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Exploring the Impact of Varying Fat Content on Milk Properties During Ohmic Heating: A PCA Study of Viscosity Changes Alongside pH Fluctuations

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ABSTRACT

Background: Some of the factors that contribute to the quality of milk include fat content, pH, viscosity and other rheological aspects. This research extensively investigated the effects of ohmic heating (OH) on milk viscosity, along with other rheological attributes and pH. **Methods:** Milk samples with varied fat constituents were subjected to OH at a constant voltage of 140V until they reached a temperature of 90°C. The pH of raw milk and heated milk was determined using an auto-pH meter, whereas the viscosity of the same was measured using a Brookfield viscometer that includes a wide range of characteristics, such as viscosity, temperature, shear stress and torque at various spindle rotation speeds. The current investigation used principal component analysis (PCA) as a key statistical approach. **Result:** The pH values exhibited little fluctuations during OH across several milk kinds, indicating mild acidification throughout the process, but the observed alterations did not reach statistical significance ($p>0.05$), suggesting that OH does not significantly affect milk pH. Viscosity measurements showed that the application of OH resulted in a decrease in the average viscosity of milk, suggesting changes in its ability to withstand shear forces. The correlation study revealed robust positive associations between viscosity and fat content (PC2-26.77%), as well as between rotational speed, shear rate and torque (PC1-53.72%) and a negative correlation with temperature, offering valuable insights into the dynamic properties of milk when subjected to thermal heat treatment.

Key words: Brookfield, Fat, Milk, pH, Rheology, Viscosity.

INTRODUCTION

Processing can affect milk's physical and chemical properties as a complex colloidal system (Kailasapathy, 2015). Milk quality and function in dairy applications depend on fat content, pH, viscosity and other rheological factors (Janahar *et al*., 2021). The viscosity of milk or milk's resistance to flow depends on its composition (Yousefi and Jafari, 2019) and it significantly affects milk processing and sensory qualities. The rheological characteristics of milk, including its viscoelastic behavior, significantly change in response to different shear rates. Thus, a thorough understanding of its viscoelastic rheology is crucial (McCarthy and Singh, 2009). Composition, temperature and processing methods like OH may influence milk's rheology, affecting stability and texture. Since milk acidity impacts stability, microbiological quality, enzyme activity, protein solubility and flavor (Murphy *et al*., 2016), pH is crucial. Cow's milk pH ranges from 6.5 to 6.7, depending on microbial activity, storage conditions and animal health and affects fermentation, coagulation and heat stability (Tsioulpas *et al*., 2007).

OH is a novel heating technique gaining attention in milk heating operations. Due to milk's natural electrical resistance, it heats quickly when heated with alternating current (Sain *et al*., 2024). Compared to standard heating methods, this technology can heat milk more uniformly and quickly, which can modify its physical and chemical properties (Sain *et al*., 2023). Understanding milk processing behavior affects quality and compatibility with a variety of dairy products (Parmar *et al*., 2018). Ohmic-treated milk's rheology has garnered attention due to its effects on the dairy industry. Milk products' texture and taste depend on viscosity, which measures a fluid's resistance to flow. Heat processing alters milk's pH and rheology, slightly acidifying and changing its physical properties (Parmar *et al*., 2018; Anema, 2009). Research shows that OH affects milk flow and shear strength (McCarthy and Singh, 2009). The rheological qualities of milk are directly related to its fat content. Iqbal *et al*. (2024) found that an increase in fat content increases the viscosity of milk. The present study can be important for determining the extent to which the viscosity of milk, with multiple fat content, changes after OH.

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Considering those above, the current study seeks to bridge a gap in our understanding of dairy science by focusing on the precise impacts of OH on milk's rheological properties and pH changes after heat treatments. Conventional heat processing affects milk's rheology, but the effects of OH are unclear. This work addresses this gap and offers new insights that could revolutionize dairy processing. The research also explores the relationship between milk fat and the rheological attributes associated with milk. This factor is vital because milk types with different fat percentages may react differently. Understanding this link is essential for adapting processing methods to other milks and making OH more effective in dairy processing. This study uses PCA, a sophisticated analytical tool, to reduce complex correlations between variables and uncover the main factors that affect them. This advanced statistical technique helps unravel complex issues and present more precise results.

