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INFLUENCE OF METAL IONS ON THE OXIDATIVE STABILITY OF HEATED MILK: STUDY BY ATR-FTIR SPECTROSCOPY AND CHEMOMETRICS

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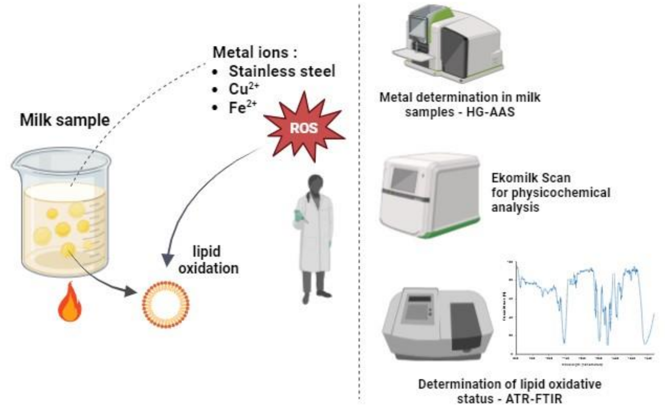

The present study demonstrates the effect of metal ions (stainless steel, Cu^{2+}, and Fe^{2+}), and their catalytic activity in the lipid oxidation of the milk samples. Milk fat is one of the complex fats and highly sensitive to auto-oxidation. To amplify the effect of metal ions, milk is subjected to temperature treatment, leading to various chemical and biochemical changes in the lipid fraction of the heated milk. Metal release were monitored by HG-AAS for the metal determination in milk samples. In this current investigation, the effect of metals ions on heated milk was determined using Ekomilk Scan for physicochemical analysis and ATR-FTIR Spectroscopy for the determination of lipid oxidative status, in combination with the chemometric PCA (Principal Component Analysis), which was not reported in earlier investigations. The heating process resulted in decreases in the areas of intensity ratios of 1746/2923 and 1737/2923 cm^{-1}. Principal component analysis and hierarchical cluster analysis confirmed that stainless steel has a higher effect on lipid oxidation than copper and iron. These results demonstrate that the metal in milk solution and their ions impact lipid oxidation during the heating. This research also indicateds that oil oxidation could be easily and rapidly monitored by ATR-FTIR spectroscopy.

INTRODUCTION

The presence of metals in milk can cause various chemical alterations. From this perspective, the presence of metal can compromise the safety and quality of milk due to its catalytic activity in several interactions between natural components, such as protein denaturation, Maillard reactions, lipid oxidation, among others.[1,2]

During the heating of milk, several chemical changes occur, which have a strong impact on the quality of the heated milk. In addition to the

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chemical changes likely caused by interactions among the natural constituents of milk, other interactions that can also alter milk stability are the effects of metal ions present in milk, especially iron, zinc, manganese and copper.[3]

Nowadays, consumer demands and concerns are directly reflected in the contamination of milk by metals during thermal treatment. There are two possible sources of exposure: cookware equipment and industrial equipment. Both sources can transfer metals to the milk, resulting in interactions that lead to various chemical changes.[4]

However, ion release from metals usually occurs at high levels due to thermal factors, which is crucial for increasing the release of the metals. This allows metals to engage in catalytic interactions, which is also an important factor because their chemistry directly impacts lipids.

The most utilized materials in the food industry and equipment are stainless steel, from which metal release has also been reported, as well as from other pure metals such as nickel and chromium.[5]

Metals release from stainless steel has also been demonstrated by,[6] which reports a high level of released of three metals (Fe, Cr, Ni) in whey protein solution and simulated milk solution at pH 6.8. Effects from metals on the chemical changes of milk have been reported, and studies involve interactions with protein, which means metals present in milk are involved in several interactions with natural milk components.[7]

Lipid oxidation is a natural phenomenon, often observed during storage, causing chemical changes in fatty acids and the stability of triglycerides (TGs).[8, 9] Research into lipid oxidation in cow's milk fat, which comprises 98 % TGs, has outlined various types of TGs that are susceptible to oxidation under thermal conditions.[10,11] This process involves complex reactions between triglycerides and oxygen, catalyzed by various metals.[12]

Studies have shown significant changes in parameters such as protein content, density, and non-fat solid components, which, when catalyzed, trigger a cascade effect of subsequent changes.[13,14]

Therefore, a more in-depth understanding of thermal oxidative stability and changes in the composition of milk lipids is necessary, especially concerning alterations during heating in the presence of various metals.[15,16] In this context, this research aimed to study the possible release of metals into the milk solution during heat treatments and their influence on the chemical behaviour of cow's milk, particularly in the milk fat domain.

