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APPLICATION OF THE THERMOGRAPHIC METHOD FOR THE MONITORING GROWTH OF SELECTED MICROORGANISMS

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Summary. All living organisms produce heat as a result of metabolic activity, and therefore, measuring heat energy can help in effective detection and quantification of bacteria. The aim of the study was to use real-time infrared thermography to measure temperature changes for determining the presence of microorganisms, which will enable the development of a fast and noncontact method for testing the microbiological quality of food in the future. In the study, the growth of *Saccharomyces cerevisiae* yeasts and *Aspergillus niger* and *Penicillium roqueforti* molds was monitored. The colonies of the examined microorganisms exhibited a different temperature compared to the medium, which may form the basis for the development of an alternative diagnostic method.

Key words: thermovision, infrared thermography, *Saccharomyces cerevisiae*, *Aspergillus niger*, *Penicillium roqueforti*

INTRODUCTION

Food products provide an ideal environment for the development of microorganisms such as bacteria, yeasts, and molds, as they contain all the substances necessary to maintain the vital functions of the microbes. The growth of microorganisms in raw materials and food products results in the loss of valuable ingredients from food, cause spoilage, and can also lead to food poisoning. This justifies that the microbiologi-

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cal quality of food should be continuously monitored. Diagnostic methods that are commonly used in food microbiology to study the degree of microbiological contamination, as well as to identify the different groups of microorganisms (including the pathogenic ones), are costly and time-consuming as they involve multiple stages. Thus, there is a need to develop new, faster, and effective methods for the detection of harmful microflora. Due to the invasive nature of traditional methods, modern and noncontact temperature measurement techniques, such as infrared thermography, play an increasingly important role in the industry, as they allow determining the surface temperature field by measuring the amount of infrared radiation emitted by a given surface [Oliferuk 2008, Vadivambal et al. 2011]. Thermography, also called thermovision, is a process of imaging the infrared band with wavelengths ranging from 0.9 to 14 μm in the medium. It involves the transformation of thermal radiation, recorded by a thermal imaging camera, into a thermographic image, called thermogram which is a graphic representation of the temperature distribution on the examined surface [Veraverbeke et al. 2006].

Thermal imaging technology is currently used in power engineering, construction, metallurgy, and medicine, as well as in scientific research, to obtain information on thermodynamic phenomena and heat exchange. This method is characterized by high practicality as it does not require direct contact with the examined object and due to its mobility, convenience of use, and the possibility of processing digital information [Amon et al. 2008, Baranowski et al. 2005a, Da-Wen 2008]. Thermal imaging can be used in many industrial processes, including agri-food production, during which heat is generated and lost in time and space [Hellebrand et al. 2002]. The possible applications of thermography include the detection of mechanical damage caused by water stress in plants, control of maturity and quality of products of plant origin, and control of temperature conditions during storage [Baranowski et al. 2005b, Veraverbeke et al. 2006, Baranowski 2008, Horabik 2011]. The thermal imaging camera can also be used to control the packaging process and the state of packaging as well as to detect foreign bodies in food products [Ginesu et al. 2002, Liu et al. 2002]. Moreover, with the growing demand for ready-to-eat food, especially of animal origin, the need to control the temperature of products on the production line is rapidly increasing [Ibarra et al. 2000, Walczycka 2005]. This means that noncontact techniques, which do not affect the properties of the tested products and allow for faster monitoring of the quality and efficiency of processing [Ma et al. 2003], are an excellent solution. Thermography, which provides reliable and easily interpretable results at a high speed and does not require contact with the tested products, meets all the requirements discussed. Moreover, it does not pose any risk of food contamination [Ma et al. 2003], may contribute to reducing costs, and has a high probability of repeatability, which means that it can even be used for new applications [Gowen et al. 2010].

The aim of this study was to use the measurements obtained from a thermal imaging camera to monitor the presence of yeasts and molds on the growth media under model conditions.

