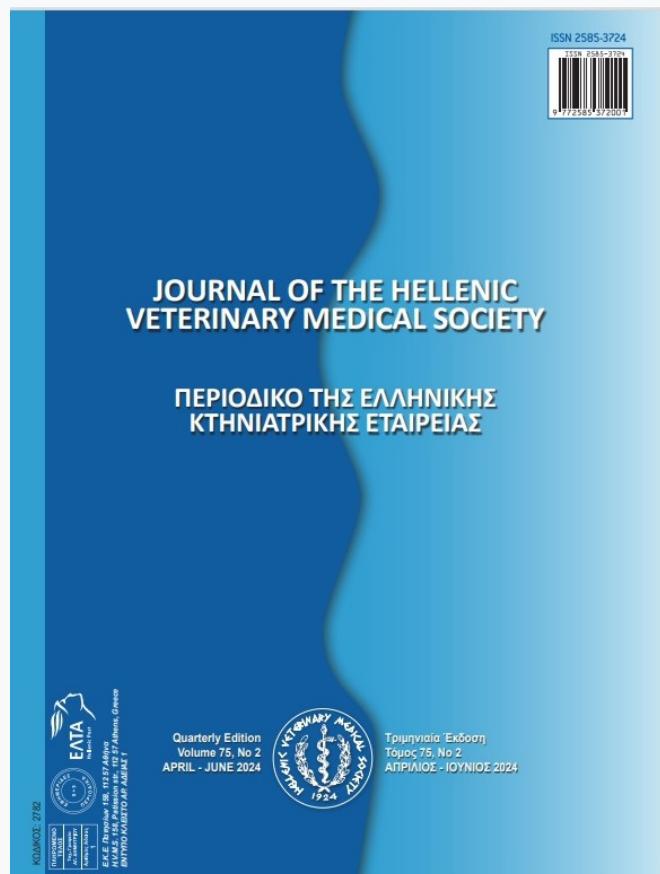


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Characterization of normal and mastitic milks using Raman Spectroscopy by application of SERS method

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Characterization of normal and mastitic milks using Raman Spectroscopy by application of SERS method

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ABSTRACT: Rapid, non-destructive analysis of the milk by Raman Spectroscopy is used in different analyses and has enabled the determination of biological samples without using additional chemicals. In the study, it is aimed to investigate the molecular structures of normal and mastitic milks using Raman spectroscopy by application of Surface Enhanced Raman Spectroscopy (SERS) method and this study is the first study done for this purpose. In the study, the presence or absence of functional groups of the spectra obtained from the milk samples was compared with the reference spectra in other studies. It was observed that the peaks obtained from the biochemical structures of normal and mastitic milks were consistent with the literature. In addition, it was found that the biochemical components (such as fat, proteins, carbohydrates and minerals) of subclinical mastitis and normal milk did not change at the molecular level.

It was thought that there was no change at the molecular level as there was no visible deterioration in the milk with subclinical mastitis and the factor caused by the infection in the breast tissue.

Keywords: Raman Spectroscopy; SERS method; mastitis; milk

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INTRODUCTION

Milk, 87% of which is water, contains fat, protein and carbohydrates as well as minerals and vitamins (McGoverin et al., 2010; El-Abass et al., 2011; Mazurek et al., 2015; Thiago et al., 2016). The chemical composition of the cow milk has an average of 87% water, 4-5% lactose, 3-4% fat, 3% protein, 0.8% mineral and 0.1% vitamin. Chemical composition of milk changes depending on animal species, environmental conditions, and animal feeding (Li-Chan, 2007, Pecka et al., 2016). One of the problems the dairy industry and cattle breeders that causes major economic losses due to reduced milk yield and quality is, "Mastitis" (Albenzio et al., 2002; Bastan et al., 2010; Zigo et al., 2014). Mastitis covers physical and chemical changes in milk, and pathological changes in mammary secretory tissue caused mainly by bacteria (Atasever and Erdem, 2008; Pyörälä and Taponen, 2009; Darbaz and Ergene, 2015; Zigo et al., 2019).

It has been reported that mastitis occurs clinically and subclinically, but in most cases (70%) subclinical mastitis occurs. Subclinical mastitis is mastitis in which there is no visible deterioration of the udder and milk (Philpot and Nickerson, 2000; Atasever and Erdem, 2008; Baştan, 2013; Darbaz and Ergene, 2015; Hisira et al. 2023).

