phenolic content. The authors attributed the initial increase in TPC to the enhanced activity of phenylalanine ammonia-lyase (PAL), a key enzyme in the phenylpropanoid pathway. PAL activity is stimulated by the thermal stress which can be generated by PL up to a certain temperature threshold.

Furthermore, a study examining the effect of PL on the phytochemical composition of pineapple juice found that TPC losses ranged between 12% and 47%. This study treated 50 mL of

pineapple juice at different voltage levels (1.8, 2.1, and 2.4 kV) and durations (30, 60, and 120 s), highlighting how treatment conditions influence TPC retention (Vollmer et al., 2020).

4.4.4. Antioxidant activity

The antioxidant activity of apple juice is primarily attributed to its polyphenol content and vitamin C, along with contributions from carotenoids and vitamin E. Polyphenols are the primary contributors, accounting for approximately 80% of the antioxidant activity, while vitamin C contributes about 5% (Arya et al., 2023). In the present study, antioxidant activity was assessed using the % DPPH scavenging activity. Figure 4.6 provides a 3D representation of the changes in antioxidant activity in apple juice subjected to agitation-assisted PL over various treatment durations. The figure reveals slight variations in antioxidant activity between treatments, which were not significantly different from the control (untreated apple juice). Interestingly, higher agitation speeds resulted in a statistically significant reduction in antioxidant activity. However, these differences between agitation speeds were minimal, and the overall decrease cannot be conclusively confirmed, as it may be attributed to differences in the initial antioxidant activity of the untreated samples used for the different agitation speeds examined.

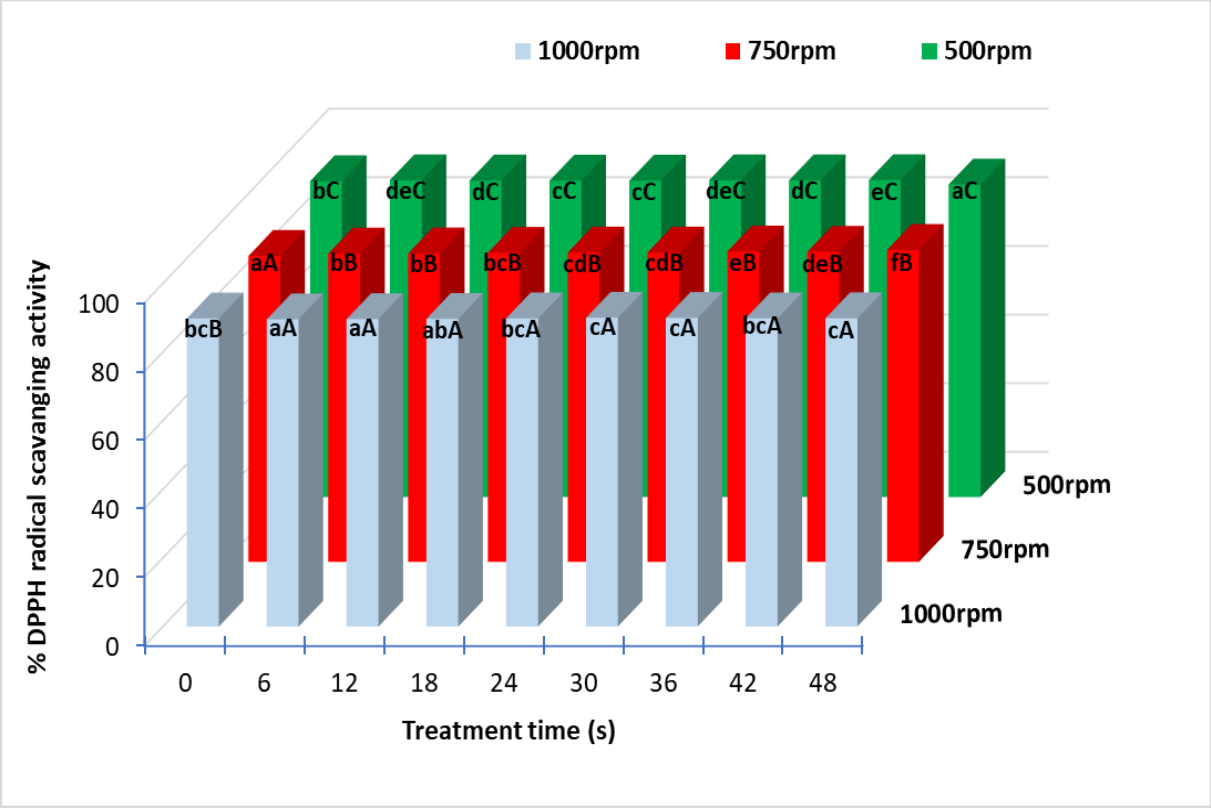

Figure 4.6. 3D visualization of antioxidant activity in apple juice subjected to PL treatment under different processing conditions.

Table 4.5 shows the antioxidant activity across different treatment durations (6-48 s) for PL-treated apple juice at varying agitation speeds. Under 500 rpm, the antioxidant activity ranged between 91.1% and 92.4%, starting from an initial value of 92.1%. For treatments at 750 rpm, the antioxidant activity varied from 89.9% to 90.9%, compared to an initial value of 89.2%. Similarly, at 1,000 rpm, the antioxidant activity ranged from 89.6% to 90.0%, starting from an initial value of 89.8%. These slight variations indicate that antioxidant activity remained relatively stable during the PL treatments, regardless of agitation speed.

Table 4.5. Antioxidant activity of apple juice under different PL treatment conditions

Treatment time (s)	DPPH radical scavenging activity (%)		
	500rpm	**750rpm**	**1,000 rpm**
0	92.05 ± 0.01^{bC}	89.17 ± 0.05^{aA}	89.81 ± 0.03^{bcB}
6	92.34 ± 0.01^{deC}	89.86 ± 0.21^{bB}	89.55 ± 0.02^{aA}
12	92.30 ± 0.01^{dC}	89.87 ± 0.07^{bB}	89.61 ± 0.02^{aA}
18	92.22 ± 0.01^{cC}	90.06 ± 0.09^{bcB}	89.65 ± 0.03^{abA}
24	92.25 ± 0.01^{cC}	90.15 ± 0.02^{cdB}	89.81 ± 0.13^{bcA}
30	92.34 ± 0.01^{deC}	90.15 ± 0.08^{cdB}	89.99 ± 0.04^{cA}
36	92.32 ± 0.01^{dC}	90.45 ± 0.07^{eB}	89.96 ± 0.06^{cA}
42	92.37 ± 0.01^{eC}	90.36 ± 0.04^{deB}	89.82 ± 0.02^{bcA}
48	91.09 ± 0.04^{aC}	90.89 ± 0.02^{fB}	89.86 ± 0.11^{cA}

Note: Values represent the means (n=3) ± SD of DPPH radical scavenging activity (%). Different lowercase letters indicate, for each evaluated agitation speed, significant differences ($p < 0.05$) among treatment times. Different uppercase letters indicate, for each treatment time, significant differences ($p < 0.05$) among the evaluated agitation speeds.

Consistent with the findings of this study, Muñoz et al. (2012) reported no significant impact on antioxidant capacity at a fluence level of 5.1 J/cm². Similarly, Palgan et al. (2011) found that the antioxidant activity of reconstituted apple juice processed with PL for 2 and 4 s (equivalent to 7 and 14 J/cm², respectively) was not significantly different from the control. However, a 5.5% decrease ($p < 0.05$) in antioxidant activity was observed after 8 s of treatment (28 J/cm²). Also, in the study conducted by Vollmer et al. (2020), the antioxidant capacity of fresh pineapple juice was measured at 16.4 ± 0.1 mg GAE/100 mL. The application of 47 pulses, regardless of the voltage level (160–375 J/cm^2), did not significantly impact the antioxidant capacity. However, a significant reduction in antioxidant capacity ($p \leq 0.05$) was observed with an increased number of pulses (94 and 187 pulses; 325-1479 J/cm^2). The largest reduction, 14%, was recorded under the most intense treatment conditions (2.4 kV/187 pulses).

