Microbiological quality analysis of organically grown oilseed products

Podravac, Dijana; Jager, Nina; Lenart, Lidija

MICROBIOLOGICAL QUALITY ANALYSIS OF ORGANICALLY GROWN OILSEED PRODUCTS

Dijana Podravac*, Nina Jager, Lidija Lenart

Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Franje Kuhača 20, 31000 Osijek, Croatia
*corresponding author: dijana.podravac@ptfos.hr

Original Research Article
UDC 631.576.3 : 664.66
664.66 : 543.95

SUMMARY
In recent years, seed bread has become one of the favourite breads for consumers, due to its better taste, nutritional value, and positive health effects. This paper illustrates the process of the microbiological quality analysis of organically grown oilseeds, from which many different products can be obtained by cold pressing, including seeds and their semi-products that can be added to the bread. Since these products are not thermally treated in any way, their microbiological safety is questionable. Therefore, the goal of this paper was to implement microbiological control of those products and to compare the obtained parameters with the norms prescribed by the Food Hygiene and Food Microbiological Laws (NN 81/2013). The conducted microbiological methods conform to the requirements prescribed by Croatian norms which are based on the ISO-9001 quality standards. A total of 40 samples, including 6 samples of cold pressed oils, 9 organic butters, 6 organic protein flours, 8 kinds of seeds and nuts, and 11 mixtures of oilseeds and edible nuts for bread and crackers, were collected and analysed on a number of aerobic mesophyllic bacteria, bacteria of the *Enterobacteriaceae* family, moulds and yeasts. The samples were also screened for the presence of pathogenic *Salmonella* and *Listeria monocytogenes* species, and sulphite-reducing clostridia, dependent on the norms prescribed by the Law. It was shown that the measured values did not exceed the prescribed normative values in all samples.

Keywords: seed bread, oilseeds, edible nuts, organic protein flour, butter

INTRODUCTION

Oilseeds are plants whose fruits, seeds and other plant organs contain high amounts of fat and are suitable for processing into vegetable oils (Rac, 1964). Organic production is on a steady rise. Concerns about health and safety and influence on nature biodiversity and environment have the greatest impact on farmer´s decision regarding the conversion to the organic type of agriculture (Cranfield et al., 2010; Hole et al., 2005).

Cold pressed vegetable oils are produced by pressing, without heating, in order to maintain the full quality and nutritional value of the oil. The raw material must undergo the process of cleaning, peeling and milling. In order to get the best quality cold pressed vegetable oil, purification can be done exclusively by washing with water, precipitation, filtration and centrifugation. Such processing helps to preserve antioxidant ingredients and affect the total quality of oil (Mandawala et al., 2012).

Cereals, fruits, vegetables, whole nuts and seeds are the main sources of dietary fibre that have a wide range of positive health effects. Soluble dietary fibres affect the slower discharge of stomach contents, thus contributing the feelings of satiety and regulating body mass. Due to its viscosity and volume in the intestine, it binds itself to the cholesterol from the intestine, which is beneficial to the health of the bloodstream system (Salas-Salvadó et al., 2006). Walnuts have so far been associated with reduced risk of cancer, heart disease and several other chronic diseases also associated with changes in the gut microbial communities. Byerley et al. (2017) studied the effect of consuming walnuts on gut microbiome and found changes that included the growth of probiotic bacteria and reduction of opportunistic pathogens. Bread with the addition of sunflower seeds had a better taste than the control bread and was nutritionally more acceptable due to the composition of essential fatty acids, copper, zinc, tocopherols and other components that could improve nutritional status (Škrbić and Filipčev, 2007).

