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Scattering and fluorescence spectra of cow milk

T.L. Dimitrova[1*], T.A. Eftimov[1], V.G. Kabadzhov[1], P.T. Panayotov[2], P.B. Boyanova[2]

[1]*Plovdiv University "Paisii Hilendarski", 24 Tzar Assen str., 4000 Plovdiv, Bulgaria*

[2]*University of Food Technologies (UFT), 26 Maritsa Blvd, 4000 Plovdiv, Bulgaria*

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The purpose of this study is to investigate the influence of fat content on the scattering and fluorescence spectra of milk. The composition analyses (content of proteins, fat, carbohydrates and minerals, caloricity), are obtained by Ekomilk-M Milkana KAM 98-2A milk analyzer. For all studied samples we have found out well expressed fluorescence pikes around of 335 nm and around of 373 nm what may be attributed to the presence of aromatic amino acids, nucleic acids and tryptophan residues. One of the samples has shown weak peak around of 500 nm probably due to the riboflavin fluorescence. Plots of the ratio: fluorescence intensity toward scattering intensity as a function of the pumping wavelength, have shown certain maxima with different values for samples of different fat content and different producers. The experimental results suggest that fluorescence and scattering spectra of milk can be used for the identification of different producers of milk and for obtaining information about milk chemical composition.

Keywords: Food control, milk, optical scattering, fluorescence

INTRODUCTION

Production of milk and milk derivates directly depends on the raw milk quality which is defined by the European Committee for Standardization (CEN) in EN ISO 8420:2005 [1]. The large variety of milk based nutrition is rapidly increasing and, in the same time, its components consciously get replaced by improper constituents. For example, milk fat gets replaced by vegetable fat, milk proteins - by other kinds of proteins, and carbohydrates - by certain products improving the texture and test qualities. In accordance with that, rises the necessity to find out adequate methods and equipment for quality control of milk products.

The classical microbiological and chemical analysis of milk and its derivates gives objective, precise and comparable results. However, it requests highly qualified specialists and needs expansive consumables. On the other side, these kinds of analyses are time consuming, and, most often, they use destructive methods. The use of optical devices is an alternative based on different non-destructive physical principles. Food and agriculture industries use mainly optical instruments in the ultraviolet (185-210 nm) and near infrared range (750-2500 nm) where typical chemical groups present in nutrients (C-H, N-H and O-H) get absorbed. In this study we have combined in an experimental setup measurement of transmission, scattering and fluorescence spectra of milk in the optical range of 200-1100 nm.

During the last decades optical transmission and scattering in the Far Ultraviolet (FUV) and Middle Ultraviolet (MUV) range (185-210 nm) and in the Near Infrared (NIR) range (750-2500 nm) have been largely applied in cases where the typical C-H, N-H and O-H chemical groups contained in food products show absorption. Due to the high water absorption, Infrared (IR) range is not appropriate for studying samples with high content of an advantage of optical transmission and scattering in comparison with the others spectral methods is that there is no need for preliminary chemical treatment, dilution or components separation. This permits the use of the same samples for further analysis. Because of different spectral absorption of the main milk components (water, fat, proteins and sugar), they can be studied contemporaneously using Near Inftared Spectroscopy (NIRS) [2].

Milk fat is composed of about 96% triglycerides (1 molecule glycerin and three fatty acids), 2-3% of diglycerides, 1% of phospholipids, essential polyunsaturated fatty acids such as Linoleic acid (LA) and Linolenic acid, as well fat-soluble vitamins (A, B, D, E and K), cholesterol, carotenoids [3]. The other contained molecules are smaller than the wavelength in the visible and near infrared range. When the fat is removed from the milk, the main particles that scatter the shorter wavelength light are the casein micelles and this causes the milk bluish colour. Casein micelles are

* To whom all correspondence should be sent.
E-mail: tldimitrova@abv.bg

the biggest particles in the milk liquid phase. They represent protein molecules linked through calcium phosphate nanoparticles. Casein micelles have spherical form. The size distribution of casein micelles is very broad (20–250 nm in diameter) [4]. There are four different kinds of casein phosphoproteins (αS1, αS2, β, κ) taking almost 76-86% of all milk proteins [2,5]. The biggest part of the casein proteins are linked in micelles. Milk contains also some other kinds of proteins and enzymes, which have higher solubility and smaller sizes than the casein molecules.

