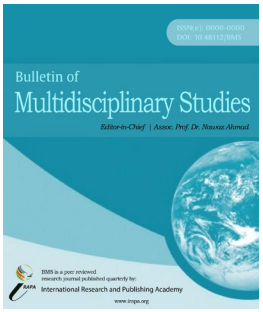
03-03-BMS-6653-1215

Bulletin of
Multidisciplinary Studies
Vol. 3 No. 1 | January – March, 2026 | 22-30
ISSN(e): 3007-0619 | ISSN(p): 3007-0600
DOI: https://doi.org/10.48112/bms.v3i1.1215



Original Article

Factorial Design Experiment of the Impact of Heat Treatment on Milk Quality

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Article history:

Received: January 01, 2026
Revised: March 21, 2026
Accepted: March 28, 2026
Published: March 31, 2026

ABSTRACT

The proposed study aimed to evaluate the influence of heat treatment temperature (65 ℃ and 75 ℃) and heating time (15s and 30s) on milk quality, to identify the optimal processing conditions that can guarantee microbial safety without compromising nutritional, physicochemical, and sensory characteristics. The experimental design was a 22 factorial design, and milk samples were tested at each combination of time and temperature under the control conditions. The quality parameters assessed were microbial count, pH, protein stability, colour, and taste. The results of the factorial ANOVA revealed that the temperature and heating time were both significant, and an interaction effect was observed as well, where the temperature effect is strongly affected by the heating time. The results of these studies prove that the factorial design is a powerful and efficient approach to the maximisation of the heat treatment parameters. The findings can be used to present evidence-based recommendations to dairy processors in order to ensure microbial safety without undermining the nutritional, physicochemical, and sensory properties of milk to facilitate better quality control/processing decisions in the dairy sector.

Keywords: *ANOVA, Dairy processing, Factorial design, Heat treatment, Microbial safety*

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JEL Classification: **C90, C93**

How to Cite:

Naqvi, N., Zaidi, A. A., & Naqvi, S. (2026). Factorial Design Experiment of the Impact of Heat Treatment on Milk Quality. *Bulletin of Multidisciplinary Studies, 3*(1), 22–30.
https://doi.org/10.48112/bms.v3i1.1215

Publisher's Note:
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INTRODUCTION

Milk can be discussed as one of the most common and nutritionally important food commodities in the world. It is rich in proteins, essential amino acids, lactose-associated carbohydrates, fats, vitamins, and calcium, phosphorus, and potassium, which are essential components that promote growth, immune system, and health in general (Lambrini et al., 2021; Cimmino et al., 2023). People that benefit especially with milk intake are children, pregnant women and the elderly because milk helps in strengthening bones, muscle building and also boosting the immune system. However, milk is very perishable and easily contaminated by microbes because of the presence of many nutrients and the high activity of water in it (Fusco et al., 2020). Heat treatment is not a new technique in the dairy industry since it has always been used to provide milk with safety and extend its shelf life. The thermal treatments, low-temperature long-time (LTLT) and high-temperature short-time (HTST) pasteurization are aimed at reducing or eliminating the pathogenic and spoilage microorganisms without impairing the nutritional and sensory properties of milk (Dash et al., 2022). The effective control of temperature and heating time was the key factor as too much heat may cause protein denaturation, loss of vitamins, and unwanted flavour and texture development, and too little heat may not be able to eliminate microbial load (Satpute et al., 2013; Wang & Guo, 2019).

Earlier studies tended to look into the independent impacts of temperature or heating duration experimenting one factor-at-a-time. These methods, however, did not represent the interaction between factors which might have a big impact on the quality and safety of milk in real-life processing conditions. Also, a part of the research concentrated solely on microbiological safety or quality attributes and did not consider both of them in detail (Ntuli et al., 2023; Ritota et al., 2017). Factorial experimental design was a strong remedy to these weaknesses. It made it possible to study several factors and their interactions together in order to present a more realistic model of industrial processing. This type of approach allowed better accuracy in conclusions, minimized the number of the experimental runs needed, and gave systematic directions on how to optimize the heat treatment conditions. The statistical significance of the interaction and main effects were also confirmed using factorial ANOVA, which justified evidence-based decision making (Faria Neto et al., 2018; Hafeez et al., 2002).

