5

Assessment of Physicochemical and Microbial Properties of Milk Processed with an Annular UV-C Reactor: A Comparative Analysis with Pasteurization

5.1 Introduction

Food safety is an essential regulatory requirement for the food industry and food services, as well as a hygienic requirement for the end consumer. The World Health Organisation (WHO) estimates that every year 4,20,000 people die, and 600 million people, or about one in ten people globally, become unwell after consuming contaminated food or drink [Mehlhorn, 2016]. Therefore, producing high-quality food without endangering consumers' health is a major challenge and a necessity for the food industry. This challenge is even more significant for the dairy industry, which deals with the most perishable food commodity, providing the most ready nutrition source to humans and all other microorganisms. Milk is a natural food, and humans benefit significantly from milk's intrinsic nutritional value and abundance of other bioactive compounds [Rumbold et al., 2022]. Similarly, milk is a perfect substrate for nutrition to germs like *Campylobacter*, *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus* [Sharma et al., 2014]. Consuming raw or improperly processed milk leads to human dairy-related illnesses, such as brucellosis, tuberculosis, and listeriosis [Ramos et al., 2008] [Doran et al., 2009] [Oliver et al., 2009]. Without altering its nutritional properties, removing bacteria from milk has long been a challenge [Morris et al., 2007]. The most common method of pasteurization deployed in the Indian dairy industry is through a plate heat exchanger in a continuous flow system [Deeth, 2017] [Karlsson et al., 2019].

Unfortunately, pasteurization has two limitations:

(i) It can only be accomplished on a large scale within the industrial sector [Fatih et al., 2021],

(ii) it requires a lot of energy and has expensive installation and running costs [Tigabe et al., 2022].

Heat treatments are effective, but they can also have a negative impact on food by altering its flavour, texture, and colour and denaturing many important vitamins in the milk [Barba et al., 2012] [Pestana et al., 2015]. This “High Temperature” problem is further exacerbated in India as the raw milk reaching the organized processing (pasteurization) is so contaminated that the pasteurization temperature needs to be raised from a minimum of 72 °C to a temperature as high as 85 – 88 °C. This, in addition to nutrition loss, also leads to unintended caramelization of milk. It causes poor-quality consumer-level milk, stripped of a lot of nutrition eaten away by bacteria pre-pasteurization, and denaturing many bioactive components, vitamins, and protein, among other nutritional compositional changes [Moreno-Vilet et al., 2018].

Non-thermal technologies, such as pulsed electric fields, high hydrostatic pressure, ultrasonication, non-thermal plasma and ultraviolet (UV) radiation, have been researched to replace conventional thermal/heat processing in milk [Gadodia et al., 2024] [Cameron et al., 2010]

[Krishnamurthy et al., 2007a] [Trujillo, 2002] [Almeida et al., 2024]. However, there are many obstacles to overcome before non-thermal solutions can be scaled up for commercial use, including poorer efficiency, lengthy treatment durations, the need for specialized equipment, and processing costs. UV light treatment is an emerging technology with the potential to be employed as a pasteurization method in certain processing scenarios, such as milk collection centers (to prevent milk spoilage) and small-scale milk processing operations. Several studies have demonstrated the efficacy of UV radiation in combating several disease-causing microbes, making it a promising technique for treating milk [Manoharan et al., 2021] [Chawla et al., 2021a]. It is commonly recognized that UV-C, which has a wavelength between 200 and 280 nm, has antibacterial qualities and can potentially render certain bacteria and viruses inert [Ahlawat et al., 2022]. The implementation of the "Cold Chain" milk preservation technology is practically non-existent among farmers due to the expensive initial expenditure and the significant power requirement.

This chapter focuses on creating a small-scale non-thermal technology that can be developed to extend shelf life and enhance microbiological safety without compromising milk quality at the farmer level. To do so, we have designed and developed an annular UV reactor for continuous treatment of whole milk. The reactor was designed to allow the raw milk to get maximum UV radiation and improve the efficiency of processing whole milk by optimizing the operational temperature and UV dosages. The methylene blue reduction test (MBRT) was used to perform a microbiological investigation of whole milk before and after treatment. In milk samples, an investigation was carried out to study the inactivation of two foodborne bacteria, *Salmonella typhimurium* and *Staphylococcus aureus*. Lastly, to evaluate the effectiveness of the designed reactor, a comparison is made between the results of the proposed reactor and the commercially available pasteurization process.

5.2 UV Reactor Design and Experimental Setup

For continuous treatment of milk at a small scale by farmers, an annular UV photo reactor has been designed and developed using an 11 W UV-C lamp and 222 nm excimer lamp and housed in a Teflon cylindrical vessel. The Teflon cylindrical vessel has an inner diameter (ID) of 16.2 mm and a total length of 22.6 cm and also has a spiral screw inside with a pitch of 6 mm and a depth of 2 mm. A UV-C lamp (253.7 nm) having a diameter of 16 mm and length of 226 mm, emitting UV-C radiation of intensity 2.6 mW/cm^2, has been fitted inside the Teflon cylinder. Most of the milk flow happens through thread gapping that allows the milk to follow the same flow pattern. The used lamp has a quartz filter to filter out the 185 nm wavelength, which is generally considered the ozone-generating wavelength. For the comparison purpose, a 222 nm excimer lamp has also been fitted inside the Teflon cylinder, which is already described in detail in Chapter 4.

