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Research Article

Determination of CO_2 Content in the Headspace of Spoiled Yogurt Packages

Bojana Danilović,[1] **Dragiša Savić**,[1] **Lorenzo Cocola**,[2] **Massimo Fedel**,[2] **and Luca Poletto**[2]

[1]*Food Technology and Biotechnology Department, Faculty of Technology, University of Niš, Bulevar Oslobodjenja 124, 16000 Leskovac, Serbia*
[2]*National Research Council, Institute for Photonics and Nanotechnologies, via Trasea 7, 35131 Padua, Italy*

Correspondence should be addressed to Lorenzo Cocola; lcocolao@dei.unipd.it

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The CO_2 formation during food storage can often be correlated with the increase in yeast population. Yogurt and other dairy products are susceptible to yeast contamination. Accumulation of CO_2 in the headspace of yogurt packages can lead to the eventual blowing off of the package. Therefore, determination of CO_2 in the yogurt packages can indicate eventual unsafety of the product. The aim of this paper was to determine CO_2 concentration in the headspace of different yogurt containers contaminated with yeast at the levels of 1 and 5 CFU/ml. Yeast *Candida kefyr*, previously isolated from spoiled yogurt, was used for contamination. Contaminated and control samples of yogurt were incubated at 30°C. A device based on tunable diode laser absorption spectroscopy was used for the measurement of CO_2 concentration. The CO_2 content in all analysed samples changed in a similar manner with slow increase to the value of 6% during the first 30 h and, after that, rapid accumulation to 17–20%. The initial level of yeast contamination did not have significant influence to the CO_2 content trend. The increase in the number of yeast was observed after 10 h of incubation, and the final value of 6-7 log·CFU/cm^3 was reached after 40 h of incubation. The significant increase in the yeast number can be correlated with the CO_2 content in a way that CO_2 concentration of 6% can be considered as critical for microbial spoilage. Since the TDLAS technique is simple and nondestructive, it can be a promising possibility for detection of the microbial spoilage in food.

1. Introduction

Food safety has become one of the main global concerns due to the rapid economic growth and industry development. This required the use of different monitoring techniques in the field of food quality and safety [1]. The parameters that usually reflect the food quality, shelf life, and safety are temperature, pH, freshness, presence of indicator microorganisms, and leakage or content of gases (CO_2 and O_2). Conventional techniques of food safety determination are mostly time consuming, laborious, and involve the destruction of the sample. Also, pretreatment of the sample is required which leads to difficulties in real-time and online monitoring [2]. Development of high-performance, inexpensive, nondestructive method for food safety analysis has become one of the most important problems in food research.

The use of different imaging techniques in food quality monitoring gained a lot of interest in the past few years. Many in-line and off-line applications of Raman spectroscopy technique, hyper spectral imaging, magnetic resonance imaging, soft X-ray imaging, ultrasound imaging, thermal imaging, fluorescence imaging, and odour imaging in food analysis have been reported [2, 3]. Here, we focus on tunable diode laser absorption spectroscopy (TDLAS) that is inherently a noninvasive method as it measures gas content in closed containers by passing through the headspace inside the package. Due to simplicity, high selectivity, and sensitivity, TDLAS can be applied in different industrial processes, gas pipelines, and power generation plants, as well as

in beverage, agro-food, and pharmaceutical sealing processes [4, 5].

TDLAS is based on the fact that each molecule has a unique light absorption characteristic that absorb the laser beam at a very specific wavelength. The concentration, temperature, pressure, and in some cases even velocity and mass flux of gases in a gaseous mixture can be determined by measuring the absorption profile through a diode laser beam that is wavelength tuned to scan along the whole line profile. At wavelengths slightly different from these narrow absorption lines, there is no absorption from the particular gas. TDLAS enables noninvasive measurement through a wide range of closed containers, from bags to trays and cups, and a wide range of materials, from transparent to almost dark films [6, 7]. In food industry, TDLAS technique has been already applied for checking closure tightness and leak detection, for in-line measurement and monitoring of fermentation process in beer, sparkling wine, and soft drink industry, and for analyzing packages in modified atmosphere [8–13].

Yogurt is a firm or liquid fermented milk product to be consumed up to a few weeks after the production before a reduction in the sensory characteristics occurs [14]. Despite the low pH value (3.8–4.5), yogurt can be a selective environment for the growth of contaminants, especially fungi which can cause significant economic losses due to off-flavour and changes in visual appearance [15]. The spoilage of yogurt by yeasts and consequential CO_2 accumulation can induce swelling and, in the bottom line, blowing off of the container. Therefore, indirect measurement of CO_2 content in a headspace of the yogurt packages can refer to yeast contamination.

