

EFFECT OF ADDITIVES TO MICROBIOLOGICAL QUALITY OF YOGURTS

Roman Pytel, Olga Cwíková, Sylvie Ondrušíková, Šárka Nedomová, Vojtěch Kumbár

ABSTRACT

The objective of this work was to study the effect of addition chia flour, quinoa flour, nopal powder, apple fibre and bamboo fibre BAF 40 in yogurt to microbiological quality. Yogurts were made with 1, 3 and 5% of addition of these additives. The milk used for manufacturing was heated up to 85 °C for 5 min and flour, powder and fiber were irradiated for 20 min in three replicates. It was monitored: Lactic acid bacteria (LAB) – 72 hours at 30 °C (ISO 13721:1998) and yeasts and moulds – 5 days at 25 °C (ISO 21527-1:2009). During storage, the number of LAB was increased to match the initial concentration of yogurt with addition chia flour (concentration 1, 3, 5%) and quinoa flour (1%). The addition of nopal powder, apple or bamboo fiber to yogurt showed a tendency to decrease the number of LAB compared with its initial concentration. All samples were compared with the control yogurt without addition whatever flour, powder or fiber. The amount of yeasts and moulds was increased with the increasing addition of fiber in yogurts. The lowest amount of yeasts and moulds was in yogurt with the bamboo fiber. On the other hand the highest amount was in yogurt with chia flour.

Keywords: chia; quinoa; nopal; fiber; dairy product

INTRODUCTION

The term yogurt encompasses a wide range of products. Yogurt is a fermented dairy product, which is generally manufactured from pasteurized milk. High-temperature pasteurization of the yogurt mix is employed to obtain a smooth and firm body. Non-fat dry milk or stabilizers may also be added to increase the water-holding capacity and therefore improve its body (Marth and Steele, 2001). Yogurt is manufactured from the milk and common commercial cultures composite from *Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus*. The microorganisms used in the production of yogurt accomplish briefly two tasks: production of lactic acid and flavour components (Yildiz, 2010). Regarding the chemical composition of milk and yogurt, there is no significant difference between gross composition of milk and fermented milk. However, the fermentation process causes a beneficial effect on yogurt (Walstra et al., 2006). Fermentation is carried out by bacteria, moulds and yeasts which produced the enzymes. These enzymes caused that organic substances are broken down to smaller compounds. As a result, these processes cause that milk is more digestible, stable and flavoured (Yousef and Carlstrom, 2003). The addition of oat fiber did not significantly influence fermentation time, pH evolution, or orotic acid consumption by the starter bacteria during fermentation (Fernández-García et al., 1998).

Orange fibers presence in fermented camel milks also enhanced bacterial growth and survival of probiotic bacteria (Ibrahimand and Khalifa, 2015). Food fortification is one of the processes which have influence to increase food quality and quantity. Fortification of yogurt is very effective because consumption rate of dairy products such as yogurt is very high (Hashemi Gahrue et al., 2015). More authors add the fiber to yogurts but most of these works follow up rheological properties and sensory profiles as Staffolo et al. (2004). They studied the effect of apple, wheat, bamboo fibers, or inulin on sensory and rheological properties of yogurt. The chia seed is good source of valuable protein fraction and antioxidant compounds (Kačmárová et al., 2016). Chia seed is the best known plant source to maintain a balanced serum lipid profile (Nitrayová et al., 2014). According Remeňová et al. (2017) yogurt with addition of pressed flax seed and honey can have beneficial effects on human body, but addition of pressed flax had no effect on sensory properties of yogurt. Hashim et al. (2009) shown, that fortifying yogurt with 3% of date fiber produced an acceptable product with potential beneficial health effects.

The set yogurts are more safe from the microbial aspect than stirred yogurt. The set yogurt fermented at higher temperature and shorter time, producing more lactic acid and thus prohibited the growth of contaminating

microbiota eg. coliforms. The stirred yogurt fermented at lower temperature and longer time, the fermentation is slower and thus allowed a growth of mesophilic and coliforms bacteria (Görner and Valík, 2004). According to Decree no. 397/2016 Coll. yogurt must contain a minimum 7 log CFU.g⁻¹ and must be made from protosymbiotic mixture of *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*.

Moulds contamination is almost exclusively caused by Mucoraceae family which had very strong proteolytic and lipolytic activity which leads to intensive odour. The spores of Mucoraceae are spreading very rapidly (Holec et al., 1989). *Saccharomyces cerevisiae* and *Kluyveromyces fragilis* are one of the most common yeasts which changed the taste of yogurt. The typical yogurt which is contaminant by yeasts is yeast taste and bubble in the coagulum. These changes are most often seen in flavoured yogurt (Görner and Valík, 2004). If the yogurt is by the “good manufacturing practice”, it should contain no greater than 1 yeast cell.g⁻¹. When products are correctly stored in refrigerator (5 °C), the shelf life of this yogurt is 3 or 4 weeks (Suriyarachchi and Fleet, 1981). Presence of yeasts or moulds in yogurt also indicates poor sanitary practices in manufacturing or packaging. Yogurts with added sugar or fruits especially are susceptible to yeast growth (Arnott et al., 1974) but the consumption of flavoured cream yogurts is increases (Habánová et al., 2010).

Scientific hypothesis

There was monitored the effect of addition chia flour, quinoa flour, nopal powder, apple fibre and bamboo fibre BAF 40 in yogurt to Lactic acid bacteria (LAB) and yeasts and moulds during the storage.

MATERIAL AND METHODOLOGY

This research was carried out at the Department of Food Technology at Mendel University in Biotechnology Pavilion M, financed by the OP VaVpl CZ.1.05/4.1.00/04.0135 project.

The yogurt for the research was prepared from milk of Holstein dairy cows from South Moravia region. The bovine milk content: 3.50% of fat by Gerber's acidobutyrometric method (ISO 2446:2008), 3.42% of protein by Kjeldahl's method (EN ISO 8968-1:2002), 4.50% of lactose and titratable acidity 6.7 SH according Czech state standart no. 57 0530 (1974). The milk was heated up to 85 °C for 5 min and then cooled down to 36 °C. Then cooling was added into pasteurized milk of starter for making original Bulgarian yogurt (*bulgaricus*.cz, GENESIS LABORATORIES, Bulgaria). It was used a lyophilized started for preparing original Bulgarian yogurt. Before used was prepared the starter (1 g of this starter was inoculated in 1 L of milk), after fermentation was added 2% of this prepared starter. The addition of starter to the milk was such that the resulting concentration in the yogurt was 8 log CFU.g⁻¹. This mixture was fermented at 36 °C for 18 hours. After fermentation, the coagulum was stirred for 5 min and divided into 16 groups. In these groups were added chia flour, quinoa flour, nopal powder, apple fiber and bamboo

fiber. Each addition was made from three concentrations: 1, 3 and 5% of addition of these fibers. Before the addition, fibers in yogurt were irradiated for 20 min in three replicates. One group of yogurt was made as natural (control). The samples of final yogurt were stored at the 4 °C for 28 days.