Pathogens that play a role in the formation of subclinical mastitis are classified as contagious (*S. aureus*, *S. agalactia* and *S. bovis*) and environmental (*E. coli*, *P. aeruginosa*, *S. uberis*, *S. chromogenes* and other coagulase-negative staphylococci) (Albenzio et al., 2002; Ozbey et al., 2022). In recent years, Coagulase Negative Staphylococcus (CNS) has attracted interest among the agents that cause subclinical mastitis (Leitner et al., 2000; Yağcı and Göğüş, 2008; Pyörälä and Taponen, 2009).

Since milk and milk derived products are consumed by a large part of the World population, the characterization of the milk and the number of components has become important. Several research methods are used to detect the biochemical structure of milk. Raman spectroscopy is used in many food analysis, including dairy products, and has the potential to answer some questions about the molecular structure of the sample studied (Chalmers et al., 2012; Vaskova et al., 2016). Raman spectroscopy is one of the analytical methods for vibrational fingerprinting of molecules for the purpose of making a milk biochemical analysis (Petersen et al., 2021). Raman spectroscopy has been widely used in the study of biological specimens over the

past two decades. Because, it is a powerful analytical method capable of providing useful biochemical information on living cells depending on the interaction with toxic agents or drugs, disease, cell death and differentiation. The method is used to characterize and define the chemical structure of the sample at the molecular level. A unique vibrational spectrum of each molecule is obtained by Raman spectroscopy (Muik et al. 2003; Heise et al., 2005; Baeten et al., 2005; El-Abassy et al. 2009; El-Abassy et al., 2010; Açıkgöz et al., 2018). Also being non-invasive, it has been widely used in food industry in recent years due to the fact that very few samples are sufficient for examination and water is used as a solvent (Açıkgöz et al., 2018). As a result of the examination of biological samples by Raman spectroscopy, weak Raman peaks are obtained. Therefore, using the Surface Enhanced Raman Spectroscopy (SERS) method eliminates the mentioned disadvantage and provides stronger peaks. As we could reach the literature, this was the first study to compare the molecular structures of normal and mastitis milk by applying the Raman spectroscopy-based SERS method. We aimed to try how effective this method would be in the comparison and characterization of mastitis and normal milk.

MATERIAL AND METHODS

Sample preparation

Three to five-year-old Holstein-Friesian cow's milks from a dairy farm, in the same period of lactation, were included in the study. Milk samples (n=100) collected from the different mammary quarters of the cows were tested by California Mastitis Test (CMT) and grouped based on their score (normal/mastitic). After the CMT, the teat was cleaned with 70% alcohol, and after the first 4-5 squirts of milk were discharged, 10 ml of milk was collected from each quarter. Milk samples were stored in a transfer box at +4°C and transported to the laboratories within 2 h of sampling.

SCC was performed on milk samples using a somatic cell counter (BactoCount IBC, Bentley Instruments Inc., Chaska, MN, ABD) and the samples were grouped as normal ($7-177 \times 10^3$ cells) and mastitic milks ($376-1918 \times 10^3$ cells). Sodium azide (0.05 ml, 24%) (Sigma-Aldrich) was added to the milk samples in order to preserve the cells.

Microbiological examination in milk samples

Milk samples (n=100) were plated on Blood agar

(BA), MacConkey agar (MCA) and Sabouraud Dextrose agar (SDA). For bacterial isolation BA and MCA plates were incubated in aerobic medium for 2-3 days at 37°C; then the growth of colonies was identified using by biochemical tests (Gram staining, catalase, oxidase, coagulase, sugar fermentations, haemolysis, motility, Kimya lab. Turkey). Seven samples of the milks which were only coagulase negative staphylococcus (CNS) positive were involved as mastitis, whereas 7 of the milks which were pathogen free were accepted as normal milk samples. In addition, for yeast and fungi isolation SDA plates (Merck) were incubated at 25°C for 7-10 days under aerobic conditions. Species-level identification of *Aspergillus* spp. was performed by microscopic examination after lactophenol cotton blue staining and *Candida* spp. species-level identification performed by microscopic examination after Gram staining (Schultz et al., 2004; Quinn et al., 1994).