MATERIALS AND METHODS

Test microorganisms, microbiological media, and preparation of *inoculum*

Two species of mold (*Aspergillus niger* ATCC 9142 from the American Type Culture Collection, *Penicillium roqueforti* E31 IBA from the Institute of Biotechnology and Antibiotics in Warsaw) and one species of yeast (*Saccharomyces cerevisiae* ATCC 9763 from the American Type Culture Collection) were used in the study. Yeast was cultured on Sabouraud Dextrose Agar (SDA; BTL, Poland) at 28°C for 48 h. Yeast inoculum was prepared using a hemocytometer in a sterile solution of 0.85% NaCl to obtain approximately ~1×10^6 colony forming units (CFU)/ml. Mold conidia and spores were obtained from the mycelium cultivated on SDA after incubation at 28°C for 14 days. The suspensions were prepared using a hemacytometer in 0.85% NaCl with 0.1% Tween 80 to obtain ~1×10^6 spores/ml. Czapek-Dox and YPD media (both from BTL, Poland) were used to study the growth of the microbial cultures.

Model tests of microbial growth using a thermal imaging camera

The scope of the study was to use the measurements performed with the VIGOcam v50 thermal imaging camera to monitor the growth of the test microorganisms under model conditions.

After adding the inoculum, the Czapek-Dox and YPD were incubated for 5 days at 27°C. In the case of active thermal imaging, additional thermal stimulation is applied. Culturing was carried out for 11 days, and after 4 days of incubation, the biological material was transferred from the thermostat (temperature 30°C) to a refrigerator (temperature 6°C). The colonies grown afterward were tested with the VIGOcam v50 thermal imaging camera from the VIGO System. While testing with the thermal imaging camera, the temperature in the laboratory was 23.8°C and the air humidity was 46%. The camera was set in a tripod so that the front of the lens was about 40 cm above the test plate. Petri dishes containing mold colonies were placed under the thermal imaging camera, and pictures were taken with the camera and a Sony Xperia M mobile phone having a 5-megapixel digital camera. The study has been din three series.

RESULTS AND DISCUSSION

Growth testing of S. *cerevisiae yeasts* with the thermal imaging camera

Saccharomyces cerevisiae colonies (Fig. 1A), which multiplied over 5 days on the YPD medium, were thermographically analyzed (Fig. 1B). The results showed that the colonies exhibited a different temperature than the medium.

The maximum temperature recorded was 27.06°C, and the minimum was 26.26°C. The difference between the temperatures was 0.80°C.

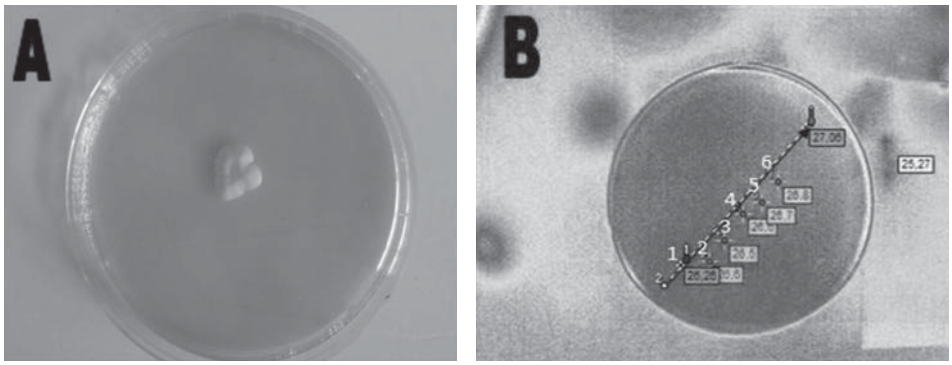

Fig. 1. Colony of *Saccharomyces cerevisiae* yeast multiplied on the YPD medium (A – actual image, B – thermogram)

Rys. 1. Kolonia drożdży *Saccharomyces cerevisiae* namnożonych na podłożu YPD (A – obraz rzeczywisty, B – termogram)