The microbiological analysis was carried out in microbiological lab at the Department of Food Technology at Mendel University. Samples were taken from three different crucibles.

For all samples were determined: Lactic acid bacteria (LAB) – 72 hours at 30 °C (ISO 15214:1998) and yeasts and moulds – 5 days at 25 °C (ISO 21527-1:2009).

The 1st analysis was carried out 24 hours after yogurt manufacturing, 2nd (7 days after storage), 3rd (14 days after storage), 4th (21 days after storage), 5th (28 days after storage).

Statistic analysis

The results were statistically processed by program MS EXCEL version 2010 (Microsoft) and STATISTICA CZ version 12 (StatSoft, Czech Republic). It was used: the calculation of basic statistical parameters, the simple sorting method of analysis of variance (ANOVA, Duncan's test) and regression analysis.

RESULTS AND DISCUSSION

Chia flour addition

The control yogurt had 8.6 log CFU.g⁻¹ at the start and, during the storage, the amount of LAB has fallen to 7.9 log CFU.g⁻¹. The amount of lactic acid bacteria was higher than is given by legislation (more than 7 log CFU.g⁻¹ in Decree no. 397/2016 Coll). According the Lengyelová et al., 2010 the amount of LAB in tested yogurt was higher than Slovak Food codex limits. The addition 1, 3 or 5% of chia flour had influence to lower amount of LAB than in the control yogurt at the start sampling, one week later the amount of LAB was higher than amount of LAB in control yogurt. The third week sampling the amount of LAB was reduced, the tendency for bacterial growth therefore decreased as well as in the control yogurt (Figure 1). There was no statistically significant difference ($p > 0.05$) between addition of 1, 3 or 5% of chia flour to amount of LAB into yogurt.

The total of yeasts and moulds in control yogurt during three weeks storage were undetectable. The amount the yeasts and moulds was higher than 2 log CFU.g⁻¹ in 1% addition in yogurt. The addition of 3% of chia flour in the yogurt had influence on total amount of yeasts and moulds in yogurt. After three weeks of storage, the amount of yeasts and moulds was higher than 3.2 log CFU.g⁻¹. This amount is comparable with 3.5 log CFU.g⁻¹ which was detectable in the yogurt with 5% addition of chia flour after three weeks of storage (Figure 2). The shelf life of yogurt with chia flour was affected with high contamination of chia flour by the yeasts and moulds. The shelf life of these yogurts was 2 weeks. This storage time is shorter than presented Suriyarachchi and Fleet, 1981. The total amount of yeasts and moulds were increased during all time of storage. These changes were statistically significant ($p < 0.05$).

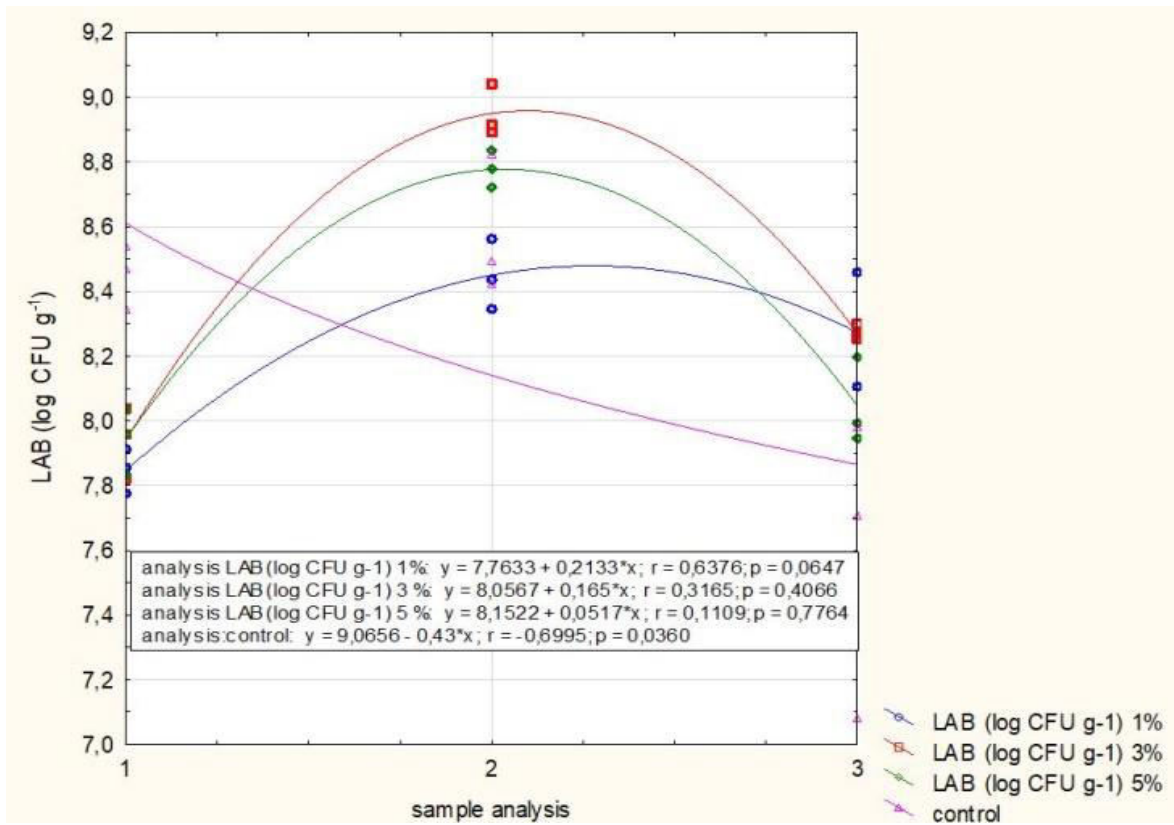


Figure 1 Changes in the amount LAB (log CFU.g-1) during the storage – analysis: 1st (24 hours after yogurt manufacturing), 2nd (7 days after storage), 3rd (14 days after storage) in yogurt with chia flour.