Nuts and nut products are at low risk from microbial contamination because of thick exterior structure and low water activity, but contamination by pathogenic bacteria can still occur and cause foodborne illness outbreaks (Li and Schneder, 2016; Danyluk et al., 2007). Nuts and cereals are often dried to the water activity lower than 0.70, which prevents survival and growth of microorganisms that could cause spoilage and disease. Humid environmental conditions during growth and harvest season contribute to contamination with moulds or *Salmonella* spp., beacuse shell becomes moist and susceptible to microorganism migration toward the core of nuts (Danyluk et al., 2007). Crops are exposed to microorganism from soil, manure, wild animals and irrigation water during their growth. Microbial contamination can also happen after harvesting, during the processing and storage. Some seed products can be dried using heat treatment, but others are minimally processed and some are treated with temperature not high enough to kill all pathogenic bacteria (Willis et al., 2009).

Oilseeds meals or cakes are important sources of protein and lipid in animal nutrition. In the diet of humans only a few meals and cakes are used. Sesame seed meal, coconut cake and meal from edible nuts are used in pastry and bakery. When oilseed cakes are used for human consumption, they must meet microbiological standards (Rac, 1964). The aim of this study was assessment of the microbiological safety of organic grown oilseeds, edible nuts and products obtained by their processing.

MATERIALS AND METHODS

A total of 40 samples were collected, 6 of which were cold pressed oils, 9 were organic butters, 6 samples were products from organic protein group, 8 samples were different kind of seeds and nuts, and 11 were mixtures for bread and crackers. Cold pressed oils were made of hemp, flaxseeds, pumpkin, apricot kernel, black cumin and coconut oil. Organic butters were made of hazelnuts, walnuts, pumpkin seeds, sesame seeds, sunflower seeds, almonds, peanuts, pistachios and Indian nuts. Four types of organic proteins have been analyzed, including flaxseed, pumpkin, hemp, sunflower seeds and pumpkin and sunflower cake left after pressing. In category of whole seeds and nuts, samples of pumpkin, flaxseeds, sunflower seeds without shell, sesame seeds, black cumin, walnuts, hazelnuts and almond seeds were analyzed. Bread mixtures were also analyzed under the names of producer: Nut, Fruit, Classic, Mediteran, Spacy, Univerzal and cracker mixtures: Nut, Fruit, Classic, Mediteran and Spacy that were tested according to the same requirements of the Microbiology Criteria for Food. The mixtures contained various ingredients such as: sunflower seed, flaxseed, pumpkin seeds, sesame seeds, coconut flour, ground almond, psyllium, cranberries, mulberries, garlic powder, dried tomatoes, basil and rosemary, walnuts, chia seeds, black pepper, cayenne powder, smoked paprika powder and sodium bicarbonate and the mixtures are foreseen for the thermal treatment for 1 hour on 160 °C.

The samples were tested based on the following parameters: the total number of aerobic mesophilic bacteria (AMB), the total number of bacteria of *Enterobacteriaceae* family (E), moulds and yeasts (M&Y), sulphite-reducing clostridia (SRK), *Salmonella* spp. (S) and *Listeria monocytogenes* (L.m.) species that are prescribed by the Food Hygiene Act and Microbiological Criteria for Food (NN 81/2013).

Microbiological parameters were determined according to the following ISO standards: ISO 21528-2:2008, ISO 4883-1:2013, ISO 4833-2:2013, ISO 15213:2003, ISO 6579:2002, ISO 11290-1:2008 and ISO 21527-1:2008.

RESULTS AND DISCUSSION

Obtained test results are shown graphically, except for *Listeria monocytogenes* and *Salmonella* because those microorganisms were not present in samples. The number of microorganisms are expressed in log cfu/mL in case of oil samples or in log cfu/g for results of butter analysis. Samples from **Figure 1** belong to group of Edible vegetable and animal fat, while samples shown in **Figure 2** belong to Edible fat and products and each product group has precisely prescribed microbiological safety requirements. According to the Microbiological Criteria for Food, samples from **Figures 1** and **2** are considered to be microbiologically safe if number of aerobic mesophilic bacteria is 10 cfu/g or 10 cfu/mL, moulds and yeasts are under 10 cfu/g or 10 cfu/mL, bacteria from *Enterobacteriaceae* family are allowed in concentration of 10 cfu/g or 10 cfu/mL or less. This group also has to be tested for

Listeria monocytogenes and has to be negative when analysing 25 g or mL of sample.