SERS method

Silver nanoparticles (AgNPs) were synthesized according to the Lee and Meisel method (1982). Raman measurements were performed without any physical or chemical pretreatment of samples, and the samples were stored at 40°C until analysis. 20 µl of milk samples was mixed with 20 µl AgNPs and this mixture was transferred into CaF₂ slides for the SERS (Renishaw plc, Wotton-under-Edge, UK) measurement. Then, the mixture was waited for 1 hour at room temperature.

Raman Spectroscopy

SERS spectra of all milk samples were measured by using Renishaw in Via Raman Spectroscopy with a 785 nm laser and Charge-Coupled Device (CCD) detector (modulated to Renishaw in Via Raman Spectroscopy, Wotton-under-Edge, UK). Spectral range was set as 100-3200 cm⁻¹ in the earlier stages of the study. The acquisition time, laser power and exposure time were the same for all measurements and they were 15s, 5 mW and 1s, respectively. Acquiring data, spectra preprocessing, analysis, and plotting were performed using the software WiRE 3.2 (Renishaw plc, Wotton-under-Edge, UK), MATLAB® (Matlab 7.13, The Mathworks, Natick, MA).

RESULTS

Milk fat is composed of more than 400 fatty acids and is presented in trace amounts. Changes in the intensity of Raman spectra are known to be caused by differences in fat. At the same time, as well as the lipids in the milk, fat-soluble vitamins, nucleic acids and essential fatty acids also play an important role (Månnsson, 2008).

The basic peak values of normal milk sample were represented in Table 1. The SERS spectra of the peaks of normal and mastitic milk samples were given in Figure 1, Figure 2, Figure 3 and Figure 4, respectively. The highest and lowest Raman peaks in milk samples were found to belong to many fatty acid bands. Vibra-

Table 1. Assignment of typical bands in Raman spectra of normal milk sample

Assignment	Raman peak [cm ⁻¹]	Vibrational mode
Lactose	357	
Glucose	450	δ (C - C - C) + τ (C - O)
Amino acids	760	ν (C - S)
Phospholipid head group	860	Phosphatidic acid
Ring-breathing (phenylalanine)	1005	
Carbohydrate	1080	ν (C - C)
Carotenoids	1150	ν (C - C)
Amide III	1265	δ (N- H); ν (C - N) Amide III
PC, PI and PS	1265	C=C <i>cis</i> unsaturation
PC, PI and PS	1297	C-H in-plane bending of ethylene groups
Cholesterol,	1442	δ (CH ₂) twisting unsaturation
PC, PI and PS		Saturated fatty acid
Carotenoids	1525	δ (CH ₂) scissoring
PC, PI and PS	1651	ν (C=C) <i>cis</i> unsaturation
Amid I	1651	ν (C - O) Amide I; ν (C - C)
Triacylglycerol	1745	Ester ν (C=O)
Acyl chains in liquid state	2850	CH ₂ symmetric stretching
Cholesterol, cholesterol ester	2890	CH ₃ symmetric stretching
Acyl chains	3008	<i>cis</i> -unsaturated =CH stretching

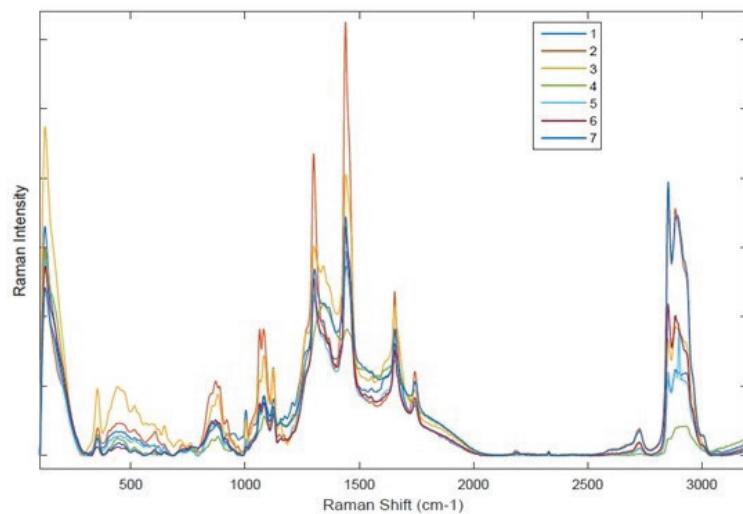


Figure 1. SERS spectra of normal milk (7 milk samples determined as normal milk are numbered from 1 to 7). The lines shown in different colors show the SERS spectra of different normal milk samples.