RESULTS AND DISCUSSION

Based on the physicochemical analysis of the milk samples, numerous chemical changes have been observed, as detailed in Table 1. Pure fat exhibits higher values, but when exposed to metals, it levels decrease, with the most significant changes noted in samples treated with stainless steel. Protein also undergoes decomposition, resulting in a reduction in its overall level, as also observed.[17]

Additionally, other measured parameters exhibit alterations in the same samples, all which are correlated with changes in chemical compositions. The increase in conductivity, confirming the release of ions from metals in a milk solution with a pH conducive to the released of these metals.[18] Other changed parameter are pH and density both of them slightly decreased this is one other confirmation of the chemical changes which occur at all monitored samples compare with pure milk samples. These chemical changes involve the released of ions from metals, triggering robust chemical reactions, evidenced in heated milk samples.[19]

However, it is still necessary to differentiate the metals released from those initially present in the milk samples. To achieve this, the studied metals were monitored in all samples by Atomic Absorption Spectroscopy. From the results obtained (Table 1), in the sample containing stainless steel, the concentrations of Cr, Fe and Ni increased significantly. Same trends of changes were observed and in two other milk samples with the presence of copper respectively iron. This is almost signicantly higher that observed in the heated milk sample without added metals. Finally, from stainless steel were released three metals probably this three metals are the major metal components of stainless steel.

Table 1

Physicochemical properties on the heated milk with different compositions

Physicochemical properties and heavy metals conc.	Pure Milk (Sample 1)	Milk with stainless steel (Sample 2)	Milk with copper (Cu) (Sample 3)	Milk with iron (Fe) (Sample 4)
Total Fats (%)	3.90 ± 0.02^{a}	2.20 ± 0.10^{b}	3.20 ± 0.10^{a}	3.10 ± 0.05^{a}
Total Proteins (%)	3.40 ± 0.10^{a}	2.80 ± 0.12^{b}	3.10 ± 0.15^{a}	3.05 ± 0.05^{ab}
Solid non fats (%)	8.33 ± 0.01^{a}	7.40 ± 0.03^{a}	7.60 ± 0.35^{a}	7.9 ± 0.55^{a}
Density (g mL^{-1})	1.032 ± 0.01^{a}	1.021 ± 0.01^{a}	1.025 ± 0.03^{a}	1.025 ± 0.02^{a}
pH	6.58 ± 0.15^{a}	6.48 ± 0.03^{a}	6.47 ± 0.50^{a}	6.51 ± 0.10^{a}
Conductivity (mS/cm)	3.98 ± 0.12^{a}	4.21 ± 0.10^{a}	4.15 ± 0.20^{a}	4.17 ± 0.20^{a}
Cu (mg L^{-1})	0.24 ± 0.02^{a}	0.26 ± 0.12^{a}	0.42 ± 0.11^{a}	0.23 ± 0.1^{a}
Fe (mg L^{-1})	0.9 ± 0.035^{a}	1.65 ± 0.11^{a}	1.02 ± 0.11^{a}	2.88 ± 0.12^{a}
Cr (mg L^{-1})	0.79 ± 0.072^{a}	1.68 ± 0.1^{a}	0.84 ± 0.12^{a}	0.81 ± 0.12^{a}
Ni (mg L^{-1})	1.36 ± 0.12^{a}	2.35 ± 0.11^{a}	1.32 ± 0.11^{a}	1.41 ± 0.12^{a}

Note: Different lowercase letters within the same row indicate a significant difference between the samples ($p \leq 0.05$). All values are expressed as the mean ±SD (standard deviation).

Chemical changes were observed in all the milk samples heated in the presence of metals. The interactions of the metal ions influence the observed chemical variations. For a better understanding, this study prioritized the monitoring of lipid oxidation as a precursor to chemical changes. To this end, the lipid content of milk fat was analysed using FTIR-ATR spectroscopy, and the corresponding IR spectra are shown in Table 2. In pure raw milk, peaks at 1737 cm^{-1} and 1746 cm^{-1} were observed, indicating characteristic vibrations of the carbonyl group in triglycerides.