MATERIALS AND METHODS

The current research study is a part of PhD research work (January 2022- January 2024), which was conducted in the Department of Dairy Engineering, ICAR-NDRI, Karnal, India. The milk was procured from the Experimental Dairy Plant, ICAR-NDRI, Karnal. The Pearson square method was used to standardize the milk with varied fat (0.5, 1.5, 3, 4.5 and 6%) (Bird, 1993). The milk was heated using the OH technique as per the method adopted by Priyanka *et al*. (2018). The milk was heated up to 90°C and once the temperature was attained, pH and viscosity were immediately analyzed at the maintained temperature (90°C±1.2) by keeping it in an insulated container. The analysis was made for the raw milk samples at a temperature 25°C ±0.5 and ohmically processed samples at a temperature of 90°C±1.2.

Determination of viscosity and pH of different milk samples

The pH was measured using an auto pH meter of make Lab Junction® (LJ1611), whereas a precise tool capable of measuring fluid viscosity under controlled circumstances, the Brookfield viscometer of make IKA® (ROTAVISC lo-vi) with an ELVS-SP spindle, was used to perform the viscosity measurements of the different milk samples. The analysis included a wide range of characteristics, such as viscosity, temperature, shear stress and torque at various spindle rotation speeds (60 to 100 rpm) with a consecutive interval of 10 rpm.

Statistical analysis

This research used PCA as a key statistical approach to shed light on the relationships between several rheological parameters, such as viscosity, shear rate, temperature, shear stress and torque (Table 1). Origin-2016 software was used to do the analysis. Independent sample t-test was done using SPSS 24 software. Trials were done in triplicates.

RESULTS AND DISCUSSION

Examination of the pH levels in several milk samples

There were small changes in pH after OH. Ohmic heated skimmed and double-toned milk with a minimum variance of ±0.05 revealed a pH of 6.67. A minor reduction in pH (6.7±0) from their respective raw states was shown, suggesting slight acidification during the OH process, although no statistically significant difference was found ($p>0.05$). Ohmic heated toned milk (6.63±0.05), ohmic heated standardized milk (6.53±0.05) and ohmic heated full cream milk (6.53±0.05) all exhibited a consistent pH level, slightly lower than their raw counterparts, but not significantly different from one another ($p>0.05$).

Minor acidification may occur throughout the OH process because certain chemicals break down or release acidic byproducts. Temperature and milk concentration affected calcium and phosphate distribution between the soluble and colloidal phases. Raising temperatures caused them to swiftly enter the colloidal phase and lower pH (Anema, 2009; Janahar *et al*., 2021). These findings suggest that the OH treatment did not significantly change the pH of the milk sample ($p>0.05$), as shown in Fig 1.

One important conclusion was that heating milk below the critical temperature of 100°C had no discernible effect on its pH. Because of its reversibility, it is likely that the structural alterations or chemical reactions that take place in the milk when it is heated below 100°C would not result in a significant enough change in acidity over time (Ma and Barbano, 2003). Due to this, the pH levels of the various milk samples before and after OH were consistent in this study, suggesting that the heating technique had no appreciable effect on the milk's acidity or alkalinity. This is the reason the pH was disregarded for further investigation and analysis.

Examination of the descriptive statistics for several milk sample parameters

The OH caused distinct modifications in raw milk (Table 2) and ohmic heated milk (Table 3) at 90°C. Ohmic heated milk showed a lower average viscosity (2.04 mPa.s) than raw milk (2.13 mPa.s). The average viscosity decreased after OH, suggesting that the milk's shear pressure resistance had changed. After thermal treatment, the standard deviation of viscosity increased from 0.13 mPa.s for raw milk to 0.18 mPa.s for heated milk, indicating a larger range. Heating milk to 90°C ohmically increased viscosity variability from 0.39 mPa.s in raw milk to 0.82 mPa.s in heated milk. During viscosity measurements, raw milk samples have an average shear rate of 99.06 1/s. After OH, the shear rate averaged 99.06 1/s. This indicates that the heat treatment did not substantially affect the milk's resistance to flow (Cooper *et al*., 2010). Raw milk's average torque of 30.52 N-m reflected viscosity test requirements.

Post-OH torque averaged 30.488 N-m, quite stable. This suggests that the OH process did not significantly modify the rotating force needed to measure viscosity.

Table 1: Various parameters for PCA analysis.

