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Photoluminescence Spectral Patterns and Parameters of Milk While Souring

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Abstract: For the efficient production and processing of milk, it is important to control its quality indicators. Optical spectroscopy, in combination with statistical analysis methods, can be a useful method of evaluation due to its speed, non-invasiveness, and relative cheapness. This investigation is aimed at studying of the interrelations of the spectral patterns, the absorption parameters, and the photoluminescence values of cow's milk during its souring. The spectral characteristics of excitation and photoluminescence were measured on a diffraction spectrofluorometer in the range of 200–500 nm. For establishing an effective control procedure during milk souring, the most informative method is found to be the use of the excitation wavelengths of 232 nm, 322 nm, 385 nm and 442 nm. These ranges correspond to the amino acids of milk proteins, the fatty acids of milk fat, and the aromatic fragments of vitamins. When using the photoluminescence flux ratios Φ_{232}/Φ_{322} and Φ_{385}/Φ_{442}, linearly approximated dependences on acidity can be obtained with determination coefficients of 0.88–0.94. The proposed photoluminescent method can be used as a non-destructive and fast-acting tool for monitoring the properties of milk during fermentation, as well as for the subsequent creation of a portable and inexpensive sensor based on this method.

Keywords: cow's milk; souring; photoluminescent analysis; UV-Vis range; linear regression models

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1. Introduction

According to the International Dairy Federation (IDF), the medical norm for the consumption of dairy products should be 392 kg per person per year, of which whole milk should equal 116 kg. The top countries for milk consumption per capita per year are as follows: 1. Australia—97.2 kg/person, 2. USA—67.8 kg/person, 3. European Union—64.8 kg/person, 4. Russia—57.6 kg/person, and 5. India—48.3 kg/person.

Milk is an emulsion of fat globules and casein micelles dispersed in an aqueous medium in which lactose, whey proteins, and some minerals are dissolved. An accurate quantification of milk components is important at various stages of the production/processing of the dairy products for proper technological control and quality assurance. There are four categories of inspection at the processing plant: visual, organoleptic, chemical, and microbiological.

The following conditions are important for efficient milk production and processing: the quality monitoring of the products obtained; the control and operability of the technological equipment; the control/management of the technological processes of milking and

primary milk processing; and the optimization of energy and other economic costs during cow milking and primary/advanced milk processing [1,2].

The above-listed tasks can be achieved by the use of the data obtained as a result of an accurate milk quality indicator measurement.

Modern industrial milk processing is a complex activity involving consistently performed interrelated chemical, physicochemical, microbiological, biochemical, biotechnological, thermophysical, and other labor-intensive and specific technological processes., Monitoring the serviceability of technological equipment for milking, primary processing, and final processing of milk, as well the management of these technological processes with the optimization of energy and economic costs, is especially relevant in the field of product quality monitoring.

According to the traditional technological scheme (Figure 1) for the production of milk and dairy products, milk quality control is provided in the production area at the dairy plant, i.e., positions 6 and 9. At the same time, as a rule, in the farm area during milking operations and primary milk processing, milk quality parameters are not monitored due to the lack of an instrument base. However, farmers are very interested in controlling milk quality parameters in positions 2 and 4 (Figure 1).

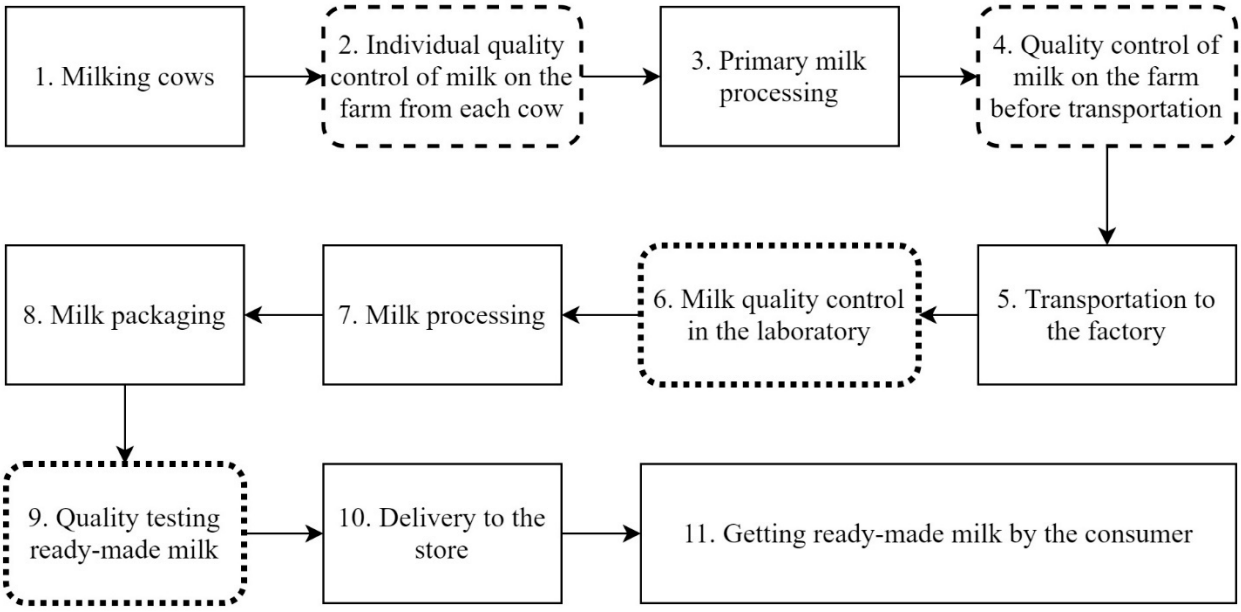

Figure 1. Traditional technological scheme of milk and dairy product production with control points for measuring milk quality indicators: 6, 9—existing measurement points; 2, 4—measurement points proposed by the authors of the article.