Light scattering effects are significant due to the milk turbidity. Therefore, Mie scattering theory may be applied [6]. Thus, the scattering cross section $\sigma_g = \pi R^2$ (where R is the scattering particle radius) is maximal at smaller wavelength. That allows evaluating the protein molecules sizes according to the formula:

$$\lambda_p = 2\pi R, \quad (1)$$

where λ_p is the wavelength at the pick scattered intensity.

Another non-destructive technique giving additional information for milk composition is fluorescence. However, data interpretation of fluorescence spectra of milk is ambiguous due to absorption by other molecular groups [3]. During the manufacturing process of milk occur biochemical reactions, for example: Maillard reaction, riboflavin degradation, tryptophan modification. For this reason, we consider more appropriate the use of comparative studies by changing one only parameter of the sample at time.

EXPERIMENTAL

In this study we have combined in an experimental setup measurement of transmission, scattering and fluorescence spectra of milk [7,8]. Milk is turbid liquid with significant optical absorption what makes difficult the use of standard spectrophotometer cuvettes. For this reason we have opted for a fiber optic setup. The optical range of 200-1100 nm is covered by two light sources: a halogen and a deuterium lamp. Light is directed through the sample (milk drop) placed on an aluminum base via optical fiber. The transmitted light is conducted by another fiber to the spectrometer AvaSpec 2048 with resolution of 8 nm. The two fibers are fixed on the board at a distance of 200 μm, equal to the fiber core diameter. The scattered light is caught by a third fiber along the direction orthogonal to the transmitted light. In this case the fluorescence spectra, even less intensive because of the low incident light intensity, cannot be separated from the scattered spectra. For evaluation of the fluorescence phenomena we have used a number of narrow waveband light sources UVTOP deep ultraviolet LEDs by Roithner Lasertechnik GmbH [9] with the next wavelengths: 245 nm, 255 nm, 275 nm, 285 nm, 295 nm, 305 nm.

Here we report some experimental data on the influence of fat contents on the scattering and fluorescence spectra of milk. We have chosen three different kinds of commercial cow milk labeled as Sample1, Sample2 and Sample3, and from each of them - samples with of 0.1%, 1.5% and 3.0% fat content. The commercial marks are not named here with the aim to avoid conflict of interest with the producers even more, for our study is important to demonstrate that the fluorescence provoked in the chosen optical range is able to give useful information for distinguishing the present of fat independently of the milk origin. The composition analysis (content of proteins, fat, carbohydrates and minerals, caloricity), has been obtained by Ekomilk-M Milkana KAM 98-2A, Bulteh 2000 Ltd, Stara Zagora, Bulgaria. Some more important parameters are presented in Table1.

Table 1. Composition analysis of milk with different content of fat.

Samples	Milk fat %	Milk-solids-nonfat (MSNF) %	Protein %	Density kg/dm^3
1	0.1	8.02	3.00	1.0295
2	1.5	8.43	3.16	1.0298
3	3.0	7.93	3.00	1.0265

RESULTS AND DISCUSSION

Turbidimetry is based on measuring the loss of intensity of the transmitted light due to scattering from the suspended in the studied medium particles. In this sense, the fiber-optical setup described above can be used for evaluating the absorption properties of milk. In Figure 1a are presented transmission spectra of Samples1 with fat content respectively of 0.1%, 1.5% and 3.0%. In the optical range of 200-1100 nm and in Figure 1b are shown the corresponding absorption curves calculated in the ideal case of lack of scattering light. The well expressed absorption maxima may be explained by Mie scattering from spherical particles with sizes ~ 0.1λ where λ is the wavelength of the absorbed light.