This paper reports the determination of the CO_2 content in the headspace of yogurt cups and bottles during storage. In order to examine the process, the samples were contaminated with the yeast *Candida kefyr*. CO_2 content was continuously measured by a TDLAS device specially constructed for these measurements.

2. Materials and Methods

2.1. CO_2 Measurement. The content of gaseous CO_2 in the headspace of closed yogurt containers is measured by a device specially realized for these measurements and based on TDLAS.

The device is based on a VCSEL source operating on the 2 μm CO_2 absorption band and is meant to measure the content of CO_2 in the headspace of axially symmetrical containers such as bottles and cups. The sample to be measured is placed on a rotating table with adjustable height, in order to transmit light from the source through a diameter of the sample volume within its headspace and to collect emerging light with an extended InGaAs detector placed on the opposite side (Figure 1). Sample rotation during the measurement is required to smooth out irregularities in the sample itself as well as to reduce optical fringing due to light interference.

Wavelength modulation spectroscopy technique is used to obtain maximum sensitivity [16]. This is done by overlapping a high-frequency sinewave carrier over the linear wavelength scanning signal and doing a demodulation at the receiver side that is synchronous with the carrier frequency and its harmonics. This kind of processing is implemented on a fully software-defined basis by a single data acquisition card, which synchronously controls the laser source while acquiring photodetector signal. An embedded computer provides control on the signals and measurements, while performing lock-in demodulation and signal processing of the recorded absorption profiles to evaluate the gas concentration.

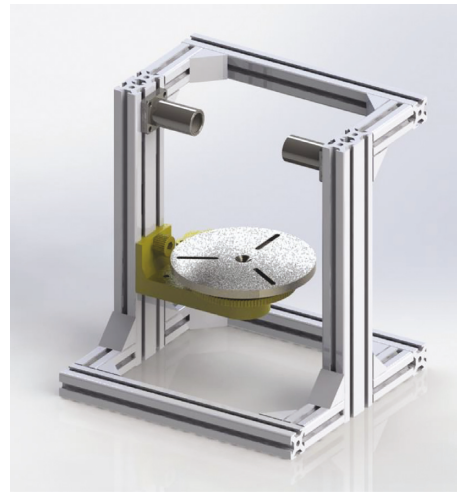

Figure 1: Schematic of the instrument optical front end: (a) laser source; (b) photodetector; (c) rotating table; (d) height adjustment.

The optical front end of the instrument and sample management frame were designed to be easily integrated in an incubator to allow continuous measurement on thermally controlled samples. Additionally, a calibration protocol was identified and validated so that the instrument could be easily calibrated on the field and applied to a wide range of available samples. To calibrate the device, dummy packages were placed in the instrument and flushed with known, calibrated gas mixtures. This procedure also provides a direct assessment of the output linearity. Measurements during the initial validation process reported linearity up to 25% CO_2 volume, a minimum detectable threshold of 60 ppm (in a controlled environment), and a resolution of 160 ppm over a 5% CO_2 volume reference level [17]. As in scattering samples, such as the majority of food containers, the actual optical path length may differ significantly from the geometrical source-to-detector distance, and it is important to note that calibration is container dependent, and thus, calibration has to be taken on every considered type of package.

The accuracy of the measurement was validated by measuring CO_2 content in the containers flushed with a gas mixture containing 6% CO_2 and 94% N_2, custom made from Messer gas factory (Pancevo, Serbia).

2.2. Yogurt. Liquid yogurt "Vedro, 2.8% milk fat" ("Niška mlekara," AD Niš, Serbia), filled in PVC cups (180 g yogurt/205 cm^3 total volume) and PET bottles (500 g/525 cm^3 and 1000 g/1025 cm^3), was used for the experiment.