Based on the results of the analysis of the literature references, the following classification of the instrumental methods for the measurement of the physical/chemical properties of milk is proposed (Table 1):

Table 1. Classification of methods for studying the physical/chemical parameters of milk.

Physical/Chemical Parameter of Milk	Applied Measurement Method
1. Fat	1.1. Nephelometry 1.2. Turbidimetry 1.3. Fluorimetry 1.4. Infrared spectroscopy 1.5. Ultrasonic examination 1.6. Infrared spectrophotometry

Table 1. *Cont.*

Physical/Chemical Parameter of Milk	Applied Measurement Method
2. Protein	2.1. Fluorimetry 2.2. Infrared spectroscopy 2.3. Colorimetry 2.4. Refractometry 2.5. Titrimetry 2.6. Infrared spectrophotometry
3. Milk solids non-fat (MSNF)	3.1. Infrared spectroscopy 3.2. Refractometry 3.3. Ultrasonic examination 3.4. Infrared spectrophotoscopy
4. Water content	4.1. Infrared spectroscopy 4.2. Infrared spectrophotoscopy 4.3. Dielmetry 4.4. Thermogravimetry 4.5. Viscosimetry 4.6. Density measurement
5. Acidity	5.1. Titrometry 5.2. Conductometry 5.3. Ionometry/Direct potentiometry (pH) 5.4. Scatterometry/Reflectometry 5.5. Spectrofluorimetry
6. Milk naturality	6.1. Ionometry (pNa, pNH_4) 6.2. Cryoscopy
7. Viscosity and consistency/texture	7.1. Rotational method 7.2. Capillary method 7.3. Vibration method 7.4. Penetration method
8. Bacterial contamination	8.1. Colonies counting 8.2. Microorganisms counting 8.3. Lactate estimation 8.4. Bioluminescence
9. Microorganisms concentration	9.1. Colonies counting 9.2. Bioluminescence 9.3. Turbidimetry
10. Number of somatic cells	10.1. Conductometry 10.2. Viscosimetry 10.3. Ionometry (pCl) 10.4. Fluorimetry

Optical spectroscopy, combined with the statistical analysis methods, can be a useful evaluation method, as it is fast, non-destructive, and cheap as compared to any chemometric methods.

Currently, among various optical methods for the product quality estimation of milk and dairy products, near-infrared reflective spectroscopy has become the most widespread [3]. The infrared spectra of milk have been studied for changes detected during milk coagulation [4], the presence of impurities [5], fat and protein amount estimation [6], etc. Near-IR spectroscopy methods are used for analyzing milk powder [7], goat's milk [8], buttermilk/yogurt [9], cheese [10], melted butter (ghee) [11], and ice cream [12]. For the implementation of these methods, a number of milk detectors-analyzers have been developed based on the reflected radiation. These include the following: a detector based on a multispectral sensor covering 18 wavelengths [13]; a detector (with a fiber-optic probe) based on a microspectrometer in the range of 650–1100 nm [14], and a transmission analyzer in the range of 960–1690 nm [15].

Much less attention has been paid to the spectroscopy of the visible and ultraviolet ranges. This method is known for detecting melamine in milk when the photoluminescence in the milk is excited by the radiation wavelength of 457 nm and registered by the luminescence at the wavelength of 540 nm [16]. Another method was proposed for the water content estimation in milk when visible radiation was transmitted [17]. The light scattering and transmission by milk was analyzed using visualization [18]. The analysis sizes and refractive indices of the scattering components in the visible [19] and ultraviolet ranges was carried out. In the study [20], a multiple linear regression model was used to predict the percentage of non-concentrated and concentrated milk fat, pH, viscosity, density, and moisture content, depending on the color components (L*, a*, and b*).

The luminescent analysis in the ultraviolet and visible ranges is effectively used in order to estimate and control the dry bulk food product parameters [21,22], including the fats in sheep's, goat's, and cow's milk [23], as well as the quality of yogurt and ice cream [24]. This method is highly accurate, non-contact, non-destructive, and does not require any expensive equipment for its final practical introduction.

The world dairy market is mainly dominated by devices operating in the infrared range, while a smaller proportion utilized ultrasound diagnostics, which are more affordable and possess many analogues. Devices based on the principle of fluorescence have also become widespread.

The operation of the Milkotronic Lactoscan SCC analyzer is based on the method of fluorescent microscopic cell counting. Thanks to the fluorescent dye, LED optics, and CCD technology for creating images, this method of analysis of somatic cells in milk is accurate, reliable, and fast, and the method of fluorescence microscopy is recognized as effectual.

The DeLaval DCC device is an analyzer of somatic cells in milk. DCCs are designed to measure the counting concentration of somatic cells in raw cow's milk. The principle of operation of the analyzers is based on the automatic counting of somatic cells labeled with fluorescent dye in the analyzed milk sample, which fluoresce when they are irradiated with ultraviolet radiation.

In this paper, we investigate the feasibility of the optical methods using some milk parameter estimation as the milk sours; upon the souring of the milk, the previously conducted work was taken into account [25].

When milk sours, several processes occur. The effect of lactobacilli on milk protein molecules is that long protein molecules break down into shorter ones. The fermentation of milk sugar–lactose with the formation of lactic acid increases the acidity of milk and gives a characteristic sour taste to the milk and fermented milk products.

This study is especially relevant in the field of the product quality monitoring, as it monitors the serviceability of technological equipment used for milking cows and nursing animals. It also monitors the primary processing and final processing of milk, as well as in the management of these technological processes with the optimization of energy and economic costs. For example:

1. According to the recommendations, it is better to milk cows with high-acidity milk in a strictly defined range.
2. On a dairy farm, it is necessary to monitor the serviceability of the technological equipment (refrigeration equipment) and its management.
3. On small dairy farms, it is often problematic to maintain and not exceed the normative permissible indicators of milk quality after the evening milking until morning (in order to sell it on the market) while incurring minimal energy and economic costs without the use of refrigeration equipment.
4. In the production of various products (kefir, butter, etc.) based on milk processing, it is required to control the milk's acidity at different stages of the technological process.