In contrast, Pihen et al. (2024) noted a 14.5% reduction in DPPH inhibition in fresh apple juice treated with a higher fluence of 176.4 J/cm^2 for a short duration of 70 s. This substantial reduction can likely be attributed to the significantly higher energy dose used in their study,

which is considerably greater than the maximum fluence of approximately 5.04 J/cm^2 (48 s) applied in the present research. Overall, these findings suggest that bioactive compounds, such as antioxidants, are generally preserved under short PL exposure durations or low energy doses (low fluence).

4.4.5. Total soluble solids

The total soluble solids (TSS, °Brix) were measured across all PL treatment durations (6-48 s) at three different agitation speeds (500 rpm, 750 rpm, and 1,000 rpm). As illustrated in Figure 4.7, it is evident that TSS remained unaffected by any of the PL treatments, regardless of the treatment durations or agitation speeds examined.

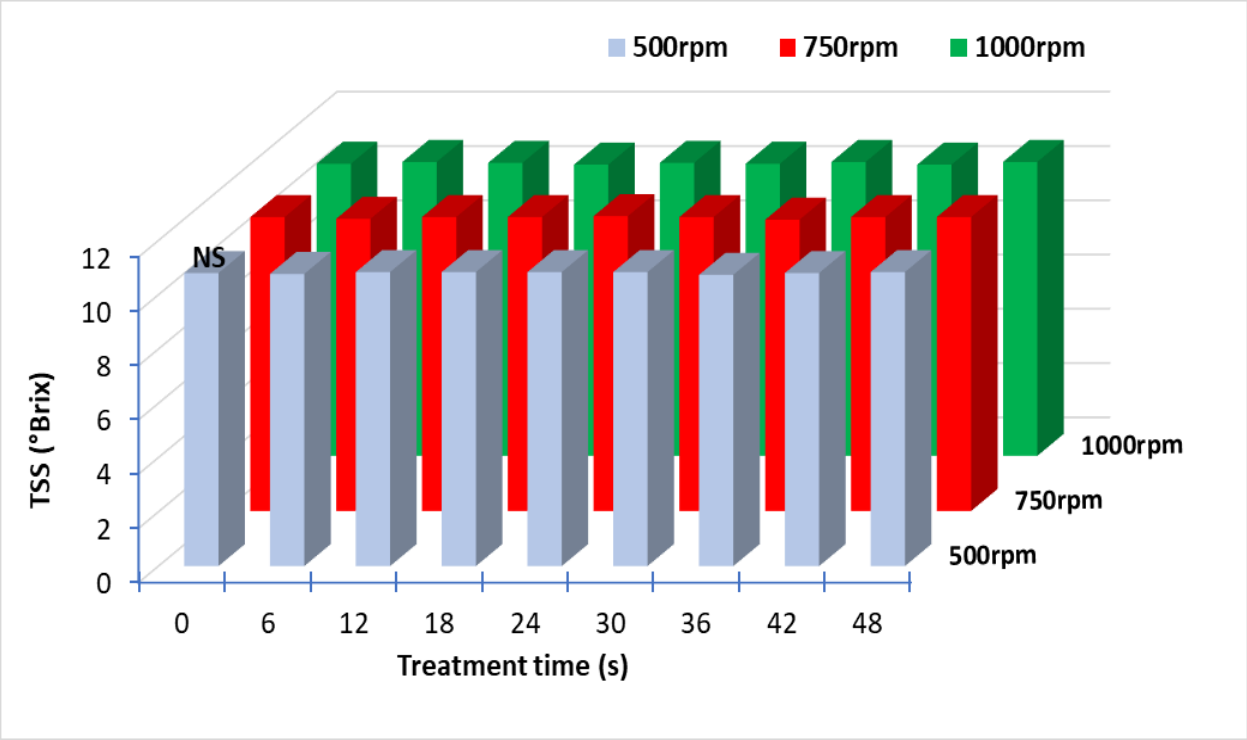

Figure 4.7. 3D graph of TSS in apple juice treated at various durations and agitation speeds.

Note: NS: non-significant difference in TSS among the evaluated agitation speeds and treatment times.

As shown in Table 4.6, the total soluble solids (TSS) of untreated apple juice, measured at 10.8 ± 0.4 °Brix at a treatment time of 0 s, remained unchanged throughout all PL treatments conducted in this study. Several studies have similarly examined the impact of PL treatment on TSS in various liquid foods. For instance, Pihen et al. (2024) found that the soluble solids (°Brix) in apple juice samples remained consistent with the control sample at 14.40°Brix, despite undergoing PL treatment. Likewise, Kasahara et al. (2004) observed no significant differences in

the TSS of clarified apple juice when exposed to pulsed UV light at energy dosages ranging from 1850 to 3354 mJ/cm^2 compared to the control. Additionally, a study by Chakraborty et al. (2020) demonstrated that PL treatments had no significant effect ($p > 0.05$) on the TSS of Indian gooseberry juice (20 mL) subjected to different voltages (2.7, 2.8, and 2.9 kV) and treatment times (3, 4, and 5 min). Similarly, Jayachandran et al. (2024) reported that the TSS of sugarcane juice (100 mL samples) remained unaffected across all samples processed under varying voltages (2.1-2.7 kV) and treatment durations of 100, 150, and 200 s, corresponding to 100, 150, and 200 pulses, respectively.

Table 4.6. Total soluble solids in apple juice under various PL treatment conditions

Treatment time (s)	Total soluble solids, TSS (°Brix)		
	500 rpm	**750 rpm**	**1,000 rpm**
0	10.8 ± 0.4^{aA}	10.8 ± 0.1^{aA}	10.8 ± 0.1^{aA}
6	10.8 ± 0.1^{aA}	10.8 ± 0.1^{aA}	10.8 ± 0.1^{aA}
12	10.8 ± 0.1^{aA}	10.8 ± 0.2^{aA}	10.8 ± 0.2^{aA}
18	10.8 ± 0.1^{aA}	10.8 ± 0.1^{aA}	10.7 ± 0.2^{aA}
24	10.8 ± 0.1^{aA}	10.9 ± 0.1^{aA}	10.8 ± 0.1^{aA}
30	10.8 ± 0.3^{aA}	10.8 ± 0.1^{aA}	10.8 ± 0.1^{aA}
36	10.7 ± 0.1^{aA}	10.7 ± 0.1^{aA}	10.8 ± 0.1^{aA}
42	10.8 ± 0.1^{aA}	10.8 ± 0.1^{aA}	10.7 ± 0.2^{aA}
48	10.8 ± 0.4^{aA}	10.8 ± 0.2^{aA}	10.8 ± 0.1^{aA}

Note: Values represent the means (n=3) ± SD of TSS. Different lowercase letters indicate, for each evaluated agitation speed, significant differences ($p < 0.05$) among treatment times. Different uppercase letters indicate, for each treatment time, significant differences ($p < 0.05$) among the evaluated agitation speeds.

4.4.6. pH

The pH of apple juice was measured across all PL treatment durations (6-48 s) at three different agitation speeds (500 rpm, 750 rpm, and 1,000 rpm). Figure 4.8 presents a 3D visualization of the pH values, clearly indicating that the pH remained unchanged under all PL treatments, regardless of the treatment durations or agitation speeds.

As shown in Table 4.7, the initial pH of untreated apple juice, recorded as 3.26 ± 0.01 (control), remained consistent throughout all PL treatments performed in this study. Similar results have been reported in other research investigating the effects of PL treatment on the pH of various liquid foods. For example, Palgan et al. (2011) found that the pH of apple juice samples remained steady at 3.66, matching the control, even after PL processing for 2, 4, and 8 s (corresponding to 7, 14, and 28 J/cm^2, respectively).