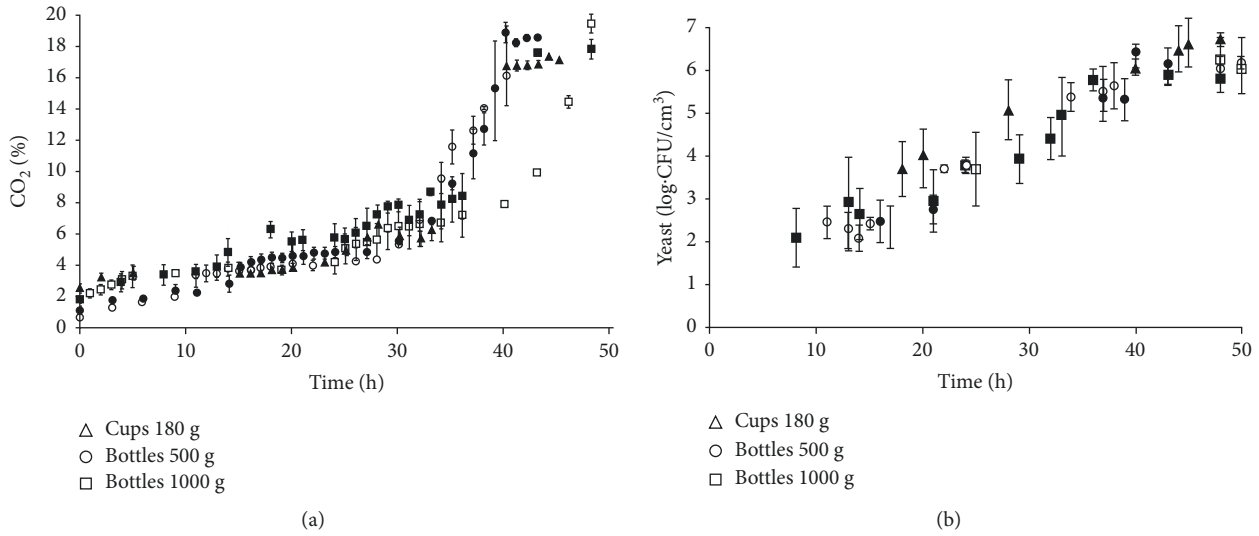

Figure 2: Changes in the CO_2 content (a) and the number of yeasts (b) during the incubation (30°C) of yogurt ("Vedro," 2.8% milk fat) packed in PVC cups (180 g) and the PVP bottles (500 g, 1000 g) contaminated with the yeast *Candida kefyr* with initial concentration of 1 CFU/cm^3 (open symbols) and 5 CFU/cm^3 (black symbols). The error bars represent standard deviation on experimental data.

2.3. Microorganism. Yeast *Candida kefyr* was previously isolated from spoiled yogurt (kept for 30 days at room temperature) and identified by VITEK 2 Systems Version: 06.01. Sabouraud maltose agar ("Torlak," Serbia) with the addition of chloramphenicol ("Sigma-Aldrich," Germany) (0.05 g/dm^3) was used for enumeration of the yeasts, while de Man, Rogosa and Sharpe medium ("Torlak," Serbia) was used for enumeration of lactic acid bacteria.

2.4. Experimental Conditions. A total number of 50 samples (9 samples of cups of 180 g, 21 samples of bottles of 500 g, and 20 samples of bottles of 1000 g) were used for the experiment. The experiments were performed in triplicate.

The samples were supplied directly from the production line and aseptically inoculated with the isolated yeast strain at a level of 1 CFU/cm^3 for bottles of 500 g and 1000 g, and 5 CFU/cm^3 for cups and bottles of 500 and 1000 g. Samples were inoculated with a suspension of yeast for which turbidity was adjusted to be in accordance with 0.5 McFarland standard. A few uncontaminated cups and bottles were used as control samples. The samples were incubated and CO_2 measurements were performed during the incubation process, while the temperature was kept at 30°C.

Every sample which showed a significant change in the CO_2 content in the headspace was analysed for a number of yeasts, lactic acid bacteria (by serial dilution method), and pH value (by the pH meter "Hanna HI 92240").

2.5. Statistical Analysis. The error bars were calculated in Excel (Microsoft, USA) as standard deviation on experimental data. Prediction area ellipses were determined based on correlation between the number of yeast cells and CO_2 content using Statistica 7.0 (StatSoft Inc., USA) for Windows. The meaning of the ellipses is that the probability of a new pair of measurement values falling within the marked area is estimated to be 90%.

3. Results and Discussion

Along with the mould contamination, contamination with yeasts is the largest microbiological problem in fermented milk products. Yeasts are generally well recognized as a typical type of spoilage microorganisms in yogurt and yogurt-based drinks [18]. The yeast used in this study, isolated from spoiled yogurt and identified as *Candida kefyr*, is well recognized as a spoilage microorganism in dairy products [19].