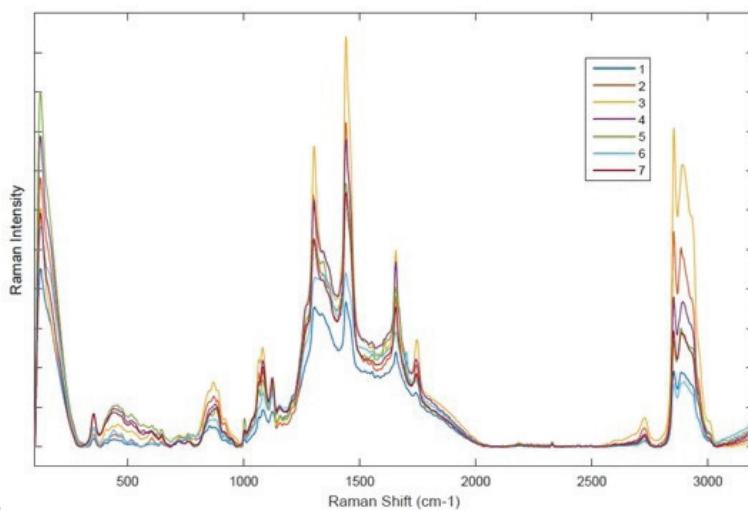


Figure 2. SERS spectra of mastitic milk (7 milk samples determined as mastitic milk are numbered from 1 to 7). The lines shown in different colors show the SERS spectra of different mastitic milk samples.

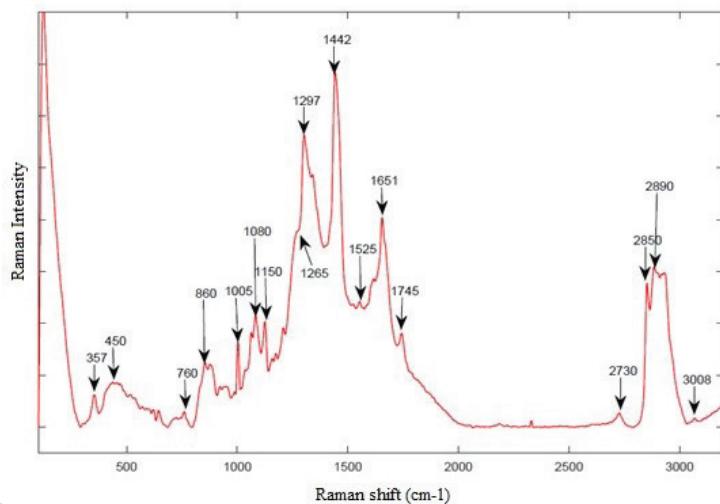


Figure 3. SERS spectrum of normal milk in the region 100 to 3200 cm^{-1} .

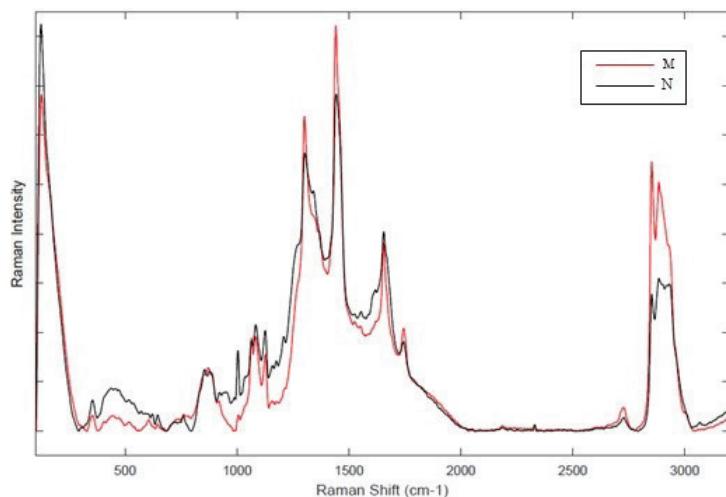


Figure 4. SERS spectra of normal (black line) and mastitic milk (red line) in the region 100 to 3200 cm^{-1} . When the spectra are examined, it is seen that normal and mastitic milks have different intensity of peaks in the Raman bands.

