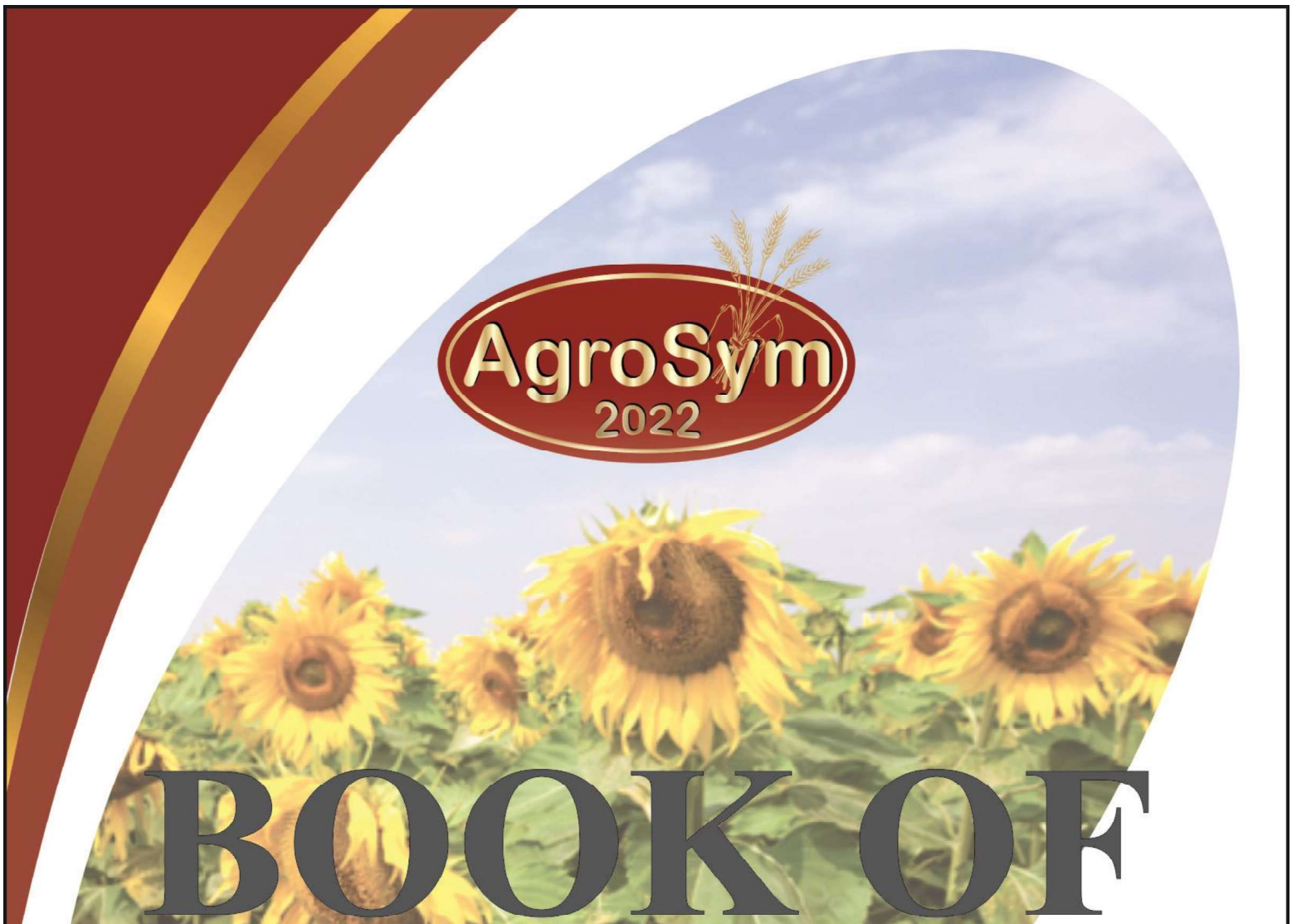

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MICROBIOLOGICAL QUALITY OF COLD PRESSED PUMPKIN AND WALNUT OILS

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Abstract

The aim of this study was to determine the content of crude oil in seeds and the microbiological quality of cold pressed oils of eight genotypes of pumpkin and walnut from Central and Western Serbia. The oil content in pumpkin seeds of different genotypes ranged from 16.44% to 35.13% per dry matter of whole seed, in the walnut kernel was between 50.17% and 60.41% per weight of the walnut kernel. Genotypes of pumpkin seeds differ significantly in oil content - genotype with the lowest oil content contains only 47% crude oil compared to the genotype with the highest oil content. Among the walnut samples, there is less deviation in the oil content of different genotypes. The number of total bacteria in walnut oil was less than 100 cfu/g. The number of yeasts and mold in walnut oil was very low, even in 3 samples the presence of this microorganisms were not detected, in 4 samples the number was lower than 10 and in the 1 sample the number was between 10 and 100 cfu/g. Two samples of pumpkin oil had a number of bacteria more than 100, but in other samples the number of bacteria less than 100 cfu/g. The number of bacteria, yeasts and molds in the oils of all genotypes was lower than the limit values prescribed in the "Guide to the application of microbiological criteria for food". Sulfite-reducing clostridia were not detected in any oil sample.

Keywords: *crude oil, local genotypes, microbiological quality, pumpkin, walnut.*

Introduction

In recent years, consumer interest in non-refined vegetable oils has been increasing worldwide, mainly because of growing evidence these oils have nutritional and health benefits. In the group of non-refined oils, a special place belongs to so-called "cold pressed oils". Cold pressed oils to have a high nutritional value due to the presence of ω-3 and ω-6 fatty acids and non-nutritive components such as tocopherols, sterols, phenolic compounds, squalene end carotenoids. Pumpkin oil and walnut oil belong to the group of edible oils that are produced exclusively by mechanical means and which can be included in the group of functional food products (Patel and Rauf, 2017). In addition to the nutritional and functional properties of walnuts and pumpkin seeds and oil, microbiological quality is also very important. The safety and shelf life of foods depend upon the interaction of chemicals, physical and microbial factors (Falola et al., 2011). Microbial food safety programs for raw and minimally-processed agricultural products have become an essential