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# Formation and Cumulation of $CO_2$ in the Bottles of the Fermented Milk Drinks

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**Abstract.** As in majority of food during storage, the development of $CO_2$ in headspace of yogurt is mainly the result of contamination and yeast growth. Thitherto, the monitoring of the changes of $CO_2$ concentration in the yogurt package's atmosphere can be an indicator of the product quality and safety. The aim of this paper is to determine the accumulation of the $CO_2$ in the headspace of bottles of fermented milk products: yogurt ''Vedro 2.8% milk fat'' and fermented milk drink with probiotics ''FidoBe 0.5% milk fat'', both produced in ''Niška mlekara'', AD Niš, Serbia. To examine these phenomena, the products were incubated at 30$^{o}$C and contaminated with yeast at the levels of 1 and 5 CFU/ml. The strain of yeast used for the contamination was isolated from the spoiled yogurt. During the incubation, the $CO_2$ measurements were continuously performed with a device specially constructed for this purpose. The instrument was based on Tunable Diode Laser Absorption Spectroscopy with a Wavelength Modulation Spectroscopy technique. The number of lactic acid bacteria and the number of yeasts were monitored, too. Also the pH value was measured.

The content of $CO_2$ in the headspace of contaminated (initial yeast concentration -1 CFU/ml) bottles of yogurt ''Vedro'' slightly increased from the start value to cca 5,0% at the 30th hour of incubation reaching yeast level of cca 4 logCFU/ml. After that, the $CO_2$ content and the number of yeasts increased faster until the 40th h and reached the values of cca. 16% and 6 logCFU/ml, respectively.

During the incubation of the contaminated bottles of fermented milk drink ''FidoBe'', the $CO_2$ level slightly increased from the initial cca 1% to cca 5% during 36 h in bottles contaminated with 1 CFU/ml, and after 30 h in the bottles contaminated with 5 CFU/ml. Hereupon, the increase of $CO_2$ content was faster reaching 20% after 40h in experiment with the initial 5 CFU/ml yeasts, and after 48h in experiment with the initial 1 CFU/ml of yeast. During the incubation, the yeast level increased until the final level of 6.0 logCFU/ml.

**Keywords:** TDLAS, carbon dioxide, headspace, yogurt.

## 1. Introduction

Processed and raw foods can frequently be contaminated with different pathogens and spoilage microorganisms. So, the determination of the critical control points in factories and automated control systems is essential in order to achieve elimination or minimization of contamination [1]. The shelf life of fermented milk products (FMP) is restricted to the time the product remains safe to eat and its sensory properties remain acceptable to consumers. Fresh yogurt is the best in the period of first few weeks of shelf life and after that, the reduction in the sensory characteristics occurs [2]. The storage period which ensures the constant sensory characteristics under refrigerating conditions for probiotic yogurt is about 3 weeks [3]. Spoilage is, primarily, a consequence of the growth of microorganisms which survive thermal treatment and/or postprocessing microbial contamination [2].

Despite the low pH, FMP are susceptible to microbial contamination, especially by fungi which grow and reproduce in acidic environments. Yeast and moulds are shown to be the determinant of the shelf life of

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pasteurized cream and their count can be linked to doming of yogurt containers [4]. Despite the off-flavour and changes in visual appearance of dairy products, fungal contamination can lead to the significant economic losses [5]. Additionally, the introduction of fruit into yogurts can amplify the risk of spoilage by yeasts by providing additional sources of contamination and fermentable substrates. As a consequence, the control of yeasts contamination has become one of the main concerns of FMP producers. Since the yeast generally produce $CO_2$ during their growth on fermentative substrates, the spoilage of yogurts by yeasts can be recognized by the swelling and eventual blowing off of the container. Hereof, indirect measurement of gas content in headspace of FMP could be an indicator of contamination and refer to unsafe product.

The measurement of gas content in the headspace of food products has been already applied in order to monitor gas mixtures in packages with MAP. A simple non-destructive and contactless technique based on Laser Absorption Spectroscopy has been applied in Soligo dairy (Latteria di Soligo Societ à Agricola Cooperativa, Italy) for in-line measuring of oxygen content in the headspace of bags with Mozzarella cheese [6].