tional band of fatty acids were found at 1265 cm^{-1} , 1297 cm^{-1} , 1442 cm^{-1} , 1651 cm^{-1} and 1745 cm^{-1} , respectively. The band at 1265 cm^{-1} can be assigned to (δ twist (CH₂, R1) CH = CH CR cis double bonds), the band at 1297 cm^{-1} can be assigned to cm^{-1} (CH-CH₂ twisting groups), the band at 1442 cm^{-1} can be assigned to (δ (CH), -CH₂ scissoring bonds), the band at 1651 cm^{-1} can be assigned to (ν C1 = C1) double bonds of cis(RHC = CHR), and the band at 1745 cm^{-1} can be assigned to (ν (C = C ester)). The strongest peak obtained from the 1442 cm^{-1} is one of the saturated fatty acids of cholesterol.

Lipid identification in milk both normal and mastitis was performed in the Raman spectrum; high lipid ratio was found at 2730-3010 cm^{-1} and strong wide Raman bands were found to be between 400-1800 cm^{-1} . The globules membranes of milk lipids contain a complex mixture of phospholipids. A different vibration mode for complex membrane phospholipids was found in both normal and mastitis milk samples.

The basic peak of phospholipids was around at 860 cm^{-1} , and wide Raman peaks were observed at 845-895 cm^{-1} . The reason for this is that the complex structure of phospholipids is thought to be due to the overlap of different polar head groups (Figure 3).

The highest intensity obtained from normal and mastitic milk samples (Figure 4) of SERS spectra represent the fat content. Triglycerides formed by C = O stretching of the peak ester groups were obtained at 1745 cm^{-1} in the Raman spectrum. In addition, the spectral band at 1005 cm^{-1} in normal and mastitis milk samples was found to be an indicator of the pro-

tein which is the ring structure of the phenylalanine. Raman spectra of proteins in liquid milk were found to be much weaker than expected.

Raman peaks of the carotenoids, which are found in very small amounts in milk, are shown in both normal and mastitis milk at 1150 cm^{-1} and 1525 cm^{-1} . The peaks obtained at 2850 cm^{-1} and 2890 cm^{-1} in the Raman spectrum show the ν (C-H) vibrations of the symmetric and asymmetric CH₂ and CH₃ groups, respectively. The band at 3008 cm^{-1} (C - H) corresponds to the symmetrical shear mode. The band at 3008 cm^{-1} (C - H) corresponds to the symmetrical scissoring mode. These modes are the origin of the acyl oil chains in the CH₂ and CH₃ groups, and the Raman spectra at 2730-3010 cm^{-1} are indicative of saturated fatty acids. The common peaks obtained from normal and mastitis milk is shown in Figure 3.

It was found that there was almost no difference between Raman spectra obtained from milk both normal and mastitic. Significant protein peaks in Raman spectra; ν (C-S) at 680-760 cm^{-1} represents the vibration modes of various amino acids, 1265 cm^{-1} Amide-III mode and 1651 cm^{-1} represents the amide-I mode. Differences in the intensity of spectral vibration modes of proteins in mastitis milk were expected. However, it was observed that it exhibited a spectral peak like in normal milk (Figure 4). The reason for this is that the bacteria that need to be found in the diseased milk do not grow or it is thought to be caused by the clinic of the disease. At the same time, Amide-II, Amide-III proteins and various amino acids such as tryptophan have the same spectral bands in milk and bacteria. Therefore, it was not possible to distinguish

between the Raman spectra of milk with both normal and mastitis milks. Thus, it should be noted that different cellular responses, biochemical changes, and Raman spectra of different toxic substances will show markedly spectral bands.

In the study, it was determined that the spectral band obtained from 1080 cm^{-1} belonged to carbohydrates. In addition, in the study was found to 357 cm^{-1} peak lactose, 450 cm^{-1} peak belong to the glucose. In the results obtained, the peak densities of the vibrational modes in lactose and glucose spectral bands are the same in milk with normal and mastitis (Figure 4). However, lactose production will be reduced due to changes in lactase enzyme in mastitis milk. Therefore, it was expected that there would be no minimal spectrum or no spectrum depending on the clinic of the disease. It is thought that there is no change in carbohydrate metabolism as in proteins. It was determined that the biochemical components did not change at the molecular level between the milk with subclinical mastitis and normal milk.