Milk samples	Fat (%)	Speed (rpm)	Viscosity (mPa.s)	Shear rate (1/s)	Temperature (°C)	Shear stress (Pa)	Torque (N-m)
Raw skim milk (RS)	0.5	60	2.03	74.37	24	0.12	18.2
Raw skim milk (RS)	0.5	70	1.99	86.82	24	0.17	22.1
Raw skim milk (RS)	0.5	80	1.97	99.76	24	0.2	25.7
Raw skim milk (RS)	0.5	90	1.95	111.23	24	0.22	28.9
Raw skim milk (RS)	0.5	100	1.92	123.1	24	0.25	33.8
Ohmic heated skim milk (OHS)	0.5	60	1.92	74.37	90	0.13	19.2
Ohmic heated skim milk (OHS)	0.5	80	1.88	99.76	90	0.21	25.2
Ohmic heated skim milk (OHS)	0.5	70	1.87	86.82	90	0.16	22.6
Ohmic heated skim milk (OHS)	0.5	90	1.84	111.23	90	0.22	27.6
Ohmic heated skim milk (OHS)	0.5	100	1.8	123.1	90	0.26	32.1
Raw double-toned milk (RDT)	1.5	60	2.14	74.37	24	0.11	21.8
Raw double-toned milk (RDT)	1.5	70	2.1	86.82	24	0.17	25.6
Raw double-toned milk (RDT)	1.5	80	1.99	99.76	24	0.2	28.2
Raw double-toned milk (RDT)	1.5	90	1.96	111.23	24	0.21	30.4
Raw double-toned milk (RDT)	1.5	100	1.93	123.1	24	0.24	32.7
Ohmic heated double-toned milk (OHDT)	1.5	60	2.03	74.37	90	0.12	21.7
Ohmic heated double-toned milk (OHDT)	1.5	70	2.62	86.82	90	0.16	26.4
Ohmic heated double-toned milk (OHDT)	1.5	80	1.89	99.76	90	0.19	29.3
Ohmic heated double-toned milk (OHDT)	1.5	90	1.86	111.23	90	0.22	31.7
Ohmic heated double-toned milk (OHDT)	1.5	100	1.83	123.1	90	0.25	34.5
Raw-toned milk (RT)	3	60	2.21	74.37	24	0.1	25.8
Raw-toned milk (RT)	3	70	2.19	86.82	24	0.15	29.1
Raw-toned milk (RT)	3	80	2.14	99.76	24	0.19	32.5
Raw-toned milk (RT)	3	90	2.11	111.23	24	0.2	37.6
Raw-toned milk (RT)	3	100	2.08	123.1	24	0.23	38.9
Ohmic heated toned milk (OHT)	3	60	2.11	74.37	90	0.11	24.7
Ohmic heated toned milk (OHT)	3	70	2.09	86.82	90	0.14	28.3
Ohmic heated toned milk (OHT)	3	80	2.05	99.76	90	0.19	33.5
Ohmic heated toned milk (OHT)	3	90	2.01	111.23	90	0.21	36.8
Ohmic heated toned milk (OHT)	3	100	1.96	123.1	90	0.25	38.8
Raw standardized milk (RSt)	4.5	60	2.28	74.37	24	0.11	25.9
Raw standardized milk (RSt)	4.5	70	2.25	86.82	24	0.19	30.1
Raw standardized milk (RSt)	4.5	80	2.22	99.76	24	0.24	33.5
Raw standardized milk (RSt)	4.5	90	2.18	111.23	24	0.27	36.2
Raw standardized milk (RSt)	4.5	100	2.16	123.1	24	0.29	37.8
Ohmic heated standardized milk (OHSt)	4.5	60	2.17	74.37	90	0.11	26.7
Ohmic heated standardized milk (OHSt)	4.5	70	2.13	86.82	90	0.18	30.1
Ohmic heated standardized milk (OHSt)	4.5	80	2.1	99.76	90	0.22	34.7
Ohmic heated standardized milk (OHSt)	4.5	90	2.08	111.23	90	0.25	36.4
Ohmic heated standardized milk (OHSt)	4.5	100	2.06	123.1	90	0.28	38.4
Raw full cream milk (RFC)	6	60	2.31	74.37	24	0.13	26.7
Raw full cream milk (RFC)	6	70	2.29	86.82	24	0.18	30.2
Raw full cream milk (RFC)	6	80	2.27	99.76	24	0.23	33.2
Raw full cream milk (RFC)	6	90	2.25	111.23	24	0.26	38.9
Raw full cream milk (RFC)	6	100	2.22	123.1	24	0.28	40.2
Ohmic heated full cream milk (OHFC)	6	60	2.21	74.37	90	0.16	25.7
Ohmic heated full cream milk (OHFC)	6	70	2.19	86.82	90	0.18	29.4
Ohmic heated full cream milk (OHFC)	6	80	2.17	99.76	90	0.21	32.3
Ohmic heated full cream milk (OHFC)	6	90	2.15	111.23	90	0.24	36.7
Ohmic heated full cream milk (OHFC)	6	100	2.13	123.1	90	0.28	39.4