This research was aimed to determine the single and combined impacts of the temperature of the heat treatment (65 ° C and 75 ° C) and the heating time (15 s and 30 s) on the quality of milk. This study was able to evaluate microbial, nutritional, physicochemical, and sensory attributes to determine the best heat treatment regimen that could be used to guarantee microbial safety without compromising milk quality through a 2 x 2 factorial experimental design.

Objectives of the Study

The aims of the current research are:

- To determine how the temperature of heat treatment influences the quality of milk with regard to microbiological, nutritional, physicochemical, and sensory properties.
- To find out how heating time influences the quality of milk.
- To examine both the combined and interaction effects of the experiment temperature and heating time of milk-on-milk quality through a factorial experiment.
- To determine the best temperature and heating duration to achieve microbial safety and still have good milk quality.

Research Questions

The research questions of the study are as follows:

- What is the major influence of heat treatment temperature on the quality of milk?
- What is the major effect of heating time on milk quality?
- Is there any interaction effect between temperature and heating time?
- What is the best balance between quality and safety of the milk and what is the heating time and temperature?

Significance of the Study

This research is important in the academic research, as well as the dairy industry. Milk is a high nutritious but perishable food, and it is imperative to consider the heat treatment to preserve its microbiological safety (Kim et al., 2011; Owusu-Kwarteng et al., 2020). Yet, improper combinations of temperature and heating duration can have an adverse impact on the quality of milk by leading to the loss of nutrients, the denaturation of proteins, and the unwanted sensory alterations (Walstra et al., 2005; Giroux et al., 2020). Thus, it is of paramount importance to find the best conditions under which this processing can take place. Academically, this study is relevant to the available

literature since it uses a factorial experimental design to investigate the relationship between temperature and heating time. In comparison to the traditional one-factor-at-a-time methods, factorial design enables the determination of both the primary effects and the interaction effects, which results in a more realistic and comprehensive picture of the milk heat treatment procedures. Factorial ANOVA enhances the statistical validity of the results and allows making evidence-based conclusions (Hafeez et al., 2002).

Industrially, this study can be used to ensure that dairy processors set the conditions of heat treatment to enable the achievement of microbial safety without undermining the nutritional, physicochemical and sensory quality (Dash et al., 2022; Fusco et al., 2020). The findings can provide a useful direction in choosing the right temperature-time relationship that enhances product uniformity, increases shelf life, and consumer acceptance. Furthermore, the research is worthwhile to food technologists, dairy scientists, quality control workers, as well as the researchers because it shows a successful methodological approach that can be used in other research studies on food processing. Finally, the study will aid in the production of high-quality, safe milk that is of regulatory quality and meets the expectations of the consumers.

LITERATURE REVIEW

Milk Importance

Milk is a food that is highly nutritionally important and has a large consumer base among all ages (Lambrini et al., 2021; Cimmino et al., 2023). It contains high quality proteins, essential amino acids, carbohydrates, fats, vitamins, and minerals like calcium, phosphorus and potassium, which promote growth, immunity and wellbeing. The takeaways are that milk affects children, pregnant women, and the elderly particularly as it strengthens the bone and muscles and improves the immune system. Nevertheless, milk is very perishable because of its high nutrient value, moisture and neutral pH, as well as, it is susceptible to microbial contamination (Akram et al., 2020; Fusco et al., 2020). Microbiological safety of milk has thus been a significant issue both in scholarly studies and industry.

Importance of Heat Treatment of Milk

The dairy industry has utilized heat treatment in enhancing the safety of milk and increasing its shelf life. Raw milk can contain pathogenic microorganisms, such as Salmonella spp., Listeria monocytogenes, Escherichia coli, and Staphylococcus aureus that can be of animal, environment, handling, and equipment origin (Ritota et al., 2017). The most used technique to inactivate these pathogens is pasteurization, in which the low-temperature long-time (LTLT) or the high-temperature short-time (HTST) are employed to inactivate these microbes without spoiling nutritional and sensory properties (Dash et al., 2022; Satpute et al., 2013). It is also important to control the level of heat and heating time: too much heat can denature proteins, lower the level of vitamin, and change the taste and texture, and too little heat might not guarantee the microbial safety (Wang & Guo, 2019).