DISCUSSION

In the literature, there are studies about Raman spectroscopy and chemometric analysis in determining the biochemical structure of milk (Baeten et al., 1998; Yang and Irudayaraj, 2001; Yang et al. 2005; Li-Chan, 2007; Argov et al., 2008; El-Abassy et al. 2011; Rodrigues et al., 2016). However, there are no studies conducted with Raman spectroscopy on the molecular level of normal and mastitic milk. Li et al. (2016) showed the characteristic bands of carbon-oxygen-carbon stretching vibration at 1085 cm^{-1} for the determination of lactose in milk by Raman spectroscopy. In their study, Raman spectroscopy was a simple and fast method for the determination of lactose in milk. McGoverin et al. (2010) measured the amount of lactose, protein and fat in fatty and skimmed milk powder by Raman spectroscopy and applied partial least squares (PLS) method to obtained data.

The low wave number at 1745 cm^{-1} $\nu(\text{C}=\text{O})$ modes represents milk fats. The phenylalanine ring breathing band at 1005 cm^{-1} was found to be an indicator of protein. The peaks at 355 cm^{-1} and 445 cm^{-1} are belong to lactose, the peaks are $860\text{--}920\text{ cm}^{-1}$ $\nu(\text{C-C}), \nu(\text{C-O})$ are correspond to the specific modes of various amino acid found in frequencies. Raman spectroscopy is effective in determining the amount of protein and fat in lean and whole milk powder. It is an important role in the determination of additives

(calcium carbonate) (El-Abassy et al., 2009; 2010). In another study, fat content was measured in liquid homogenized milk samples prepared by different methods. Fat content of milk was measured by Raman spectroscopy and the results were analyzed by PLS method. The quantitative analysis of the fat concentration was obtained using an $800\text{--}3050\text{ cm}^{-1}$ Raman spectral band. As a result, they reported that Raman spectroscopy has a high potential as a fast, simple and non-destructive tool for chemometric analysis (El-Abassy et al., 2011).

Vaskova et al. (2016) compared the fat content of milk and milk products with the results obtained by Röse-Gottlieb gravimetric and butyrometric methods to verify the measurability of Raman spectroscopy. They showed that the band at 1748 cm^{-1} obtained in the molecular structure of milk contained represents the $\text{C}=\text{O}$ stress of the ester groups of triglycerides. The phenylalanine ring breathing band obtained at 1005 cm^{-1} was found to be an indicator of proteins. In the determination of the fat content in the samples, spectral bands obtained in 1303 cm^{-1} , 1443 cm^{-1} and 1748 cm^{-1} were evaluated in three important groups. They determined that CH_2 deformation vibrations at 1303 cm^{-1} , 1443 cm^{-1} saturated fatty acids and $\text{C}=\text{C}$ at 1654 cm^{-1} for unsaturated fatty acids in cis configuration are specific band. They concluded that Raman spectroscopy is a vibrational spectroscopic method with a potential related to chemical details of molecular structure, which makes this technique definitely proper for material identification.

CONCLUSIONS

In the study, the presence or absence of functional groups of the spectra obtained from the samples was compared with the reference spectra in other studies. It was observed that the peaks obtained from the biochemical structures of normal and mastitis milk were consistent with the literature. In addition, it was found that the biochemical components of subclinical mastitis and normal milk did not change at the molecular level. We established that there is no change in the molecular level as there is no visible deterioration in the milk with subclinical mastitis and the factor caused by the infection is found in the breast tissue. We believe that even very few components can be analyzed both qualitative and quantitative and this can be achieved by the application of one of the chemometric methods PLS based on data obtained from Raman spectroscopy.

DECLARATION OF CONFLICTING INTERESTS

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. There has been no significant financial support for this work.

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REFERENCES

Açıkgoz G, Hamamci B, Yıldız A (2018) Determination of Ethanol in Blood Samples Using Partial Least Square Regression Applied to Surface Enhanced Raman Spectroscopy. *Toxicol Res* 34(2): 127-132.