The steady shear rate and minute torque fluctuations suggest that OH has complicated effects on interior flow behavior. The concentrate's viscosity changed after OH because heating the milk broke down whey proteins and increased their voluminosity (Anema *et al*., 2014). These minor fluctuations show OH's distinct rheological changes at high temperatures, revealing how temperature influences milk's properties.

Analysis of the correlation between the different milk samples

According to the correlation matrix (Fig 2), a strong positive correlation (0.66) between viscosity and fat content indicates that greater fat content is linked to higher viscosity. Other researchers have discovered similar findings, stating that there was a positive correlation between an increase in temperature and viscosity (Sutariya *et al*., 2017). OH was used in this research to raise the temperature to 90°C. As a result, the whey protein denaturation process occurred, resulting in a link between heating and viscosity. Fat had a positive connection with both shear stress (0.20) and torque (0.50), suggesting that increased fat content also tended to increase both parameters. When speed is examined, there is a very significant correlation (1.00) between it and shear rate, which supports the basic link between these dynamic characteristics. The link between rotational speed and shear

Table 2: Descriptive statistics of different parameters of the milk samples before OH.

Parameters	Viscosity (mPa.s)	Shear rate (1/s)	Shear stress (Pa)	Torque (N-m)
Average	2.13	99.06	0.1976	30.56
Standard deviation	0.13	17.59	0.06	5.84
Sample variance	0.02	309.57	0.00	34.15
Range	0.39	48.73	0.19	22.00
Minimum	1.92	74.37	0.10	18.20
Maximum	2.31	123.10	0.29	40.20
Sum	53.14	2476.40	4.94	764.00

Table 3: Descriptive statistics of different parameters of the milk samples after OH.

Parameters	Viscosity (mPa.s)	Shear rate (1/s)	Shear stress (Pa)	Torque (N-m)
Average	2.05	99.06	0.1972	30.49
Standard deviation	0.18	17.59	0.05	5.68
Sample variance	0.03	309.57	0.00	32.21
Range	0.82	48.73	0.17	20.20
Minimum	1.80	74.37	0.11	19.20
Maximum	2.62	123.10	0.28	39.40
Sum	51.15	2476.40	4.93	762.20

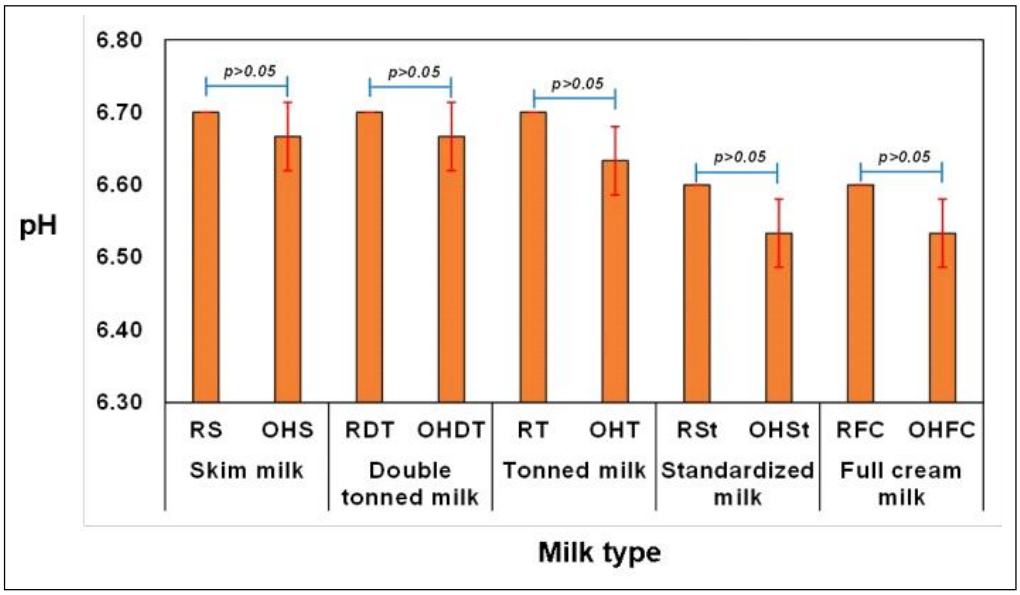

Fig 1: pH of different types of milk samples before and after OH.