Unprocessed milk was used to ensure no homogenization, pasteurization or standardization during the experiment. These processes alter the temperature-sensitive milk constituents, potentially affecting the inactivation of pathogens and reducing the MBRT value. This is because fat can envelop pathogens, thereby protecting them. The unprocessed milk has been exposed to different dosages of UV light by adjusting the number of flows through the UV reactor. All milk handling and sample collection are done in a biosafety cabinet (BSC) to prevent contamination before and after UV treatment. The transfer and filling of treated milk into test tubes are also carried out in BSC. Figure 5.1 depicts a detailed schematic representation of the milk treatment system that utilizes a UV source. Milk was pumped through the designed UV-C reactor using a peristaltic pump at a flow rate of 1 L/hr for conducting physicochemical and microbiological analysis. Before the experiment, the UV-C lamp (253.7 nm) was switched on for 2.5 minutes as a warm-up time and to ensure no external contamination was present within the experimental reactor, while the 222 nm lamp operated directly because it does not require any warm-up. In addition, the unit was disinfected by flushing ethanol (50% vol/vol) through it for 3-4 minutes, followed by flushing sterilized water for an additional 4-5 minutes to eliminate any

remaining ethanol residue. Following the application of UV-C treatment to milk, the Oxonia solution is then passed through the reactor for 5 minutes. Oxania is utilized as it is a well-recognized disinfectant that utilizes hydrogen peroxide/peracetic acid. It effectively disinfects surfaces by rendering a variety of microorganisms inactive [33].

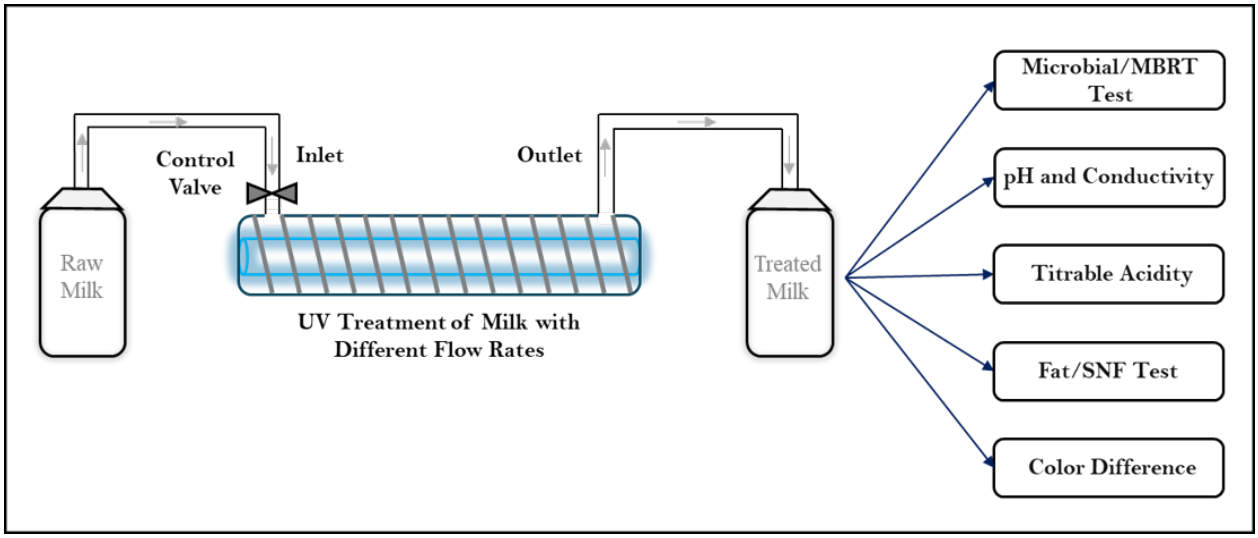


Figure 5.1: Schematic diagram of UV light-based continuous milk treatment system.

5.3 Results and Discussion

5.3.1 UV Dosage using Actinometry Method

Figure 5.2 depicts the UV dosage supplied to milk samples at different passes through the developed UV reactor. At different passes, i.e., one, two, three, and four, the approximate UV dosages are 1.29 J/ml, 3.07 J/ml, 4.48 J/ml, and 6.16 J/ml, respectively. Moreover, the residence time for one, two, three, and four pass/passes through the UV-C reactor is 8.1, 16.2, 24.3, and 32.4 seconds, respectively.