In order to enable research within the ''Packsensor'' joint Italian-Serbian project regarding the shelf life of the dairy products and contamination in the FMP packages, a device for $CO_2$ measurement was constructed based on Tunable Diode Laser Absorption Spectroscopy (TDLAS) in the Wavelength Modulation Spectroscopy (WMS) variety [7], [8]. The device is designed to perform $CO_2$ concentration measurement in the headspace of the packages on non-destructive manner without compromising the seals.

The aim of this paper is to determine the releasing and accumulation of the $CO_2$ in the headspace of bottles of FMP. To examine the phenomena, the products were contaminated with the yeast isolated from spoiled yogurt. The measurements of $CO_2$ were continuously performed with a device specially constructed for this measurements.

# 2. Experimental

## 2.1. Fermented Milk Products

The experiment were conducted with liquid yogurt ''Vedro 2.8% milk fat'' and fermented milk drink with probiotics ''FidoBe 0.5% milk fat'', both produced in ''Niška mlekara'', AD Niš, Serbia, and bottled in 500 ml bottles.

## 2.2. Microorganism

The strain of yeast used for the contamination of FMP was isolated from spoiled yogurt (storage one month at room temperature) and the identification is in progress. The number of yeasts was detected on SMA medium (''Torlak'', Serbia). The number of lactic acid bacteria was determined on MRS medium (''Torlak'', Serbia).

## 2.3. $CO_2$ Measurement

The instrument for the detection and evaluation of gaseous $CO_2$ in the headspace of closed containers is based on Tuneable Diode Laser Absorption Spectroscopy with a Wavelength Modulation Spectroscopy technique in order to accomplish a non-invasive measurement inside closed containers. The instrument's optical front end has been designed to be integrated in a thermally stabilized incubator (Fig. 1).

## 2.4. Experimental Conditions

The bottles were obtained directly from the Dairy's product line and were contaminated with the yeast strain at the level of 1 CFU/ml (''Vedro'') and 1 CFU/ml and 5 CFU/ml (''FidoBe''). Also, a couple of bottles were separated as controls (not contaminated). The Device was placed and all measures were done in the incubator at 30$^{o}$C. During the night, the one sample was put on the Device, and the measurement program was set to do 3 measurements every 15 minutes. If the change of $CO_2$ content determined in the headspace of the bottles was significant, one of the bottles was taken out of the incubator and opened for determination the number of lactic acid bacteria and number of yeasts. The pH value was measured using the "Hanna hi 92240" pH meter.

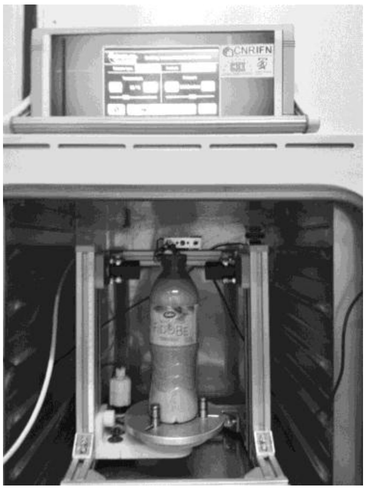

Fig. 1: The laboratory device constructed for non-destructive measurement of $CO_2$ content in packages of fermented milk products.

## 3. Results and Discussion

Fig. 2 shows the changes of $CO_2$ content in headspaces and the number of yeasts in the contaminated with 1 CFU/ml and no contaminated liquid yogurt ‘’Vedro, 2.8% milk fat’’. The initial values of $CO_2$ in contaminated bottles were lower (1.5-2.0%) than in control bottles (3.0-4.0%) since the control bottles were not opened to be contaminated. During next 38 h of incubation, the $CO_2$ content in the control bottles slightly and linearly increased to cca 5% and the bottles were not bloated. The yeast could not be detected in the control yogurt samples at the beginning and after 11h of incubation, but at the end of monitoring, the yeast were detected at the level of 2.9 logCFU/ml. One control bottle showed higher level of $CO_2$ (7,3%) as a consequence of presence of higher number of yeasts (4.9 logCFU/ml on Fig. 2B). The high and inadequate temperature of the yogurt storage (30$^o$C) caused the growth of yeasts and spoilage of one control bottle with the increase of the content of $CO_2$ in the headspace (the rest control bottles were not affected during 40 h of incubation).