The titrated acidity (in Turner degrees) does not correspond to the fully active acidity, as it indicates a pH shift from 6.3 to 8.5, and does not show the presence of any alkalis. Thus, fresh milk can have high titratable and low active acidity. This is due to the fact that the pH does not change for a certain period of time due to the buffering properties of milk

(the content of proteins, phosphates, nitrites). When a certain amount of alkali is added to the milk, the pH will remain the same, and the titrated acidity changes. The change in active acidity occurs only when the acid and amide groups of the amino acids of proteins are neutralized.

If the milk has just been milked, it has an acidity index of 16–18 °T. The acidity increases after two hours, if the milk has not been cooled. As the microorganisms develop, the fermentation process takes place, and the acidity also increases.

An increase in acidity causes proteins to become less resistant to heating, so milk with an acidity of 21 °T is unsellable, and with an acidity of 22 °T, it is already on the verge of souring and is not subject to delivery to dairies. Titrated acidity is one of the criteria for determining the milk grade. For the highest grade, the acidity should be 16–17 °T, for the first grade—no more than 19 °T, and for the second grade—no more than 20 °T.

This investigation is aimed at studying of the interrelationships of the spectral patterns, the absorption parameters, and the photoluminescence values of cow's milk during its souring.

2. Materials and Methods

2.1. Milk Used

Pasteurized cow's milk produced at a local agricultural enterprise was used for this study. According to the manufacturer, the fat content of at least 3.2% was required. The initial parameters of the milk were measured using the device ExpertProfi (Russia), revealing that the proportion of fat was 3.55%, and the proportion of protein was 3.0%. The titratable acidity was 17 °T. During the research, milk samples were stored in a darkened space at a temperature of about 20 °C. Before each measurement, the milk was checked for souring using the organoleptic method, and then mixed.

The milk souring was carried out in 50 mL containers in summer at a natural ambient temperature.

2.2. Acidity Control

The acidity of the milk under study was determined by a titrimetric method based on the neutralization of the acids contained in the product using a NaOH solution in the presence of a phenolphthalein indicator.

For each experiment, a standard solution containing 10 mL of milk, 20 mL of distilled water, and 1 mL of $CoSO_4$ solution is prepared in a 100 mL flask. The ingredients must be thoroughly homogenized. Then, at the analysis stage, 10 mL of milk, 20 mL of distilled water, and 3 drops of phenolphthalein are poured into a flask of the same volume (100 mL). The components are then thoroughly mixed. The resulting mixture is titrated with a NaOH solution until a light pink staining appears; in the case of milk, in accordance with the reference color standard, this light pink tincture should not disappear until at least 1 min had passed. The acidity of the solution in Turner degrees (°T) is determined by multiplying the coefficient 10 by the volume (in mL) of the NaOH solution consumed by neutralizing the acids contained in a certain volume of the product.

In this method, the permissible absolute error of the measurement result is ±1.9 °T, with an assumed confidence probability of $p = 0.95$ and three-fold repetition.

2.3. Equipment and Procedure of Spectral Measurements

The spectral characteristics of excitation and photoluminescence were measured in this experiment using a Fluorat-02-Panorama (Russia) spectrofluorometer using the built-in Panorama-Pro program. The light-optical system of the spectrofluorometer consists of a xenon lamp operating in pulsed mode, along with a photomultiplier as a photodetector with a pulse duration of (1.5 μs) and a frequency of 25 Hertz. A monochromator with a Cherni-Turner diffraction system was used for spectral scanning.

During the studies, the excitation spectra of η_e (λ) were measured. The spectral range was 200–500 nm. The method is described in more detail in the reference [21]. The

photomultiplier supply voltage was set to an average level; the adjustment interval of the monochromators was set to 1.0 nm, and the results were averaged over 10 flashes of the radiation source. First, a search (synchronous) scan was performed, in which the excitation and luminescence monochromators were rebuilt in parallel with a delay of 60 nm.

Based on the results of the measurements of the η_e (λ) spectra, their maxima were determined, to which the excitation monochromator was tuned, after which the photoluminescence spectrum φ_l (λ) was measured. Subsequently, the obtained characteristics were processed in the PanoramaPro software package.

The measurements of all spectra showed sevenfold repeatability.

2.4. Spectrum Parameters Calculation

For the obtained spectral characteristics, the integral absorption capacity H was determined by the formula:

$$H = \int_{\lambda_1}^{\lambda_2} \eta_e(\lambda) d\lambda, \quad (1)$$

where η_e (λ) is the dependence of the absorption energy efficiency on the wavelength, and λ_1–λ_2 are the boundary wavelengths of the absorption range.

Integrals of spectral characteristics φ_l (λ), which have a physical equivalent of luminescent fluxes Φ, were calculated by the formula:

$$\Phi = \int_{\lambda_1}^{\lambda_2} \varphi_l(\lambda) d\lambda, \quad (2)$$

where φ_l (λ) is the dependence of the radiation flux density on the wavelength, and λ_1–λ_2 are the boundary wavelengths of the radiation range.

The error of the calculated parameters for a sevenfold repetition did not exceed 5%.

3. Results and Discussion

On the first day of the experiment, two measurement sessions of the excitation spectra η_e(λ) were carried out at synchronic scanning (the first measurement at 0 h after pasteurization; the second measurement in 6 h). For each measurement, 7 containers of 50 mL each, or a total of 350 mL of pasteurized milk obtained from the farm, were used. The next day, the organoleptic control showed that the milk was not yet sour. After preliminary stirring, the measurements η_e (λ) were performed again, marking the third measurement session; this measurement was performed 21 h after the start of the souring process (after the package opening). On the third day, the milk became sour. With the souring of the milk, the excitation spectrum changed quantitatively, but not yet qualitatively (judging from the curve peaks). The fourth measurement session was performed 45 h and the fifth session 54 h, respectively, after the start of souring. The results of the measurements of the milk parameters are shown in Table 2, and the excitation spectra are shown in Figure 2.