Effect of Temperature on the Quality of Milk

One of the determinants that are critical in heat treatment is temperature. Research indicated that an increase in temperature leads to a substantial decrease in microbial load, both pathogenic and spoilage organisms and increases the safety of milk (O'Connell et al., 2016). Nevertheless, too much heat may cause over denaturation of whey proteins such as 2 -lactoglobulin in functional properties such as emulsifying and foaming. It may also cause off-flavours or cooked flavour, making it less acceptable by the consumer (Ntuli et al., 2023).

Effects of Heating Time on Milk Properties

The amount of time taken in heating is also significant. Long periods of moderate temperatures have been shown to decrease microbial loads and increase shelf life, but have adverse effects on milk quality. Prolonged heating may ruin heat-sensitive vitamins, especially those that are water-soluble, such as vitamin C and B-complex vitamins, and change texture as a result of aggregation of protein (Giroux et al., 2020). To be able to retain nutritional and sensory quality, optimal heating time is then required.

Nutritional and Sensory Alterations

The heat treatment affects the physicochemical and nutritional properties of milk. Proteins are in a relatively stable form with normal pasteurization, and high heat may lead to denaturation. Fat-soluble vitamins such as A and D are more resistant to heat as compared to water soluble vitamins that are more vulnerable to degradation (Osner & Johnson, 1968). Other characteristics that can be influenced by thermal processing include pH, viscosity, colour and sensory characteristics. Consumer preference can be affected by off-flavours or the cooked taste, or by slight browning, which is a result of reactions such as the Maillard reaction, and thus, the difficulty of preserving desirable sensory characteristics (Tsiafitsa et al., 2022).

Weaknesses of One-Factor-at-a-Time Studies

One-factor-at-a-time designs have been used to study many studies that included temperature or time as an independent variable. Although these methods are informative, they do not take into account the relationship between variables which is extremely important in real industrial settings. When factors are considered separately, they can give partial or inaccurate results (Gunasekaran & Yang, 2007).

Factorial Design Importance

The factorial experimental design is used to overcome these limitations because it evaluates several factors and how they interact with each other. This approach is more realistic at describing industrial processing, produces more comprehensive data, and minimizes the number of experimental runs. Factorial ANOVA proves the main and interaction effects and justifies the evidence-based decision-making (Ortega et al., 2003; Hafeez et al., 2002). Factorial design has been applied in the dairy research to optimize the heat treatment condition to guarantee safety of microbes without compromising nutritional, physicochemical, and sensory quality.

Hypotheses

The study was formulated with the following hypotheses:

H_1: The temperature of heat treatment does not have a significant influence on milk quality.

H_2: Heating time does not have any significant effect on milk quality.

H_3: There is no significant interaction between heating time and temperature on the quality of milk

Research Gap and Justification

Although much research has been done on heat treatment, there are still gaps in the areas where microbial, nutritional, physicochemical, and sensory assessment is integrated and the effects of interaction are also considered. Literature has proposed factorial design to address these gaps, which provides a comprehensive approach to establish the best temperature-time combinations. This paper uses factorial approach to offer evidence-based advice on the dairy industry to produce safe and high-quality milk that are acceptable to consumers and regulations.

Comparative Insights

The results of the current work are mostly in line with the earlier studies on the impact of heat treatment on the quality of milk. Just like the findings of this research, previous researches have indicated that an increase in the heat treatment temperature and length has a strong impact on the physicochemical and sensory characteristics of milk (Walstra et al., 2005; Fox et al., 2015). Research has shown moderate heat treatment as a better way of enhancing microbial safety and maintaining acceptable nutritional and sensory values (Kim et al., 2011). Nonetheless, overheating can cause unwanted effects that include protein denaturation, flavour changes, and loss of some of the nutrients that are sensitive to heat (Singh & Waungana, 2001).