In the bottles contaminated at the initial level of 1 CFU/ml, the content of $CO_2$ slightly increased from start value to cca 5,0% at the 30th hour of incubation reaching yeast level of cca 4 logCFU/ml. After that, the $CO_2$ content and the number of yeast increased faster until the 40th h and reached cca. 16% and 6log CFU/ml, respectively (Fig. 2A).

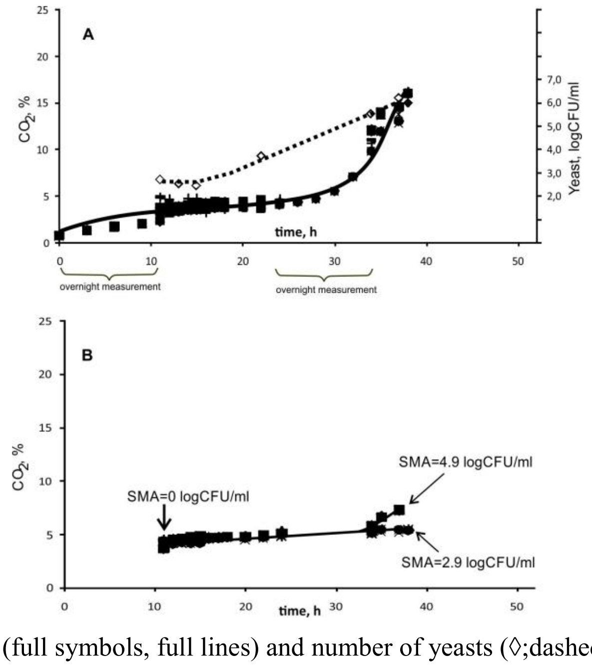


Fig. 2: Changes of $CO_2$ content (full symbols, full lines) and number of yeasts (◊;dashed line) during incubation (30$^o$C) of contaminated (1 CFU/ml) with yeast (A) and no contaminated (B) yogurt (Vedro, 2.8% milk fat, PET bottles, 500 ml). Different full symbols represent different bottles.

During the experiment with contaminated yogurt, the number lactic acid bacteria slightly decrease from the initial 8.0 logCFU/ml to the final cca 6.0 logCFU/ml. The pH of yogurt decreased from initially 4,5 to 4,1-4,2 after 12 hours of incubation and then remain same to the end of monitoring.(data not shown)

Another FMP with probiotics ''FidoBe'' was also contaminated, but at two initial levels (1 CFU/ml and 5 CFU/ml). During the incubation of the contaminated bottles, the $CO_2$ level slightly increased from the initial cca 1% to cca 5% during 36 h in the bottles contaminated with 1 CFU/ml (Fig. 3A), and 30 h in bottles contaminated with 5 CFU/ml (Fig. 3B). After that, the increase of $CO_2$ content was faster reaching 20% after 40h in experiment with initial 5 CFU/ml yeasts, and after 48h in experiment with initial 1 CFU/ml of yeast. In the bottles contaminated with 1 CFU/ml the $CO_2$ content continued to increase, while in one bottle contaminated with 5 CFU/ml remained on the level of 20%. This is probably because in the later bottle $CO_2$ leak out. During the incubation, the yeast level increased until final level of 6.0 logCFU/ml (Fig. 3B).