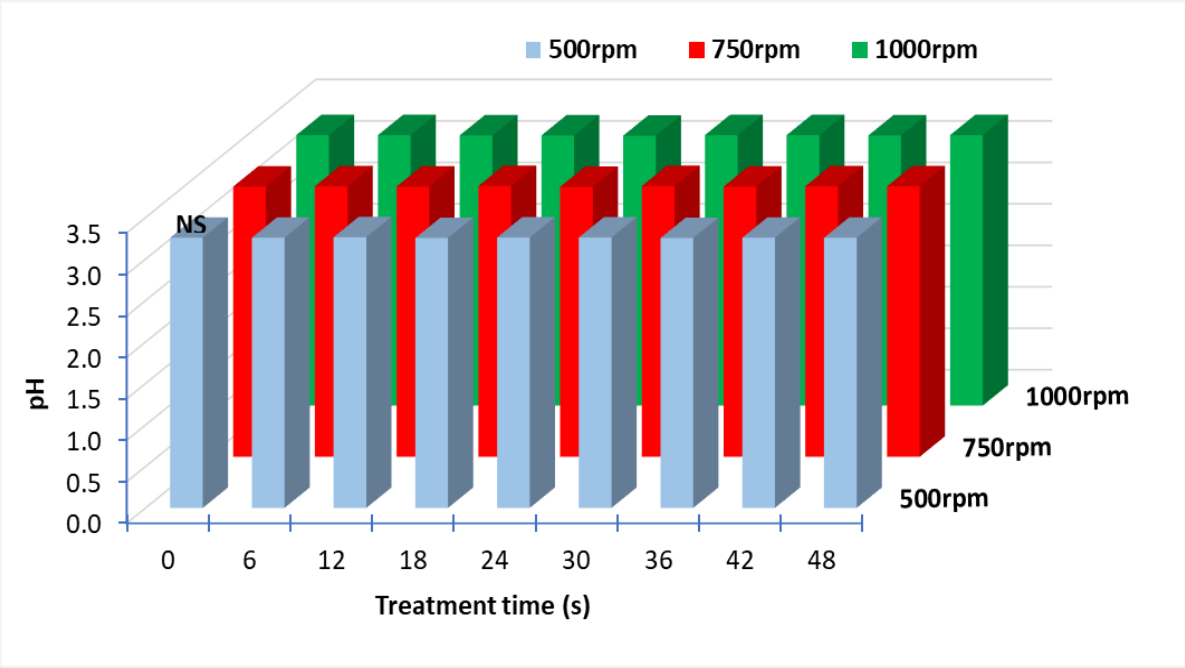

Figure 4.8. 3D representation of pH levels in apple juice treated for varying durations at different agitation speeds.

Note: NS: non-significant difference in pH among the evaluated agitation speeds and treatment times.

Similarly, Cai et al. (2024) reported no significant changes in the pH of apple juice treated with pulsed light. In their study, 5.65 mL samples (solution thickness of 2 mm) were exposed to PL at fluences of 6.75, 13.50, 20.25, 27.00, 33.75, 40.50, 47.25, and 54.00 J/cm², with total processing times of 1 minute 5 seconds, 2 minutes 10 seconds, 3 minutes 15 seconds, 4 minutes 20 seconds, 5 minutes 25 seconds, 6 minutes 30 seconds, 7 minutes 35 seconds, and 8 minutes 40 seconds, respectively. These findings align with those of Qi et al. (2023), who observed no impact of PL on the pH of apple juice.

Similarly, Chakraborty et al. (2020) reported that PL treatments had no significant effect ($p > 0.05$) on the pH of Indian gooseberry juice (20 mL) subjected to varying voltages (2.7, 2.8, and 2.9 kV) and treatment durations of 3, 4, and 5 min. Likewise, Jayachandran et al. (2024) noted that the pH of sugarcane juice (100 mL samples) remained unaffected across all treatments involving different voltages (2.1-2.7 kV) and durations of 100, 150, and 200 s, equivalent to 100, 150, and 200 pulses, respectively.

Table 4.7. pH levels of apple juice subjected to different PL treatment conditions

Treatment time (s)	pH		
	500 rpm	**750 rpm**	**1,000 rpm**
0	3.26 ± 0.01^{aA}	3.26 ± 0.01^{aA}	3.26 ± 0.00^{aA}
6	3.26 ± 0.00^{aA}	3.26 ± 0.00^{aA}	3.26 ± 0.00^{aA}
12	3.26 ± 0.01^{aA}	3.26 ± 0.01^{aA}	3.26 ± 0.01^{aA}
18	3.25 ± 0.01^{aA}	3.26 ± 0.01^{aA}	3.26 ± 0.01^{Aa}
24	3.26 ± 0.01^{aA}	3.25 ± 0.01^{aA}	3.25 ± 0.01^{Aa}
30	3.26 ± 0.00^{aA}	3.26 ± 0.01^{aA}	3.26 ± 0.00^{aA}
36	3.25 ± 0.01^{aA}	3.26 ± 0.01^{aA}	3.26 ± 0.00^{aA}
42	3.26 ± 0.01^{aA}	3.26 ± 0.00^{aA}	3.26 ± 0.01^{aA}
48	3.26 ± 0.01^{aA}	3.26 ± 0.00^{aA}	3.26 ± 0.01^{aA}

Note: Values represent the means (n=3) ± SD of pH. Different lowercase letters indicate, for each evaluated agitation speed, significant differences ($p < 0.05$) among treatment times. Different uppercase letters indicate, for each treatment time, significant differences ($p < 0.05$) among the evaluated agitation speeds.

4.4.7. Color

The color of juice significantly influences its sensory appeal, making it essential to study the effects of pulsed light (PL) on juice color. In the current study, the impact of PL on the color of treated apple juice was analyzed under varying treatment durations and agitation speeds. For each treated sample, along with the untreated control, color parameters L^* (lightness/darkness), a^* (redness/greenness), and b^* (yellowness/blueness) were recorded to evaluate color variations, and these values were used to calculate the total color change (ΔE^*).

As indicated in Table 4.8, liquid mixing influenced color retention, with increased agitation speeds significantly ($p < 0.05$) reducing total color change for the same treatment duration. This suggests that higher agitation speeds promote better color retention in PL-treated apple juice. However, when comparing treatments conducted at the same agitation speed, color changes varied with treatment durations (6-48 s). ΔE^* values ranged between 5.917 ± 0.021 and 8.555 ± 0.606 at 500 rpm, 1.204 ± 0.027 and 1.824 ± 0.028 at 750 rpm, and 0.493 ± 0.045 and 1.244 ± 0.015 at 1,000 rpm. Based on the ΔE^* values, color changes were classified: 'not noticeable' ($\Delta E^* < 0.5$), 'slightly noticeable' ($0.5 \leq \Delta E^* < 1.5$), 'noticeable' ($1.5 \leq \Delta E^* < 3.0$), 'well visible' ($3.0 \leq \Delta E^* < 6.0$), and 'great' ($6.0 \leq \Delta E^* < 12.0$)) (Cserhalmi et al., 2006; Wibowo et al., 2015). Accordingly, the color change in this study was categorized as slightly noticeable (0.5–1.5) under PL treatments at 1,000 rpm, noticeable (1.5–3.0) at 750 rpm, and great (6.0–12.0) at 500 rpm.

