

MICROBIOLOGICAL RISK ESTIMATION AT WALNUTS LONG-TERM STORAGE

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Abstract. The paper presents a bibliographic and experimental study of the microbiological incidence of *Juglans regia* L. walnuts at long-term storage. It was effectuated the microbiological control of autochthonous walnuts stored at different ambient conditions. We found that an improper storage leads to an increased risk of nuts infection with various microorganisms such as fungi, bacteria and acaridae. It is important to mention that the walnuts are vulnerable to fungi attack with *Aspergillus flavus* and *A. parasiticus*, species that produce aflatoxins, considered to be carcinogenic. Other isolated fungi in walnuts are: *Alternaria*, *Penicillium*, *Phoma*, *Botryosphaeria*, *Fusarium*, *Cladosporium*, etc. Microorganisms which are presented in walnuts may produce lipolytic enzymes that degrade the product quality and safety; also the same impact can be reflected on other foods based on these fruits. There are many factors that have a great influence on walnuts quality and safety. The most important are climatic conditions, varieties, cultivation methods and storage methods.

Key Words: microbial contamination, risk, long-term storage, fungi attack, walnuts quality and safety

I. Introduction

Various nuts are used as a raw material in many industries as well as for a direct consumption. Walnuts *Juglans regia* L. are rich sources of unsaturated fatty acids (56.2%, Ω3, Ω6, Ω9), proteins (15-25.2 %), carbohydrates (0.2 -15%), vitamins (B₁, B₂, B₃, B₆, B₉, C, E, PP, β-carotene, A), minerals (Fe, K, Ca, Mg, Na, S, P, Cl, I₂, Co, Mn, Cu, Zn) etc. [8]. They contain an important amount of proteins and fat and their products have wide acceptance as food throughout the world. Due to the extremely high fat, proteins and low water content (3.8 - 4.5) of various nuts such as hazelnut, almonds, walnuts, these products are quite refractory to spoilage by bacteria. Molds can grow upon them if they are stored under conditions that permit sufficient moisture for their propagation [6].

Nuts quality is determined by the content of unsaturated fatty acids, proteins, vitamins, minerals, antioxidants and the presence and activity of enzymes [5, 11-13]. Microbial incidence reduces nuts quality and safety [1, 11-13]. Microbial stability of nut varieties, cultivated in Moldova, is less studied. In this context we present a bibliographic and experimental study of *Juglans regia* L. walnuts.

II. Materials and methods

2.1. Plant material

The study was conducted on autochthonous walnuts *Juglans regia* L., harvested in Telenesti, the

central part of Moldova, during 2010 and 2011. Walnuts were purchased from commercial network and stored at room temperature (25°C). Microbiological control was conducted on 100 walnuts in each year.

2.2. Microbiological analyses

A twenty-five g analytical unit taken from the 100g sample unit was used in aerobic bacterial count, mold count. Plating for total bacterial count was carried on standard nutrient agar and for molds on Sabouraud dextrose agar [10, 13]. There were studied isolated colonies after incubation period of 3 days at 28°C for bacterial count and 7 days at the same temperature for mold count.

Also, total mold, yeast contamination and bacterial counts of the samples were determined using a dilution plate method. About 25g walnut samples (whole nuts, kernels) were suspended in 225ml of 0.1% peptone solution and homogenized. Homogenized samples were diluted with 0.1 % peptone solution to concentrations of 10⁻² and 10⁻³. Each dilution (100μl) was dispensed and spread onto sterile BA/Sabouraud in Petri plates, followed by incubation at 28°C for 3/5 days. Total mold and yeast counts were estimated by back-calculation based on observed mold for each dilution and was expressed as the number of colony forming units (CFU) per gram in each sample.

III. Results and discussion

Bibliographic study indicates that the nuts microbiota depends on variety, geographical conditions, climate, collection, processing and storage [1-13]. The climatic parameters (temperature, relative humidity, precipitations and UV irradiation), the composition of leaf surface (senescence) and the availability of nutrients change over the microbial colonization [1]. Molds of many genera may be found on examined nuts [5]. The mould genera, occurring most frequently in shelled and whole nuts, were: *Aspergillus*, *Penicillium*, *Rhizopus*, *Mucor* and *Cladosporium*. *Aspergillus* and *Penicillium* species predominated in all tested shelled nuts being (32-39%) *Aspergillus* and (25 to 31%) *Penicillium*, while *Aspergillus* was dominant in all the whole nuts (41-50%). Other genera were found to be in low percentage (*Rhizopus*, *Mucor* and *Cladosporium*). The results [13] are in accordance with Smith and Arend [6] who stated that *Aspergillus*, *Penicillium*, *Rhizopus*, *Mucor* and *Cladosporium* represented the common genera in nuts.

Data reported by M.J. Sejny et al. [5] show the qualitative and quantitative distribution of different groups of bacteria present in the shelled and whole nuts. It was found that Gram positive spores forming bacilli, Gram positive Micrococcus, Gram positive rods and Gram negative short rods generally contaminated the samples. Their quantitative distribution varied through the tested nuts. The most dominant bacteria appearing in the whole nuts were Gram positive spores forming bacilli being 55% and 68% in shelled and whole walnuts respectively, while Gram positive Micrococcus were 24% and 29% respectively.

