Fluorescent method of bacterial contamination control on meat surface

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Abstract. This work presents the fluorescent method of meat bacterial contamination control based on the influence of E. coli metabolites on the fluorescence spectrum of meat surface. The experiment revealed that proposed method allows detecting bacterial contamination with concentrations above $10^6$ CFU/cm² on a beef surface.

1. Introduction
Food safety and quality control are the most disturbing problems of our time. Consumption of the contaminated food causes diseases associated with infected products, which are growing from year to year [1]. Thereby quality assurance is the fundamental task for preventing foodborne diseases. Conventional techniques are:
1. the culture and colony counting methods based on bacteria counting [2],
2. immunology-based methods based on antigen-antibody interactions [3],
3. polymerase chain reaction (PCR) method [4].
Although these well-established techniques are highly sensitive to low bacterial concentrations they are very time consuming (from a few tens of hours to few days) [5]. Moreover, most of the methods require a special sample preparation and/or specific reagents application [6] that increases detection time and makes it necessary to accomplish all the measurements by the specialists in the lab conditions.
Other methods should be determined to reduce time consumption and requirements for measurement conditions. Most suitable one is presumed optical taking into account its rapid response and potentially high sensitivity. In this work optical methods of diagnostics will be considered.

2. Brief overview of methods
There are four widely spread optical methods for diagnosis food contamination. All of these methods operate in real-time but vary in sensitivity, complexity of design of sensors and cost of implementation. Further, there is a brief overview of them.
- Surface Plasmon Resonance (SPR) methods, which are extremely sensitive (allow to measure small concentrations of bacteria less than 10 CFU/cm² [7]) but complex in the design to the same extent.
- Interferometric methods that are potentially quite sensitive but need to prepare testing sample transparent to the light. Such a requirement automatically makes this method time consuming [8].
Fiber-optic methods that can be implemented in simple design using photodetector, light source and sensing fiber. The significant drawback is invasiveness of probing since fiber must be placed into the sample [9] that greatly increases the risk of contamination of the sample by fiber.

Spectral methods are Raman, Fourier-transform infrared spectroscopy and fluorescence. Fourier-transform infrared spectroscopy is insensitive to low concentrations of bacteria. According to the literature, the best result is the detection of concentrations of \(10^3\)–\(10^4\) CFU/cm\(^2\) [10]. Raman spectroscopy allows high sensitivity but requires a precisely aligned optical setup [11]. That imposes strong restrictions on the use of both methods in production environment.

Fluorescent methods stand out among spectral methods because in prospect they may be sensitive to low concentrations of bacteria and to different bacteria species but quite easy to implement. In fact, methods have instantaneous measurement time. The current state of the art technologies allow you to refuse to use fluorescence tags for labeling samples because the electronics market offers spectrum analyzers with high sensitivity [12, 13] and powerful narrow-band light emitters. Thus, nowadays fluorescent methods are most appropriate methods to control food contamination among optical spectral methods, which can be created in portable and low cost implementation.

3. The fluorescent method of the food contamination control

Bacterial contamination control based on the fluorescence method which uses the fact that infected and uninfected biological surfaces have different spectra of the fluorescence in the visible range of electromagnetic radiation. Differences in spectra are caused by the presence or absence of metabolites of bacteria E.coli on that surface. Moreover, the shape of the fluorescence spectrum, which is excited by the powerful narrow-band light source, changes with the increasing of the amount of E.coli metabolic products that was confirmed while making experiments. Thereby comparison the radiation intensities at some narrow bands of the fluorescence spectrum gives an opportunity to detect the presence of pathogenic or opportunistic bacteria (Salmonella typhimurium and Escherichia coli) on the surface of the sample.

The measurements are conducted in conditions of an uneven biological surface, a varying distance from surface to the optical probe and the changing angle of incidence of radiation. Therefore, the intensity of fluorescence fluctuates from one measurement to another and so criterion for controlling contamination is to be independent on intensity. In this article task of unambiguous determination of bacterial contamination of the test sample is solved on the basis of the proposed criterion of estimating the bacterial contamination by the ratio of particular spectrum marker wavelengths.