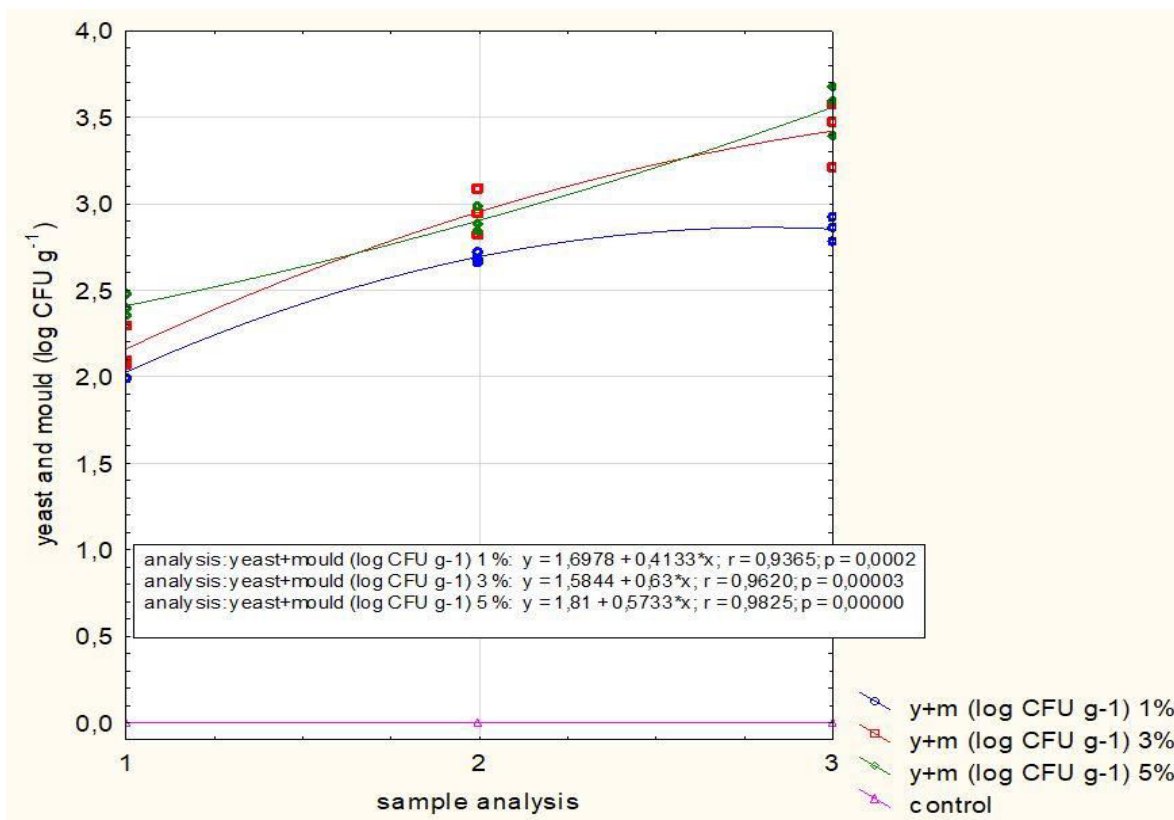


Figure 2 Changes in the amount yeasts and moulds (log CFU.g⁻¹) during the storage – analysis: 1st (24 hours after yogurt manufacturing), 2nd (7 days after storage), 3rd (14 days after storage) in yogurt with chia flour.

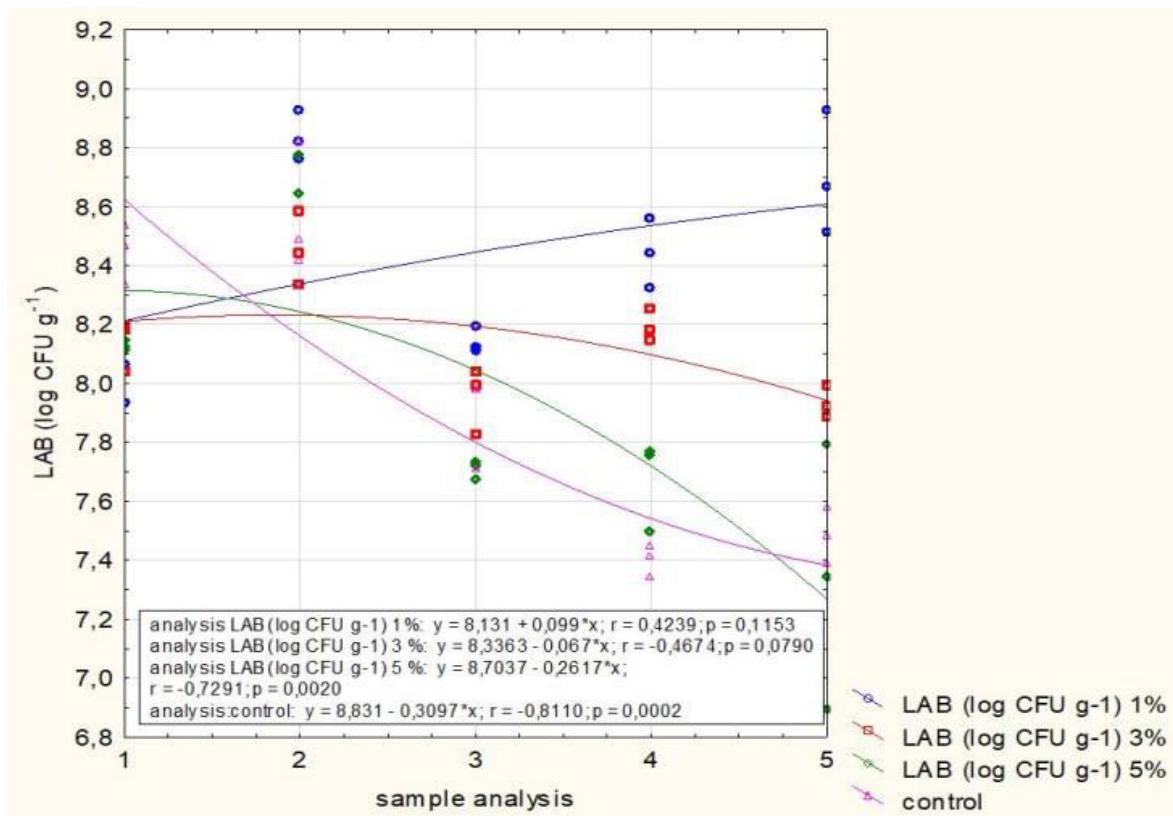


Figure 3 Changes in the amount LAB (log CFU.g⁻¹) during the storage – analysis: 1st (24 hours after yogurt manufacturing), 2nd (7 days after storage), 3rd (14 days after storage), 4th (21 days after storage), 5th (28 days after storage) in yogurt with quinoa flour.