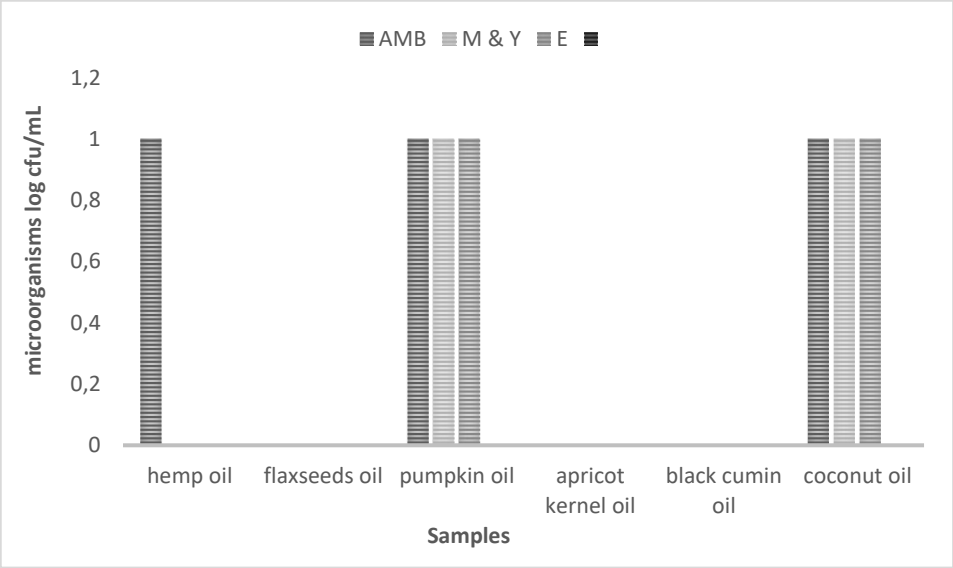

Figure 1 Microbiological analysis of cold pressed oils

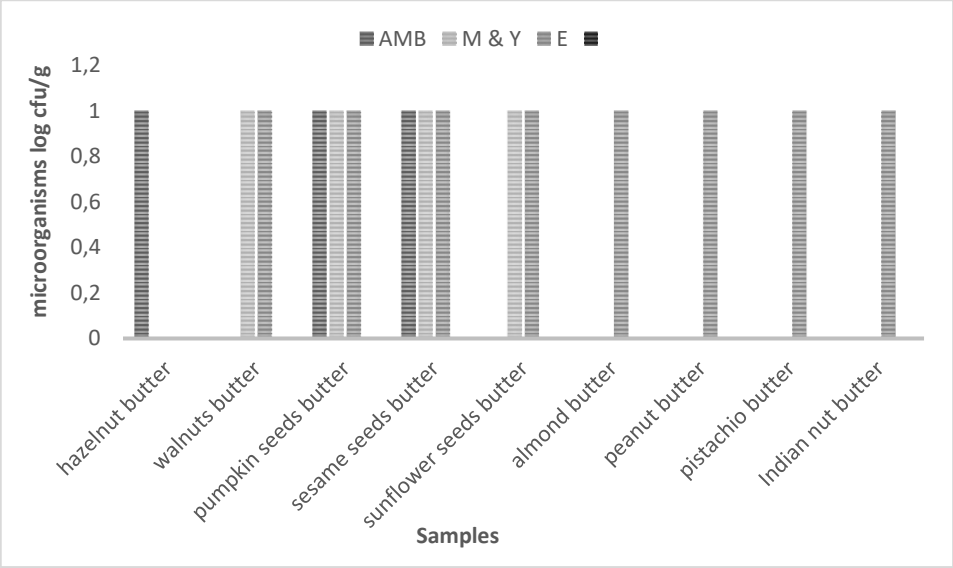

Figure 2 Microbiological analysis of organic butters

Samples which are displayed in **Figures 3, 4, 5** and **6**, belong to the group of protein products of herbal origin (based on soybean, other oilseeds, grain, yeast, etc.). According to Microbiological Criteria for Food maximum, the recommended number of aerobic mesophilic bacteria in that group is 10^4 cfu/g, moulds and yeasts are allowed in concentration of 10^2 cfu/g and bacteria from *Enterobacteriaceae* family are allowed in concentration of 10^2 cfu/g. This group has to be tested for