The changes in the CO_2 content in the headspace of the different packages (PVC cups: 180 g; PET bottles: 500 g and 1000 g) and the number of yeasts in contaminated yogurt ("Vedro, 2.8% milk fat") are presented in Figure 2. The yogurt samples were contaminated with the yeast *Candida kefyr* at two levels of initial cell concentrations: 1 CFU/cm^3 (bottles) and 5 CFU/cm^3 (cups and bottles). In order to discuss and compare changes in the CO_2 content in the headspaces of two types of yogurt, the results of the previous experiment [20] with the bottles (500 g) contaminated at the initial level of 1 CFU/cm^3 are also included.

Regardless of the type of container and initial yeast concentration, the CO_2 contents in the headspaces of the samples changed in a similar manner in the first 30 hours of monitoring, that is, CO_2 content slightly and linearly increased from the initial value of 1–3% to about 6%. From that moment, the CO_2 content increased faster reaching the level of 17–20% CO_2 at the end of the monitoring. Slightly slower increase in CO_2 content was noticed only in the 500 g bottles contaminated at the initial yeast level of 1 CFU/cm^3, while in all other samples, regardless of the type of container or initial yeast number, the changes in the content of CO_2 are similar (Figure 2(a)).

Table 1: Values (±SD) of pH, CO_2 content, and number of lactic acid bacteria (LAB) and yeast in yogurt not-contaminated (Ø) and contaminated with the yeast *Candida kefyr* (initial cell concentrations 1 and 5 CFU/cm^3), and packed in cups and bottles at the beginning (0) and the end of monitoring (end).

		Cup, 180 g		Bottle, 500 g			Bottle, 1000 g		
		Initial concentration (CFU/cm^3)							
	t, h	Ø	5	Ø	1[23]	5	Ø	1	5
pH	0	4.3 ± 0.04			4.4 ± 0.05			4.4+0.07	
	End	4.2 ± 0.09	4.2 ± 0.09	4.3 ± 0.04	4.3 ± 0.04	4.3 ± 0.03	4.3 + 0.05	4.3 ± 0.05	4.2 ± 0.1
LAB* (log·CFU /cm^3)	0	6.4 ± 1.1			6.8 ± 1.3			6.1+0.4	
	End	6.1 ± 0.8	5.5 ± 0.6	5.7 ± 0.3	5.9 ± 0.6	5.6 ± 0.8	7.2 + 0.6	6.2 ± 0.4	7.1 ± 0.5
Yeast** (log·CFU /cm^3)	0	—	0.7	—	0	0.7	—	0	0.7
	End	2.1 ± 0.9	6.7 ± 0.5	2.9 ± 0.4	6.2 ± 0.3	6.1 ± 0.2	1.9 + 0.9	6.1 ± 0.6	5.8 ± 0.3
CO_2 content (%)	0	3.6 ± 0.5	2.4 ± 0.2	3.3 ± 0.4	2.3 ± 0.3	2.1 ± 0.5	3.6 + 0.5	2.1 ± 0.3	1.9 ± 0.4
	End	5.6 ± 0.9	17.7 ± 0.2	6.2 ± 0.3	16.2 ± 0.6	18.6 ± 0.2	5.3 + 0.2	20.1 ± 0.5	18.6 ± 0.5

*Determined on MRS agar, **determined on SMA agar with chloramphenicol.

The number of yeast changed similarly regardless of the initial number and the type of the container (Figure 2(b)). Linear increase in the yeast number was noticed during the first 40 h of incubation from about 2 log·CFU/cm^3 (after 10 h of incubation) to about 6-7 log·CFU/cm^3 after 40 h and then remained nearly on the same level till the end of monitoring. Higher yeast number was detected in cups in the period 18–28 h, but after that the number is in accordance with the rest of samples (Figure 2(b)). The maximum yeast number determined in all samples was in accordance with the previous investigation which reported increase in the number of psychotropic yeasts to 6 log·CFU/g after 2, 10, and 20 days for the samples stored at 25, 15, and 5°C, respectively [21].

During the period of incubation of control samples under the same conditions, the CO_2 content slightly and linearly increased to about 5-6% (Table 1) and the containers were not bloated. The yeast could not be detected by the applied method in the control yogurt samples at the beginning and after 10 h of incubation, but after certain time of incubation, the yeast concentration increased to the detectable level due to increased temperature of the incubation. At the end of monitoring period, the detected yeast concentration was in the range of 1.9–2.9 log·CFU/cm^3 (Table 1).

The LAB microbiota seemed not to be influenced by the presence of yeast since the number of LAB was not significantly changed during the incubation of contaminated and not-contaminated yogurt (Table 1). The LAB number during the conduction of the experiment was in the range of 5.5–7.2 log·CFU/cm^3 in all samples which is in accordance with number of lactic acid bacteria normally present during the storage of the yogurt [22]. Also, the pH values in the samples were generally unchanged during the period of the experiment, ranking 4.2–4.3 which is in compliance with the literature [22].