Table 4.8. Total color change of apple juice subjected to various PL treatment conditions

Treatment time (s)	Total color change, Δ*E**		
	500rpm	**750rpm**	**1,000 rpm**
6	5.917 ± 0.021^{aC}	1.692 ± 0.027^{cdB}	0.493 ± 0.045^{aA}
12	6.057 ± 0.020^{aC}	1.824 ± 0.028^{dB}	0.902 ± 0.043^{cA}
18	7.259 ± 0.010^{bcC}	1.649 ± 0.124^{cdB}	1.244 ± 0.015^{dA}
24	7.491 ± 0.051^{bcC}	1.574 ± 0.096^{bcdB}	0.637 ± 0.171^{abA}
30	6.797 ± 0.076^{abC}	1.732 ± 0.135^{dB}	0.762 ± 0.041^{bcA}
36	7.076 ± 0.642^{bcB}	1.281 ± 0.044^{abA}	1.204 ± 0.038^{dA}
42	7.831 ± 0.087^{cdC}	1.410 ± 0.221^{abcB}	0.615 ± 0.026^{abA}
48	8.555 ± 0.606^{dB}	1.204 ± 0.027^{aA}	0.936 ± 0.018^{cA}

Note: Values represent the means (n=3) ± SD of total color change (Δ*E**). Lowercase letters indicate, for each evaluated agitation speed, significant differences ($p < 0.05$) among treatment times. Uppercase letters indicate, for each treatment time, significant differences ($p < 0.05$) among the evaluated agitation speeds.

Similar studies have evaluated the effect of PL on the color of fruit juices. Consistent with the findings at 500 rpm in the present study, Qi et al. (2023) reported a significant ($p < 0.05$) increase in Δ*E** with longer PL treatment durations in apple juice, reaching a maximum Δ*E** value of 5.9. In a study on table grape juice, Chakraborty and Parab (2023) observed that a PL fluence of 2772 J/cm^2 caused a noticeable color change, while a fluence of 3186 J/cm^2 led to a well-visible change. Similarly, an increase in Δ*E** was noted in PL-treated bael fruit juice (Dhar & Chakraborty, 2023) and Indian gooseberry juice (Chakraborty et al., 2020). The observed PL-induced color changes may result from enzymatic browning at lower fluences (when enzymes remain active) or non-enzymatic browning at higher fluences (when most enzymes are inactivated). Non-enzymatic browning could involve PL-induced Maillard reactions, changes in pigment content, or the formation of dark or brown substances (Dhar et al., 2022; Dhar & Chakraborty, 2020; Mahendran et al., 2019).

4.4.8. Ascorbic acid

Ascorbic acid (AA), commonly referred to as vitamin C, is a vital component of the antioxidant activity in apple juice, contributing approximately 5% of the total antioxidant capacity (Arya et al., 2023). However, vitamin C is one of the least stable vitamins, being highly susceptible to degradation during processing and storage (Chakraborty et al., 2020). This highlights the importance of examining the impact of PL processing on the vitamin C content of treated apple juice. In this study, AA content was evaluated under various PL treatment conditions, involving different treatment durations and agitation speeds, with the results depicted in Figure 4.9.

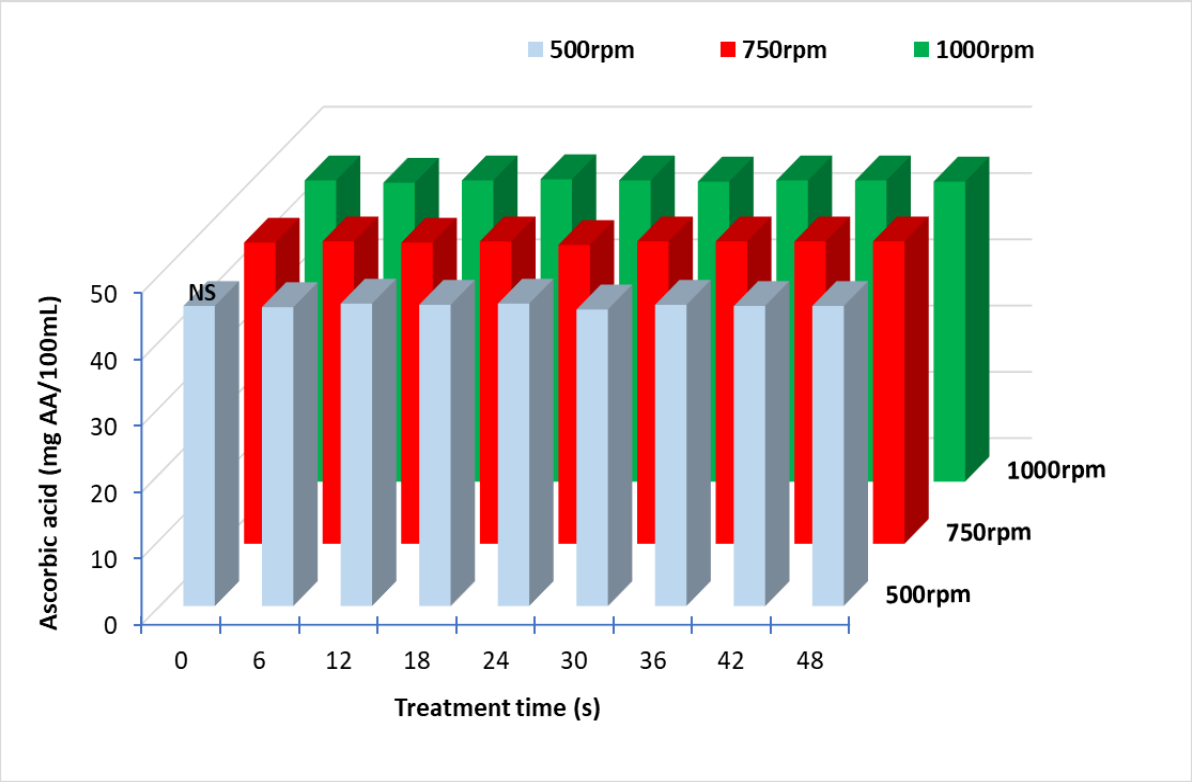

Figure 4.9. 3D graph of ascorbic acid content in apple juice subjected to PL treatment.

Note: NS: non-significant difference in AA content amongst agitation speeds and treatment durations.

The 3D visualization in Figure 4.9 compares the AA content of untreated juice (0 s) to that of treated samples subjected to treatment durations ranging from 6-48 s and agitation speeds of 500 rpm, 750 rpm, and 1,000 rpm. The figure clearly shows that AA content remained unaffected under all PL treatment conditions, irrespective of the treatment duration or agitation speed. Additionally, Table 4.9 demonstrates that the AA content of untreated juice ranged between 45.3 ± 0.6 and 45.4 ± 0.5 mg AA/100 mL, while the treated samples exhibited no significant changes (p> 0.05), with values ranging from 44.7 ± 0.8 to 45.6 ± 0.6 mg AA/100 mL.

Similar studies have investigated the impact of PL on the AA content of fruit juices. For instance, Vollmer et al. (2020) observed that mild PL treatment at 2.1 kV with 47 pulses (253 J/cm^2) completely retained the AA content in pineapple juice. However, at 2.4 kV with 187 pulses (1479 J/cm^2), a 29% reduction in AA content was recorded. Their findings indicated that AA degradation increased with higher pulse numbers and voltages during PL treatment. In a separate study, Pihen et al. (2024) reported a 38.4% decrease in vitamin C (7 mg AA/100 mL) in fresh apple juice subjected to PL treatment at a maximum fluence of 176.4 J/cm^2 for 70 s. The greater loss observed in their study may be attributed to their use of longer treatment duration (70 s) and

a higher total fluence (176.4 J/cm^2) compared to the mild PL conditions in the current study (maximum duration of 48 s and fluence of approximately 5.04 J/cm^2).

Similarly, Chakraborty and Parab (2023) reported a 12% reduction in vitamin C content in PL-treated pasteurized table grape juice (pH 3.5) when subjected to elevated fluence levels of 1152-3186 J/cm^2.