Table 2

ATR-FTIR spectral band assignments of milk fat

Wavenumber (cm^{-1})	Spectra Assignment
2923 and 2853	C-H antisymmetric and symmetric stretching vibrations of aliphatic CH_2 group of the fatty acid [20]
1737 and 1746	Ester carbonyl group (O-C=O) stretching vibrations of triglycerides [21]

Two different triglyceride vibrations likely correspond to distinct types of triglycerides present in milk, but both exhibit decrease intensity during thermal heating in the presence of metals.[22] Another indicator for triglycerides in fat is the presence of chains of saturated methylene groups, both asymmetric and symmetric. Clear observations from all selected peaks indicate significant lipid decomposition in the presence of stainless steel compared to iron and copper, where the intensity also decreases (Figs. 1 and 2).[23]

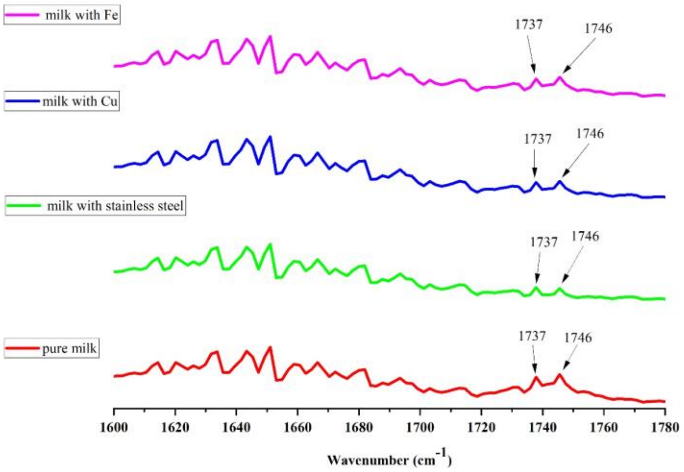

Fig. 1 – ATR-FTIR spectra of heated milk samples in the region 1600–1780 cm^{-1}.

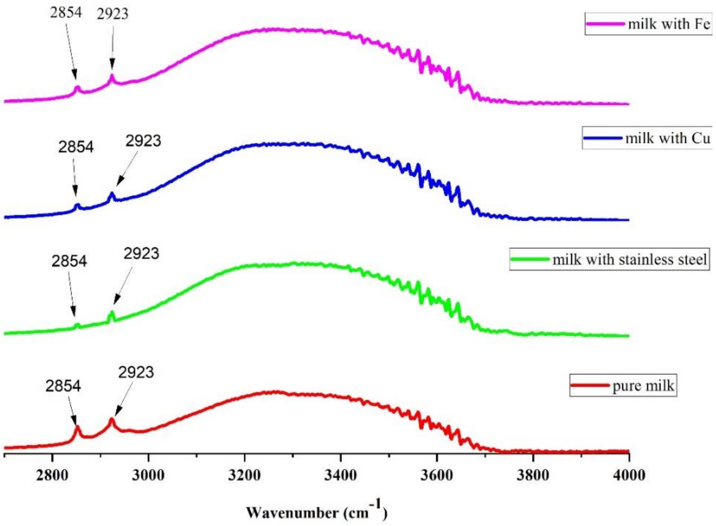

Fig. 2 – ATR-FTIR spectra of heated milk samples in the region 2750–4000 cm^{-1}.

To better understand lipid oxidation between two different triglycerides, the intensity ration was utilized.[24] The ratio of intensities between the ester carbonyl group of triglycerides at 1746 cm^{-1} demonstrates a strong correlation with the saturated aliphatic -C-H group at 2923 cm^{-1}. During the oxidative thermal transformations of triglycerides, long-chain fatty acid hydrocarbons undergo chemical interactions, indicating that a decrease in this ratio leads to the conversion of triglycerides into other oxidized compounds.[25]

Figures 1 and 2 depict the intensity of the selected peaks, which are higher in the heated pure milk sample. However, the ratio of intensities (Fig. 3) for triglyceride reveals changes in the sample with stainless steel, correlating with the greater release of metals in this sample.[26] In the samples with copper the triglyceride stability ratio decreased, although it did not exhibit significant changes compare with pure heated milk but some other changes were observed in milk sample with iron presence, exactly one of the triglyceride molecule has same level of changes like sample with copper but some difference ratio has other ratio which is indicator for decreased level of other triglyceride molecule.[22]