The fat content in milk scarcely changes over time. The content of proteins and SNF increases slightly. The dependence of acidity on the souring time is approximated by a linear function, with a determination coefficient R^2 = 0.985.

Table 2. Milk parameters during souring.

t, h	Fat, %	Solids Nonfat (SNF), %	Protein, %	Acidity, °T
0	3.55 ± 0.17	8.20 ± 0.16	3.00 ± 0.06	17
6	3.57 ± 0.08	8.16 ± 0.05	2.99 ± 0.02	18
21	3.50 ± 0.11	8.28 ± 0.04	3.03 ± 0.01	26
45	3.53 ± 0.10	8.74 ± 0.01	3.20 ± 0.01	36
54	3.63 ± 0.12	8.67 ± 0.02	3.18 ± 0.01	37

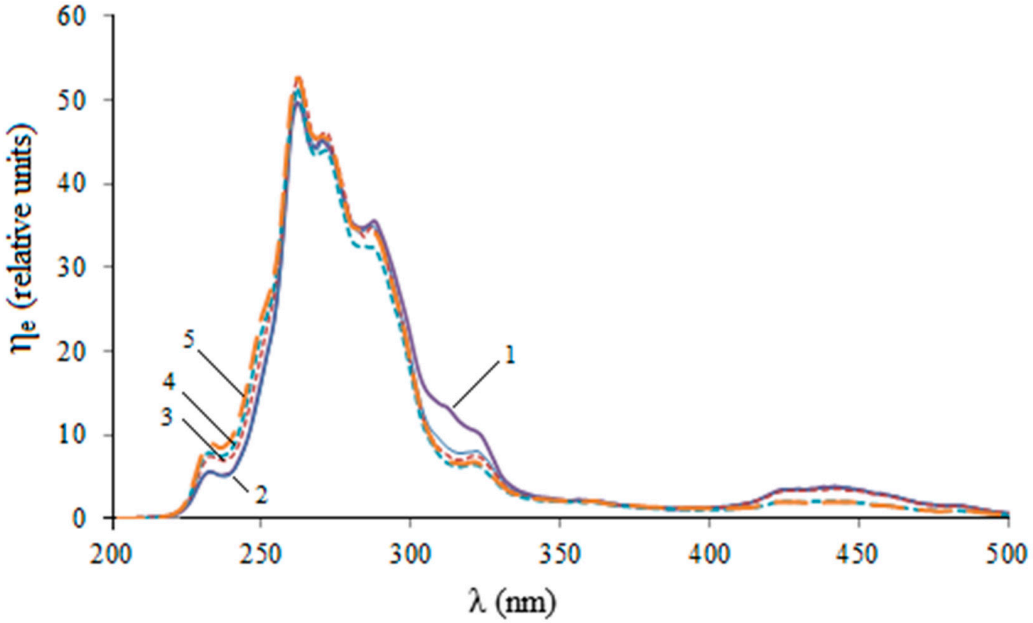

Figure 2. Spectral characteristics of milk at photoluminescence excitation: 1 = the first; 2 = the second; 3 = the third; 4 = the fourth; and 5 = the fifth measurement session.

In the spectral region with the maximum λ_e = 232 nm, the curves of the first and second measurements are the lowest, and they essentially coincide. Then, at the third, fourth and fifth measurement sessions, the height of the curves increases sequentially. The difference between the maxima η_e of the fifth and first measurements reaches 60%. In the range of 255–290 nm, neither significant qualitative nor quantitative differences are observed between the curves. The difference in the maximum at the wavelength of 262 nm between the fifth and first measurement sessions is only 6%. In the range with the maximum of 322 nm in the process of milk souring, the values η_e (λ) fall noticeably. In the range of 380–400 nm, the curves of the fourth and fifth measurements begin to slightly exceed (by 13%) the curves of the first and second measurements, while outside this range, the opposite phenomenon is observed. While the milk sours, in the vicinity of the maximum of 442 nm, the curves shift downwards. At 442 nm, the maximum value is reduced by 93%, and the integral absorption capacity H calculated by the Formula (1) for the range of 420–500 nm is reduced by 75%. The number of replications is determined using the Student's *t*-test. Using the method of successive approximations, with a reliability p = 0.95 and an interval with a given accuracy ε = 1, we determined that at least 7 repetitions of each measurement are required.

At the next stage of the research, in order to determine the photoluminescence fluxes during the milk souring, the following extended experiment was conducted. For the wavelengths of the excitation maxima λ_e equal to 232, 262, 270, 284, 290, 322, 385, and 442 nm, the luminescence spectra φ_l (λ) were measured. Subsequently, for λ_e = 290 nm, the low sensitivity of the radiation receiver was used, while for λ_e = 232, 262, 270, 284, and 322 nm, the average sensitivity was applied, and for λ_e = 385 nm and 442 nm, the high sensitivity of the radiation receiver was used. The radiation fluxes calculated by the Formula (2) are presented in Table 3. (r. u. are relative units).

Table 3. Integral parameters of milk luminescence spectra Φ_λ at various stages of milk souring.

t, h	Φ_{290}, r. u.	Φ_{232}, r. u.	Φ_{262}, r. u.	Φ_{270}, r. u.	Φ_{284}, r. u.	Φ_{322}, r. u.	Φ_{385}, r. u.	Φ_{442}, r. u.
0	520	486	3546	3753	4656	606	447	1855
6	514	418	3525	3725	4570	602	425	1674
21	517	536	3640	3797	4510	573	441	1821
45	501	577	3532	3687	4437	501	424	696
54	528	644	3710	3915	4775	516	476	750

It is seen from Table 3 that almost systematically, the photoluminescence fluxes are changed at the following excitation wavelengths: 232 nm, 322 nm, 385 nm, and 442 nm. The functional connection between the relative photoluminescence flux Φ_λ and the time of milk souring are shown in Figure 3.