Scattering particles sizes are evaluated by defining the wavelength at the peak of the

absorption intensity of Sample1 with 0.1% of fat (oiliness milk). The experimental value, obtained

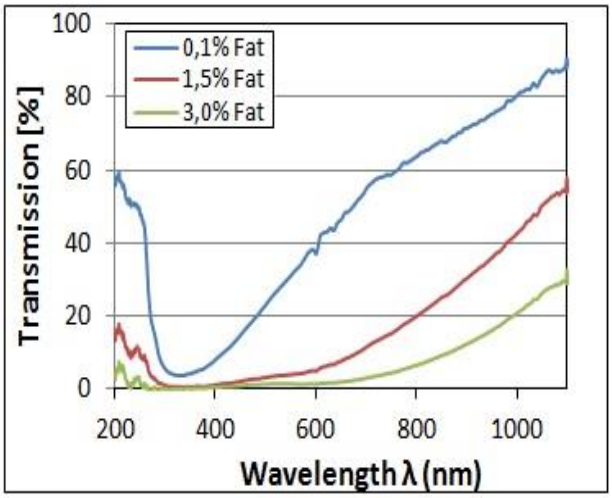

Fig. 1a. Transmission spectra of milk Samples 1 with 0.1%, 1.5% and 3.0% fat content.

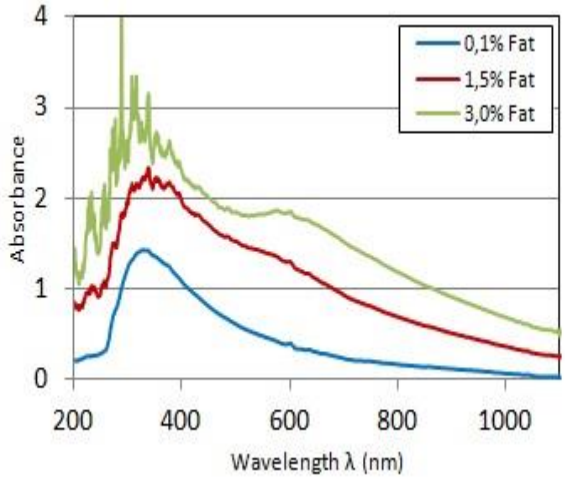

Fig. 1b. Absorption spectra of the same samples.

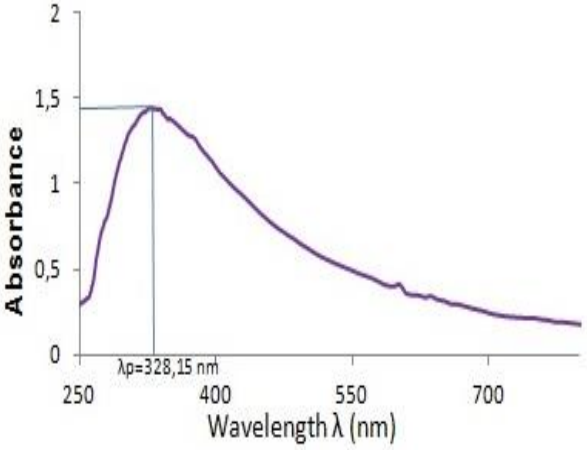

Fig. 2 Absorption spectra of milk Sample 1 with 0,1% of fat (oiliness milk).

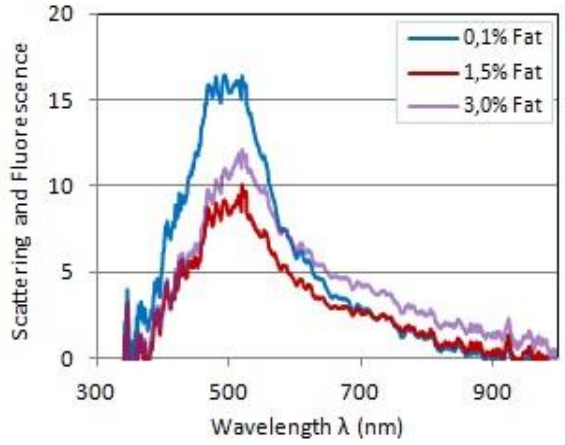

Fig. 3. Scattering and fluorescence spectra of milk Samples1 with 0,1%, 1,5% and 3,0% fat content.