sulphite-reducing clostridia that can be present no more than 10 cfu/g and test for *Listeria monocytogenes* has to be negative for 25 g of sample. But bread and cracker mixtures from **Figures 5** and **6** can also be controlled as a part of other food groups with different requirements. Depending on their ingredients, they can be grouped as Grain and mill products (AMB max 10^5 cfu/g, E max 10^4 cfu/g, M&Y max 10^4), Dried and candied fruits, fruitage and seeds, dried vegetables and mushroom (AMB max 10^4 cfu/g, S not present in 25 g, E max 10^2 cfu/g, SRK max 10 cfu/g, M&Y max 10^2 cfu/g) or as Spices, seasoning plants and their mixtures (AMB max 10^5 cfu/g, S not present in 25 g, E max 10^3 cfu/g, SRK max 10^2 cfu/g and M&Y max 10^3 cfu/g). Microbiological criteria in these food groups are not so strict since products from this group are intended for baking and high temperature that contributes to the destruction of microorganisms.

Observing the oil samples contamination in **Figure 1**, it could be noticed that pumpkin seed oil was the most contaminated oil, containing 10 cfu/mL AMB, M&Y and E but, since it is the maximum amount prescribed by the Food Hygiene Act and Microbiological Criteria for Food (NN 81/2013), from microbiological point of view, the sample is considered safe. Pumpkin oil is known for its antibacterial and antifungal activity (El-Aziz and El-Kalek, 2011), but in some experiments antimicrobial activity was not proven (Hammer et al., 1999). Flaxseed, apricot kernel and black cumin oil were not contaminated by microorganisms. This could be related to Friedman et al. (2002) research who used 96 essential oils and 23 oil compounds, including apricot and black cumin oil that were active against *Cmpylobacter jejuni*, *Escherichia coli*, *Salmonella enteritica* and *Listeria monocytogenes.* That report on antimicrobial effectiveness suggests that the use of these oils may even improve other food safety.

As could be seen in **Figure 2**, all samples of organic butter contained maximum allowed amount of bacteria of *Enterobacteriaceae* family (10 cfu/g), but were microbiologically safe. Sesame seed butter analysis showed that it was the most contaminated product in this group, and the values of AMB and M&Y were maximum allowed, but still microbiologically safe. Customary heat treatment has not been carried out so it is possible that microorganisms may be left behind in seeds and nuts prior to grinding and that their growth and multiplication have increased the initial number of microorganisms. Non-processed sesame seeds (**Figure 4**) contained a smaller number of microorganisms and that suggests that contamination occurred during or after processing.

The microbiological analysis of **Figure 3** shows the results of organic protein flours and cake after pressing. Sunflower seed cake had the greatest contamination and was potentially microbiologically dangerous. The total number of all determined microorganisms was maximum allowed number, only SRK was slightly below the limit. Cakes are used to feed domestic animals, so their contamination is indirectly dangerous to humans. Hens can eat contaminated seeds or cakes and enter *Salmonella* spp. in organism. Infected hen can transfer bacteria to the egg and then to human, but in this case, all the samples were negative for *Salmonella* spp.