rate during the viscosity measurements is highlighted by this. Shear stress (0.93) and torque (0.82) showed a high positive association with speed, indicating that as rotational speed rose, so did shear stress and torque. Shear stress (0.21) and torque (0.08) exhibited a positive connection with viscosity, meaning that an increase in viscosity is associated with an increase in shear stress and torque. This is consistent with the knowledge that greater viscosity equals greater resistance to shear forces (McCarthy and Singh, 2009). Interestingly, temperature showed minor correlations with the other factors, indicating that the relationships found were not significantly affected by temperature fluctuations within the experimental range. In another study, researchers used a water bath to study whether the viscosity of coconut milk altered at different temperatures (up to 90°C). They found that the water bath significantly affected the viscosity of the milk samples with varying fat contents (Simuang *et al.*, 2004). However, OH did not correlate with altering the various milk sample parameters in this investigation.

Analysis of the eigenvalues of the correlation matrix of the milk sample

Fig 3 shows that the first principal component (PC1), which accounted for 53.72% of the total variance, had the greatest eigenvalue of 3.76072. This implies that PC1 captured most of the main causes of variance in the sample. PC2 came in second with an eigenvalue of 1.87, making up 26.77% of the variance overall. PC1 and PC2 together accounted for almost 80.49% of the total variance, demonstrating their significant contribution to the understanding of the variation in the milk viscosity parameters. The eigenvalues drop further after PC3, with PC7 having the lowest eigenvalue of 0.0002.

These outcomes highlight the extent to which PCA captured the important patterns in the data. Regarding how OH affects milk viscosity, PC1 and PC2's dominance suggests that these elements represent the main variables that were impacted by the heating process. The total variability was less substantially contributed by the succeeding components, indicating that the first two primary components essentially describe the observed effects of OH on milk viscosity.

Analysis of the principal components loading plot of the various milk samples

With a value of 0.51 for PC1, speed was the largest contribution, followed by torque (0.46), shear rate (0.51) and shear stress (0.50) (Fig 4). These positive coefficients imply that these parameters and PC1 have a favorable relationship. Although it had a negative coefficient (-0.14) and suggested an inverse link, viscosity also contributed to PC1. With positive coefficients, fat (0.66) and viscosity (0.66) were the main factors for PC2. Shear stress (0.03) and temperature (-0.20) also had an impact, but not as much (Fig 4).

It was clear from looking at PC1, which represented the leading causes of variation, that torque, speed, shear rate and shear stress all positively impacted this principal component. This implies that changes in fat content and OH caused torque, shear rate, rotational speed and shear stress to all rise, ultimately increasing PC1. The total solids (protein, lactose and minerals) in milk samples increase as