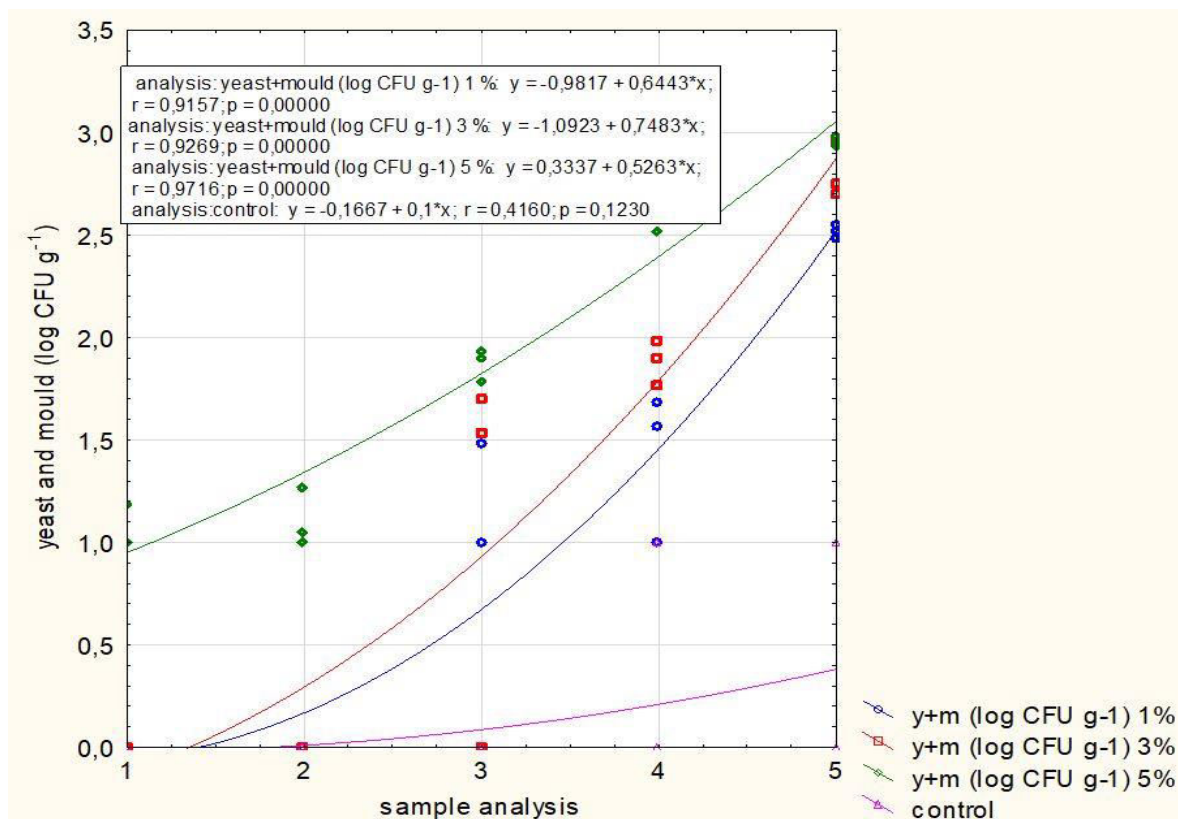


Figure 4 Changes in the amount yeasts and moulds (log CFU.g⁻¹) during the storage – analysis: 1st (24 hours after yogurt manufacturing), 2nd (7 days after storage), 3rd (14 days after storage), 4th (21 days after storage), 5th (28 days after storage) in yogurt with quinoa flour.

Quinoa flour addition

The addition 1% of quinoa flour in yogurt caused that during the storage, the amount of LAB has increased (Figure 3). In the beginning, the amount of LAB was $8.2 \log \text{CFU.g}^{-1}$ and at the end of shelf life, the amount of LAB was $8.6 \log \text{CFU.g}^{-1}$. Addition of 3 or 5% caused that the amount of LAB has fallen during storage. With addition of 3% quinoa flour, the amount of LAB decreased from $8.2 \log \text{CFU.g}^{-1}$ to $7.9 \log \text{CFU.g}^{-1}$. When there was added more quinoa flour (5%), that caused bigger decrease from the $8.3 \log \text{CFU.g}^{-1}$ to $7.3 \log \text{CFU.g}^{-1}$. Saponins are bitter compounds that are naturally present in quinoa located in the outer layers of quinoa seeds. The content saponin in quinoa is in range 0.1 to 5.0% (Valencia-Chamorro, 2003). This bigger decrease of LAB in yogurt can be caused by higher content of saponins. This decrease was statistically significant ($p < 0.05$).

There was no statistically significant difference between the amount of LAB in control and 5% addition quinoa flour in yogurt ($p > 0.05$). But the statistically significant difference ($p < 0.05$) was between 1 and 3% of quinoa flour added in yogurt.

The total amount of yeasts and moulds in control yogurt during the whole storage time was very low (less than $0.4 \log \text{CFU.g}^{-1}$ after five weeks of storage). This change was statistically insignificant ($p > 0.05$).

The amount the yeasts and moulds at the beginning was not detected. The amount of yeasts and moulds was increased to $2.5 \log \text{CFU.g}^{-1}$ in yogurt with 1% of quinoa flour addition, respectively $2.9 \log \text{CFU.g}^{-1}$ in yogurt with 3% of quinoa flour addition during the storage. The most yeasts and moulds were in yogurt with 5% addition of quinoa flour. At the beginning was $1 \log \text{CFU.g}^{-1}$ yeasts and moulds in yogurt and, after five weeks of storage, the amount of yeasts and moulds increased to $3.1 \log \text{CFU.g}^{-1}$. There was statistically significant growth trend ($p < 0.05$) of yeasts and moulds in yoghurt with 1, 3 or 5% quinoa flour addition (Figure 4). The least yeasts and moulds were in the control yogurt, while the most yeasts and moulds were in yogurt with 5% quinoa flour added regardless of the other observed factors (storage time).