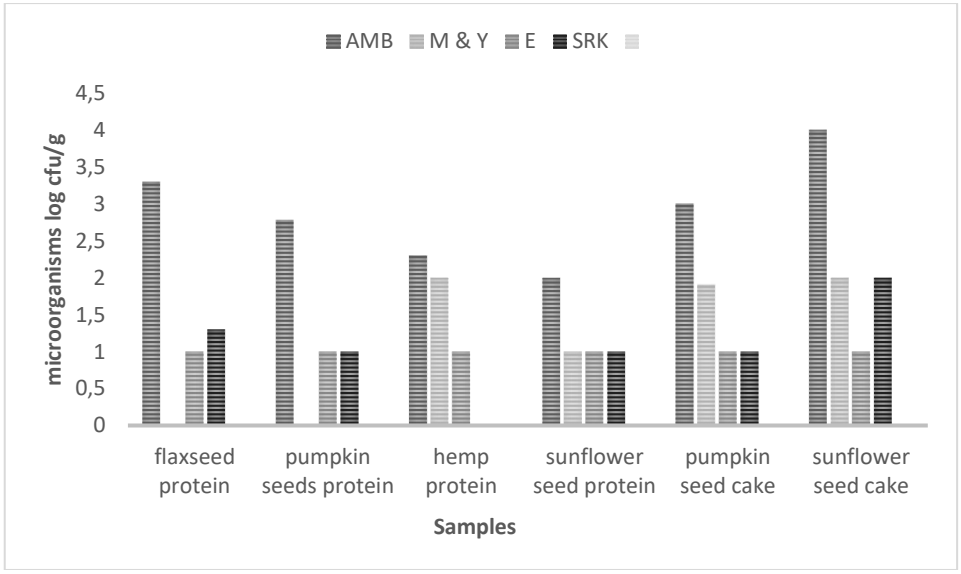

Figure 3 Microbiological analysis of organic protein flours and cake after pressing

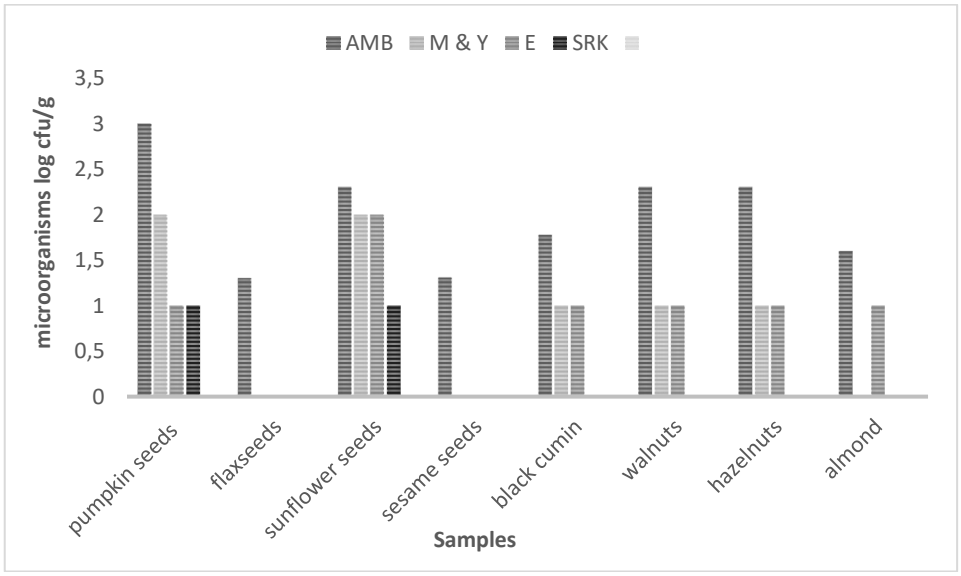

Figure 4 Microbiological analysis of whole seeds and nuts

Figure 4 shows the results of the analysis of whole seeds and nuts. It can be noticed that number of SRK in sunflower seeds was 10 cfu/g, while the sunflower cake had nearly 10^2 cfu/g. The cell wall is destroyed by pressing and it helps microorganisms to penetrate the cake easier than the whole seed. Although this contamination, compared to the Toth (2011) analysis, in which SRK was not found in sunflower seeds and cakes, this result is undesirable, but it is very low contamination when comparing to Microbiological Criteria for Food. The cake was not produced by elevated temperatures, which also affects the number of bacteria that can be

destroyed by the temperature of 121 °C. Since these bacteria are very resistant and can survive conditions that destroy vegetative bacterial cells, this SRK number is not uncommon. Organic sunflower seed butter contained bacteria from E family (10 cfu/g) and 10 cfu/g of moulds and yeasts, while there was no AMB. Based on the results, it can be concluded that the processing method affects the microbiological stability of the final product.