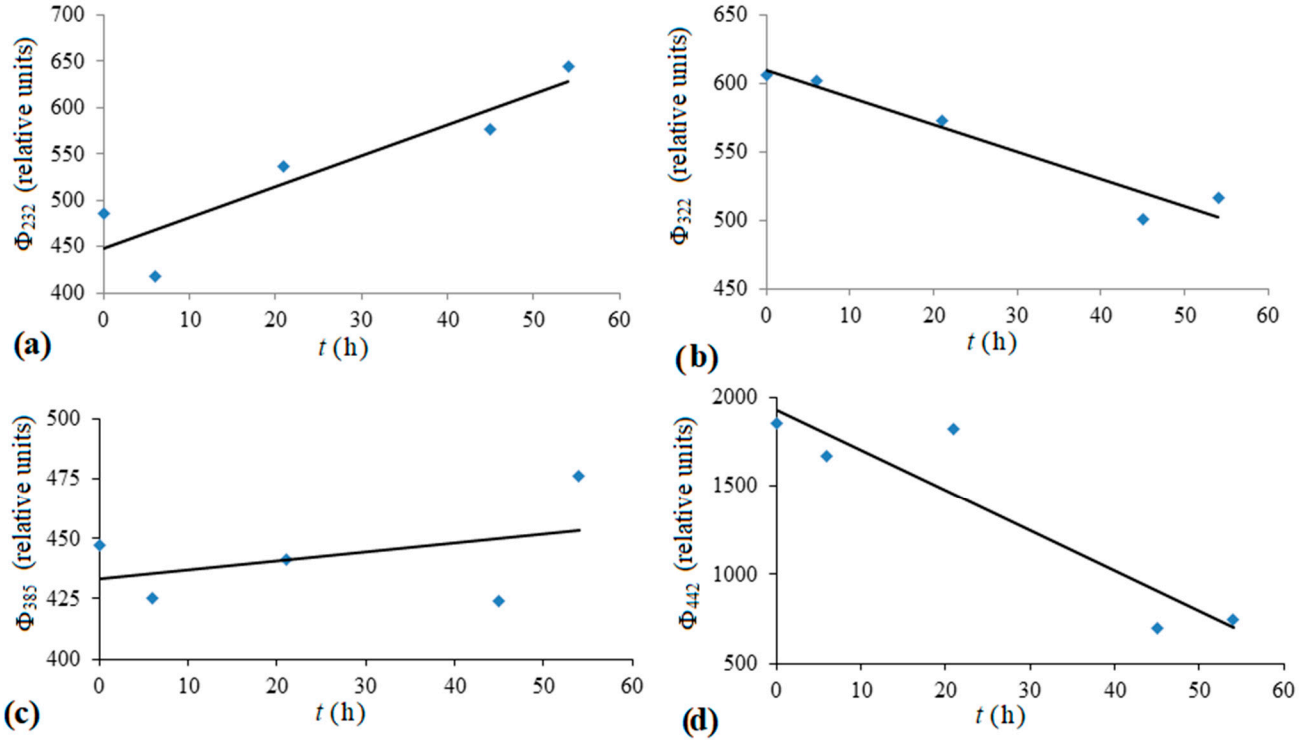

Figure 3. Dependences of relative photoluminescence fluxes Φ_λ obtained from milk souring time and their linear approximations: (**a**) $\Phi_{232}(t)$, (**b**) $\Phi_{322}(t)$, (**c**) $\Phi_{385}(t)$, (**d**) $\Phi_{442}(t)$.

The functional connections are approximated by the linear regression model as follows:

$$\Phi_{232} = 3.32t + 448.5. \quad (3)$$

The determination factor $R^2 = 0.83$.

The relative sensitivity can be calculated by the formula:

$$S = 100 \cdot \left| \frac{\Delta\Phi_\lambda}{\Delta t \cdot \Phi_{t=0}} \right|. \quad (4)$$

The function (Figure 2(a)) is equal to $S = 0.68\%$.

Similarly, for excitation by radiation of other wavelengths, the following linear regression models are used:

$$\Phi_{322} = -1.99t + 609.7, \quad (5)$$

$R^2 = 0.94$; $S = 0.33\%$.

$$\Phi_{385} = 0.369t + 433.3, \quad (6)$$

$R^2 = 0.17$; $S = 0.08\%$.

$$\Phi_{442} = -22.81t + 1934, \quad (7)$$

$R^2 = 0.85$; $S = 1.23\%$.

However, it is advisable to work with the ratio of fluxes $\Phi_{\lambda 1}/\Phi_{\lambda 2}$, rather than with the flux functions, which makes it possible to increase the sensitivity if the wavelength λ_2 is chosen for the declining dependence $\Phi_\lambda(t)$ (Figures 4 and 5). Sibsequently, for the long-wave photoluminescence, the excitation at the wavelength of 385 nm can be used, despite its relatively low sensitivity.