Nopal powder

The control yogurt had $8.6 \log \text{CFU.g}^{-1}$ at the start and, during the storage the amount of LAB has fallen to $7.4 \log \text{CFU.g}^{-1}$. The amount of lactic acid bacteria was higher after five weeks of storage than is given by legislation – more than $7 \log \text{CFU.g}^{-1}$ (Decree no. 397/2016 Coll.). At the beginning was amount of LAB lower in yogurt with 1, 3 or 5% nopal powder addition, but after 1 week of storage was the amount of LAB higher than the amount of LAB in control yogurt. After one week of storage the amount of LAB decreased also in the control yogurt. The tendency for bacteria growth decreased. For yogurt with 5% nopal powder addition, there was a typical, almost constant decline of the amount of LAB during storage (Figure 5). There was no statistically significant difference ($p > 0.05$) between the

control yogurt and yogurt with 1, 3 or 5% nopal powder addition to amount of LAB in these yogurts regardless of the other observed factors (storage time).

The change of the amount of yeasts and moulds in control yogurt was not statistically significant ($p > 0.05$). The amount of yeasts and moulds at the beginning was not detected regardless of the nopal powder addition. The amount of yeasts and moulds was increased to $2.5 \log \text{CFU.g}^{-1}$ in yogurt with 1% nopal powder addition, respectively $2.3 \log \text{CFU.g}^{-1}$ in yogurt with 3% nopal powder addition during the storage. The most yeasts and moulds were in yogurt with 5% nopal powder addition. At the beginning were not detected yeasts and moulds in yogurt and after five weeks of storage was amount of yeasts and moulds increased to $3.5 \log \text{CFU.g}^{-1}$. There was statistically significant growth trend ($p < 0.05$) yeasts and moulds in yogurt with 1, 3 and 5% nopal powder addition (Figure 6). The least yeasts and moulds were in the control yogurt, while the most yeasts and moulds were in yogurt with 5% nopal powder addition, regardless of the other observed factors (storage time).

Apple fiber

The control yogurt and yogurts with apple fiber addition had at the beginning more than $8 \log \text{CFU.g}^{-1}$ of LAB. Yogurt with 1% ($8.5 \log \text{CFU.g}^{-1}$) and 5% ($8.3 \log \text{CFU.g}^{-1}$) apple fiber addition showed that amount of LAB in these yogurts was decreased until the third sampling when the amount of LAB was increased. At the end of sampling was in yogurt with 1% apple fiber addition $8 \log \text{CFU.g}^{-1}$ and yogurt with 5% apple fiber addition $7.8 \log \text{CFU.g}^{-1}$. On the other hand, the yogurt with 3% apple fiber addition showed the opposite trend. The amount of LAB was increased until the third sampling and, after the third sampling, the amount of LAB has decreased (Figure 7). Staffolo et al. (2004) there were found the highest differences between control and yogurt with apple fiber in rheological and sensory characteristics.

There was no significant difference ($p < 0.05$) between control yogurt and yogurt with 1, 3 or 5% apple fiber addition to amount of LAB in these yogurts regardless of the other observed factors (storage time).

The amount of yeasts and moulds at the beginning was not detected regardless of the apple fiber addition. The amount of yeasts and moulds increased to $1.9 \log \text{CFU.g}^{-1}$ in yogurt with 1% apple fiber addition, $2.6 \log \text{CFU.g}^{-1}$ in yogurt with 3% apple fiber addition and $3.9 \log \text{CFU.g}^{-1}$ yogurt with 5% apple fiber addition after five weeks of storage. With increasing the addition of apple fiber has increased the amount yeasts and moulds in yogurts. There was statistically significant growth trend ($p < 0.05$) of yeasts and moulds in yoghurt with 1, 3 and 5% apple fiber addition (Figure 8). The least amount of yeasts and moulds was in the control yogurt, while the most yeasts and moulds were in yogurt with 5% apple fiber added regardless of the other observed factors – storage time ($p < 0.05$).