Table 4.9. Ascorbic acid levels in apple juice under various PL treatment conditions

Treatment time (s)	Ascorbic acid, AA (mg AA/100mL)		
	500rpm	**750rpm**	**1,000 rpm**
0	45.3 ± 0.6^{aA}	45.4 ± 0.5^{aA}	45.4 ± 0.5^{aA}
6	45.1 ± 0.6^{aA}	45.6 ± 1.1^{aA}	45.1 ± 0.3^{aA}
12	45.6 ± 0.3^{aA}	45.4 ± 0.5^{aA}	45.5 ± 0.0^{aA}
18	45.5 ± 0.5^{aA}	45.6 ± 0.6^{aA}	45.6 ± 0.8^{aA}
24	45.6 ± 1.1^{aA}	45.1 ± 1.1^{aA}	45.5 ± 0.5^{aA}
30	44.7 ± 0.8^{aA}	45.6 ± 0.6^{aA}	45.3 ± 0.8^{aA}
36	45.5 ± 0.5^{aA}	45.6 ± 0.3^{aA}	44.5 ± 0.5^{aA}
42	45.3 ± 0.8^{aA}	45.6 ± 0.6^{aA}	45.5 ± 0.5^{aA}
48	45.3 ± 0.8^{aA}	45.6 ± 0.3^{aA}	45.3 ± 0.3^{aA}

Note: Values represent the means (n=3) ± SD of ascorbic acid content. Lowercase letters indicate, for each evaluated agitation speed, significant differences ($p < 0.05$) among treatment times. Uppercase letters indicate, for each treatment time, significant differences ($p < 0.05$) among the evaluated agitation speeds.

4.4.9. Rheological properties

The impact of PL treatment on the rheological properties, such as the flow behavior index (n), consistency coefficient (m), and shear viscosity (apparent viscosity, η), was analyzed by comparing untreated apple juice samples with those subjected to different treatment durations (6-48 s) and agitation speeds (500 rpm, 750 rpm, and 1,000 rpm). The results, as presented in Table 4.10, indicate that all rheological parameters evaluated exhibited minimal variation across the untreated and treated samples. However, these differences were not statistically significant ($p > 0.05$) and were independent of the treatment duration or agitation speed applied during the PL processing.

Table 4.10. Rheological properties of apple juice under various PL treatment conditions

Treatment (s)	500 rpm	750 rpm	1,000 rpm
a. Consistency coefficient, *m* (mPa)			
0	1.80 ± 0.05^{aA}	1.80 ± 0.05^{aA}	1.80 ± 0.05^{aA}
6	2.13 ± 0.38^{aA}	1.77 ± 0.09^{aA}	1.76 ± 0.10^{a}
12	2.35 ± 0.38^{aB}	1.75 ± 0.09^{aA}	1.88 ± 0.18^{aAB}
18	2.31 ± 0.42^{aB}	1.85 ± 0.08^{abAB}	1.69 ± 0.22^{aA}
24	2.43 ± 0.54^{aA}	2.31 ± 0.45^{bA}	1.71 ± 0.14^{aA}
30	2.22 ± 0.62^{aA}	2.03 ± 0.19^{abA}	2.13 ± 0.16^{aA}
36	2.35 ± 0.53^{aA}	1.98 ± 0.20^{abA}	2.17 ± 0.68^{aA}
42	2.46 ± 0.52^{aA}	1.74 ± 0.12^{aA}	2.07 ± 0.59^{aA}
48	2.40 ± 0.50^{aA}	1.88 ± 0.28^{abA}	1.93 ± 0.09^{aA}
b. Flow behavior index, *n*			
0	1.00 ± 0.00^{aA}	1.00 ± 0.00^{aA}	1.00 ± 0.00^{aA}
6	1.00 ± 0.00^{aA}	1.00 ± 0.00^{aA}	1.00 ± 0.00^{aA}
12	0.99 ± 0.03^{aA}	1.00 ± 0.00^{aA}	1.00 ± 0.00^{aA}
18	0.99 ± 0.03^{aA}	1.00 ± 0.00^{aA}	1.00 ± 0.00^{aA}
24	0.98 ± 0.05^{aA}	1.00 ± 0.00^{aA}	1.00 ± 0.00^{aA}
30	0.98 ± 0.05^{aA}	1.00 ± 0.00^{aA}	1.00 ± 0.00^{aA}
36	1.00 ± 0.00^{aA}	1.00 ± 0.00^{aA}	0.99 ± 0.03^{aA}
42	0.97 ± 0.03^{aA}	1.00 ± 0.00^{aA}	0.99 ± 0.03^{aA}
48	0.98 ± 0.04^{aA}	1.00 ± 0.00^{aA}	1.00 ± 0.00^{aA}
c. Shear viscosity, η (mPa.s)			
0	1.80 ± 0.05^{aA}	1.80 ± 0.05^{aA}	1.80 ± 0.05^{aA}
6	2.13 ± 0.38^{aA}	1.77 ± 0.09^{aA}	1.76 ± 0.10^{aA}
12	2.46 ± 0.58^{aB}	1.75 ± 0.09^{aA}	1.88 ± 0.18^{aA}
18	2.42 ± 0.62^{aA}	1.85 ± 0.08^{aA}	1.69 ± 0.22^{aA}
24	2.61 ± 0.87^{aA}	2.31 ± 0.45^{aA}	1.71 ± 0.14^{aA}
30	2.40 ± 0.98^{aA}	2.03 ± 0.19^{aA}	2.13 ± 0.16^{aA}
36	2.35 ± 0.53^{aA}	1.98 ± 0.20^{aA}	2.29 ± 0.90^{aA}
42	2.66 ± 0.75^{aA}	1.74 ± 0.12^{aA}	2.16 ± 0.77^{aA}
48	2.53 ± 0.77^{aA}	1.88 ± 0.28^{aA}	1.93 ± 0.09^{aA}

Note: Values are reported as the mean (n=4) ± SD of three rheological properties. Lowercase letters indicate, for each evaluated agitation speed, significant differences ($p < 0.05$) among treatment times. Uppercase letters indicate, for each treatment time, significant differences ($p < 0.05$) among the evaluated agitation speeds. NS: non-significant.

Figure 4.10 illustrates the curves of apparent viscosity (η) as a function of shear rate ($\dot{\gamma}$) over a range of 0.1-100 s^{-1} under three different agitation speeds. The flow behavior comparison between untreated (control) samples and those treated at the shortest (6 s) and longest (48 s) durations revealed variations in fluid flow behavior across agitation speeds. At an agitation speed of 500 rpm (Figure 4.10.a), samples treated for 48 s exhibited, a slightly, shear-thinning behavior ($n < 1$), where viscosity decreased with an increasing shear rate. This response differed from the untreated sample, which exhibited Newtonian flow behavior. Conversely, at higher agitation speeds (750 rpm and 1,000 rpm), both untreated and treated samples showed similar Newtonian

flow behavior (Figure 4.10.b & c), indicating that viscosity remained constant across the tested shear rate range (0.1-100 s^{-1}).