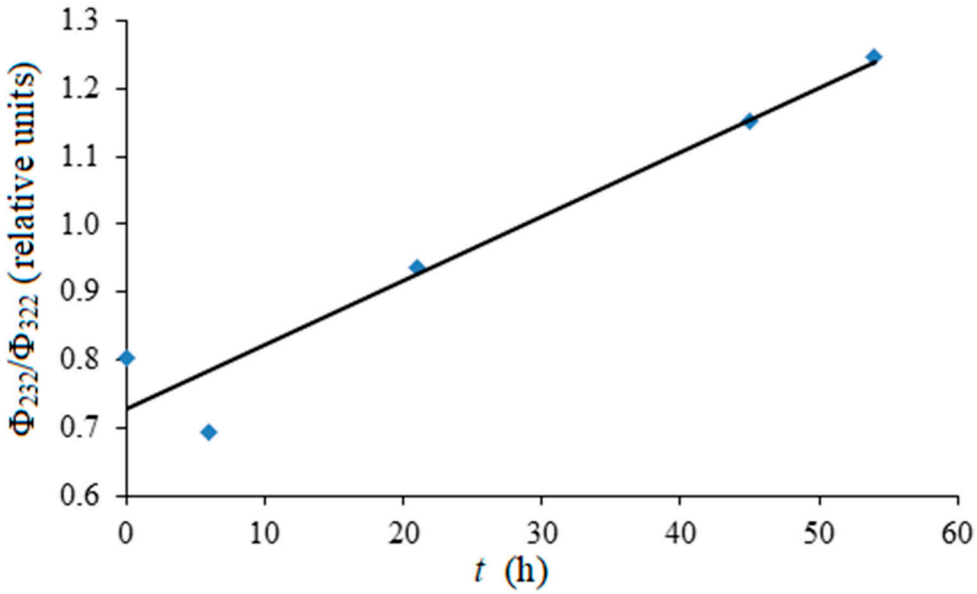

Figure 4. Functional connection between relative photoluminescence fluxes ratio Φ_{232}/Φ_{322} and milk souring time.

$$\frac{\Phi_{232}}{\Phi_{322}} = 0.0095t + 0.727, \quad (8)$$

$R^2 = 0.94$; $S = 1.18\%$.

$$\frac{\Phi_{385}}{\Phi_{442}} = 0.0082t + 0.1897, \quad (9)$$

$R^2 = 0.88$; $S = 3.4\%$.

Taking into account increments according to the Woodward–Fieser rule, the absorption maxima correspond to the following chemical structures of chromophores:

1. 232 nm: C=N;
2. 232, 262, 270 nm: C=O;
3. 322 nm: aromatic aldehydes or amino acids with aromatic substituents.

Thus, the absorption maxima of chromophores correspond to the amino acids of milk proteins, the fatty acids of milk fat, and the aromatic fragments of vitamins.

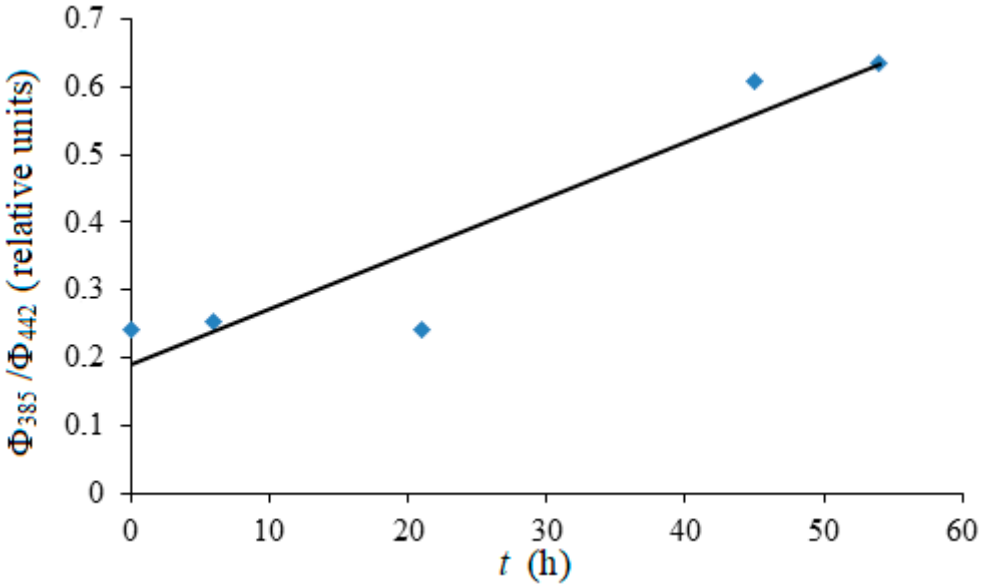

Figure 5. Functional connection between relative photoluminescence fluxes ratio Φ_{385}/Φ_{442} and milk souring time.

As for riboflavin, it has a strong and wide fluorescence peak in the range of 525–531 nm [26]. It also exhibits a wide excitation spectrum, including the maximum peaks of 385 nm and 442 nm used in this study. It is well known that when illuminated with a fluorescent light, riboflavin enters a known photochemical reaction and decomposes into various forms of the lumichrome and the lumiflavine. These compounds also fluoresce. Their emission maxima are in the range from 516 to 522 nm. This experiment confirms the hypothesis that the main change in the milk fluorescence is caused by the riboflavin photochemical degradation. The photo-degradation of the riboflavin in milk depends on the light wavelength and intensity, the exposure time, the protective effect of the packaging material, and the temperature. The degradation follows the rate of the first-order reaction, i.e., it begins immediately upon illumination.

The riboflavin plays the role of a photo-sensitizing agent. In its singlet state, under the light action, the sensitizer is transformed into an excited triplet sensitizer. The excited triplet sensitizer can react with food components, such as proteins and fat, causing the formation of free radicals and radical ions. Moreover, the released triplet sensitizer can react with the atmospheric triplet oxygen so that the singlet oxygen is formed. The singlet oxygen is very unstable and can react directly with compounds containing a large number of free electrons, such as fatty acids and fat-soluble vitamins displaying double bonds; this results in the formation of compounds with undesirable taste tinctures [26].