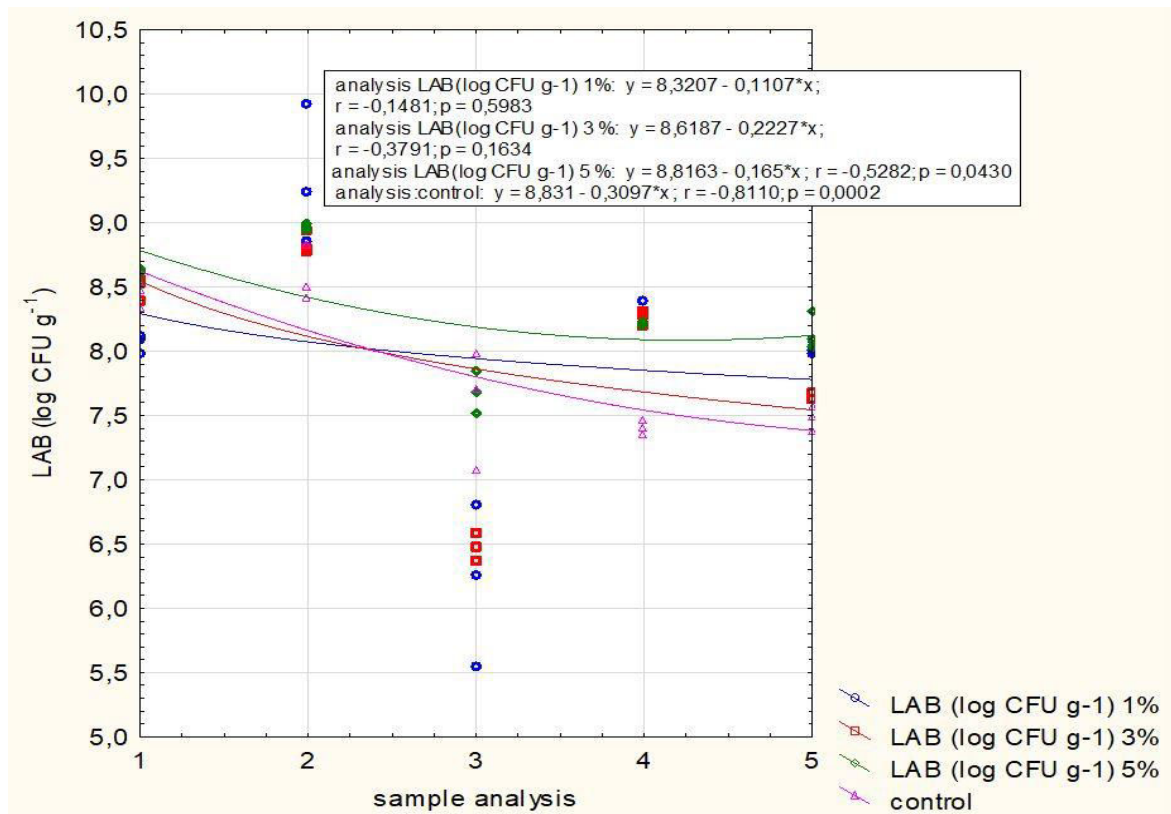


Figure 5 Changes in the amount LAB (log CFU.g⁻¹) during the storage – analysis: 1st (24 hours after yogurt manufacturing), 2nd (7 days after storage), 3rd (14 days after storage), 4th (21 days after storage), 5th (28 days after storage) in yogurt with nopal powder.

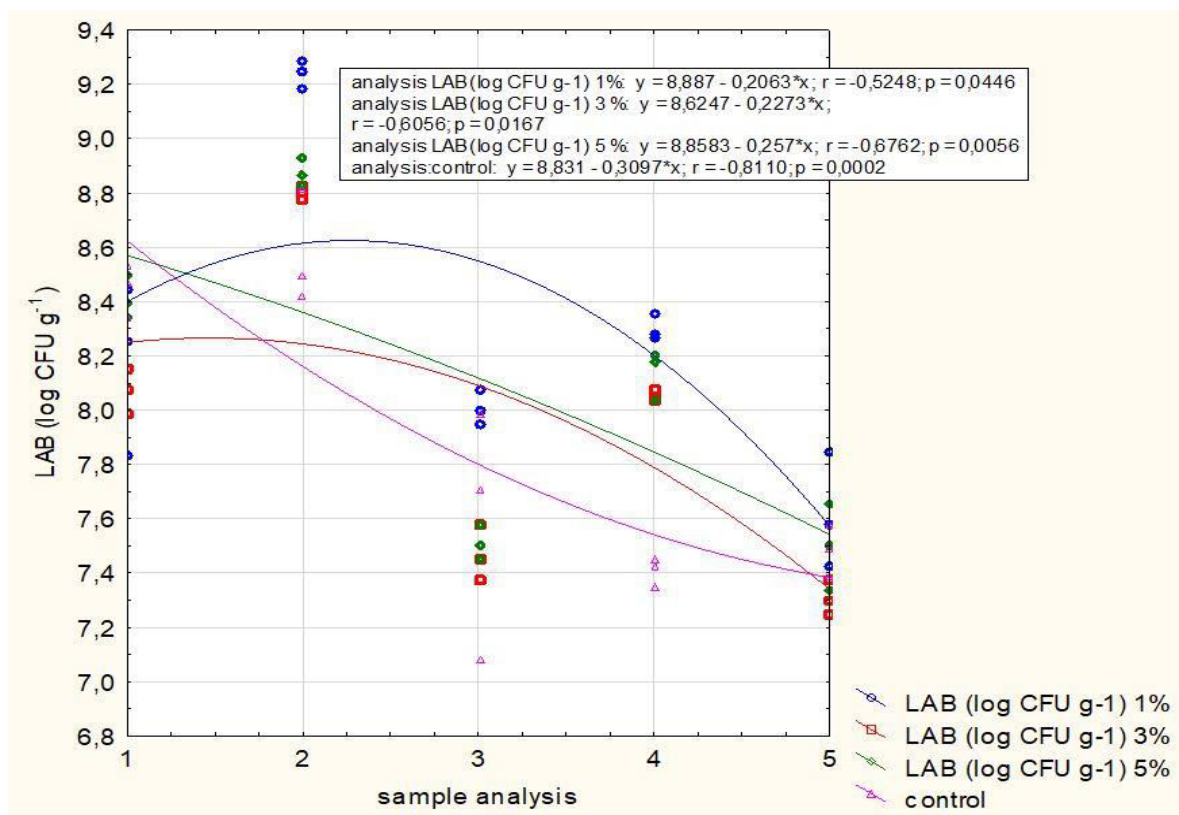


Figure 6 Changes in the amount yeasts and moulds (log CFU.g⁻¹) during the storage – analysis: 1st (24 hours after yogurt manufacturing), 2nd (7 days after storage), 3rd (14 days after storage), 4th (21 days after storage), 5th (28 days after storage) in yogurt with nopal powder.