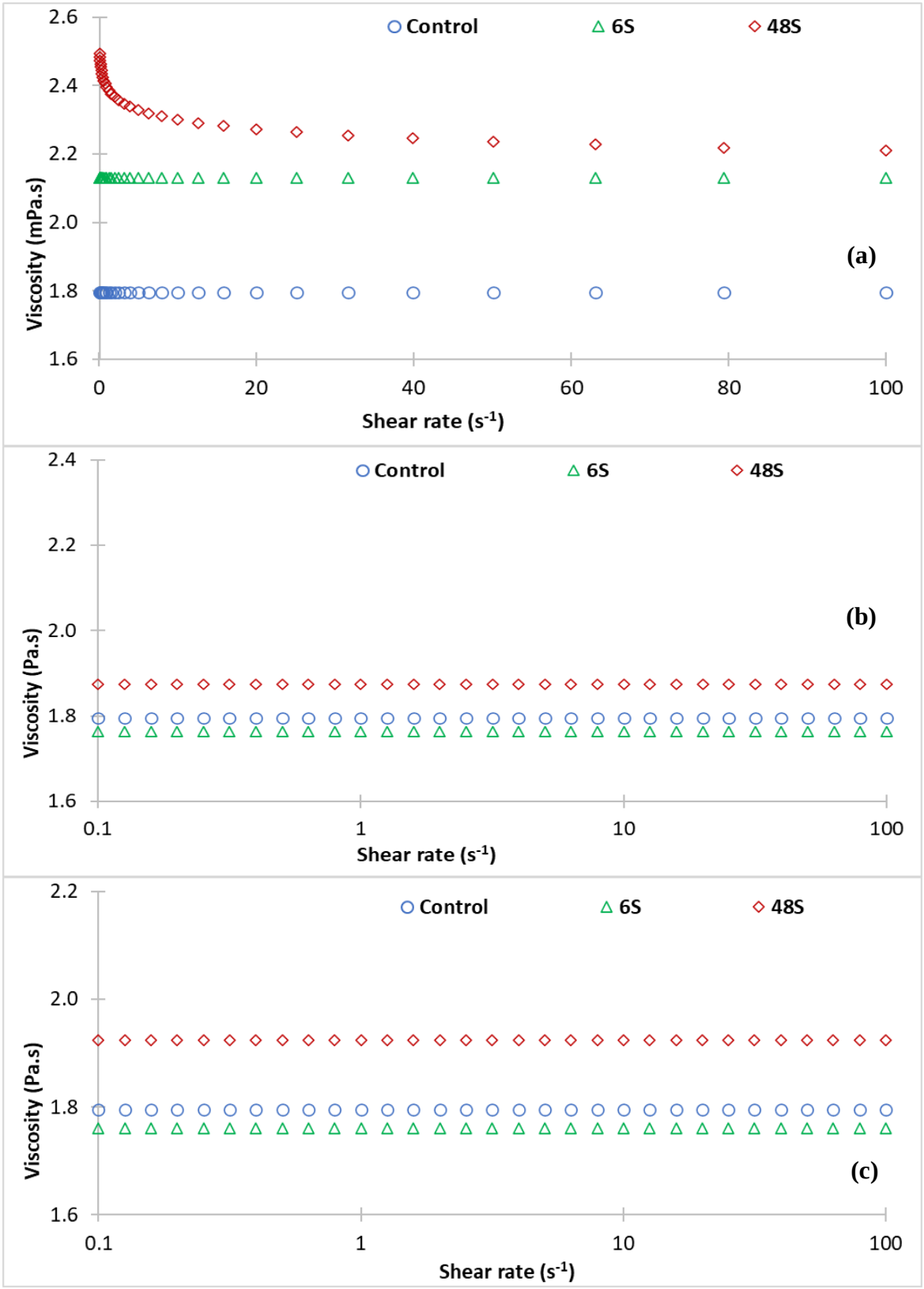

Figure 4.10. Flow behavior under various agitations: (a) 500 rpm, (b) 750 rpm, and (c) 1,000 rpm.

Overall, PL treatment did not induce any significant changes in the flow behavior of apple juice across all treatment durations (0-48 s) and agitation speeds, as the flow behavior index (*n*) values remained statistically unchanged ($p > 0.05$) between controls and treated samples.

Similar findings have been reported in previous studies investigating the effects of PL on the rheological properties of liquid foods. Consistent with the current study, Pihen et al. (2024) observed no significant changes in the viscosity of apple juice following PL treatment. Kasahara et al. (2004) also found that clarified apple juice exposed to pulsed light at fluences ranging from 1850 to 3354 mJ/cm^2 exhibited no significant viscosity differences compared to fresh samples. Furthermore, Kasahara et al. (2015) reported that goat milk samples treated with fluences of 5 J/cm^2 and 10 J/cm^2 showed only a minor, non-significant increase in viscosity.

In contrast, Pok et al. (2023) demonstrated that PL treatments (3-60 s) corresponding to fluences between 3.6 and 71.6 J/cm^2 caused a slight but significant reduction in the apparent viscosity of melon juice. Additionally, they observed shear-thinning behavior, with viscosity decreasing at increasing shear rates across all samples.

The rheological behavior of fruit juices is influenced by interactions between the dispersed phase (pulp) and the serum phase viz. soluble polysaccharides, sugars, salts, and acids (Diamante & Umemoto, 2015). According to Ibarz et al. (1996), juices containing pectin and pulp exhibit non-Newtonian behavior, while depectinised and clarified juices follow a Newtonian pattern. The minimal and non-significant changes in rheological properties observed in the current study may be attributed to the nature of the apple juice used, which lacked pectins and pulps that could promote the absorption of pulsed light and potentially trigger the depolymerization of pectins and polysaccharides.

4.5. Conclusions

The present study demonstrated that agitation-assisted pulsed light (PL) treatment serves as a promising non-thermal alternative to conventional thermal pasteurization methods for fruit juices. While PL has been previously recognized for its efficiency in microbial inactivation on food surfaces and in clear liquids, its application in non-clear liquids has been limited due to weak penetration. However, this study showed that the integration of PL treatment with liquid agitation effectively enhanced microbial inactivation in apple juice, a non-clear liquid, by facilitating uniform exposure of liquid particles to PL. This approach also allowed for the pasteurization of

larger volumes, up to 400 mL. For example, at an agitation speed of 1,000 rpm, a D-value of 4.7 s was achieved, enabling a five-log microbial reduction within approximately 24 s of treatment. In contrast, under static conditions (0 rpm), the same reduction could not be attained, as microbial counts plateaued at around 6 log CFU/mL after 24 s of PL treatment.

The agitation-assisted PL method met the criteria for nonthermal processing, as the temperature rise in apple juice samples during treatment remained well below the threshold for thermal methods. Moreover, PL treatment had minimal or negligible effects on critical quality attributes, including total phenolic content, antioxidant activity, total soluble solids, pH, color, ascorbic acid content, and rheological properties. These findings emphasize the potential of optimized PL treatment conditions to achieve the FDA-recommended five-log microbial reduction while preserving the sensory and nutritional quality of the juice. Additionally, the protocols and experimental insights from this study could guide further research, facilitate the development of treatment protocols, and support the design, scaling up, and pilot-scale testing of agitation-assisted PL systems.

4.6. References

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Chapter 5

Comprehensive Scholarly Discussions

5.1. Discussion of key findings

This study explored the microbial inactivation efficiency and quality preservation of agitation-assisted pulsed light (PL) treatment in water and apple juice, using *Escherichia coli* K-12 as a model microorganism. The research aimed to understand how agitation speed, sample volume, and treatment time affect microbial decontamination in both water and apple juice, with a particular focus on quality retention in apple juice. In the first part, sterile water inoculated with *E. coli* K-12 was treated under various PL conditions, such as different treatment durations, agitation speeds, and sample volumes, to assess the influence of these factors on PL's microbial inactivation efficiency. In the second part, pasteurized apple juice (400 mL) was treated under varying PL durations and agitation speeds to evaluate the combined effect of agitation-assisted PL on microbial inactivation and quality preservation in the juice.