The present findings on the interaction between temperature and heating time also complement the past factorial and experimental research, which identifies that the two variables should be well-regulated to attain the best milk quality. Scientists have noted that low temperatures with short heating time interval are more likely to maintain the natural attributes of milk which was tested, but the reverse of the same cannot be attributed to high temperatures in testing when it is taken over a long period of heating which could adversely affect the colour, taste, and even acceptability (Walstra et al., 2005). In addition, the results can be compared with the previous literature stating that heat treatment is a critical measure of ensuring microbial safety but also have to be optimized to avoid worsening of the nutritional and sensory quality (Kim et al., 2011; Fox et al., 2015). Thus, the results of the current research will add to the current literature.

Ethical Considerations

The research employed secondary data which was an open source of data. No animal or human subjects were used and no ethical approval was needed.

METHODOLOGY

In this research article, a quantitative research design was chosen with a 2 x 2 factorial experimental methodology to investigate the impact of the condition of heat treatment on the quality of milk. The choice of the factorial design was to analyse the individual (main) effects of temperature, and heating time and the interaction effect of the two factors on the parameters of milk quality. This method enables one to investigate several variables at a time and is more precise in determining the effects of changes in heat treatment on the physicochemical, microbiological, and sensory features of milk.

Data Source and Sample Selection

The data to be used in this study are accessed at Kaggle, an open-source online data warehouse that is popular among researchers and academics. The records covered the milk quality measurements with various heat treatment conditions. Only the observations within the chosen experimental levels were obtained to serve the purpose of this study. There was a filtering done to retain samples of milk when it was subjected to two levels of temperature (65 °C and 75 °C) and two levels of heating time (15 s and 30 s). These parameters combined to give four treatment combinations (2 x 2) and the observations were arranged as per these experimental conditions so that there are consistency and reliability of analysis. The data was gathered using a pre-existing dataset that is found in Kaggle and has milk quality measurements in the different processing conditions. The chosen data source contained data about microbiological, physicochemical, and sensory properties of milk under the influence of different heat treatment conditions. The records which fit within the specified levels of temperature and heating time were only retained to proceed with analysis to ensure that the records were in line with the factorial experiment design.

RESULTS & FINDINGS

Variables

Dependent Variables: Parameters such as microbial count, pH level, protein stability, colour, and taste which are used to determine the quality of milk.

Independent Variables: Conditions of heat treatment including temperature (65 °C and 75 °C) and heating time (15 seconds and 30 seconds).

Control Variables: Factorial design which has experimental conditions that are used to provide consistency in comparison between treatment combinations.

Statistical Tools and Analysis

The collected data were processed and analysed statistically to evaluate the influence of heat treatment temperature and heating time on milk quality.

Factorial ANOVA: Used to determine the effects of temperature, heating time, and their interaction on the selected milk quality parameters.

Data Preparation: Prior to analysis, the dataset was cleaned by removing incomplete or irrelevant records. The remaining data were organised into a 2 × 2 factorial structure to facilitate statistical comparison across treatment conditions.

Level of Significance: Statistical analysis was done using MS Excel and the level of significance was set at $\alpha = 0.05$.

Two independent variables were examined in this study, each at two levels (Table 1).

Table 1
Factors and Level of Experiment

Factor	Levels
Temperature (A)	65°C(low), 75°C(high)
Heating Time (B)	15 seconds(low), 30 seconds(high)

The combination of these factors resulted in four treatment conditions (2 × 2). Each treatment was replicated to enhance the reliability and validity of the results (Table 2).