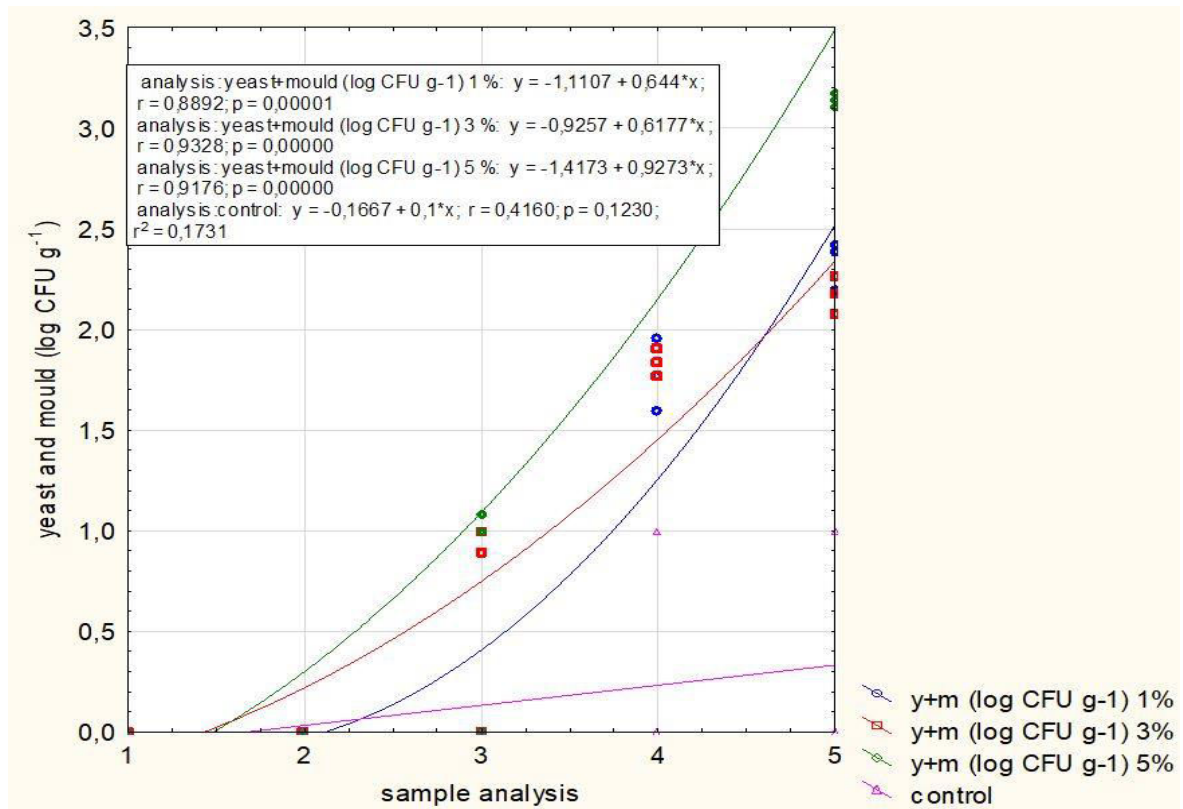


Figure 7 Changes in the amount LAB (log CFU.g⁻¹) during the storage – analysis: 1st (24 hours after yogurt manufacturing), 2nd (7 days after storage), 3rd (14 days after storage), 4th (21 days after storage), 5th (28 days after storage) in yogurt with apple fiber.

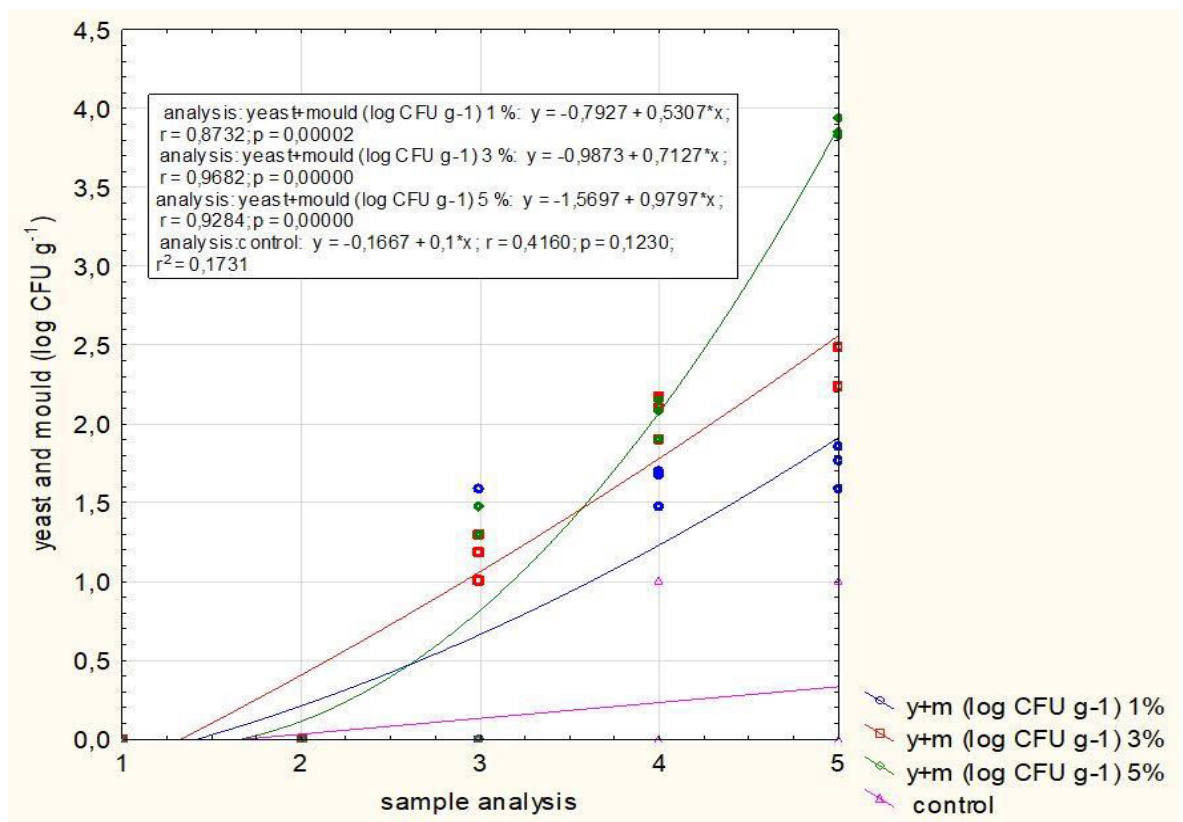


Figure 8 Changes in the amount yeasts and moulds (log CFU.g⁻¹) during the storage – analysis: 1st (24 hours after yogurt manufacturing), 2nd (7 days after storage), 3rd (14 days after storage), 4th (21 days after storage), 5th (28 days after storage) in yogurt with apple fiber.

Bamboo fiber

The addition of 1% of bamboo fiber had an influence on lower amount of LAB than in the control yogurt at the start of sampling. This situation changed after three weeks of storage when the amount of LAB increased in yogurt with 1% bamboo fiber addition. The amount of LAB in yogurt with 3% bamboo fiber addition was changed during storage time. At the beginning and at the end had this yogurt more LAB than control yogurt. These changes in yogurt with 1 and 3% addition were not statistically significant ($p > 0.05$). But yogurt with 5% bamboo fiber addition had, for the whole storage time, more LABs in yogurt than the control yogurt. Addition of 5% bamboo fiber in yogurt made a good condition for growth LAB (Figure 9). There was no statistically significant difference ($p > 0.05$) between addition of 1, 3 or 5% of bamboo fiber to amount of LAB into yogurt.

The amount of yeasts and moulds at the beginning was not detected regardless of the bamboo fiber added. This amount of yeasts and moulds was same for the whole storage time. There was no statistically significant difference ($p > 0.05$) between the control and 1, 3 or 5% bamboo fiber addition samples, regardless of the other observed factors (storage time). Just as **Ibrahimand and Khalifa (2015)** in their work, they did not detect any yeasts and moulds in yogurt with date and orange fiber during storage.