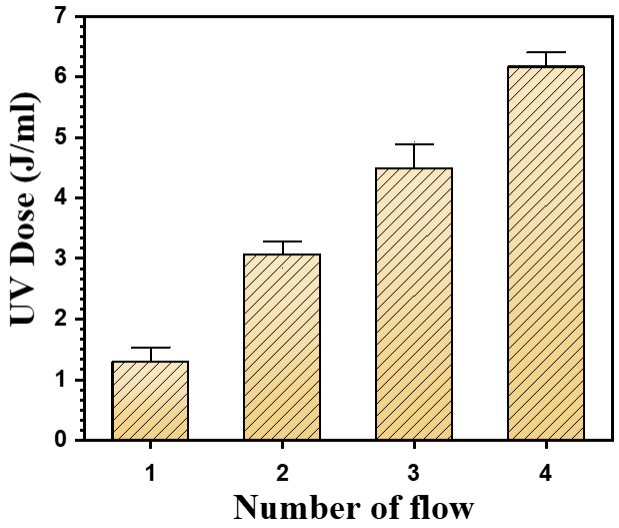


Figure 5.2: UV dosage supplied to milk at different passes.

5.3.2 Physicochemical Properties Analysis

The evaluation and preservation of milk quality depend heavily on its physicochemical properties. We must closely evaluate milk's physicochemical properties, particularly temperature, if we are looking for an alternative and effective technique for processing milk. Figure 5.3 shows the temperature of the whole milk immediately after different flows through the designed UV reactor with UV-C lamp (253.7 nm), and it is compared to the temperature before treatment. It has been observed that an increase in the number of passes through the UV-C reactor increases the milk's temperature but with a minor difference. But, in the case of 222 excimer lamps, the temperature will increase up to 60 °C, as illustrated in Table 5.1. This is due to the joule heating during UV treatment in the milk. The temperature of the untreated milk sample was 26.2 °C, and after the UV treatment with different dosages, the maximum temperature increased to 34.2 °C and 60.4 °C in the case of UV-C and 222 nm excimer lamp, respectively. The obtained high temperature in the case of 222 nm excimer lamp is due to the applied high voltage to generate the equivalent UV intensity when compared to the conventional UV-C lamp. However, the obtained temperature is significantly lower than the milk pasteurization temperature (63 °C for 30 minutes). Ponraj et al. [Ponraj et al., 2017] found that applying plasma treatment caused a significant rise in the milk temperature, reaching as high as 65 °C. The researchers utilized a water-cooling jacket to regulate the temperature and effectively kept it at a constant 42 °C. However, the current study successfully kept the temperature below 35 °C without utilizing any external water cooling. As can be seen from Figure 5.3, subjecting the milk to multiple passes through the UV reactor does not have a noticeable impact on the milk's temperature.

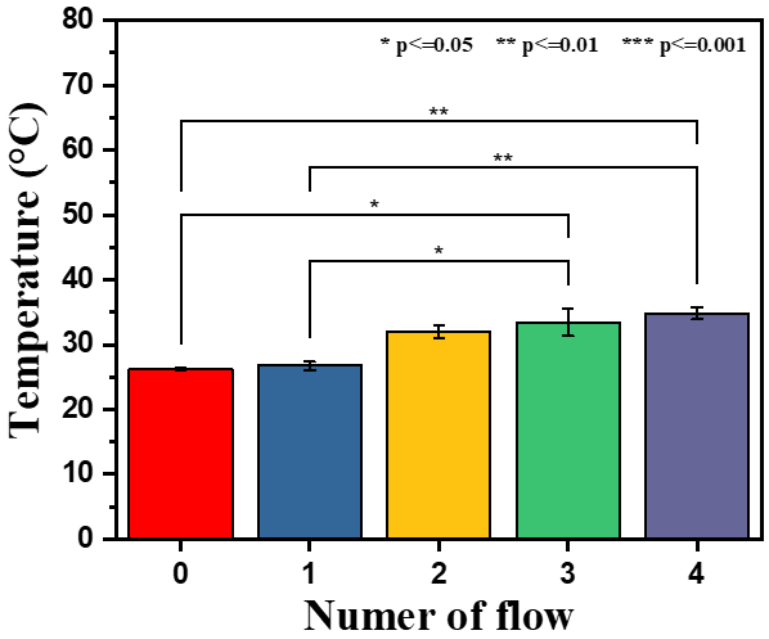


Figure 5.3: Temperature results of untreated milk after exposure to UV-C treatment at various flow rates. The error bars represent the mean±SD of the samples, while the symbols (*, **, and ***) correspond to P values less than or equal to 0.05, 0.01, and 0.001, respectively.

The impact of plasma based non-thermal technology on the physical and chemical characteristics of milk is assessed in multiple research [Wu et al., 2021] [Nikmaram et al., 2022b]. Physicochemical changes can be attributed to several factors, including enzymatic hydrolysis, oxidative reactions, and the production of microbial metabolites. Microbial metabolites are produced when bacteria metabolize milk components, resulting in the creation of unwanted chemicals [Wang et al., 2021]. Lactic acid is generated by the metabolic breakdown of lactose by lactic acid bacteria, resulting in a decrease in pH and the precipitation

of proteins [Almeida et al., 2024]. This study involved assessing many physicochemical parameters of milk, including pH, conductivity, titratable acidity, and fat/SNF.