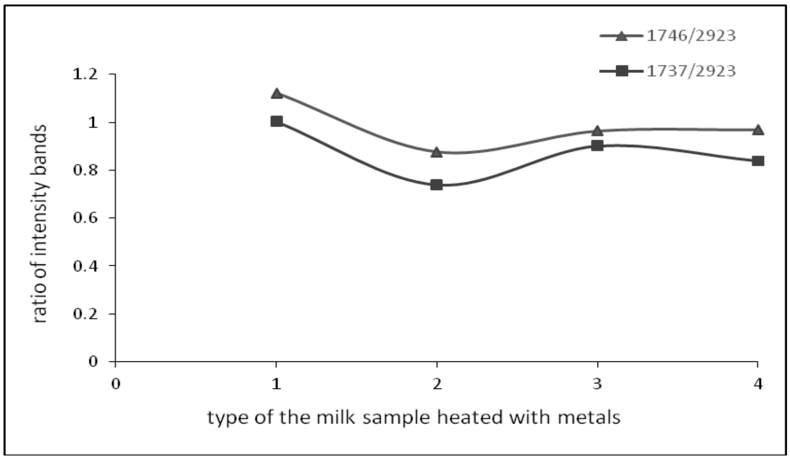

Fig. 3 – Changes in the band intensity ratio of the infrared band related to triglyceride stability obtained from heated milk samples: 1. pure milk sample; 2. milk with stainless steel; 3. milk with copper (Cu); 4. milk with iron (Fe).

Identifying which changes are crucial for triggering the cascade effect involving alterations in all other components of milk is very complex. Nevertheless, it is known that lipid oxidation is one of the main reactions, which can occur simultaneously with other possible reactions.[27]

To better classify the milk fat samples, FTIR spectra encompassing the complete spectrum, correlating with all milk components, were analyzed using multivariate PCA analysis.

In this investigation, PCA was applied to the vibrational spectra to analyse the discrimination of milk samples in the presence of different metals during thermal processing. Since the range of 4000–1000 cm^{-1} provided the best discrimination between pure milk and samples with metals presence, PCA score plots presented in Fig. 3 illustrate their classification.

However, PC 1 accounts for most of the variance, while PC 2 represents the lowest variance (PC1 + PC2 = 99.99). This indicates that PCA can perfectly classify the milk fat samples according to the metals present during thermal heating.[28]

In Fig. 4, it can be observed that all the samples are located on the positive side of PC1 but are differentiated along PC2. Only the pure milk sample is situated on the positive side of PC2, while the other three are on the negative region. The two samples heated in the presence of metals are closely positioned to each other (sample with Fe and sample with Cu), whereas the sample heated with stainless steel is distinguished from the other two samples, exhibiting a greater disparity compared to the pure milk samples. This different effect of the stainless steel could be explained by chemical composition of the stainless steel with three major metal composition has higher metal release in milk solution and chemical changes which occur in this milk samples it is completely different from two others.

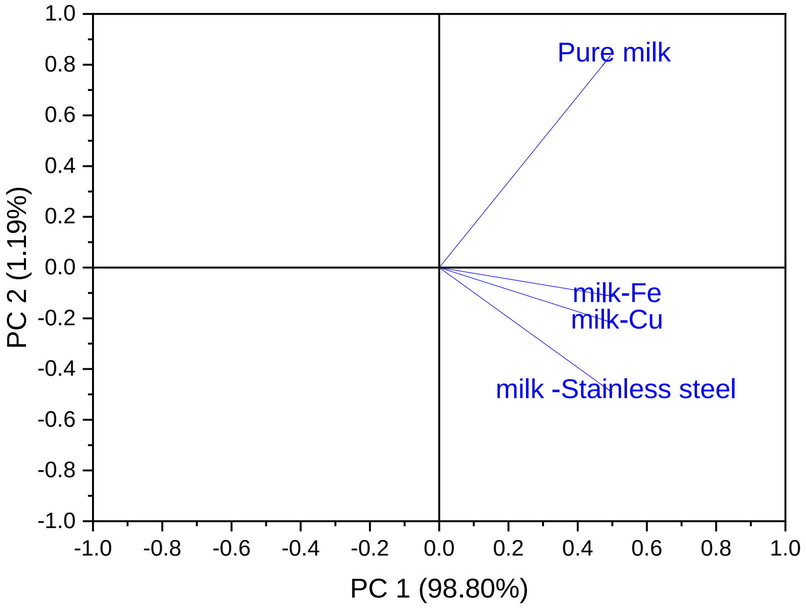

Fig. 4 – PCA score plots of ATR-FTIR spectra obtained from heated milk samples with different metal for the 4000–000 cm^{-1} spectral range.

EXPERIMENTAL

Sample preparation and reagents

Raw milk (RM) was sourced from a local farm in the Mitrovica region of north Kosovo (42° 53' N 20° 52' E) and it was refrigerated at 4–6°C until further processing.