Figure 2 shows the temperature distribution at six points that were located on the selected section crossing the area of the substrate and the yeast colony (Fig. 1B). It can be observed that as the colony moved along the designated section from its central part to its banks and substrate, the temperature increased. The higher temperature observed for the substrate can be explained in many ways. A substrate is a dead matter that only absorbs energy from the environments of higher temperature and then accumulates it. In addition, it should be assumed that the growth of colonies (producing ATP) was not indifferent to the formation of its parameters [Nicklin Graeme-Cook and Killington 2004]. On the other hand, as living organisms, microbial colonies use part of the energy resulting from metabolic processes for their basic vital functions, which can be explained by their lower temperature [Dudkiewicz et al. 2014]. An important aspect that determines the temperature difference is the thermal capacity of the substance. This parameter is dependent on the water content of the substances and their density. The substrate that has a higher thermal capacity accumulates more energy and exhibits a higher temperature. Moreover,

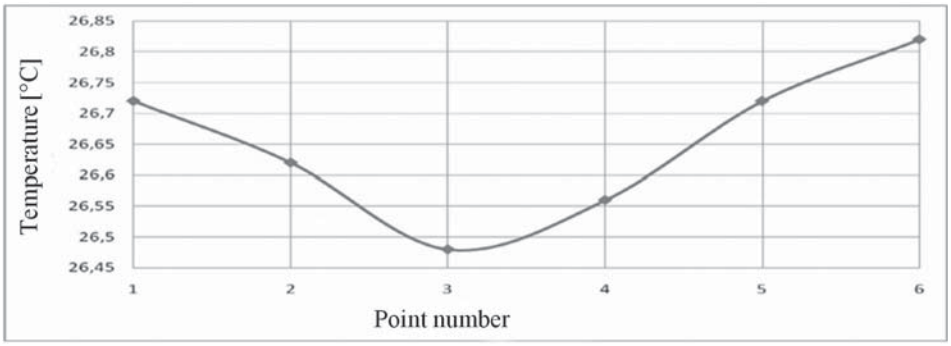

Fig. 2. Temperature distribution of the colony of *Saccharomyces cerevisiae* yeast

Rys. 2. Rozkład temperatury wzrostu kolonii drożdży *Saccharomyces cerevisiae*

the substrate and colonies have different densities and water contents [Filin 2005]. Another important aspect that determines the differences in temperature between substances (substrate and colony) is their different levels of energy emission and heat absorption [Alagić et al. 2018].

Growth *P. roqueforti* strains with thermal imaging camera

The colonies of *P. roqueforti* mold observed on the YPD medium after 5 days of cultivation (Fig. 3A) also showed a different temperature than the surrounding medium. The thermographic image is shown in Fig. 3B indicates that the maximum temperature was 27.65°C and the minimum was 25.78°C, which had a difference of 1.87°C.

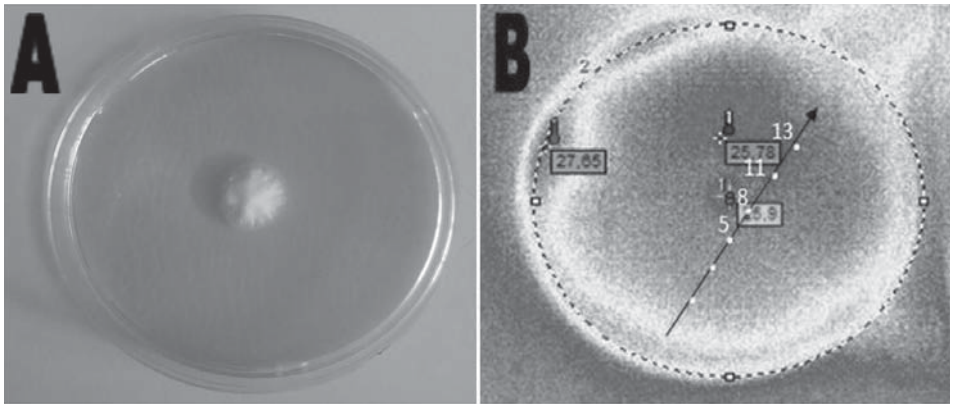

Fig. 3. Colony of *Penicillium roqueforti* mold multiplied on the YPD medium (A – actual image, B – thermogram)

Rys. 3. Kolonia pleśni *Penicillium roqueforti* namnożona na podłożu YPD (A – obraz rzeczywisty, B – termogram)