Table 5.1: *Measurement of temperature following milk post-treatment with excimer 222 nm lamp.*

Number of flow (s)	**Untreated**	**1**	**2**	**3**	**4**
Temperature (°C)	26.2±0.8	52.3±2.8	55.8±2.1	58.2±3.7	60.4±2.9

Table 5.2 displays the pH and titratable acidity values before and after treatment of whole milk at different passes. It has been observed that the both parameters remain almost unchanged after every pass, as can be seen from Table 5.2. Sepulveda et al. [Sepulveda et al., 2005] reported that sensory changes in milk occur when the concentration of lactic acid exceeds 0.2%. There is no noticeable variation in the sensory characteristics and pH level of the milk when subjected to UV treatment. The obtained results are highly consistent with the previous findings, which reported that the pH of the milk remained unchanged after undergoing UV treatment [Orlowska et al., 2013].

Table 5.2: *Measurement of pH and titratable acidity following milk post-treatment with UV-C lamp (253.7 nm) and 222 nm excimer lamp.*

Number of flow (s)	**pH**		**Titratable acidity (% lactic acid)**	
	254 nm	**222 nm**	**254 nm**	**222 nm**
Untreated	6.70±0.02	6.70±0.02	0.133±0.004	0.133±0.004
1	6.60±0.03	6.62±0.08	0.131±0.006	0.129±0.008
2	6.69±0.07	6.68±0.04	0.137±0.001	0.134±0.003
3	6.68±0.04	6.65±0.02	0.134±0.006	0.131±0.005
4	6.64±0.08	6.67±0.05	0.132±0.009	0.132±0.004

Note: The values are shown as the mean ± standard deviation (SD) of three measurements. No statistically significant change has been observed.

The electrical conductivity of milk is a critical component for the bacterial inactivation/MBRT analysis. Figure 5.4 depicts the electrical conductivity of the whole milk for untreated and UV-treated samples at different flow rates in the case of conventional UV lamp. Conductivity was found to exhibit a modest rise, reaching a maximum of 5.71 mS/cm after the fourth pass of UV treatment. Subsequently, it decreased to its initial value of 4.87 mS/cm within 15 minutes. This increase in conductivity is ascribed to the elevation in milk's temperature. Once the temperature of the milk falls back to its initial value, its electrical conductivity also reverts to its initial value. A similar result has been obtained in the case of 222 nm excimer source, as no significant change in electrical conductivity has been observed at different flow rates (see Table 5.3). Henningsson et al. [Henningsson et al., 2005] examined how temperature affects the conductivity of milk. It was determined that as the temperature increases, the presence of different salts in milk leads to an increase in its conductivity.

Table 5.3: *Measurement of electrical conductivity following milk post-treatment with excimer 222 nm lamp.*

Number of flow (s)	Untreated	1	2	3	4
Electrical Conductivity (µS/cm)	4.87±0.08	5.32±0.05	5.27±0.10	5.38±0.07	5.57±0.12

In order to better understand the sensory characteristics of the milk, the fat and SNF of both the control and UV-treated milk were also measured. The findings indicate no substantial alteration in the fat content between the two. SNF is recorded after the different milk passes through the UV reactor, in the case of both arrangements i.e., UV-C lamp assembly and 222 nm excimer lamp assembly, as shown in Table 5.4, because of the low UV dosage used for the milk treatment. The values are shown as the mean ± standard deviation (SD) of three measurements. There has been no discernible change in statistical significance.

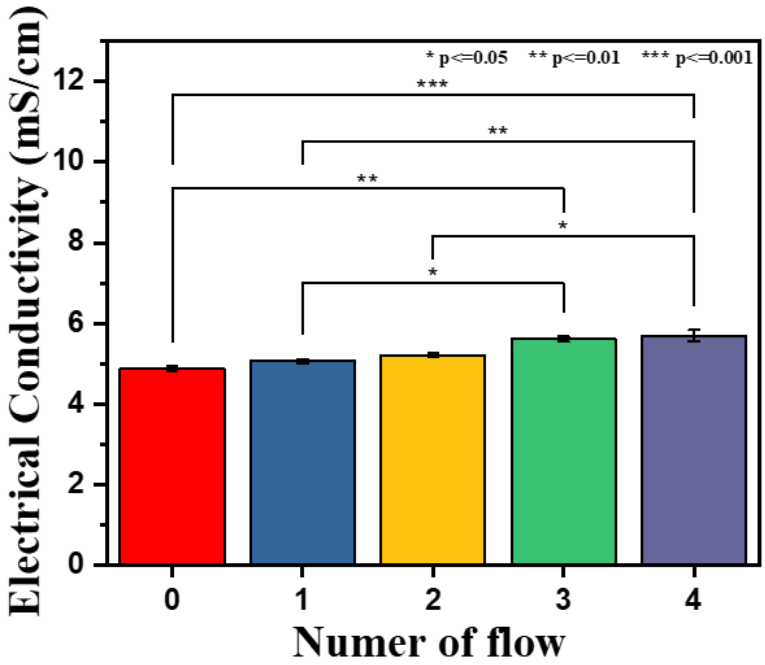


Figure 5.4: Electrical conductivity results of untreated milk after exposure to UV-C treatment at various flow rates. The error bars represent the mean±SD of the samples, while the symbols (*, **, and ***) correspond to P values less than or equal to 0.05, 0.01, and 0.001, respectively.