from Fig. 2 is $\lambda_p \approx 328$ nm. The approximate diameter $d = 2R$ of the scattering particles obtained by substitution of this value in formula (1) is:

$$2R = \frac{\lambda_p}{\pi} = \frac{328}{3{,}14} \approx 104nm \qquad (2)$$

With reference to the literature [6] we suppose that the experimental value may be attributed to the scattering from casein micelles. As the protein content is the same for all samples, represented in Figure 1, one can consider that the increase of the absorption with the fat content is due only to its quantity and, eventually, may be used for evaluating the fat percentage in milk. The fat content influences also the scattered light, as it can be seen from Figure 3 where the spectra of scattered light for milk Samples1 (0.1%, 1.5% and 3.0% of fat) are presented.

However, considering the multi-component structure of milk and taking in account the fact that the studied optical region is not well explored for analysis of this nutrient, it's hard to claim obtaining precise information about its composition and structure. Despite of that, this study is promising when looking for simplicity, low price and easy practical control of one component (for example fat or protein content).

In Fig. 4a the fluorescence spectra of milk Sample1 with 15 % of fat content are presented. As expected, different pumping wavelengths provoke different emission of specific fluorescence spectra. For all studied samples we find out well expressed fluorescence pikes around of 335 nm and around of 373 nm which may be attributed to the presence of aromatic amino acids, nucleic acids and tryptophan residues. Sample1 has shown weak peak around of 500 nm probably due to the riboflavin fluorescence [10,11]. One can notice that the quantum efficiency is higher when exiting with LEDs emitting at 275 nm, 285 nm and 305 nm.

Plots of the ratio fluorescence intensity toward scattering intensity I_F/I at 375 nm pumping wavelength for milk Samples1 with fat contents of 0.%, 1.5% and 3.0% are presented in Figure 4b.

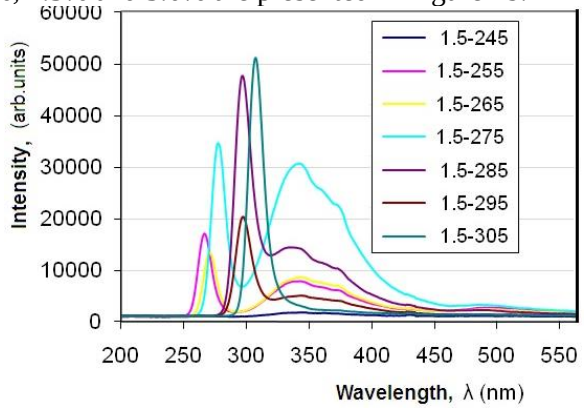

Fig. 4a) Fluorescence spectra of milk Samples1 with 1.5% fat content.

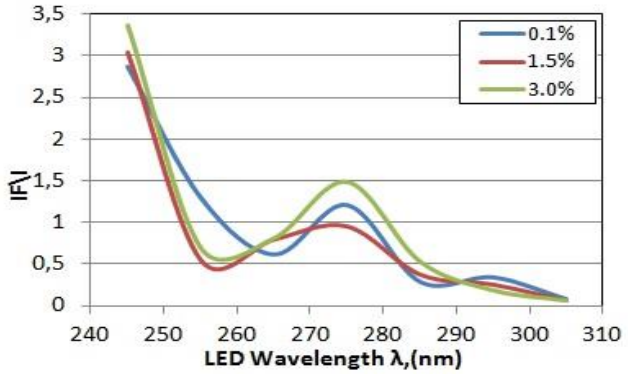

Fig. 4b) Plots of the ratio fluorescence intensity toward scattering intensity at 375 nm pumping wavelength for Sample1 with fat content of 0.1%, 1.5% and 3.0% are presented in Figure 2b.

The results for Samples2 and Samples3 (not presented here) are similar. The ratio I_F/I is highest for excitation at 245 nm. There are maxima of smaller values for 275 nm and for 295 nm excitation. For Samples 2 and Samples 3 the second maximum is at excitation wavelenght of 265 nm. The values of the maxima are different for the samples with different fat content.