Four different milk samples were prepared from the same batch of milk, each with different compositions:

1. Pure milk without any added metals (control sample).
2. Milk with the addition of stainless steel AISI 316 at a concentration of 0.1g mL^{-1} milk solution.
3. Milk with the addition of copper at a concentration of 0.1 g mL^{-1}.
4. Milk with the addition of iron at a concentration of 0.1 g mL^{-1}.

All chemicals used were of analytical grade and sourced from Sigma-Aldrich.

Determination of the total metal concentration by AAS

To determine the metal concentration in the milk, 2 grams of the milk sample were measured, and 10 mL of pure concentrated nitric acid (HNO_3) was added. All the samples were then digested for 3 hours at 210°C using a microwave accelerator and subsequently cooled to room temperature (20 ± 1°C). The concentrations of the metals of interest in the digested samples were then determined using the HG-AAS model Varian AA240.

Figures 5 depicts the schematic of milk samples preparation for subsequent analysis in this study

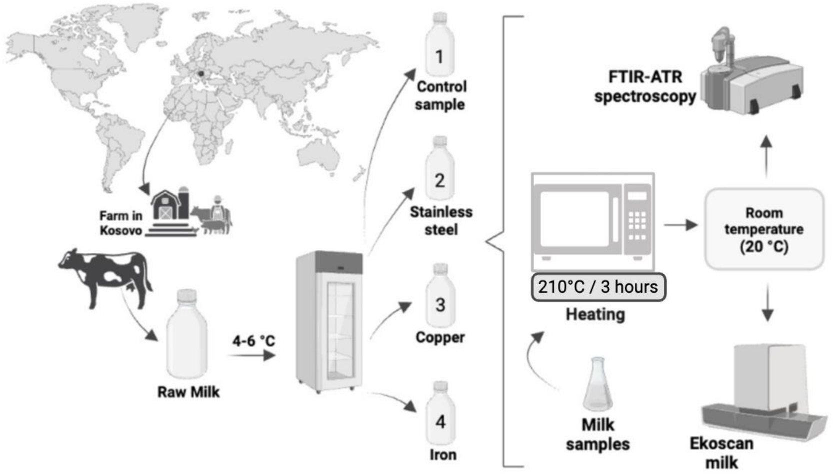

Fig. 5 – Schematic representation of the stages of preparing milk samples for subsequent analyses (FTIR and Ekoscan milk).

Milk analysis (Ekoscan)

The physicochemical composition of the milk was analysed using a rapid milk analyser (Ekoscan milk analyser, Eon Trading, Stara Zagora Bulgaria). The results were compared with the published reference reports: fat content not less than 3.25 %, protein content not less than 3.50 %, solid not-fat content not less than 8.50 %, and density ranging from 1.028–1.036 g mL^{-1}.[29]

FTIR-ATR Spectroscopy

The FTIR spectra of milk fat samples were obtained using an ATR type ZnSe accessory at room temperature (20 ± 1°C). The fat spectra were acquired in the range of 600 - 4000 cm^{-1} using an FTIR instrument (Iraffinitty-1, Shimadzu), with each spectra recorded over 32 scans. The ATR accessory was cleaned with acetone to remove any possible impurities, and a new background was recorded after each measurements using IR Solution software (Shimadzu).

Statistical analysis

Principal Component Analysis (PCA) was conducted using OriginPro 2021 software for multivariate analysis (MVA). The analyses were performed on baseline-corrected and vector-normalized spectra. The PCA results were visualized and presented as score plots. Data were analyzed using Statistica software (version 10.0, StatSoft Inc., Tulsa, USA). All measurements were performed in triplicate and reported as the mean ± standard deviation (SD). Significance among mean values was determined at 5 % level ($p \leq 0.05$) of significance by one-way analysis of variance (ANOVA) with following post-hoc Tukey’s test.[30]

CONCLUSIONS

This research investigated the thermal stability of cow's milk and milk fat under heat treatment, both in the presence and absence of metals acting as reaction catalysts. ATR-FTIR data identified structural and compositional changes in milk fat occurring in samples subjected to heat treatment with metals, primarily due to lipid oxidation reactions, compared to pure milk samples. Although similar changes were observed in different chemically identified triglycerides, the trend of changes varied depending on the metal used in this study. The results revealed that stainless steel had a more pronounced impact on oxidation and metal release compared to samples containing the other two metals (Fe and Cu), where lipid oxidation also occurred but to a lesser extent than in the stainless-steel sample. These findings demonstrate the significant effect of these metals on milk lipid oxidation and other chemical components during heating. Furthermore, it underscores the utility of ATR-FTIR spectroscopy and chemometric analysis as rapid, sensitive, and sustainable tools for monitoring lipid oxidation in milk fat.

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