5.1.1. Microbial inactivation efficiency

5.1.1.1. Effect of agitation

Agitation significantly enhanced the microbial inactivation efficiency of pulsed light (PL) treatment in both water and apple juice. The data indicated that the inactivation of *E. coli* K-12 was influenced by agitation speed, with higher speeds resulting in greater inactivation for any given treatment time. In water, at 1,000 rpm, the D-value was 2.96 s, meaning a five-log reduction could be achieved in about 15 s. In contrast, under static conditions (0 rpm), achieving the same reduction would take approximately 30 s, twice as long. A similar trend was observed in apple juice: at 1,000 rpm, the D-value was 4.7 s, and a five-log reduction could be reached in about 24 s. Without agitation (0 rpm), however, microbial counts levelled at around 6 log CFU/mL after 24 s, and further reduction was not observed. These findings suggest that different liquids respond differently to PL, but agitation effectively helped pasteurize apple juice, a result that was not achievable without it. This improvement in microbial inactivation over our previous studies, such as those by Mittal (2023), which found PL ineffective in inactivating *E. coli* in 5 mL of apple juice without agitation, can be attributed to better mixing. Agitation ensures more uniform exposure of the liquid to PL radiation, overcoming the shielding effect that occurs in deeper liquid layers, where microorganisms remain inadequately exposed to light pulses.

Continuous circulation provided by agitation ensures consistent and more effective exposure to PL treatment.

5.1.1.2. Impact of sample volume

The sample volume also impacted the microbial inactivation kinetics during pulsed light (PL) treatment. To assess the effect of sample volume on PL efficiency, water inoculated with *E. coli* K-12 (EC1-5G) was treated with PL for various durations (0-20 s) at volumes of 100 mL, 200 mL, 300 mL, and 400 mL. Smaller volumes exhibited steeper inactivation curves and lower D-values, indicating more efficient treatment in smaller quantities. Larger volumes required significantly longer exposure times to achieve the same level of inactivation due to increased optical thickness and reduced uniformity of treatment. At a constant agitation speed of 500 rpm, microbial reduction varied across treatment times. For example, after a 6-second PL treatment, the microbial load decreased from 7.0 log to 5.8 log, 5.6 log, 4.7 log, and became undetectable for 400 mL, 300 mL, 200 mL, and 100 mL samples, respectively. Additionally, smaller volumes showed faster inactivation rates. Reducing *E. coli* K-12 from 7.0 log to undetectable levels took just 4 s for a 100 mL sample, while 200 mL, 300 mL, and 400 mL samples required 10, 14, and 20 s, respectively. These findings emphasize the importance of agitation in enhancing microbial inactivation during PL treatment. Agitation induces turbulence, which improves the exposure of deeper liquid layers to PL, thereby increasing the inactivation rate. As noted by Gómez-López (2011), microorganisms on the surface are easily inactivated, while those deeper in the liquid are shielded from PL exposure. Interestingly, agitation helps overcome this by ensuring uniform mixing, ensuring all microorganisms are exposed to the light, and maximizing the inactivation efficiency.

5.1.1.3. Multi-container treatments and energy efficiency

Treating multiple smaller containers simultaneously proved to be an efficient approach, yielding microbial inactivation kinetics comparable to those observed with single-container treatments of the same total liquid volume. This strategy optimizes energy use and increases process efficiency. In addition, treating smaller containers concurrently enhances the uniformity of light exposure and the effectiveness of agitation, providing a dual benefit for microbial inactivation.

5.1.2. Quality preservation of apple juice

5.1.2.1. Temperature increase

The temperature rise observed in this study further supports the classification of pulsed light (PL) treatment as a nonthermal technology. A processing method is considered nonthermal if the temperature increase in the treated product does not exceed 30°C (Chiozzi et al., 2022). In this study, the maximum temperature increase in PL-treated apple juice was 3.13 ± 0.06°C, well below this threshold, confirming that the PL treatment remains non-thermal. This is important for preserving the sensory and nutritional qualities of liquid food products like apple juice, as heat treatments could lead to undesirable changes in flavor, texture, and nutritional value.

5.1.2.2. Total phenolic content retention

Phenolic compounds are crucial for a healthy diet, making their preservation in food essential from a nutritional perspective. This study examined the effects of agitation-assisted PL treatment on the total phenolic content (TPC) of apple juice. Results showed an initial increase in TPC up to specific treatment durations: 18 s at 1,000 rpm, 24 s at 750 rpm, and 30 s at 500 rpm. However, prolonged exposure beyond these points led to a gradual decline in TPC, eventually falling below initial levels. These findings underscore the importance of optimizing treatment conditions, as prolonged exposure can significantly reduce bioactive compound levels.

Similar reduction trends have been noted in previous research. Pihen et al. (2024) reported a 38.4% decrease in TPC in fresh apple juice subjected to a fluence of 176.4 J/cm^2 for 70 s, attributing the reduction to thermal effects. Conversely, Palgan et al. (2011) found no significant change in TPC after an 8-second PL treatment of apple juice at a fluence of 1.17 J/cm^2 per pulse, highlighting the effectiveness of shorter treatment durations for preserving phenolic compounds.

5.1.2.3. Antioxidant activity retention

Antioxidant activity in apple juice is primarily attributed to polyphenols and vitamin C, with additional contributions from carotenoids and vitamin E. In this study, antioxidant activity was evaluated using the % DPPH scavenging method. Results showed that antioxidant levels remained relatively stable throughout PL treatments, regardless of agitation speed.

At 500 rpm, antioxidant activity ranged from 91.1% to 92.37%, with an initial value of 92.1%. At 750 rpm, the activity varied between 89.9% and 90.9% (initial: 89.2%), while at 1,000 rpm, it ranged from 89.6% to 90.0% (initial: 89.81%). These minor fluctuations suggest that antioxidant

activity was largely unaffected by the PL treatment, maintaining the functional properties of apple juice. However, the influence of liquid agitation on TPC during PL treatments could not be conclusively confirmed.

Similar studies were conducted previously. Muñoz et al. (2012) found no significant effect on antioxidant capacity at a fluence of 5.1 J/cm^2. Palgan et al. (2011) noted a 5.5% decrease after an 8-second treatment at 28 J/cm^2. Vollmer et al. (2020) observed no substantial changes in the antioxidant capacity of pineapple juice treated at fluences up to 375 J/cm^2 but reported a 14% reduction under more intense conditions. These studies further emphasize the resilience of antioxidant activity under moderate PL treatments.

5.1.2.4. Total soluble solids stability

The Total Soluble Solids (TSS) content, measured in °Brix, remained consistent across all PL treatments, regardless of treatment duration or agitation speed. The TSS of untreated apple juice was 10.8 ± 0.4 °Brix, and no significant changes were detected following the treatments. Similarly, pH levels remained stable throughout, indicating that PL treatments did not compromise the sensory qualities of apple juice. These results highlight the robustness of key quality parameters under nonthermal processing methods.

These findings are consistent with previous studies. Pihen et al. (2024) reported that the TSS of apple juice remained steady at 14.40 °Brix after PL treatment. Similarly, Kasahara et al. (2004) found no significant changes in the TSS of clarified apple juice treated with energy dosages between 1,850 and 3,354 mJ/cm^2. Comparable outcomes were reported by Chakraborty et al. (2020) and Jayachandran et al. (2024), who observed no significant variations in the TSS of Indian gooseberry and sugarcane juices subjected to PL treatments. These findings further underscore the stability of TSS and antioxidant properties under non-thermal preservation techniques.

5.1.2.5. pH stability

The pH of apple juice remained stable throughout all PL treatment durations (6-48 s) and agitation speeds (500 rpm, 750 rpm, and 1,000 rpm). As shown in Figure 4.8, the pH values did not change, with the initial pH of untreated apple juice recorded at 3.26 ± 0.01. These results are consistent with previous studies, such as those by Palgan et al. (2011) and Cai et al. (2024), which also reported no significant pH changes after PL processing of apple juice. Similar findings were observed for other fruit juices, including Indian gooseberry juice (Chakraborty et

al., 2020) and sugarcane juice (Jayachandran et al., 2024), further confirming the pH stability under PL treatment. The minimal or no effect on pH suggests that PL treatment does not trigger chemical reactions that could alter the acidity of liquid foods.