CONCLUSIONS

In this work are presented some experimental results concerning the influence of fat content in milk on the scattering and fluorescence spectra in the optical range from 200 nm to 1100 nm. A compact fiber-optic setup and a spectrometer AvaSpec 2048 with resolution of 8 nm are used. The sizes of the scattering particles evaluated from the absorption pick correspond to the sizes of the casein micelles retrieved from the literature. The experimental results show that the intensity of the scattering spectra changes with the fat content. Fluorescence spectra have well expressed picks with maximum intensity at pumping wavelengths of 275 nm, 285 nm and 305 nm. The ration fluorescence intensity toward scattering intensity is also influenced from the fat content. The obtained experimental results suggest that fluorescence and scattering spectra for excitation of milk with ultraviolet light can be used for identification of different production of milk and for obtaining information about its chemical composition.

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REFERENCES

1. EN ISO 8420:2005, Animal and vegetable fats and oils - Determination of content of polar compounds (ISO 8420:2002).
2. L. Bokobza, *J. Near Infrared Spec*. **6**, 3 (1998).
3. C.M. Andersen, G. Mortensen, *J. Agric. Food Chem*. **56**, 720 (2008).
4. G.A. Morris, T.J. Foster, S.E. Harding, *Biomacromolecules*, **1**, 764 (2000).
5. Y.W. Park, Georgia Small Ruminant Research & Extension Center, Fort Valley State University and Adjunct Professor Department of Food Science & Technology University of Georgia.
6. D.W. Hahn, Light Scattering Theory, Department of Mechanical and Aerospace Engineering, University of Florida.
7. T.L. Dimitrova et al., Study of the presence of vegetable fat in cow milk by optical transmission and scattering, 7th Radionica Fotonike Conf., Kopaonik, Serbia, 10–14 March 2014, Book of abstracts, p. 42 (2014).
8. T. L. Dimitrova et al., Distinguishing of animal fat from vegetable fat in cow milk by optical transmission and scattering, 5th International Conference on Radiation interaction with materials: fundamentals and applications on 12-15 May, 2014, Kaunas, Lithuania, Conference Proc.eedings, P2-11, p.242-245 (2014).
9. Roithner Lasertechnik GmbH, http://www.roithner-laser.com/led.html
10. R. Karoui, B. Martin, É. Dufour, *Lait*, **85**, 223 (2005).
11. M. Hammami, S. Dridi, F. Zaidi, O. Maâmouri, H. Rouissi, C. Blecker, R. Karoui, *Int. J. Food Prop*., **16**, 1322 (2013).

СПЕКТРИ НА РАЗСЕЙВАНЕ И ФЛУОРЕСЦЕНЦИЯ НА КРАВЕ МЛЯКО

Т.Л. Димитрова[1*], Т.А. Ефтимов[1] Т А, В.Г. Кабаджов[1], П.Т. Панайотов[2], П.Б. Боянова[2]

[1]Пловдивски Университет „Паисий Хилендарски“, ул. „Цар Асен“ 24, 4000, Пловдив, България.
[2]Университет по хранителни технологии (УХТ), бул. „Марица“ 26, 4000 Пловдив, България.

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(Резюме)

Целта на това проучване е да се изследва влиянието на съдържанието на мазнини върху спектрите на разсейване и флуоресценция на краве мляко. Анализът на състава (съдържание на протеини, мазнини, въглехидрати и минерали, както и калоричност), са получени с помощта на млекоанализатор Екомилк-М Милкана КАМ 98-2А. За всички изследвани проби са установени добре изразени флуоресцентни пикове около дължини на вълните 335 nm и 373 nm, което може да се дължи на присъствието на ароматни аминокиселини, нуклеинови киселини и триптофанови остатъци. Една от пробите показва слаб пик около 500 nm, което вероятно се дължи на флуоресценцията на рибофлавин. На графиките, показващи отношението на интензитета на флуоресценция към интензитета на разсейване като функция от дължината на вълната на възбуждане, се наблюдават максимуми за млечни проби от отделните производители, съдържащи мазнини с различна концентрация. Експерименталните резултати показват, че спектрите на флуоресценция и разсейване на млякото могат да бъдат използвани получаване на информация за химичния състав на млякото, както и за идентифициране на производителя.