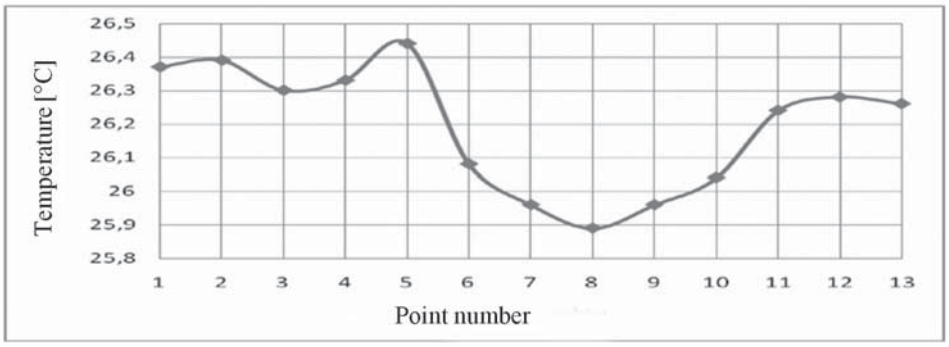

Fig. 4. Temperature distribution of the colony of *Penicillium roqueforti* mold

Rys. 4. Rozkład temperatury wzrostu kolonii pleśni *Penicillium roqueforti*

Figure 4 shows the temperature distribution at 13 points on the substrate and the multiplied mold colony. It was observed that as the colony moved along the designated section from its central part to its banks and substrate, the temperature increased. Baranovichi and colleagues [2009] analyzed the abiotic stress exerted by the pathogenic strains of *Alternaria brassicae* and *Alternaria brassicicola* on rape leaves. They observed that healthy tissues were characterized by lower temperature, whereas the infected ones had higher temperature. The changes in temperature recorded with a thermographic camera confirmed that this method can be used for monitoring the growth of microorganisms.

Growth *A. niger* strains with the thermal imaging camera

After 5 days of cultivation on Czapek-Dox medium (Fig. 5A), temperatures of the biological material and growth environment were recorded using thermal imaging camera. The thermographic image showed that the temperatures differed (Fig. 5B). The maximum temperature was 27.41°C and the minimum was 25.30°C, which had a difference of 2.11°C. Thus, the image obtained from the thermal imaging camera clearly showed the growth of mold (Fig. 5B).

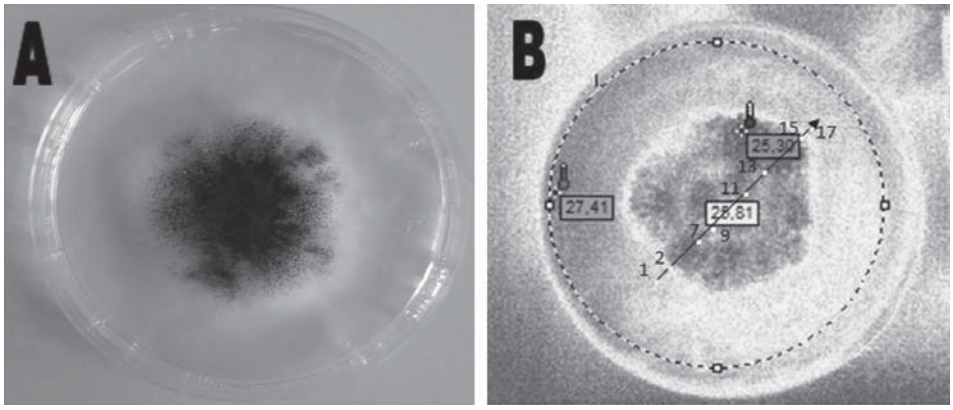

Fig. 5. Colony of *Aspergillus niger* mold multiplied on Czapek-Dox medium (A – actual image, B – thermogram) after 5 days of incubation

Rys. 5. Kolonia pleśni *Aspergillus niger* namnożona na podłożu Czapek-Doxa (A – obraz rzeczywisty, B – termogram) po 5 dniach inkubacji

Figure 6 shows the temperature distribution at 17 points selected on the area of substrate and colonies. It can be observed that the temperature in the substrate near the edges of the plate was higher than the value recorded in the colony itself and in the substrate located not far from it.