The concentration of CO_2 in the headspace of a package at the beginning of measurement was 1.9–3.6% (Table 1), which is much higher than the atmospheric concentration of 0.03% CO_2 probably because of the establishing of equilibrium with the product itself. Dissolved CO_2 depends on the partial pressure of the CO_2 and the available amount of

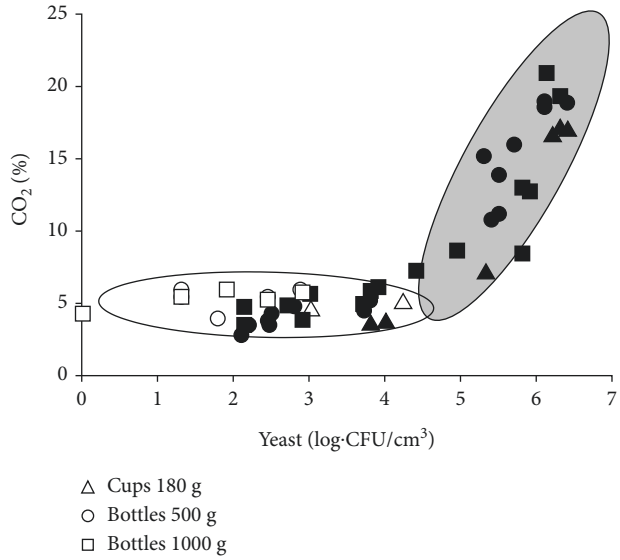

Figure 3: The relations between averaged values of CO_2 content in headspace and yeast level in the yogurt ("Vedro," 2.8% milk fat) packed in PVC cups (180 g) and the PVP bottles (500 g, 1000 g) during incubation (30°C) of not-contaminated (open symbols) and contaminated with the yeast *Candida kefyr* (black symbols) samples. Prediction area ellipses were defined by the coefficient of 90%.

CO_2. The CO_2 amount in the headspace is in relation to the headspace volume and the CO_2 concentration [23]. Compared to the quantity of yogurt (180 g, 500 g, and 1000 g), the volume of headspace in the packaging is small (about 25 cm^3 of air).

Based on the averaged values of the data presented in Figure 2, the relations between CO_2 content in the headspace of the packages (cups and bottles of different volume) and the number of yeasts (regardless of the initial level of contamination) can be observed. The relations could be divided into two areas (Figure 3). The presence of yeasts at the level of up to 5 log·CFU/cm^3 induced the accumulation of CO_2 in the range of 3–7% in the headspace of the yogurt containers (open ellipse on Figure 3) regardless of the duration of the incubation. On the contrary, at yeast counts higher than about 5 log·CFU/cm^3, there was a significantly

greater accumulation of CO_2 content above yogurt (grey ellipse in Figure 3). Since the contamination was done with the yeast isolated from spoiled yogurt, we presumed that the same strain caused the accumulation of CO_2 in contaminated and not-contaminated samples. The similar level of yeast (10^5–10^6 CFU/g) has been reported to be the cause of yeasty and fermented off-flavours and gassy appearance in yogurt [18].

According to the results, the CO_2 content above 6% can be considered critical for microbial spoilage since at this point the level of detected number of yeast is significant. This confirmed our previous state [20, 24] that this content of CO_2 in the headspace of fermented milk products is an indicator of possible contamination.

4. Conclusions

Contamination with yeasts, which induces the increase in the CO_2 content, often creates the largest microbiological problem in dairy products. Therefore, CO_2 measurement could be used as an indication of dairy products spoilage. Scarce literature data for the determination of the CO_2 content in dairy products increase the novelty of the conducted investigation with the use of TDLAS technology for measuring CO_2 content. Also, data indicated the correlation between the content of developed and accumulated CO_2 in the headspace of yogurt packages and the presence of contamination by yeasts. Since measuring of CO_2 content is much easier and more precise than yeast number determination, this efficient and contactless technique for monitoring the CO_2 content represents a simple non-destructive method for the detection of yogurt yeast contamination. The determined CO_2 content out of predefined ranges could refer to inadequate control or storage conditions during yogurt production and distribution. So, the TDLAS technique shows to be a promising novel approach in designing instruments for the CO_2 content measuring in the headspace of yogurt packages.

Data Availability

All data arising from this study are contained within the manuscript.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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