4. Results and discussions

Figure 1 shows the fluorescence imaging device with fiber-optic bundle, which was designed for the bacterial contamination control on a meat surface. The current device uses 450 nm 1.6 W laser diode (PL TB450B, OSRAM Opto Semiconductors GmbH, Germany) as the excitation light source, the multipurpose tunable spectrometer (ASP-150T, Avesta Ltd., Russia), 7x1 fiber bundle (art photonics GmbH, Germany). All measurements were conducted using this device.
Bacteria of the *Escherichia coli* (E. coli) species are the most common pathogenic bacteria that occur on food products, particularly on beef. In this regard, experiments with bacterial culture of E. coli ATCC 29522 were conducted.

All measurements were performed in research zone of Intestinal Infections Laboratory (St. Petersburg Pasteur Institute, Russia) without thermostabilization, at room temperature (25 ± 5 °C). Test samples of beef were artificially contaminated with E. coli and put into dishes.

Figure 2 depicts the fluorescence of the contaminated sample. A peak in emitted spectrum is showed on the figure and it is growing over time. This peak characterizes the found fluorescence that is radiated by the contaminated samples. Raise is caused by the increase in the number of metabolites of the bacteria during time. Fluorescence is within visible band of the electromagnetic spectrum and has an emission peak at wavelength \( \lambda = 535nm \). The microbiological measurements found concentration of bacteria to be \( 10^6 \) CFU/cm\(^2\) is on the sample after 5 hours of the experiment.

**Figure 1.** The fluorescence imaging device with fiber-optic bundle.

**Figure 2.** The impact of the bacteria on the beef fluorescent spectra of sample artificially contaminated with E. coli.
Figure 3 shows the fluorescence intensity of uninfected meat sample during the experiment. It may be noticed that there is no change in the shape of the spectrum of uncontaminated meat. This gives reason to distinguish between infected and uninfected meat.

Figure 3. Fluorescent spectra of uninfected sample.

Figure 4 shows the normalized fluorescence spectra of infected and uninfected samples after 5 hours. Normalization was carried out by the intensity of fluorescence at a wavelength $\lambda_1 = 500$ nm. Such a wavelength was chosen because it is convenient to operate two marker wavelengths: $\lambda_1 = 500$ nm and $\lambda_2 = 535$ nm which comparison of intensities ($I_{\lambda_1}$ to $I_{\lambda_2}$) determines the shape of the spectrum and indicates the contamination.

Figure 4. The impact of the bacteria on the normalized beef fluorescent spectrum: green – uninfected sample; red – sample artificially infected with E. coli bacteria ($10^6$ CFU/cm$^2$).
Thus, the essence of the proposed method is as follows. The laser irradiates the sample at wavelength 450 nm and the fluorescent spectra contain the peak with central wavelength 535 nm. The proposed criterion to decide whether the sample is infected or not is in comparison of $I_1$ to $I_2$. When $I_1 > I_2$ there is given a negative assessment (the sample’s surface is not contaminated), and if $I_1 < I_2$ there is given a positive assessment (the sample’s surface is contaminated). Figure 4 clearly illustrates that point. That ratio may be used as an obvious marker of bacterial contamination of meat surface.

5. Conclusion
As a result of the research it was investigated that infected with E. coli sample may be confidently detected 5 hours after the contamination by the fluorescence spectra of its irradiated surface. Proposed method allows detection of the bacterial infection by bacterial metabolites. Concentration of the bacteria is above $10^6$ CFU/cm$^2$ after 5 hours.

The advantage of this method is that the proposed criterion of the bacterial contamination on the meat surface is not dependent on intensity of fluorescence. In addition, the device has the simple structure and measurements are carried out contactless giving instant assessment of the bacterial contamination.

Although the proposed method is able to detect high concentrations of bacteria, it may serve the meat industry as a first stage of quality assurance. Applying fluorescent labels with dyes or time-resolved spectroscopy may be perspective to improve the sensitivity of the method.

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