Fig. 7 shows an 11-day colony of *A. niger*, which was multiplied on the Czapek-Dox medium for 4 days and then transferred from the thermostat (30°C) to the refrigerator (6°C) where it was incubated for 7 days. The colonies were examined with a thermal imaging camera, and the thermographic image showed that the difference between the

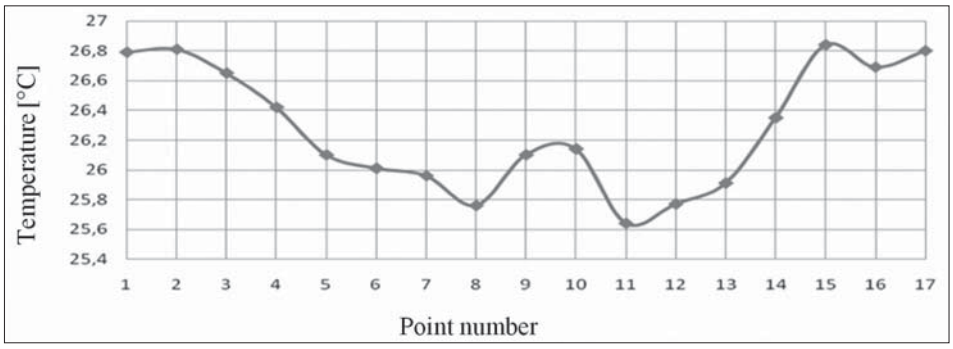

Fig. 6. Temperature distribution of the colony of *Aspergillus niger* mold on Czapek-Dox medium after 5 days of incubation

Rys. 6. Rozkład temperatury wzrostu kolonii plesni *Aspergillus niger* na podłożu Czapek- Doxa po 5 dniach inkubacji

temperature of the tested substrate and that of the biological material amounted to 3.01°C (Fig. 7B). This indicates that the image obtained from the thermal imaging camera clearly revealed the growth of mold.

Figure 8 shows the distribution of temperatures determined at 23 points located in the section cutting through the substrate and the mold colony. A similar dependency can be observed in the figure as noted for the previously tested colonies of molds and yeasts. A higher temperature was found in the area of the substrate at the edges of the plate, whereas a lower value was recorded in the areas of the colony and the substrate not far it. Due to the fact that the temperature of the tested microorganisms differed from the ambient temperature, they were clearly visible on the thermographic image, and were thus

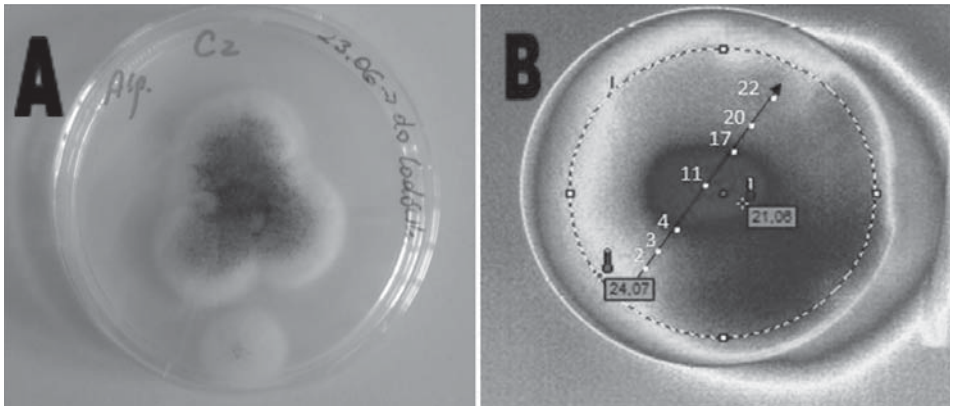

Fig. 7. Colony of *Aspergillus niger* mold multiplied on Czapek-Dox medium (A – actual image, B – thermogram) after 11 days of incubation

Rys. 7. Kolonia plesini *Aspergillus niger* namnożona na podłożu Czapek-Doxa (A – obraz rzeczywisty, B – termogram) po 11 dniach inkubacji