The colour of milk changes during preservation, which is considered relevant and influences people's perceptions. As a result, measurements of the colour changes after UV treatment are contrasted with raw milk's colour. Throughout the UV milk treatment, the L*, a*, and b* values exhibited a small decrease in all the samples compared to the control. The range of 0 - 0.05 for the total colour change (ΔE*) indicates a non-notable variation, whereas a range of 1 - 3 corresponds to a noticeable difference [Manoharan et al., 2021]. If the change in color, represented by ΔE*, surpasses the threshold of 6, it indicates a substantial alteration. The ΔE* value for all the samples in our investigation ranges from 0.01 to 0.13 and 0.01 to 0.17, in the case of conventional UV-C and 222 excimer lamps, respectively. Table 5.5 displays the variation in color of processed milk samples at various flow rates using the CIE color system following many passes through the UV reactor. There has been no discernible change in statistical significance. The

findings on physicochemical parameters align with prior research on milk subjected to non-thermal treatment [Ponraj et al., 2017].

Table 5.4: *Fat (%) and SNF (%) measurements after post-treatment of milk with UV-C lamp (253.7 nm) and 222 nm excimer lamp.*

Number of flow (s)	Fat (%)		SNF (%)	
	254 nm	222 nm	254 nm	222 nm
Untreated	4.28±0.19	4.28±0.19	8.84±0.29	8.84±0.29
1	4.38±0.22	4.43±0.17	8.57±0.29	8.71±0.22
2	4.39±0.16	4.47±0.24	8.66±0.11	8.76±0.17
3	4.46±0.23	4.39±0.17	8.73±0.11	8.81±0.24
4	4.42±0.17	4.52±0.26	8.83±0.45	8.89±0.41

Note: The values are shown as the mean ± standard deviation (SD) of three measurements. No statistically significant change has been observed.

Table 5.5: *Colour difference of UV-treated milk samples with respect to the number of flows (in case of conventional UV-C lamp) using the CIE colour system.*

Number of flow (s)	Colour			
	L*	a*	b*	ΔE*
Untreated	94.81±0.04	-1.56±0.08	10.11±0.06	--
1	94.89±0.05	-1.54±0.06	10.11±0.05	0.13±0.04
2	94.81±0.06	-1.51±0.06	10.06±0.03	0.08±0.06
3	94.89±0.04	-1.54±0.09	10.14±0.02	0.09±0.06
4	94.79±0.08	-1.52±0.09	10.13±0.05	0.08±0.05

5.3.3 MBRT and Bacterial Reduction Testing

The milk samples, both untreated and treated, were subjected to a methylene blue reduction test (MBRT) for qualitative analysis [Thornton et al., 1930]. MBRT is especially important from an industry perspective, as it is the conventional test carried out during milk collection throughout India. Figure 5.5 compares MBRT results for raw milk treated at various flow rates/UV dosages of conventional UV-C lamp. The MBRT of untreated milk was approximately 2 hours 45 minutes, and it has been enhanced to more than 5 hours and 15 minutes after the fourth pass (UV dosage of 6.16 J/ml) of raw milk through the UV reactor. It should be noted that there is a notable rise in MBRT when raw milk is exposed to UV light at various flow rates (see Figure 5.5). The photonic energy of 254 nm light and its unique behaviour in deactivating bacteria may be the source of this improvement in MBRT since this wavelength is

thought to be the most lethal range of the UV spectrum. A similar result has been obtained in the case of 222 nm excimer source and an enhancement in the MBRT value to more than 5 hours and 30 minutes has been obtained after fourth pass of raw milk through the UV reactor (see Table 5.6). This slight more change in MBRT value in the case of UV 222 nm light is due to the more photonic energy at 222 nm as compared to 254 nm light.