The authors of reference [27], upon checking the spoilage and falsification of urea in real time ,also believe that the detected luminescence in milk in the range of 520–540 nm is due toriboflavin, while the wavelength of the exciting radiation is approximately 444 nm [28]. Thus, the decrease in the photoluminescence flux we have established at λ_e = 442 nm (Figure 3d) can be explained by the quenching of photoluminescence during souring. The quenching mechanism is mainly explained by the transfer of fluorescence resonance energy and absorption competition [29].

The change in the fluorescent fluxes in the wavelength range of 420–500 nm can take place due to the reaction between primary and secondary lipid oxidation products and proteins, in which case, some fluorescent compounds are formed. Usually, such products exhibit their fluorescence in the range from 400 to 500 nm.

4. Conclusions

For establishing of an effective control procedure during milk souring, the most informative method is found to be the use of the excitation wavelengths of 232 nm, 322 nm, 385 nm, and 442 nm and the subsequent photoluminescence registration in the ranges of 290–400 nm, 360–450 nm, 440–490 nm, and 490–600 nm, respectively. For other wavelengths (262 nm, 270 nm, 284 nm and 290 nm), the dependences $\Phi_\lambda(t)$ are non-systemic, and the determination factors are $R^2 < 0.34$.

The dependences of the integral parameters of the spectra on the milk souring time are approximated by a number of the linear regression models with the determination factors ranging from 0.83 to 0.94, with the exception of the approximation during the excitation $\lambda_e = 385$ nm, where $R^2 = 0.17$. This functional connection $\Phi_{385}(t)$ may only be used with $\Phi_{442}(t)$. The dependence $\Phi_{262}(t)$ is approximated with the determination factor 0.33, while the sensitivity for such functional connection is equal to $S = 0.06\%$, which value is comparatively low. That is why in practice, it is not advisable to use this dependence, despite the fact that it exhibits the highest level of the photo signal.

The milk fluorescence can be used as a non-destructive and fast-acting tool for the measurement of the organoleptic properties associated with the souring of dairy products. Since the riboflavin light-catalyzed degradation is a precursor to foreign flavors in food products, this method may be used for the detection of early souring in a number of dairy products.

Studies of electrophysical (optical) properties using the fluorometric method regarding milk quality indicators in the process of milk souring points to a leading new direction in the field of global science prospects. Based on these studies, we plan to develop an information and control module for measuring milk quality indicators in a contactless manner.

The simplicity and rapidity of this method open up wide possibilities for the effective assessment of a number of factors affecting the souring of dairy products, including factors such as packaging materials, light sources, exposure time, and temperature. The main application of the obtained results is the development of an information-regulating module for determining the parameters of milk using a sensor operating on the principle of photoluminescence. The sensor will include narrow-spectrum radiation sources and receivers operating in the ranges obtained in this study.

The disadvantages of the developed method are the need to use two pairs of radiation sources and receivers to increase sensitivity, as well as the need for significant amplification of the received photo signal (photo signal ratio). This method of monitoring the parameters of milk during its souring, along with devices for its implementation, can be recommended to farmers, as well as to milk processing enterprises, for the monitoring of milk during storage and in the course of the nursing of calves.

Validation of the developed method, as well as the sensors based on this method, can be carried out by comparing the results obtained with those of the arbitration method (the titrimetric approach), as described in Section 2.2. Other alternative methods of validation may include conductometric, ionometric, or reflectometric approaches.