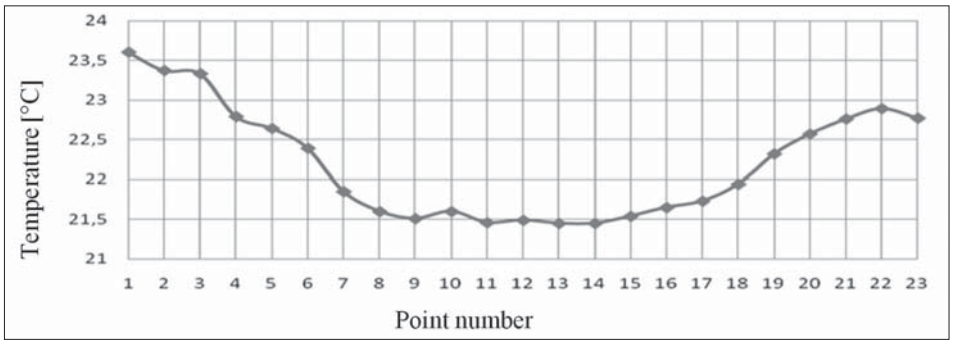

Fig. 8. Temperature distribution of the colony of *Aspergillus niger* mold on Czapek-Dox medium after 11 days of incubation

Rys. 8. Rozkład temperatury wzrostu kolonii pleśni *Aspergillus niger* na podłożu Czapek-Doxa po 11 dniach inkubacji

detectable, which indicates that the thermographic method can be applied in the monitoring of microbiological contamination.

According to Baranowski [2008], the temperature of an examined object differs from that of its surroundings, yet the temperatures remain quite close. The same fact was reported in the studies carried out to monitor the growth of mold and yeast, in which the temperature of the tested strains differed from the substrate. In addition, the results of the studies showed that in samples with multiplied biological material, which were stored for 7 days in a refrigerator and then examined with a thermal camera, greater differences in temperature were recorded between the substrate and the colony. Therefore, it was suggested that cooling the examined object before the application of active thermal imaging is more effective in the detection of microbiological contamination.

Lahiri and colleagues [2012] used infrared thermography in their study to quantify the pathogens of *Vibrio cholerae*, *Vibrio mimicus*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*. They observed that for all the tested bacterial species the temperature increased linearly with the concentration of the organisms and the growth rate differed between the species. The authors concluded that the change in the thermal energy with the concentration of pathogens depends on their morphology, which affects the mechanism of convection.

CONCLUSIONS

The observations of the study proved that using a thermal imaging camera it is possible to monitor the growth of microorganisms, and hence, in the long term, this device can be applied in the evaluation of the microbiological quality of food. It was found that *S. cerevisiae* yeasts exhibited a lower temperature than the medium and the difference between temperatures was 0.8°C. In the case of the mold species *P. roqueforti* and *A. niger*, the temperature difference between the colony and the substrate was higher with 1.87°C and 2.11°C, respectively. This difference in temperature was related to the size of the colony and its structure.

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ZASTOSOWANIE METODY TERMOGRAFICZNEJ W MIKROBIOLOGII

Streszczenie. Wykrywanie żywych bakterii ma fundamentalne znaczenie we wszystkich dziedzinach mikrobiologii i biotechnologii. Konwencjonalne metody liczenia bakterii są często czasochłonne i pracochłonne. Wszystkie żywe organizmy wytwarzają ciepło spowodowane ich aktywnością metaboliczną, a zatem pomiar energii cieplnej jest skutecznym narzędziem do wykrywania i analizy ilościowej bakterii. Celem badań było wykorzystanie termografii w podczerwieni w czasie rzeczywistym do pomiaru zmian temperatury jako wskaźnika obecności drobnoustrojów, co w perspektywie umożliwi opracowanie szybkiej i bezkontaktowej metody badania jakości mikrobiologicznej żywności. Monitorowano wzrost drożdży *Saccharomyces cerevisiae* oraz pleśni *Aspergillus niger* i *Penicillium roqueforti*. Dowiedziono, że kolonie badanych drobnoustrojów wykazywały inną temperaturę niż podłoże, co może stanowić podstawy do opracowania alternatywnej metody diagnostycznej.

Słowa kluczowe: termowizja, termografia podczerwieni, *Saccharomyces cerevisiae*, *Aspergillus niger*, *Penicillium roqueforti*