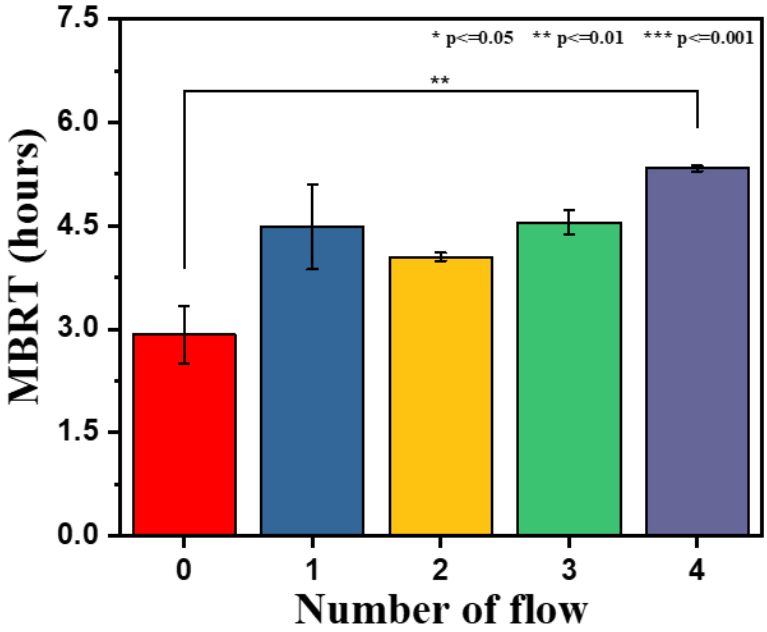


Figure 5.5: MBRT results of untreated milk after exposure to UV-C treatment at various flow rates. The error bars represent the mean±SD of the samples, while the symbols (*, **, and ***) correspond to P values less than or equal to 0.05, 0.01, and 0.001, respectively.

Table 5.6: *Measurement of MBRT following milk post-treatment with excimer 222 nm lamp.*

Number of flow (s)	Untreated	1	2	3	4
MBRT (hours)	2.45±0.10	4.15±0.20	3.45±0.10	5.15±0.10	5.30±0.15

Further bacterial research was conducted on the two specific bacteria generally present in whole milk. Specifically, *S. aureus* (a gram-positive bacteria) and *Salmonella typhi* (a gram-negative bacteria) were chosen for further examination. We checked the efficiency of UV-C light on these specific bacteria. The total bacterial population for *Salmonella typhi* was 5.05 log/ml in the untreated raw milk. Figure 5.6 (a) demonstrates a substantial reduction in the initial load of *Salmonella typhi* throughout several cycles. The number of colonies on the petri plates decreased after every consecutive pass. However, a significant difference is noticed after the first (2.95 log/ml) and second pass (0.21 log/ml), and no colonies are recorded after the third and further passes.