CONCLUSION

There was monitored the effect of addition of chia flour, quinoa flour, nopal powder, apple fibre and bamboo fibre BAF 40 to Lactic acid bacteria (LAB) and the amount of yeasts and moulds in these yogurts.

The addition of chia flour, apple fiber or bamboo fiber in yogurts caused that the amount of Lactic acid bacteria was higher than the amount of LAB in control samples of yogurt. The addition of 1 or 3% of quinoa flour in yogurt showed higher amount LAB in yogurt too. These changes were not statistically significant ($p > 0.05$). But addition of 5% of quinoa flour caused a decrease of LAB in yogurt due to a possible higher presence of saponins that may affect the condition of growth for LAB (statistically significant difference $p < 0.05$). Yogurt with the nopal powder had the same or higher amount of LAB during the storage. The statistically significant difference ($p < 0.05$) was for yogurt with 3 and 5% nopal powder addition.

The amount of LAB was not decreased below the limit $7 \log \text{CFU} \cdot \text{g}^{-1}$ during the storage. All monitored yogurts fulfilled the requirements of the Decree no. 397/2016 Coll.

The amount of yeasts and moulds in the control yogurt was very low during the whole storage time. It confirmed a “good manufacturing practice” because the total amount of yeasts and mould was not greater than $1 \text{ yeast cell} \cdot \text{g}^{-1}$. However, the problem was with the added flour, powder and fiber. The amount of yeasts and moulds increased with the higher addition of fiber in yogurts. The lowest amount of yeasts and moulds was in the yogurt with bamboo fiber. On the other hand, the highest amount was in the yogurt with chia flour.

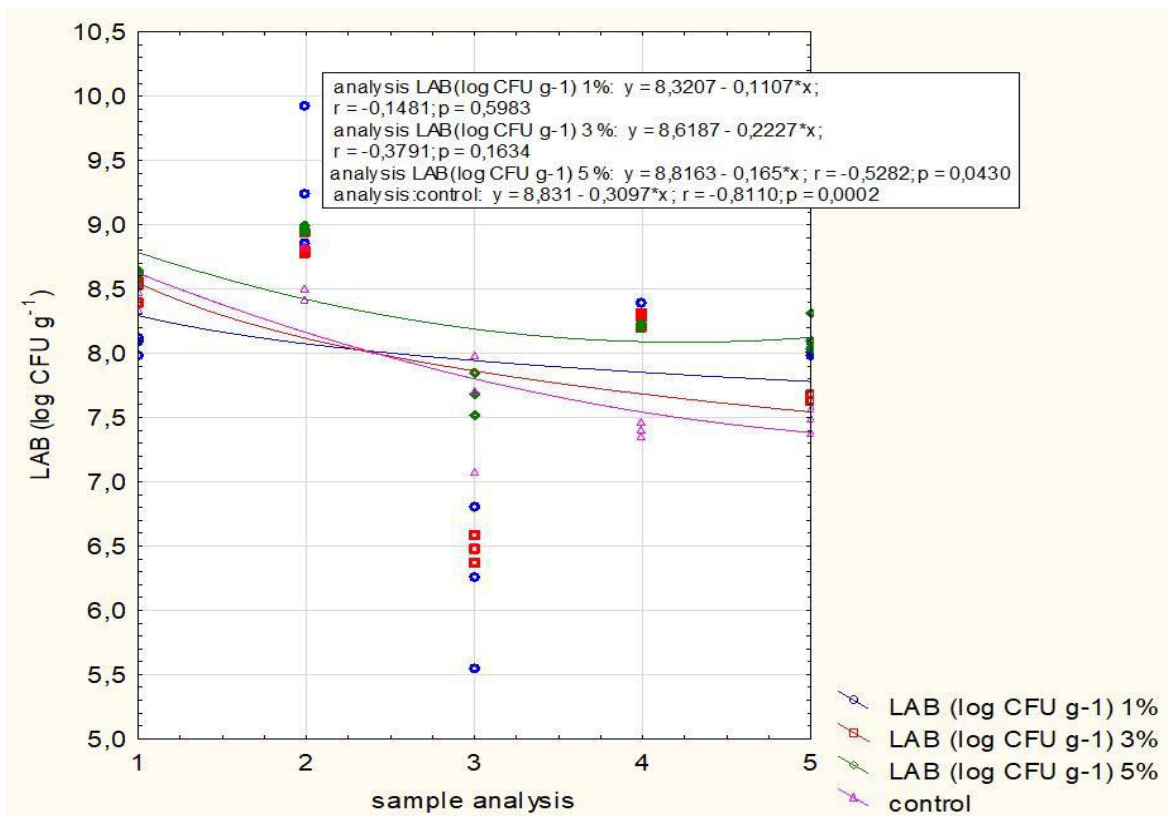


Figure 9 Changes in the amount LAB ($\log \text{CFU} \cdot \text{g}^{-1}$) during the storage – analysis: 1st (24 hours after yogurt manufacturing), 2nd (7 days after storage), 3rd (14 days after storage), 4th (21 days after storage), 5th (28 days after storage) in yogurt with bamboo fiber.

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Contact address:

Ing. Roman Pytel, Mendel University in Brno, Faculty of AgriSciences, Department of Food Technology, Zemědělská 1, 613 00 Brno, Czech Republic, E-mail: roman.pytel@mendelu.cz

MVDr. Olga Cwiková, Ph.D., Mendel University in Brno, Faculty of AgriSciences, Department of Food Technology, Zemědělská 1, 61300 Brno, Czech Republic, E-mail: cwikova@mendelu.cz

Ing. Sylvie Ondrušíková, Mendel University in Brno, Faculty of AgriSciences, Department of Food Technology, Zemědělská 1, 613 00 Brno, Czech Republic, E-mail: sylvie.ondrusikova@mendelu.cz

doc. Ing. Šárka Nedomová, Ph.D., Mendel University in Brno, Faculty of AgriSciences, Department of Food Technology, Zemědělská 1, 613 00 Brno, Czech Republic, E-mail: snedomov@mendelu.cz

doc. Ing. Vojtěch Kumbár, Ph.D., Mendel University in Brno, Faculty of AgriSciences, Department of Technology and Automobile Transport, Zemědělská 1, 613 00 Brno, Czech Republic, E-mail: vojtech.kumbar@mendelu.cz