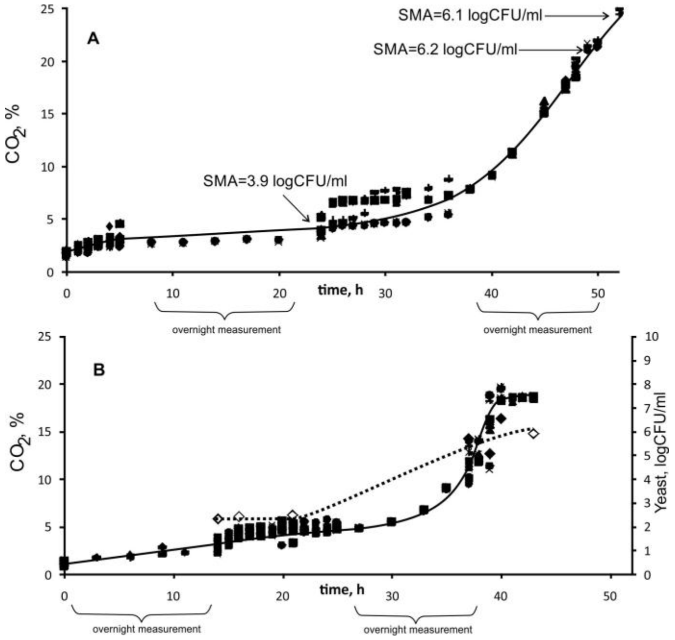


Fig. 3: Changes of $CO_2$ content (full symbols, full lines) and number of yeasts (◊;dashed line) during incubation (30$^oC$) of contaminated with 1 CFU/ml (A) and 5 CFU/ml of yeast (B) fermented drink with probiotics (''FidoBe'', 0.5% milk fat, PET bottles, 500 ml). Different full symbols represent different bottles.

Experiments with not contaminated (control) ''FidoBe'' bottles showed that $CO_2$ content slightly and linearly increased from the initial cca 2% to final cca 7% after 86 hours of incubation. The yeasts were not detected at the beginning of the experiment, but after 37h they were first detected at the level of 1,7 logCFU/ml and at this level remained to the end of monitoring. So, this level of yeast continuously increased the content of $CO_2$. (data not shown).

The results of the examining of the $CO_2$ content in the headspace of two contaminated FMPs, showed that liquid yogurt ''Vedro'' supposed to be more sensitive to the yeasts contamination. Comparing the two products contaminated with the same initial number of the yeast (1 CFU/ml), the ''FidoBe 'needed cca 36 hours of incubation at 30$^oC$ to accelerate the $CO_2$ increase (Fig. 3A), while in liquid yogurt ''Vedro'' the $CO_2$ content started to increase rapidly after cca 30 h (Fig. 2A). Also, the contamination of the ''Vedro'' on the level of 1 CFU/ml caused similar changes of $CO_2$ contents and the number of yeast compared to ''FidoBe'' initially contaminated at the level of 5 CFU/ml. The explanation for this occurrence could be in the addition of some probiotics during the production of ''FidoBe'' which can have some antimicrobial effects on the contaminants. The antifungal activity of some probiotic strains could be linked to the production of organic acids or other compounds like bacteriocins [9], [10].

Results of the investigation of the growth of yeast and moulds in concentrated yogurt indicated that the number of psychotropic yeasts increased to 6 log CFU/g after only two days storage at 25 $^oC$ [4]. The same number of psychotropic yeasts was achieved after 10 and 20 days for the samples stored at 15 and 5 $^oC$, respectively.

The initial values of $CO_2$ in the headspace of the bottles of both examined FMPs were surprisingly much higher than atmospheric (0,03%). This can be explained by the complex processes during an equilibrium being established between the headspace in a package and the products. According to Hotchkiss et al [11], the amount of $CO_2$ dissolved in water is governed by the partial pressure of the $CO_2$ above the water as well as the amount of $CO_2$ available, which is determined by both the volume of the headspace and the concentration of $CO_2$ in that headspace. In the bottles of FMPs, there is a little headspace (fewest ml of air) comparing with the volume of the products (500 ml).

## 4. Conclusions

For the authors knowledge, there is scare literature data about the determination of the $CO_2$ content in FMPs. Since the yeasts contaminants are primarily responsible for the accumulation of $CO_2$, the measurement of this gas content could be good indicator for monitoring the FMPs spoilage.

The investigation of the use of the instrument based on TDLAS for measuring $CO_2$ content in the headspaces of FMPs packages indicate the correlation between content of formatted and accumulated $CO_2$ and the presence of contamination by yeasts. In particular, $CO_2$ content in headspaces above 6% can be considered as critical for microbial spoilage since it represents the starting point of the rapid growth of yeasts. So, introducing simple and non-destructive method for measuring the $CO_2$ content in the headspaces of packages could be a solution for effective and sufficient indirect determination of the presence of yeast contamination in FMPs. This measuring could be the indicator of inappropriate production process or storage of FMPs which can be helpful in the prevention of the economic looses. The device constructed on a base of Laser Absorption Spectroscopy showed to be a promising possibility for continuous measuring and monitoring of the $CO_2$ content in the FMPs packages.

## 5. Acknowledgements

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