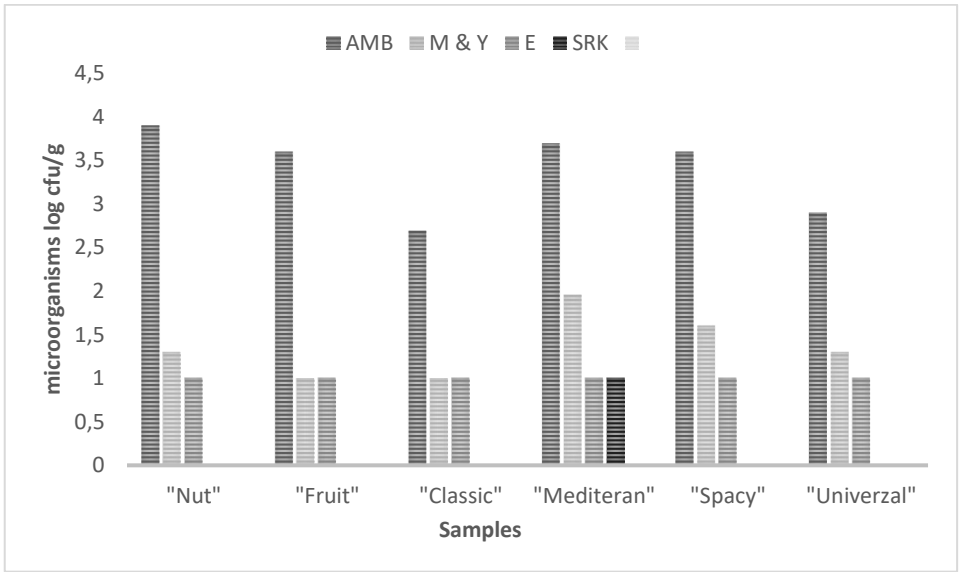

Figure 5 Microbiological analysis of bread mixtures

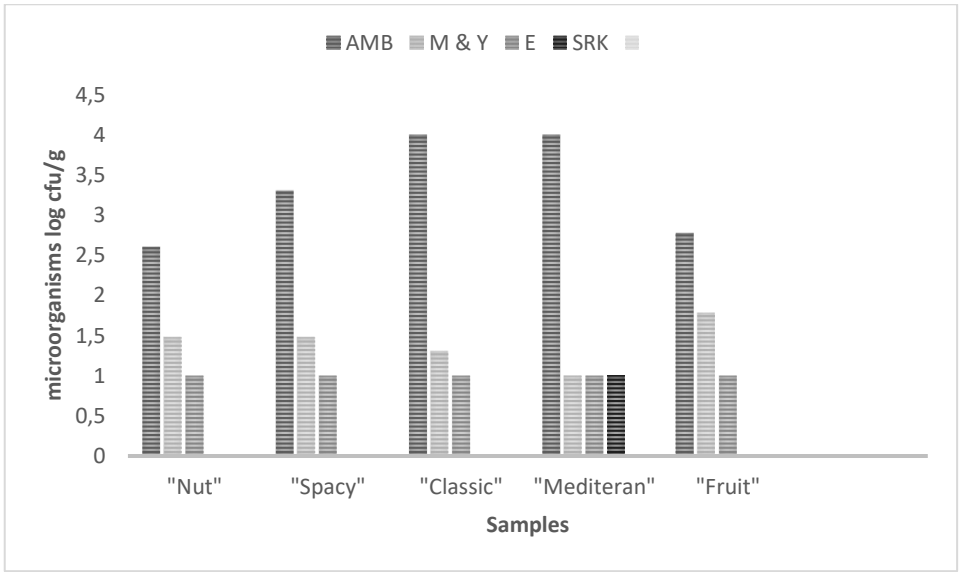

Figure 6 Microbiological analysis of cracker mixtures

Figures 5 and **6** show graphical results of bread and cracker mixtures analysis. Because they contain numerous ingredients, it is difficult to place them in the appropriate product group prescribed by the Law. The most contaminated was "Mediteran" blend for cracker with 10^4 cfu/g AMB and "Mediteran" mixture for bread with 5×10^3 cfu/g AMB. "Mediteran" mixture was also the only mixture that was contaminated with 10 cfu/g SRK in case of cracker and 10 cfu/g in case of bread mixture. All other samples proved to be negative for SRK and *Salmonella* spp. Although the results for number of AMB are more noticeable on the graph, than the results of other analysis, these figures are not of very high concern because, as mentioned earlier, these products are intended for baking at high temperatures and the number of microorganisms is acceptable.

CONCLUSIONS

Based on the results of the research conducted in this paper, the following conclusions can be drawn:

- All the analyzed samples were microbiologically correct despite the doubts since they were not thermally treated in any way.
- Microbiologically, the least contaminated were the samples of flaxseed cold-pressed oil, apricot kernel and black cumin oils because microorganisms of the species prescribed by the norms did not grow on a growth media, which is not surprising because it is widely believed that cold pressed oil on the organic method of producing plants has antimicrobial activity.
- The "Mediterranean" and "Classic" cracker mix samples were contaminated the most, with aerobic mesophilic bacteria (10^4 cfu/g) and the value was at the upper limit as prescribed by the Food Hygiene and Food Microbiological Laws (NN 81/2013), but samples were still microbiologically safe.
- The highest percentage of moulds and yeasts (10^2 cfu /g) were recorded in the sample of sunflower seeds and organic protein from hemp seeds.
- Most bacteria from the *Enterobacteriaceae* family (10 cfu/g) were found in pumpkin seed oil, in all analyzed organic butters, organic flaxseed protein and sunflower cake, but this is not of high concern as this number is allowed by norms.
- The highest number of sulfite-reducing clostridia (20 cfu/g) was measured in the sample of organic protein from flaxseed, but the normative allows up to 100 cfu/g of this type of bacteria.
- Neither the *Salmonella* spp. nor the bacterial species *Listeria monocitogenes*, were found in all 40 samples.

Oilseeds, their semi-products and products obtained by non-thermal processing may be contaminated with microorganisms during field growth, storage and all processing stages, and therefore their microbiological safety must be determined. In order to confirm the obtained results, further research should be carried out to

determine the safety of organically produced oilseeds from other producers and to compare samples with non-organic samples.