Albenzio M, Taibi L, Muscio A, Sevi A (2002) Prevalence and etiology of subclinical mastitis in intensively managed flocks and related changes in the yield and quality of ewe milk. *Small Ruminant Research* 43(3): 219-226.

Argov N, Lemay DG, German JB (2008) Milk Fat Globule structure & function; nanoscale comes to milk production. *Trends Food Sci Technol* 19(12).

Atasever S, Erdem H (2008) Relationships between mastitis and electrical conductivity of raw milk in dairy cows. *Anadolu Journal of Agricultural Sciences* 23(2): 131-136.

Baeten V, Hourant P, Morales MT, Aparicio R, Agric J (1998) Oil and Fat Classification by FT-Raman Spectroscopy. *J Agric Food Chem* 46 (7): 2638-2646.

Baeten V, Pierna JAF, Dardenne P, Meurens M, Garcia-Gonzalez DL, Aparicio-Ruiz R (2005) Detection of the Presence of Hazelnut Oil in Olive Oil by FT-Raman and FT-MIR Spectroscopy. *J Agric Food Chem* 53: 6201-06.

Baştan A (2013) İneklerde Meme Sağlığı ve Sorunları. İkinci Baskı. Ankara: Kardelen 30-141.

Baştan A, Cengiz M, Cengiz S, Polat B, Colak A, Akan M, Darbaz I, Acar DB (2010) Effects of precalving antibiotic treatment on mastitis and individual Somatic Cell Count in heifers. *Journal of Animal and Veterinary Advances* 9(8): 1245-1249.

Chalmers JM, Howell GE, Hargreaves MD (2012) Infrared and Raman spectroscopy in forensic science. 1st pub. Chichester, West Sussex, UK: Wiley.

Darbaz I, Ergene O (2015) Sürü Meme Sağlığı Yönetiminde Somatik Hücre Sayısının Önemi. Erciyes Üniv Vet Fak Derg 12(3): 203-210.

El-Abassy RM, Donfack P, Materny A (2009) Rapid Determination of Free Fatty Acid in Extra Virgin Olive Oil by Raman Spectroscopy and Multivariate Analysis. *J Am Oil Chem Soc* 86: 507-511.

El-Abassy RM, Donfack P, Materny A (2010) Assessment of conventional and microwave heating induced degradation of carotenoids in olive oil by VIS Raman spectroscopy and classical methods. *Food Res Int* 43: 694-700.

El-Abassy RM, Eravuchira PJ, Donfack P, Von der Kammer B, Materny A (2011) Fast determination of milk fat content using Raman spectroscopy. *Vibrational Spectroscopy* 56: 3-8.

Heise HM, Damm U, Lampen P, Davies AN, McIntyre PS (2005) Spectral variable selection for partial least squares calibration applied to authentication and quantification of extra virgin olive oils using Fourier transform Raman spectroscopy. *Appl Spectrosc* 59(10): 1286-94.

Júnior RPH, de Sá Oliveira K, de Almeida CER, De Oliveira LFC, Stephani R, Pinto Mda S, de Carvalho AF, Perrone IT (2016) FT-Raman and chemometric tools for rapid determination of quality parameters in milk powder: Classification of samples for the presence of lactose and fraud detection by addition of maltodextrin. *Food Chem* 1(196): 584-8.

Lee PC, Meisel D (1982) Adsorption and Surface-Enhanced Raman of Dyes on Silver and Gold Sols. *J Phys Chem* 86: 3391-3395.

Leitner G, Shoshani E, Krifucks O, Chaffer M, Saran A (2000) Milk Leucocyte Population Patterns in Bovine Udder Infection of Different Aetiology. *J Vet Med B* 47: 581-589.

Li M, Chen J, Xu J, Fu S, Gong H (2015) Determination of Lactose in Milk by Raman Spectroscopy. *Analytical Letters* 48: 1333-1340.

Li-Chan ECY (2007) Vibrational spectroscopy applied to the study of milk proteins. *Lait* 87: 443-458.

Månnsson HL (2008) Fatty acids in bovine milk fat. *Food Nutr Res* 52:10.

Mazurek S, Szostak R, Czaja T, Zachwieja A (2015) Analysis of milk by FT-Raman spectroscopy. *Talanta* 138: 285-289.