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References

1. Samarin, G.N.; Vasilyev, A.N.; Dorokhov, A.S.; Mamahay, A.K.; Shibanov, A.Y. Optimization of Power and Economic Indexes of a Farm for the Maintenance of Cattle. In *Intelligent Computing and Optimization. ICO 2019, Advances in Intelligent Systems and Computing*; Springer: Berlin/Heidelberg, Germany, 2020; pp. 679–689.
2. Pavkin, D.Y.; Dorokhov, A.S.; Vladimirov, F.E.; Dovlatov, I.M.; Lyalin, K.S. Algorithms for detecting cattle diseases at early stages and for making diagnoses and related recommendations. *Appl. Sci.* **2021**, *11*, 11148. [CrossRef]
3. Holroyd, S.E. The use of near infrared spectroscopy on milk and milk products. *Near Infrared Spectrosc.* **2013**, *21*, 3111. [CrossRef]
4. De Lorenzi, C.; Franzoi, M.; De Marchi, M. Milk infrared spectra from multiple instruments improve performance of prediction models. *Int. Dairy J.* **2021**, *121*, 105094. [CrossRef]
5. Amsaraj, R.; Dilip Ambade, N.; Mutturi, S. Variable selection coupled to PLS2, ANN and SVM for simultaneous detection of multiple adulterants in milk using spectral data. *Int. Dairy J.* **2021**, *123*, 105172. [CrossRef]
6. Katsumata, T.; Aizawa, H.; Komuro, S.; Ito, S.; Matsumoto, T. Quantitative analysis of fat and protein concentrations of milk based on fibre-optic evaluation of back scattering intensity. *Int. Dairy J.* **2020**, *109*, 104743. [CrossRef]
7. Wang, C.; Reis, M.G.; Waterhouse, G.I.N.; Hemar, Y.; Reis, M.M. Prediction of dairy powder functionality attributes using diffuse reflectance in the visible and near infrared (Vis-NIR) region. *Int. Dairy J.* **2021**, *117*, 104981. [CrossRef]
8. Drackova, M.; Hadra, L.; Janstova, B.; Navratilova, P.; Pridalova, H.; Vorlova, L. Analysis of goat milk by near-infrared spectroscopy. *Acta Vet. Brno* **2008**, *77*, 415. [CrossRef]
9. Shao, Y.; He, Y. Measurement of soluble solids content and pH of yogurt using visible/near infrared spectroscopy and chemometrics. *Food Bioprocess Technol.* **2009**, *2*, 229. [CrossRef]
10. Ozturk, M.; Dogan, M.A.; Menevseoglu, A.; Ayvaz, H. Infrared spectroscopy combined with chemometrics as a convenient method to detect adulterations in cooking/stretching process in commercial cheese. *Int. Dairy J.* **2022**, *128*, 105312. [CrossRef]
11. Antony, B.; Sharma, S.; Mehta, B.M.; Ratnam, K.; Aparnathi, K.D. Study of Fourier transform near infrared (FT-NIR) spectra of ghee (anhydrous milk fat). *Int. J. Dairy Technol.* **2018**, *71*, 484–490. [CrossRef]
12. Silaghi, F.A.; Giunchi, A.; Fabbri, A.; Ragni, L. Estimation of rheological properties of ice cream unfrozen liquid phase by FT-NIR spectroscopy. *Bull. Univ. Agric. Sci. Vet. Med. Cluj. Napoca. Agric.* **2009**, *66*, 453. [CrossRef] [PubMed]
13. Yang, B.; Huang, X.; Yan, X.; Zhu, X.; Guo, W. A cost-effective on-site milk analyzer based on multispectral sensor. *Comput. Electron. Agric.* **2020**, *179*, 105823. [CrossRef]
14. Yang, B.; Zhu, Z.; Gao, M.; Yan, X.; Zhu, X.; Guo, W. A portable detector on main compositions of raw and homogenized milk. *Comput. Electron. Agric.* **2020**, *177*, 105668. [CrossRef]
15. Diaz-Olivares, J.A.; Adriaens, I.; Stevens, E.; Saeys, W.; Aernouts, B. Online milk composition analysis with an on-farm near-infrared sensor. *Comput. Electron. Agric.* **2020**, *178*, 105734. [CrossRef]
16. Chen, J.; Lei, X.; Peng, B. Study on the fluorescence spectra of melamine in pure milk. *J. Opt.* **2017**, *46*, 183–186. [CrossRef]
17. Kucheruk, V.; Kulakov, P.; Vasilevskyi, O.; Mostovyi, D.; Kulakova, A.; Kobylyanska, I.M.; Karnakova, G.; Kisała, P. Optical method to determine the quantity of water in milk using the visible radiation range. In Proceedings of the SPIE 10808, Photonics Applications in Astronomy, Communications, Industry, and High-Energy Physics Experiments, Wilga, Poland, 26 May–4 June 2018; p. 108080Y.
18. Jain, P.; Sarma, S.E. Light scattering and transmission measurement using digital imaging for online analysis of constituents in milk. In Proceedings of the SPIE 9525, Optical Measurement Systems for Industrial Inspection IX, Munich, Germany, 22–25 June 2015; p. 95254A.
19. Stocker, S.; Foschum, F.; Krauter, P.; Bergmann, F.; Hohmann, A.; Happ, C.S.; Kienle, A. Broadband Optical Properties of Milk. *Appl. Spectrosc.* **2017**, *71*, 951–962. [CrossRef]
20. Al-Hilphy, A.R.; Ali, H.I.; Al-IEssa, S.A.; Gavahian, M.; Mousavi-Khaneghah, A. Assessing Compositional and Quality Parameters of Unconcentrated and Refractive Window Concentrated Milk Based on Color Components. *Dairy* **2022**, *3*, 400–412. [CrossRef]
21. Belyakov, M.V.; Kulikova, M.G. Control of bulk products' humidity and grinding size by the photoluminescent method photoluminescent quality control. *J. Food Process. Preserv.* **2020**, *44*, e14640. [CrossRef]
22. Kulikova, M.G.; Belyakov, M.V.; Samarin, M.D. Development of a Method of Luminescent Analysis of Grain Products Grinding Degree. *IOP Conf. Ser. Earth Environ. Sci.* **2021**, *666*, 022042. [CrossRef]
23. Fragkoulis, N.; Samartzis, P.C.; Velegrakis, M. Commercial milk discrimination by fat content and animal origin using optical absorption and fluorescence spectroscopy. *Int. Dairy J.* **2021**, *123*, 105181. [CrossRef]
24. Muhammad, N.; Huma, N.; Sahar, A.; Ahmad, M.H.; Rahman, U.U.; Khan, M.A.; Ishaq, A. Application of fluorescence spectroscopy for rapid and noninvasive quality determination of yoghurt ice cream using chemometrics. *Int. J. Dairy Technol.* **2020**, *73*, 437–446. [CrossRef]
25. Bunkin, N.F.; Glinushkin, A.P.; Shkirin, A.V.; Ignatenko, D.N.; Chirikov, S.N.; Savchenko, I.V.; Meshalkin, V.P.; Samarin, G.N.; Maleki, A.; Kalinitchenko, V.P. Identification of Organic Matter Dispersions Based on Light Scattering Matrices Focusing on Soil Organic Matter Management. *ACS Omega* **2021**, *5*, 33214–33224. [CrossRef] [PubMed]

26. Wold, J.P.; Jørgensen, K.; Lundby, F. Nondestructive Measurement of Light-induced Oxidation in Dairy Products by Fluorescence Spectroscopy and Imaging. *J. Dairy Sci.* **2002**, *85*, 1693–1704. [CrossRef] [PubMed]
27. Pandey, G.; Joshi, A. Riboflavin as an internal marker for spoilage and adulteration detection in milk. *Food Chem.* **2021**, *357*, 129742. [CrossRef]
28. Han, J.; Zou, H.Y.; Gao, M.X.; Huang, C.Z. A graphitic carbon nitride based fluorescence resonance energy transfer detection of riboflavin. *Talanta* **2016**, *148*, 279–284. [CrossRef]
29. Fan, L.; Li, J.; Sun, C.; Zhang, J.; Zhao, Y.; Li, W.; Chang, Z. An ultra-sensitive fluorescent sensor based on Zn-MOF for selective detection of riboflavin in food. *J. Solid State Chem.* **2022**, *316*, 123616. [CrossRef]