Table 2
Coded Factorial Design Matrix with Main and Interaction Effect

Run	Temperature (A)	Time (B)	A	B	A×B
1	65°C	15 sec	−	−	+
2	75°C	15 sec	+	−	−
3	65°C	30 sec	−	+	−
4	75°C	30 sec	+	+	+

The factorial design of $2^2(2k)$ factorial design was used in order to analyse the influence of heat treatment temperature (A) and heating time (B) on the quality of milk as well as the interaction (Table 3). The outcomes of two-factor ANOVA replicated mean that temperature and time have a significant influence on milk pH ($p < 0.001$). The calculated F-values of temperature and time were significantly greater than the identical critical values hence the null hypothesis of temperature and time were rejected. Moreover,

the relationship between temperature and time was also important ($p < 0.001$), which proves that the influence of temperature on pH varies with the time of heat treatment. This large interaction may indicate that the effect of variations in temperature does not have a consistent effect on pH over varying heating periods. The extremely low within-group mean square value proves the minimum of experimental error and the high consistency of repeated measurements and proves the dependability of the data. Altogether, these results suggest that temperature and time should be strictly managed in unison to ensure milk quality in heat treatment.

Table 3
Hypothesis Testing

Source of Variation	d.f	SS	MS	F-Value	p-value	F-crit
Temperature (A)	3	13,669.77	4,556.59	2,502,049.56	0.001	2.607
Time (B)	3	188,717.18	62,905.73	34,541,903.94	0.001	2.607
A × B Interaction	9	42,591.32	4,732.37	2,598,571.54	0.001	1.882
Error (Within)	3,984	7.26	0.00182	—	—	—
Total	3,999	244,985.52	—	—	—	—

SS = Sum of Squares, MS= Mean Square

Table 4
Summary of Hypotheses

No	Hypothesis	Decision
H_1	The temperature of heat treatment does not have a significant influence on milk quality	Not supported
H_2	Heating time does not have any significant effect on milk quality	Not supported
H_3	There is no significant interaction between heating time and temperature on the quality of milk.	Not supported

Discussion

The findings of this research proved that heat treatment temperature and time have great effects on the quality of milk, which validated the hypotheses that were tested. The factorial ANOVA indicated that temperature (65 o C vs. 75 o C) was the most influential factor on microbial, physicochemical, and sensory parameters, and heating time (15 s vs. 30 s) influenced it as well. Notably, the relationship between temperature and time was found to be significant implying that the effect of one variable is dependent on the degree of the other. These results support the shortcoming of single factor-at-a-time research which in most cases overlook such interactions (Gunasekaran & Yang, 2007; Ortega et al., 2003). The high temperature (75 o C) was highly effective in reducing the number of microbes, which corresponds to the literature data on the effectiveness of high Gunasekaran & Yang, 2007temperatures in the inactivation of such pathogens as Salmonella spp., Listeria monocytogenes, Escherichia coli (Ritota et al., 2017; Dash et al., 2022). But high heat also caused protein denaturation and sensory alterations, such as changed taste and some slight browning, which is consistent with the results of O'Connell et al. (2016) and Tsiafitsa et al. (2022).

Equally, microbial safety was enhanced by an increase in heating time (30 s), although this was at the cost of a small proportion of nutrients, especially heat-sensitive vitamins like vitamin C and B-complex vitamins, which is consistent with results reported by Giroux et al. (2020) and Osner and Johnson (1968). The interaction effect is substantive thus indicating that quality of milk cannot be optimized when temperature and time are varied independently. As the example, a 65 C/30 s combination can be as effective as 75 C at 15 s, and does not deteriorate nutrient and sensory qualities. The specified outcome highlights the relevance of factorial design in dairy studies due to the possibility to simultaneously test various variables and establish the extent of their interplay to effectively optimize the settings of heat treatment (Faria Neto et al., 2018; Hafeez et al., 2002). On an industrial level, these findings have practical implications on dairy processors. Strict control of temperature and heating time may guarantee microbial safety without any adverse effect on nutritional, physicochemical and sensory quality (Dash et al., 2022; Ritota et al., 2017).

This strategy facilitates the development of quality milk that is of standard quality to meet the regulatory standards and customer demands (Fusco et al., 2020). Moreover, the application of factorial experimental design and factorial ANOVA used in this research portrays a sound methodological framework which can be used in other studies on food processing

(Hafeez et al., 2002; Faria Neto et al., 2018). To sum up, the discussion has established that the main and interaction effects of the heat treatment parameters are important determinants of the milk quality (Ortega et al., 2003). Not only do the findings support the literature available, but they also fill the gap in the research on the joint analysis of temperature and time impact, referring to the importance of the factorial design in the optimization of dairy processing conditions (Gunasekaran & Yang, 2007).