Walnut trees are a habitat for a wide variety of fungal and (to a lesser extent) bacterial taxa some of which can pose a serious threat to plant health. Measures should be taken further to prevent the accumulation of microbial inoculums, e.g. removal of leaf debris [1]. Microbial biodiversity associated with the walnut *Juglans regia* L. is reported in several studies [1-7, 13]. Study [1] includes microflora analysis on walnuts trees in South Tyrol (Italy). From a total of 3.880 isolated cultures, the wide majority of the isolates (3.742) belonged to fungi (96.4%); only 138 (3.6%) were bacteria. Fungal isolates were classified into 30 genera, *Alternaria*, *Penicillium*, *Phoma*, *Botryosphaeria*, *Fusarium*, *Cladosporium*, *Phyllosticta* and *Epicoccum* being the most taxa. The most isolates were obtained from leaves 45.3% than from twigs 31.8% and fruit 23.0%. Microbial growth depends on variety of environmental parameters. Among

them temperature and relative humidity play a predominant role [5, 6].

Infestation rate of walnuts samples *Juglans Regia* L., stored during one year and two years, was different. The results of the investigation show that the nuts storage led to an increased microbial infestation in nearly 25-30 % of analyzed nuts.

The results of microbiological control of walnut *Juglans regia* L., grown in Moldova are the limits reported in bibliographic study. It was found that longer storage of nuts contributes to an increased microbial infestation. The rate of infestation abundance of walnuts harvested in 2010 amounted to fungi and yeasts 50% and 30% Gram positive and Gram negative 20%. Nuts harvested in 2011 were less infested. Heavy infestation rate being only 10% for fungi, yeasts, Gram positive and Gram negative bacteria.

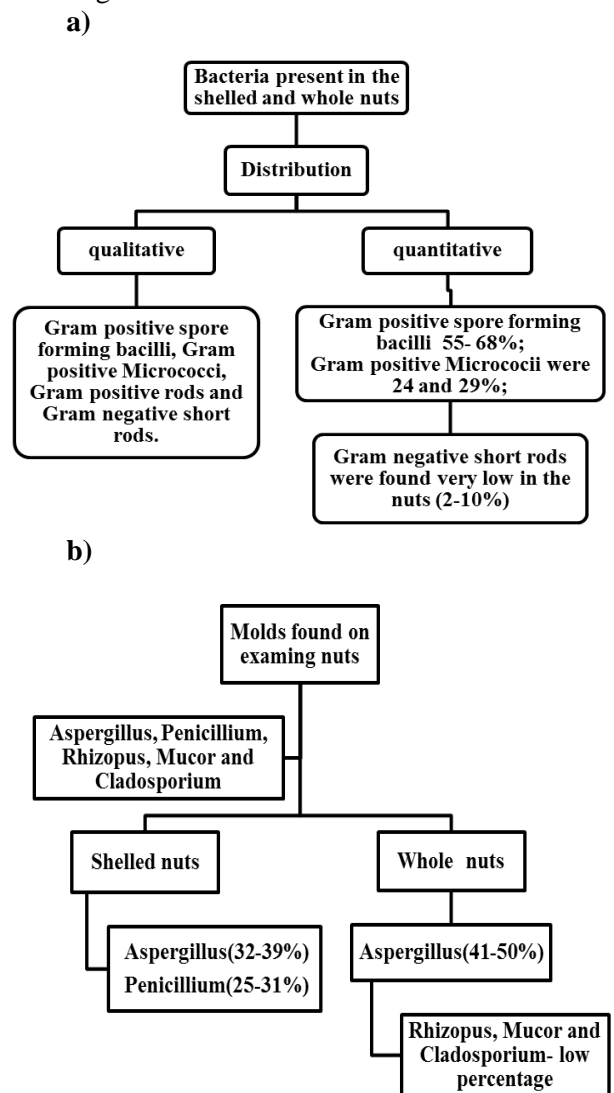


Figure 1. Microbial contamination of *Juglans regia* L walnuts stored in Moldova
a) Contamination with bacteria, b) Contamination with molds

In Figure 2 we present some images of colonies of microorganisms found in walnuts *Juglans regia* L., collected in Moldova, stored 22 months at temperature 25°C.



Figure 2. Isolated colonies after incubation period of 7 days at 28°C

The data obtained show that shelled walnuts prevailed infestation of fungi *g. Aspergillus* and *g. Penicillium* and the nuts were shelled frequently isolated molds *g. Aspergillus*. The article also aims at qualitative distribution of bacteria isolated nuts investigated, which formed following sequence: **Gram-positive bacilli > Micrococcus Gram positive > Gram-negative bacilli.**

Producing Quality Walnuts Food [9] denotes: walnut growers can minimize the potential for food borne illness outbreaks resulting from crop contamination by following good management practices. Potential for contamination of walnuts with these organisms is highest during harvest when the nuts are dropped to the ground. Procedures that can be applied to minimize potential for on-farm contamination of walnuts:

- Irrigation and Water Quality considerations
- Nutrient application
- Harvest
- Good manufacturing practices in the plan
- Proper storage.

IV. Conclusions

Bibliographic and experimental study shows that walnuts *Juglans regia* L. can be infected with fungi, yeasts and bacteria, which minimize their quality. Infection rate depends on climatic parameters (temperature, relative humidity, precipitations and UV irradiation), variety nuts and storage conditions. Good storage practices should be implemented to minimize the levels of insects and fungi in storage facilities.

To store walnuts should be taken into account Code of Practice for the prevention and reduction of aflatoxins contamination in nuts (CAC/RCP 59-2005) [3].

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