McGoverin CM, Clark AS, Holroyd SE, Gordon KC (2010) Raman spectroscopic quantification of milk powder constituents. *Analytica Chimica Acta* 673(1): 26-32.

Mendes TO, Junqueira GMA, Porto BLS., Brito CD, Sato F, de Oliveira MAL, Anjos V, Bell MJV (2016) Vibrational spectroscopy for milk fat quantification: line shape analysis of the Raman and infrared spectra. *Raman Spectrosc* 47: 692-698.

Muik B, Lendl B, Molina-Díaz A, Ayora-Canada MJ (2003) Direct, reagent-free determination of free fatty acid content in olive oil and olives by Fourier transform Raman spectrometry. *Anal Chim Acta* 487: 211-220.

Ozbey G, Cambay Z, Yilmaz S, Aytekin O, Zigo F, Ozçelik M, Otu B (2022) Identification of bacterial species in milk by MALDI-TOF and assessment of some oxidant-antioxidant parameters in blood and milk from cows with different health status of the udder. *Pol J Vet Sci* 25(2): 269-277.

Pecka-Kiełb E, Vasil M, Zachwieja A, Zawadzki W, Elećko J, Zigo F, Illek J, Farkašová Z (2016) An effect of mammary gland infection caused by *Streptococcus uberis* on composition and physicochemical changes of cows' milk. *Pol J Vet Sci* 19(1): 49-55.

Petersen M, Yu Z, Lu X (2021) Application of Raman Spectroscopic Methods in Food Safety: A Review. *Biosensors* 11(6): 187.

Philpot WN, Nickerson SC (2000) Winning The Fight Against Mastitis. Naperville, USA: Westfalia Surge 28-40.

Pyörälä S, Taponen S (2009) Coagulase-negative staphylococci—Emerging mastitis pathogens. *Veterinary Microbiology* 134 (1-2): 3-8.

Quinn PJ, Carter ME, Markey BK, Carter GR (1994) *Clinical Veterinary Microbiology*. Mosby-Year Book Europe Limited, Lynton House, London WC1H9LB, England. 209-236.

Schultz R, Smith PJ, Hogan KL, Love JS (2004) Antimicrobial susceptibility of mastitis pathogens from first lactation and older cows: *Veterinary Microbiology* 102: 33-42.

Sharma N (2007) Alternative Approach to Control Intramammary Infection in Dairy Cows: A Review. *Asian Journal of Animal and Veterinary Advances* 2(2): 50-62.

Vaskova H, Buckova M, Zalesakova L (2016) Spectroscopic analysis of milk fat and its mathematical evaluation. *International Journal of Biology and Biomedical Engineering* 10.

Yağcı S, Göğüş F (2008) Response surface methodology for evaluation of physical and functional properties of extruded snack foods developed from food-by-products. *Journal of Food Engineering* 86 (1): 122-132

Yang H, Irudayaraj J (2001) Comparison of near-infrared, fourier transform-infrared, and fourier transform-raman methods for determining olive pomace oil adulteration in extra virgin olive oil. *J Am Oil Chem Soc* 78: 889.

Yang H, Irudayaraj J, Paradkar MM (2005) Discriminant analysis of edible oils and fats by FTIR, FT-NIR and FT-Raman spectroscopy. *Food Chem* 93: 25-32.

Zigo F, Elečko J, Farkašová Z, Zigoová M, Vasil M, Ondrašovičová S, Lenka K (2019) Preventive Methods in Reduction of Mastitis Pathogens in Dairy Cows. *J Microbiol Biotech Food Sci* 9 (1): 121-126.

Zigo F, Farkasová Z, Elecko J, Lapin M, Chripková M, Czerski A (2014) Effect of parenteral administration of selenium and vitamin E on health status of mammary gland and on selected antioxidant indexes in blood of dairy cows. *Pol J Vet Sci* 17(2): 217-23.

Hisira V, Zigo F, Kadaši M, Klein R, Farkašová Z, Vargová M, Mudroň P (2023) Comparative Analysis of Methods for Somatic Cell Counting in Cow's Milk and Relationship between Somatic Cell Count and Occurrence of Intramammary Bacteria. *Vet Sci* 10(7): 468.