part of production and processing systems. Identifying sources, mode of contamination, and potential hazards are essential to reduce of foodborne disease (Heaton and Jones, 2008). The microbiological quality of cold pressed oils is directly related to the quality of the raw material. Seed contamination can occur during pre-harvest, harvest and post-harvest processing (CDC, 2006; Heaton and Jones, 2008). During the pre-harvest, growing plants are

susceptible to a wide range of microbial contamination sources. Soil and irrigation water especially those contaminated with industrial and domestic wastes, also animal excrement has been a common sources of microbial contamination (Mapanda et al., 2005). Harvesting includes collecting, classification, packaging and transportation which represent of critical points. In the post-harvest, possible sources of contamination are materials and equipment, lack of hygiene and event handlers and environments (Chitrakar et al., 2019). Bacillus sp., Pseudomonas sp., Penicillium sp., Mucor sp., and Aspergillus sp., were reported as soil/ environmental contaminants, *Staphylococcus aureus* as normal flora of human skin and opportunistic microorganism, and *Escherichia coli* as indicative organisms for fecal contamination/poor sanitary conditions (Ike et al., 2015). The Pseudomonas species (*Pseudomonas oryzihabitans, Pseudomonas putida, Pseudomonas syringae, P. viridiflava* and *Pseudomonas fluorescens)* and Bacillus species (*Bacillus subtilis, Bacillus flexus, Bacillus weihenstephanensis, Bacillus psychrodurans, Bacillus siralis, Bacillus indicus, Bacillus gibsonii and Bacillus firmus)* were detected in different microhabitats of oil pumpkin (Fürnkranz et al., 2012). Literature dates show that several microbes were isolated in pumpkin seed composite flours: Bacillus sp., *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli,* Penicillium sp., Mucor sp., and Aspergillus sp. (Ike et al., 2020). These isolates could be linked as either environmental contaminants, unhygienic processing contaminants or as inherent microflora.

Pumpkin and walnut are often grown as secondary crops of cultivation in rural areas of Central and Western Serbia, for that reason, the goal of this study was to investigate crude oil content in seeds and the microbiological quality of pumpkin and walnut oil.

Material and Methods

Plant material

The walnut fruits and pumpkin of local genotypes were collected in Central and Western Serbia, which were harvested during the 2021 crop year. The walnut fruits were dried and stored in the shell at room temperature until the beginning of the analysis. Samples of different genotypes of whole pumpkin seeds were stored at room temperature also.