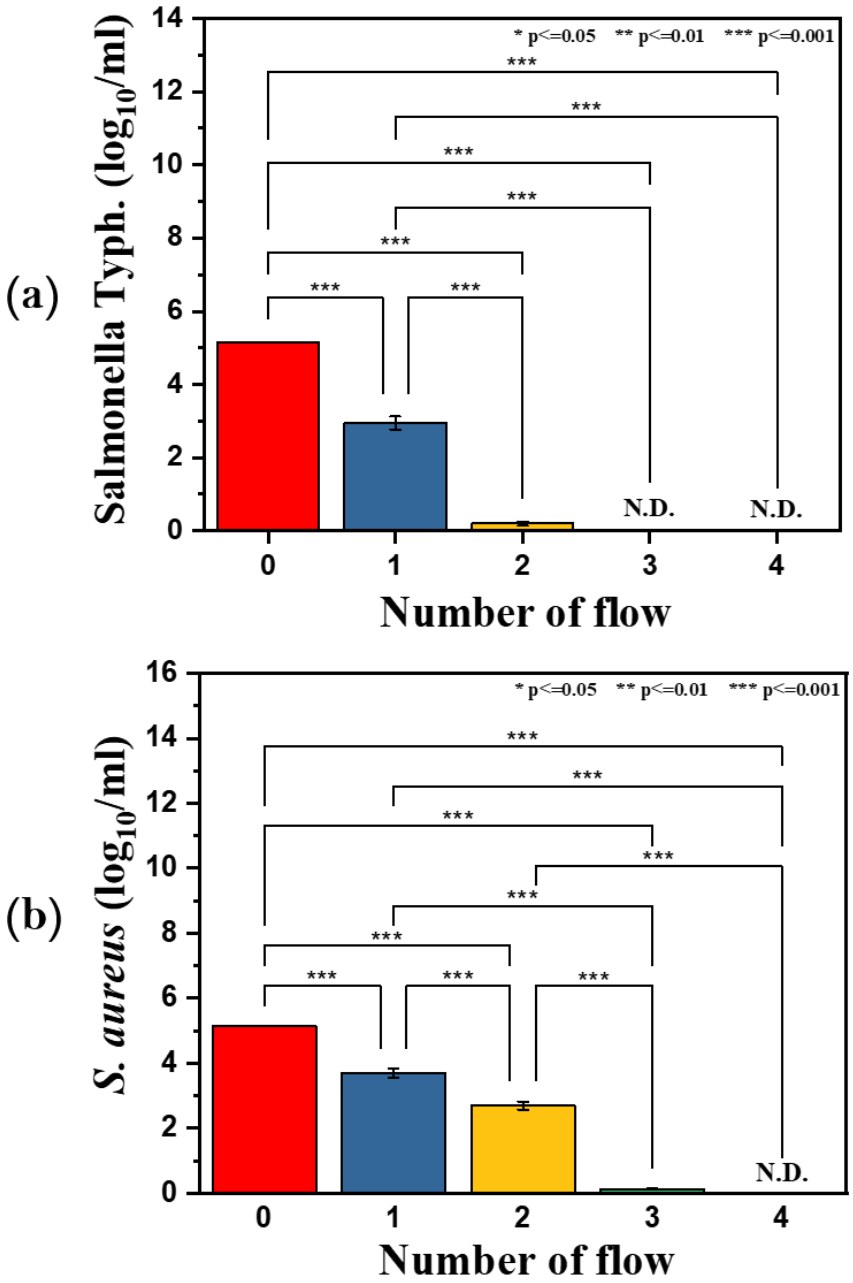


Figure 5.6: (a) *Salmonella typhi* counts and **(b)** *S. aureus* counts in milk following UV treatment at different passes. The error bars represent the mean±SD of the samples, while the symbols (*, **, and ***) correspond to P values less than or equal to 0.05, 0.01, and 0.001, respectively.

The initial population for *S. aureus* was 5.15 log/ml in the raw milk. Figure 5.6 (b) depicts that after the first and second pass of whole milk through the UV reactor, the initial concentration of *S. aureus* decreases approximately 3.68 log/ml and 2.69 log/ml, respectively. However, after the third pass, around 5 log reduction in the *S. aureus* is achieved, and after the fourth pass, no colonies are detected on the petri plates. In a study, bovine milk was exposed to various dosages of UV-C light, resulting in 2.52 log and 3.01 log reductions in *S. aureus* and *Salmonella typhi.*, respectively, when a UV-C dosage of 90 J/ml was used [Atik et al., 2021b]. In this study, the 5-log decrease in both types of bacteria was achieved with a very minimal UV dosage of approximately 5 J/ml due to the specially designed UV reactor. The results are consistent with our prior investigation, showing that the UV-C light is more effective for gram-positive than gram-negative bacteria [Ahlawat et al., 2024b].

5.4 Comparison with the Standard Pasteurization Process

To validate the effectiveness of UV treatment as a replacement for heat-based pasteurization, physicochemical and microbiological findings are compared with the result obtained from the pasteurization method.