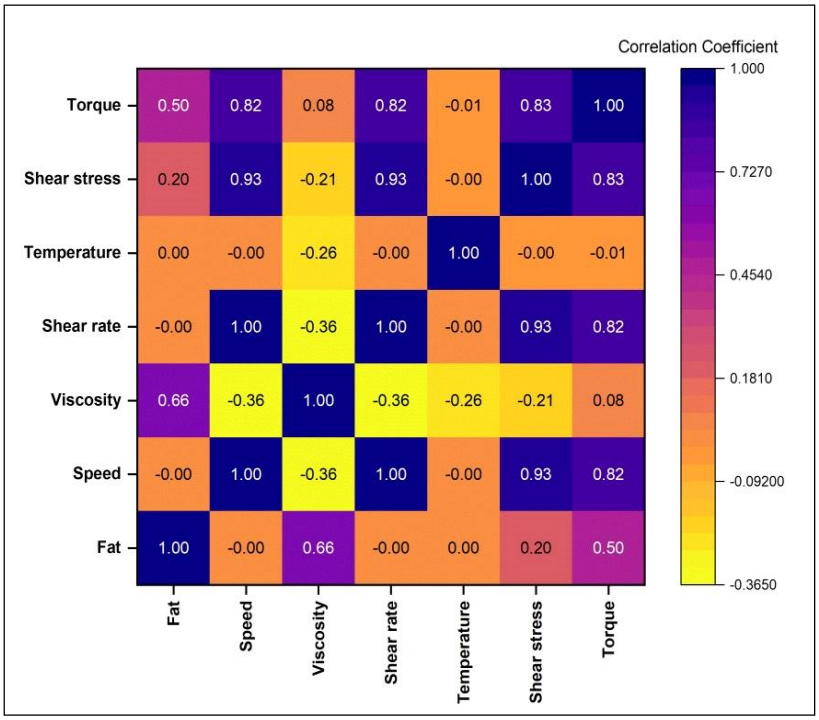

Fig 2: Correlation heat map of the different parameters of the various milk samples.

their fat content rises, leading to an overall increase in viscosity (Liu *et al*., 2023). These variations in dynamic parameters demonstrated the influence on the fluid's flow and deformation properties and indicated the rheological reaction of milk to OH. Temperature has a little positive coefficient (0.01), indicating that PC1 only contributes slightly to the variation that is explained. This suggests that, although it was limited, temperature was positively correlated with the total response of the parameters in the context of PC1. This implies that the response of the observed parameters represented by PC1 may rise slightly concurrently with temperature.

The positive coefficients in PC2, where fat and viscosity were significant contributors, suggested that OH contributed to both fat content and viscosity. This emphasizes how the heating process alters the composition. The precipitation of protein upon heating to 90°C may have caused these modifications (Kumbár and Nedomová, 2015). temperature had a negative coefficient (-0.20). This negative correlation implies an inverse link between temperature and the parameters represented by PC2. In this case, the responsiveness of the parameters denoted by PC2 may decrease as the temperature rises. The effects of OH on milk viscosity are explained scientifically by the eigenvectors. Changes in milk composition were indicated by the contributions of fat and viscosity to PC2, while the positive coefficients in PC1 indicated changes in dynamic characteristics.

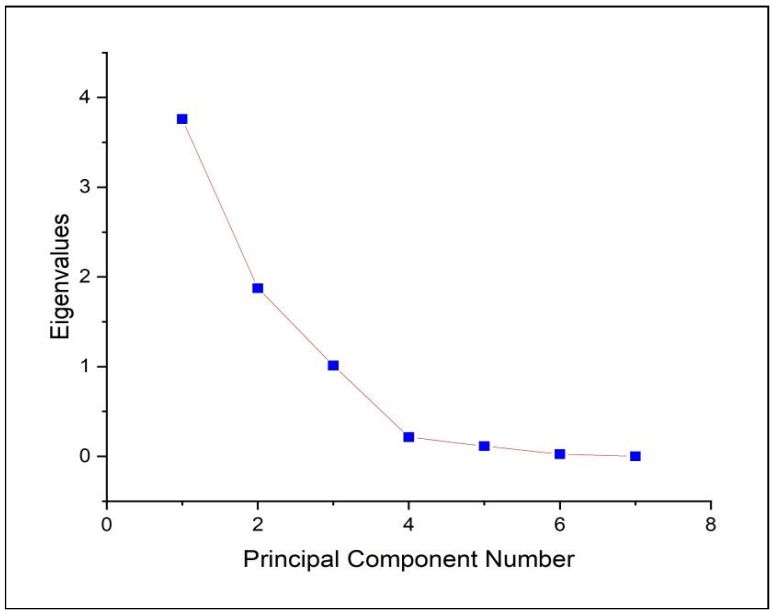

Fig 3: Scree plot of eigenvalues based on the different parameters of various milk sample.