Cold pressed oil

Cold pressed pumpkin and walnut oils were obtained by oil press (OP650W, Gorenje Group, Slovenia) with a temperature below 50 °C during the pressing. Whole pumpkin seeds were partially chopped before pressing. The color of obtained pumpkin oils varied depending on the genotype of light, dark green, with shades of brown and red, while the color of walnut oil did not vary significantly in different genotypes and was golden yellow. The samples of obtained oils were stored at -18 °C until analysis. These samples were used for microbiological analysis of the oil.

Determination crude oil content

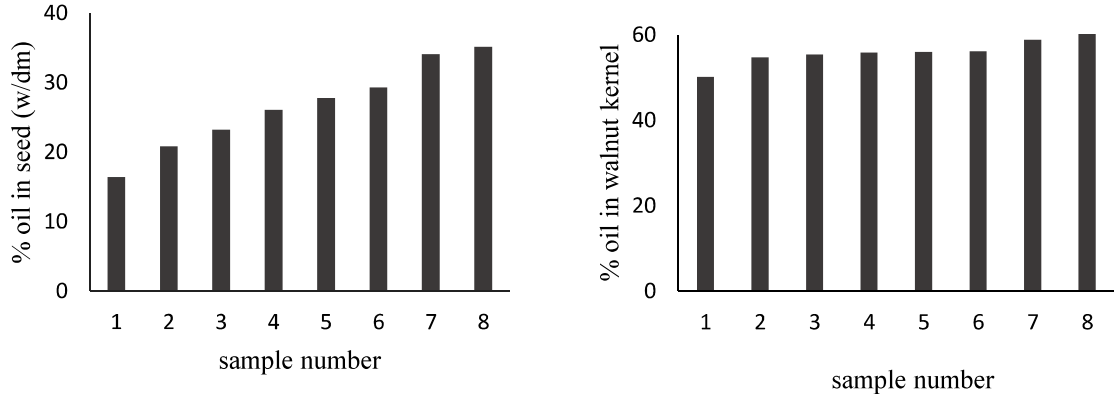
The crude oil content in pumpkin seed and walnut kernel was determined by extraction with petroleum ether in a Solvent extractor (Velp Scientifica ser 148, Italy). The plate temperature was 110 °C. Whole pumpkin seeds/walnut kernels were thoroughly chopped before extraction. Extraction oil from pumpkin seed implied the following conditions: immersion for 30 min and washing for 60 min; for oil from walnut kernel there were 90 minutes of immersion and 30 minutes of washing. The residual amount of petroleum ether evaporated in an oven at 105 °C.

Microbial Evaluation of oil

The number of aerobic mesophilic bacteria, the total number of molds and yeasts and the presence of sulfite-reducing bacteria were determined in the microbiological analysis of the oil. Enumeration of aerobic microorganisms and enumeration of molds and yeasts was determined using the standard microbiological plating method (ISO methods 4833 and 21527-1). An aliquot of 1 mL from each sample was inoculated aseptically into labeled agar plates of the media (agars: Nutrient Agar was used for the total number of aerobic mesophyll bacteria; and Sabouraud Dextrose Agar for cultivation and isolation of yeasts and molds). For the determination of the presence of sulphite-reducing clostridia, test tubes with 1 mL of basic dilution were heated in a water bath for 10 min at 80 °C, and then the Sulphite agar was poured into the tubes (Đukić et al., 2017). The height of the agar in the tubes was > 14 cm and the agar distance from the closure <1 cm. Petri dishes were incubated at 37 °C ± 2 °C for 24 to 48 hours, except for molds where the incubation period was 3 to 5 days, at 28 °C ± 2 °C. At the end of the incubation period, colonies were numerated. All media (agars) were prepared according to the manufacturer's instructions and autoclaved at 121 °C for 20 minutes.

Results and Discussion

The oil content in pumpkin seeds of different genotypes ranged from 16.44% to 35.13% per dry matter of whole seed (Figure 1 a). It is clear that genotypes differ significantly in oil content and the genotype with the lowest oil content contains only 47% crude oil compared to the genotype with the highest oil content. It is also known that the oil content in pumpkin seeds varies depending on the variety and growing conditions. Devi et al. (2018) showed that the average oil content in whole pumpkin seeds was 31.75%.



a b

Fig.1. The oil content in: a- whole pumpkin seeds and b-walnut kernels

Among the walnut samples, there is less deviation in the oil content of different genotypes. The oil content ranged from 50.17% to 60.41% per weight of the walnut kernel. Walnut kernels with the lowest oil content contain 83% oil from walnut kernels with the highest oil content. Beyhan et al. (2016) showed that contein of crude oil varide from 55.38% to 65.15% in different genotypes of walnuts.