REFERENCES

1. Byerley, L.O., Samuelson, D., Blanchard, E., Luo, M., Lorenzen, B.N., Banks, S., Ponder, M.A., Welsh, D.A., Taylor, M. (2017): Changes in the gut microbial communities following addition of walnuts to the diet. *The Journal of Nutritional Biochemistry*, 48, pp. 94-102. https://doi.org/10.1016/j.jnutbio.2017.07.001
2. Cranfield, J., Henson, S., Holliday, J. (2010): The motives, benefits, and problems of conversion to organic production, *Journal of Agriculture and Human Values*, 27 (3), pp. 291-306. https://doi.org/10.1007/s10460-009-9222-9
3. Danyluk, M., Harris, L., Sperber, W. (2007): Nuts and Cereals. In: Food Microbiology: Fundamental and Frontiers, Third Edition, Doyle M., Beuchat L. (ed.), ASM Press, Washington, USA, pp. 171-183.
4. El-Aziz, A.B.A., El-Kalek, H.H.A. (2011): Antimicrobial proteins and oil seeds from pumpkin (*Cucurbita moschata*), *Nature and Science*, 9 (3), 105-119. http://dx.doi.org/10.7537/marsnsj090311.16
5. Food Hygiene Act and Microbiological Criteria for Food (NN 81/2013). https://www.zakon.hr/z/641/Zakon-o-higijeni-hrane-i-mikrobiolo%C5%A1kim-kriterijima-za-hranu [Accessed Semptember 7, 2017]
6. Friedman, M., Henika, P.R., Mandrell, R.E. (2002): Bactericidal Activities of Plant Essential Oils and Some of Their Isolated Constituents against Campylobacter *jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*, *Journal of Food Protection*, 65 (10), pp.1545-1560. https://doi.org/10.4315/0362-028X-65.10.1545
7. Hammer, K.A., Carson, C.F., Riley, T.V. (1999): Antimicrobial activity of essential oils and other plant extracts, *Journal of Applied Microbiology*, 86 (6), pp. 985-990. https://dx.doi.org/10.1046/j.1365-2672.1999.00780.x
8. Hole, D.G., Perkins, A.J., Wilson, J.D., Alexander, I.H., Grice, P.V., Evans, A.D. (2005): Does organic farming benefit biodiversity?, *Biological Conservation* 122 (1), pp. 113-130., https://doi.org/10.1016/j.biocon.2004.07.018
9. ISO, International Standards Organization: Horizontal method for the enumeration of microorganisms - Part 1: Colony count at 30 degrees C by the pour plate technique. ISO 4833-1:2013.
10. ISO, International Standards Organization: Horizontal method for the enumeration of microorganisms - Part 2: Colony count at 30 degrees C by the surface plating technique. ISO 4833-2:2013.
11. ISO, International Standards Organization: Horizontal method for the enumeration of sulfite-reducing bacteria growing under anaerobic conditions. ISO 15213:2003.

12. ISO, International Standards Organization: Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 1: Detection method. ISO 11290-1:2008.
13. ISO, International Standards Organization: Horizontal method for the enumeration of yeasts and molds - Part 1: Colony count technique in products with water activity greater than 0,95. ISO 21527-1:2008.
14. ISO, International Standards Organization: Horizontal methods for the detection and enumeration of Enterobacteriaceae -- Part 2: Colony-count method. ISO 21528-2:2004.
15. ISO, International Standards Organization: Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella spp*. ISO 6579:2002.
16. Li, Y., Schneider, K.R. (2016): Microbiological Hazard Outbreaks in Nuts and Nut-Related Food. In: Foodborne Diseases: Case studies of Outbreaks in Agri-Food Industries, Soon J.M., Manning L., Wallace C.A. (ed.), CRC Press, Boca Ranton, Florida, USA, pp. 63-72.
17. Mandawala, S.R.P., Kochhar, S.P., Dutta, P.C. (2012): Lipid components and oxidative status of selected specialty oils, *Grasas Y Aceites* 63 (2), 143-151. http://dx.doi.org/10.3989/gya.083811
18. Rac, M. (1964): Ulja i masti, Poljoprivredni pregled, Beograd, Srbija, pp. 93-95.; pp. 261-264.
19. Salas-Salvadó, J., Bulló, M., Pèrez-Heras, A., Ros, E. (2006): Dietary fibre, nuts and cardiovascular diseases, *British Journal of Nutrition* 96 (2), 45-51. http://dx.doi.org/10.1017/BJN20061863
20. Toth, Z. (2011): Changes of Microbial Population During Sunflower Meal Production. Postgraduate Specialist Thesis. Faculty of Food Technology, Osijek
21. Škrbić, B., Filipčev, B. (2007): Nutritional and sensory evaluation of wheat breads supplemented with oleic-rich sunflower, *Food Chemistry* 108, 119–129. doi:10.1016/j.foodchem.2007.10.052
22. Willis, C., Little, C.L., Sagoo, S., de Pinna, E., Threlfall, J. (2009): Assesment of the microbiological safety of edible dried seeds from retail premises in the United Kingdom with a focus on *Salmonella* spp., *Food Microbiology* 26, 847-852. http://dx.doi.org/10.1016/j.fm.2009.05.007