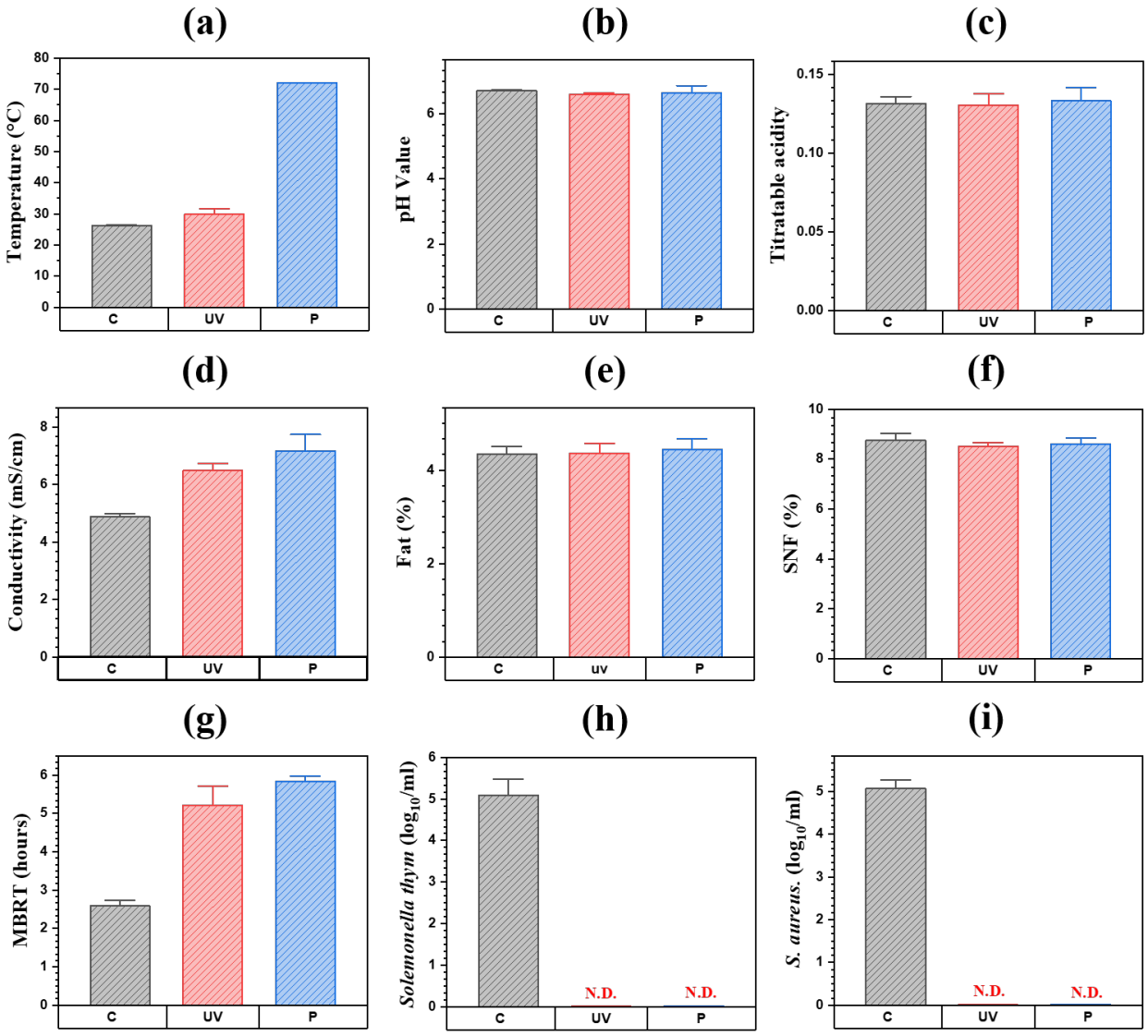


Figure 5.7: Comparison of before and after UV treatment (after the fourth pass) physico-chemical and microbiological parameters of raw milk with the pasteurization process **(a)** temperature, **(b)** pH, **(c)** titratable acidity, **(d)** electrical conductivity, **(e)** fat, **(f)** SNF, **(g)** MBRT, **(h)** *Salmonella typhi* reduction, and **(i)** *S. aureus* reduction.

Most of the physical and chemical parameters such as pH, titratable acidity, fat, and SNF do not exhibit any significant variations following pasteurization and UV treatment, as depicted in Figure 7 (b), 7 (c), 7 (e), and 7 (f), respectively. Pasteurization of milk involves raising its temperature (up to 63 °C) compared to UV treatment. As a result, the electrical conductivity, which is directly correlated with temperature, exhibited an increase, as indicated by the data in Figure 7 (a) and 7 (d). However, the temperature rise in UV treatment remains significantly below 35 °C, indicating the non-thermal characteristics of the designed reactor.

Compared to MBRT analysis, the pasteurization method has a little greater MBRT than the control and UV treatment methods, as shown in Figure 7 (g). However, an MBRT value of 5 hours and above indicates very good quality milk after the treatment with a UV reactor. Also,

Figure 7 (h) and 7 (i) demonstrate that UV treatment and pasteurization process has significantly reduced the number of bacteria ~5 logs for *S. aureus* and *Salmonella typhi*, respectively, for gram-positive and gram-negative bacteria and results are almost identical that is quite significant for cold process.

Besides above, as shown in Figure 5.7 (a) and 5.7 (d), the conductivity values of milk treated with UV-C light at 254 nm (using an annular flow reactor at ~ 30 °C) and conventional pasteurization at 70 °C appear quite similar, despite the significant difference in processing temperatures. This similarity can be attributed to the photochemical effects induced by 254 nm UV radiation. The germicidal UV-C light interacts with milk proteins and minerals, leading to modest structural alterations, oxidation of amino acid residues, and disruption of ionic equilibria. These changes can result in the release of ions such as calcium, magnesium, and phosphate, thereby increasing milk's electrical conductivity. In pasteurization (conducted here at 70 °C for 30 seconds, following the HTST standard), thermal effects cause protein denaturation and salt redistribution, especially from micellar casein structures, which also results in ion release into the serum phase. Although the mechanisms are different –thermal degradation in pasteurization versus photo-induced molecular changes in UV-C treatment –the net effect on conductivity is comparably close due to the release of similar ionic species into the aqueous phase of milk. This supports the idea that UV-C processing at 254 nm, even at lower temperatures, is capable of inducing physicochemical modifications in milk similar to thermal treatments, though to a lesser extent and with lower energy input.

5.5 Conclusion

In summary, this research demonstrates the potential of a specifically engineered UV reactor as an effective and viable method for milk sterilization. The reactor successfully reduces bacterial contamination while preserving the milk's physicochemical properties, offering a promising alternative to traditional pasteurization. Notably, the reactor operates efficiently at low temperatures and requires minimal power, making it an energy-saving solution. With just four passes of milk through the reactor, the MBRT increased by over five hours, highlighting the reactor's effectiveness in prolonging milk's freshness. Furthermore, a significant reduction in bacterial load was observed, achieving approximately 5 log reductions in *Salmonella typhi* and *Staphylococcus aureus* after only three passes of whole milk from the UV reactor. Interestingly, a very low UV dosage of 5 J/ml was sufficient to achieve total bacterial elimination. While further studies on milk shelf life are necessary to assess the long-term impact and viability of UV treatment as a substitute or complement to pasteurization, the results indicate that this UV reactor offers can effectively improve milk quality without altering the physico-chemical characteristics.