Microbiological evaluation of walnut and pumpkin oils

The results of the microbiological analysis of pumpkin and walnut oils are presented in Tables 1 and 2. In 5 of the 8 tested samples of walnut oils, the number of bacteria was less than 10 cfu/g, and in the remaining samples was between 10 and 100 cfu/g (Table 1).

Table 1. Microbial evaluation in walnut oil

Samle of walnut oil	Bacteria cfu/g	Yeasts and molds cfu/g	Sulphite-reducing clostridia
1	10–100	non detected	non detected
2	<10	non detected	non detected
3	<10	10–100	non detected
4	<10	<10	non detected
5	10–100	<10	non detected
6	<10	non detected	non detected
7	<10	<10	non detected
8	10–100	<10	non detected

Generally, the number of aerobic mesophilic bacteria was low. The number of yeasts and mold in the walnut oils was very low, even in 3 samples the presence of these microorganisms was not detected, in 4 samples the number was lower than 10 and in the 1 sample the number was between 10 and 100 (Table 1). In the tested walnut oil variants, the number of yeasts and molds was lower than the limit values prescribed in the "Guide to the application of microbiological criteria for food". Oil is an inhibitory substance that limits the growth of aerobic microorganisms. It functions by sealing up the air pores through which air could flow in to support the growth of aerobic microorganisms. Microbiological analyzes showed that the presence sulphite-reducing clostridia were not detected in any of the examined samples of walnut oil.

The number of aerobic mesophilic bacteria in pumpkin oil was low, two samples had a number more than 100, and other samples the number of bacteria was between 10 and 100 cfu/g (Table 2). According to this agency, the maximum acceptable limit of bacteria count in food products is 10^3 cfu (ICMSF, 1995). The number of molds and yeasts was very small. Sulphite-reducing clostridia were not detected in any of the examined samples pumpkin oil.

Table 2. Microbial evaluation in pumpkin oil

Samle of pumpkin oil	Bacteria cfu/g	Yeasts and molds cfu/g	Sulphite-reducing clostridia
1	>100	10–100	non detected
2	10–100	10–100	non detected
3	10–100	<10	non detected
4	10–100	<10	non detected
5	10–100	<10	non detected
6	10–100	<10	non detected
7	>100	<10	non detected
8	10–100	non detected	non detected

Traditional pumpkin seed oil is obtained by pressing previously treated seeds at 110–130 °C for 30–60 minutes, these temperatures are sufficient to inactivate a large number of microorganisms, but in this work, no pre-treatment was performed by high temperatures, but the oil was directly squeezed from seeds. The seeds were not peeled before grinding. The shell can be a source of microbiological contamination. The microbiological quality of cold pressed oils is directly related to the microbiological quality of the raw material. Also, microbial populations present in raw nuts depend on production, harvesting and handling practices. Tree nuts often come in contact with the soil during harvest their microbiota may be influenced by the microorganisms present in the soil. Although it is unknown how or when orchard soils become contaminated with pathogenic organisms. Inadequately treated compost, unsanitary irrigation water, wildlife or domestic animals have previously been addressed as potential sources of introduction into these environments (Duffy et al., 2005). The number of microorganisms on the seed can be very different. Silva et al. (2022) state that the total number of bacteria on pumpkin seeds was between 3.6×10^2 and 6.8×10^5 cfu/g. In the case of seed peeling and thermal treatment, the microbiological analysis of the obtained oil in this experiment would be different. It is known that temperatures higher than 80 °C impaired the seeds quality, using the temperature of the 60 °C for several minutes, which would be required to ensure a significant reduction in the numbers of pathogenic organisms. For example, at 65 °C, it has been demonstrated that numbers of pathogenic microorganisms like Salmonella can be reduced by 1 log/min (Adams and Moss, 1995). The general improvements in production and hygiene practices during the production /processing chain are necessary to prevent and reduce microbial contamination of the final product (Ike et al., 2015).

Conclusion

The content of crude oil in pumpkin and walnut seeds depends on the genotype. The total number of aerobic mesophilic bacteria and the number of yeasts and molds in oil were within the eligibility criteria. Sulfite-reduction clostridia were not detected in any of the tested samples of pumpkin and walnut oil, although the fact that neither raw materials (seeds) nor oils were thermally treated at any stage of production obtaining cold-pressed oils.

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