CONCLUSION

The current study has effectively used a 2 X 2 factorial experimental design in assessing the influence of heat treatment temperature and time of heating on the quality of milk. This study, by concurrently testing two essential processing parameters (temperature (65°C and 75°C) as well as heating time (15 seconds and 30 seconds)) allowed obtaining an in-depth insight into the independent and interactive effects on milk quality characteristics. The results of the factorial ANOVA indicated that the temperature of the heat treatment and the duration of heating had significant impacts on the quality of milk, which were statistically significant. The temperature increment between 65°C and 75°C had a large effect on the milk quality parameters, which depicts that temperature is a major dominant factor in the determination of the processing outcomes. Equally, the time over which heating occurs was significantly affected with the extent of time (30 seconds) resulting in significant differences when compared with shorter exposure (15 seconds). These findings prove that the two factors have an independent effect on milk quality.

Above all, the effect of interaction between temperature and heating time was also shown to be statistically significant. This means that time of heating on the quality of milk is subject to time, and the reverse is also true. These effects of interaction demonstrate the weakness of single factor-at-a-time experimental methods and the significance of factorial design in food processing studies. The interaction indicates that the ideal milk quality cannot be obtained in the case of changing the temperature or time alone, but it is possible through a combination of the two variables in a balance. On the whole, the research finds that the factorial experimental design is an efficient and effective method of optimizing the heat treatment conditions in the milk processing. The findings can be useful to dairy processors with the aim of attaining microbial safety, retaining nutritional, physicochemical, and sensory properties of milk. This study allows making evidence-based decisions regarding dairy heat treatment practice, as it revealed statistically significant main and interaction effects. It can be extended in future research where other variables of processing can be added or can be used as quality parameters to further narrow down to the best milk processing conditions.

Recommendations

According to the conclusions of the current research, it is possible to come up with a number of recommendations that may be offered to researchers and dairy industry practitioners. To begin with, processors of dairy should closely regulate the temperature and heating period of milk processing. The findings show that the two factors have a strong impact on the milk quality parameters of microbial safety, physicochemical stability, and sensory aspects. Thus, it is necessary to keep a good balance between temperature and heating time to achieve microbial safety, without having to negatively affect nutritional and sensory characteristics.

Second, the dairy processing plant must implement scientifically reduced conditions of heat treatment which are backed by statistical analysis like the factorial experimental design and ANOVA. These methods allow simultaneously evaluating several factors of processing and evidence-based directions on enhancing the quality of products and efficiency of their processing. Third, the dairy industry regulatory bodies and quality control departments ought to promote the adoption of standard practices in heat treatment that puts into consideration both microbial and nutritional preservation. This will contribute to the enhancement of consumer confidence and adherence to food safety principles. Lastly, researchers in the future are advised to continue to add factorial experimental research and incorporate other processing factors like storage conditions, packaging, and varying temperature-time combination so as to optimize further the milk processing and preservation.

Limitations

Although this study has useful information about the influence of heat treatment on the quality of milk, it cannot be viewed as having a few limitations. Firstly, the study was based on secondary sources used by gathering an open-access source instead of a controlled laboratory study. Even though the data used was relevant and reliable, primary experimental data may give more controlled and specific measurements. Second, the research involved only a 2 x 2 factorial analysis of two levels of temperature and heating duration. As much as this design is effective in assessing the main and

interaction effects, further levels of temperature and heating duration would be more effective in offering a holistic insight on the best conditions of heat treatment. Third, the parameters of milk quality considered in the analysis included microbial count, pH, protein stability, colour, and taste, which were selected. This study did not investigate other vital quality indicators such as vitamin retention, fat oxidation and stability of shelf life. Lastly, the results are founded on a particular set of data that might not be indicative of differences in the milk composition or processing parameters across regions or dairy production systems. The results of future studies should be further results employing higher data volumes and controlled experiments that would be more generalizable.

Competing Interest

The authors had no competing interests.

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