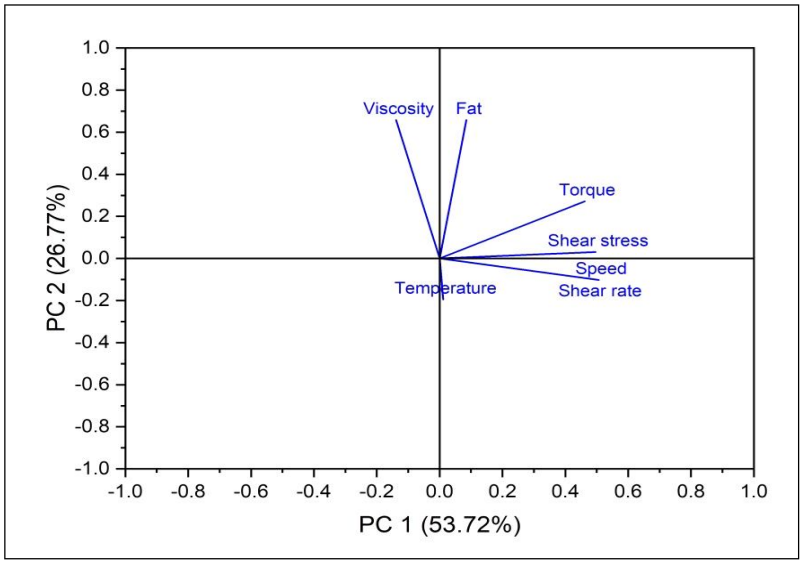

Fig 4: PCA loading plot of different parameters of the raw and ohmic heated milk samples.

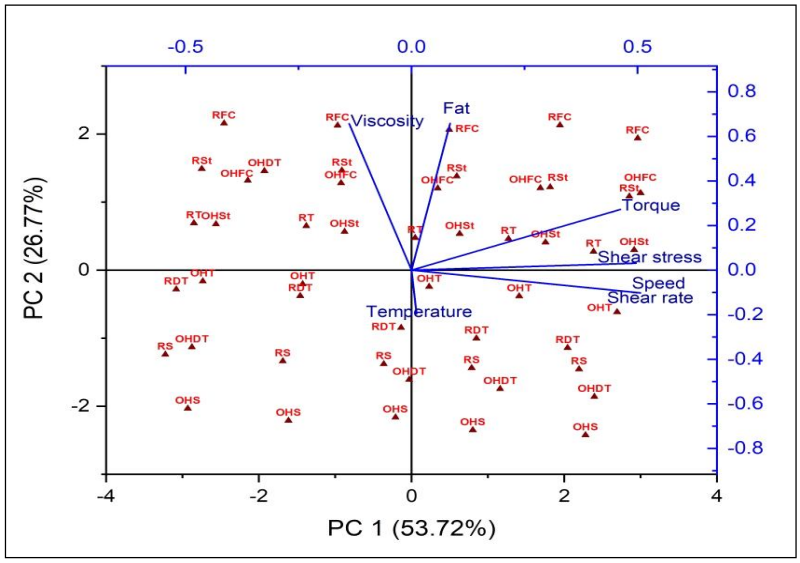

Fig 5: PCA bi-plot of different parameters of the raw and ohmic heated milk samples.

Analysis of the principal components of the biplots of the various milk samples

The distinctive patterns of various milk kinds to the experimental settings were reflected in the varied pattern shown in Fig 5. Variations in the amounts of specific parameters were responsible for the split along PC1, which significantly adds to the dataset's total variance. For example, the reactions of raw skim milk, raw double-toned milk and raw full-cream milk clustered together in the negative range of PC1. Similar properties like viscosity, shear stress and fat content also might have an impact on this alignment.

On the other hand, ohmic heated full-cream milk, ohmic heated skim milk, ohmic heated double-toned milk and ohmic heated standardized milk were all included in the positive range of PC1. The unique clustering of these ohmic heated samples suggests that the procedure had caused comparable modifications to their characteristics and may be associated with the effects of OH on protein denaturation, lipid emulsification, or other structural changes.

The separation along PC2, where ohmic heated toned milk was in the negative range and raw standardized milk and raw toned milk were positioned in the positive range, points to the possibility of other variables impacting the responses. These might involve variations in shear rate, temperature sensitivity and other parameters.

CONCLUSION

This work highlights the complex impact of OH on the flow characteristics of milk. This study offers valuable insights into the relationship between fat content and the viscosity of milk and showcases the possibility of OH as a means to alter milk properties minimally. The treatment did not demonstrate any statistical significance, indicating a negligible influence of OH on the pH of milk. It was found that the application of OH had little effect on the viscosity of milk, but it did not have a substantial impact on rheological characteristics, including shear rate and torque. This suggests that OH has a complicated but limited influence on the flow behavior of milk. The results have significant implications for the dairy sector, namely in milk processing and quality control and provide a scientific foundation for optimizing heating methods to preserve the desirable characteristics of milk products.

Conflicts of interest

The authors declare that they have no conflicts of interest or competing interests.

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