5.1.2.6. Color preservation

Color is an essential sensory attribute of fruit juices, and the study found that agitation during pulsed light (PL) treatment had a significant effect on color retention. Higher agitation speeds (750 rpm and 1,000 rpm) reduced total color change (ΔE^*) for the same treatment duration. According to ΔE^* value classification (Wibowo et al., 2015), color changes were considered slightly noticeable (0.5-1.5) at 1,000 rpm, noticeable (1.5-3.0) at 750 rpm, and great (6.0-12.0) at 500 rpm. These results are consistent with previous studies by Qi et al. (2023) and (Chakraborty & Parab, 2023) Chakraborty and Parab (2023), who also reported color changes in fruit juices. According to these studies, the changes are likely attributed to enzymatic or non-enzymatic browning reactions triggered by PL exposure.

5.1.2.7. Ascorbic acid retention

Ascorbic acid (vitamin C) is an essential antioxidant in apple juice. The study revealed that its content remained stable across all PL treatment conditions, regardless of treatment duration or agitation speed. Untreated juice had AA levels between 45.3 ± 0.6 and 45.4 ± 0.5 mg AA/100 mL, while treated samples ranged from 44.7 ± 0.8 to 45.6 ± 0.6 mg AA/100 mL. These findings are in line with previous research by Vollmer et al. (2020) and Pihen et al. (2024), which also reported minimal or no degradation of AA under mild PL conditions. The results emphasize that mild PL treatments (maximum duration of 48 s and fluence of approximately 5.04 J/cm^2) are effective in preserving the nutritional quality of apple juice.

5.1.2.8. Rheological properties

The study also examined the rheological properties of apple juice, including the flow behavior index (n), consistency coefficient (m), and apparent viscosity (η). Minimal and statistically insignificant differences ($p > 0.05$) were observed between untreated and treated samples across all PL durations (6-48 s) and agitation speeds (500 rpm, 750 rpm, and 1,000 rpm). At 500 rpm, samples treated for 48 s exhibited slight shear-thinning behavior, where viscosity decreased with increasing shear rate. In contrast, untreated samples exhibited Newtonian flow behavior. At higher agitation speeds (750 rpm and 1,000 rpm), both untreated and treated samples

demonstrated similar Newtonian flow behavior, indicating stable viscosity across the tested shear rate range (0.1-100 s^{-1}). These results are consistent with studies that found minimal changes in viscosity after PL treatment of apple juice (Pihen et al., 2024) and goat milk (Kasahara et al., 2015). The findings suggest that PL treatment does not disrupt the structural components responsible for the flow behavior of apple juice.

5.1.3. Mechanistic insights into PL-agitation synergy

The enhanced performance of agitation-assisted PL treatments can be explained by several synergistic mechanisms: *Enhanced mixing*: Agitation ensures continuous movement of the liquid, preventing the formation of stagnant zones that would be poorly exposed to PL radiation. *Uniform exposure*: Agitation helps eliminate the shielding effect caused by the uneven distribution of PL radiation in static systems, ensuring more uniform microbial inactivation throughout the sample. *Heat dissipation*: Agitation promotes better heat dissipation, particularly at higher speeds, which may prevent localized overheating and reduce the potential for thermal effects on microbial cells.

5.1.4. Practical and industrial implications

The findings of this study offer valuable insights into the food and beverage industry. Incorporating agitation-assisted PL treatments in production lines can enhance microbial inactivation rates while preserving product quality. From an operational perspective, optimizing treatment parameters, including sample volume and agitation speed, is crucial for maximizing efficiency. Additionally, utilizing small-volume treatments in multi-container setups presents a promising strategy to boost process efficiency and reduce energy consumption.

5.2. Future recommendations

Extended storage studies are essential to assess the growth patterns of spoilage microorganisms and potential pathogens in PL-treated fruit beverages compared to those treated with conventional pasteurization. This evaluation will help determine the microbiological safety and shelf-life benefits of PL treatment, which is crucial for its commercial viability. Additionally, assessing the impact of PL on sensory attributes will ensure that the treated products are acceptable to consumers.

To optimize the efficiency of PL treatment, continuous flow systems with various configurations can be utilized to maximize liquid particle exposure to PL. This method would enhance

absorption without increasing the surface dose, allowing for higher-load applications of the technology.

Although this study primarily investigated *Escherichia coli* K-12, future research should broaden the microbial spectrum to include yeasts, molds, and acid-tolerant pathogens commonly found in fruit juices. The first-order kinetic model was selected for its simplicity and widespread application in initial studies, enabling straightforward comparisons of treatment efficacy. However, it is important to acknowledge that this model assumes a constant inactivation rate and may not adequately represent nonlinear inactivation patterns, such as tailing or shoulder effects, which are often observed in complex food matrices like fruit juices. In contrast, the Weibull model offers greater flexibility in capturing such deviations due to its ability to accommodate both upward and downward concavities in survival curves. As such, future research should consider incorporating alternative modeling approaches, like the Weibull model, to provide a more accurate and comprehensive understanding of microbial inactivation dynamics.

Following the success of agitation-assisted PL in treating water and apple juice, future studies should explore its application to more turbid liquid foods, like pulp-based fruit juices and milk. Additionally, applying the treatment to larger sample volumes, beyond 400 mL, would provide valuable insights into scaling up the technology for commercial use.

PL's established efficiency in water treatment suggests it could also be applied to wastewater treatment as an alternative to sodium hypochlorite. This could drive greater demand for PL equipment, reduce initial investment barriers, and make technology more accessible to various industries.

Finally, enhancements in PL equipment design should aim to integrate mechanisms that generate turbulence or active mixing within liquid samples, while ensuring comprehensive 360° exposure to pulsed light. This could involve the use of high-shear mixers to enhance fluid dynamics and the optimization of lamp arrangements to enable multi-angle light delivery. Such improvements would streamline the treatment process and facilitate the commercial scalability of PL technology, particularly for processing opaque or high-viscosity liquid products.

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Chapter 6

General Conclusions

The initial phase of the study demonstrated that pulsed light (PL) treatment is a promising method for water decontamination due to water's high responsiveness to PL. Agitation speed significantly influenced the treatment's effectiveness, with higher speeds enhancing microbial inactivation, as evidenced by decreasing D-values. The findings also indicated that PL treatment was more effective with thinner liquid layers, as smaller sample volumes resulted in better inactivation. Furthermore, treating multiple containers aligned along the lamp's length achieved uniform microbial inactivation, comparable to treating a single container, suggesting energy efficiency advantages when processing larger volumes, and extending the treatment exposure to the full length of the PL Xenon tube. These insights provide a valuable basis for developing treatment protocols, conducting pilot-scale tests, and scaling up applications. A major finding of the study is adaptation possibilities for scale up. Using a PL tunnel, a continuous system can be visualized. While including a magnetic stirrer is not practical, there are other ways of container agitation to facilitate mixing of the contents deriving the same or similar benefits as the use of magnetic stirrers.

Building on these findings, the study assessed PL treatment as an alternative to traditional thermal pasteurization for fruit juices, which possess distinct optical properties compared to clear water. In apple juice, increased agitation speeds resulted in lower D-values, signifying enhanced microbial inactivation. The agitation-assisted PL treatment did not affect quality parameters such as total soluble solids, pH, and ascorbic acid content. However, minor but statistically insignificant changes were observed in total phenolic content, antioxidant activity, color, and rheological properties. This suggests that higher fluence levels might cause noticeable alterations, as observed in other studies, emphasizing the importance of optimizing treatment conditions for each liquid product. Additionally, an observed temperature increase during treatment was proportional to the treatment duration but remained within acceptable limits for non-thermal methods, further emphasizing the importance of optimizing treatment conditions.

These results form a foundation for more detailed studies, supporting the development of treatment protocols, process design, scaling up, and pilot-scale trials. Considering apple juice's popularity and the ongoing concern about *E. coli* contamination in water, this research emphasizes the potential of agitation-assisted PL treatment to improve the safety and extend the shelf life of liquid foods.

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