ASSESSMENT OF BACTERIA AND FUNGI ASSOCIATED WITH THE INSTANT NOODLES AND ACCOMPANYING SEASONING PACKETS

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Abstract

Instant noodles and the accompanying seasonings have gained popularity because of its convenience and affordability among young population in most country of the World. In this study the microbial quality (bacteria and fungi) of three different brands of noodles (designated as A, B and C) with their accompanying seasonings commonly marketed and consumed in Romania were investigated. The samples were serially diluted and poured in Petri plates. One gram of each brand of noodles and seasonings was aseptically transferred into 9 ml of sterile distilled water. Potato dextrose agar (PDA) in different compositions (classic, with streptomycin and rose-bengal stain) were the media used in this research. The least microbial load was obtained by heating samples at 100°C for 10 min. Sample B had the highest bacterial count of 16×10^3 cfu/g for cold noodles, and also the highest count of 6.6×10^3 cfu/g for hot noodles. For the seasonings, the total bacterial count varied from 6.6×10^3 cfu/g (sample A) to 33×10^3 cfu/g (sample B). The total fungal count of all samples was slightly higher than that of the bacterial counts. Microbial analysis showed the presence of Gram negative bacteria as predominant bacteria type (e.g. *Pseudomonas* spp), while *Aspergillus*, *Rhizopus*, *Penicillum* were the three isolated genera of fungi. *Penicillum* was the most frequently isolated genera of fungi in case of all brands of noodles.

Key words: bacteria, fungi, instant noodles, seasoning packets

Instant noodles have become a controversial global food product in the last decade, as their nutritional qualities are questioned, but despite this fact, approximately 106 billion servings were consumed globally in 2019, (instantnoodles.org), with increasing tendency for 2020. Such high demand requires rigorous control over the microbial quality. The growth of microorganisms in foods is facilitated, prevented or restricted by many factors; water activity, pH and temperature are the most important (Akhigbemidu W. *et al*, 2015).

The physical, chemical and microbiological spoilage of cereal products influences the flavor, aroma, leavening, presentation and overall consistency of the final consumer product (Cook F.K., Johnson B.L., 2009).

Instant noodles (dried or precooked) are commercially available either in cups with the seasoning sprinkled over the noodles or in pouches with packets of flavoring including seasoning oil (Hou G., 2001).

Instant noodles are especially popular among young people and have become a convenient alternative for lunch or dinner for its "quick to cook" properties and affordable price.

After being boiled or immersed in boiling water for 2-5 minutes, dry noodles are normally consumed, while precooked noodles may be reheated or eaten straight from the box. A single instant noodle portion is high in fat and carbohydrates, but low in fiber, vitamins and minerals (Lee E.T., 2009).

Thanks to their preservation, fine dried noodles (moisture content less than 14%) have accounted for the majority of noodle production for a long time. However, the taste, texture and nutritional properties of noodle products may be destroyed by these deep drying processes (Li M. *et al*, 2012).

Despite the new technologies, there is still a risk of contaminants coming from the compounds used in the manufacturing process, as well as those accidentally introduced from other sources. Pathogenic microorganisms that manage to survive throughout the production chain and end up being consumed, occasionally have the ability to induce food poisoning.

The aims of this study are to assess the microbial quality of three brands of noodles with their accompanying seasonings, respectively and

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to isolate and identify the bacteria and fungi present in the noodles and the seasonings.

MATERIAL AND METHOD

Three brands of noodles with their packets of flavoring, commonly marketed and consumed in Romania, were purchased randomly from different retail outlets. To ensure that they were intact (no tear or damage) and not expired, the sachets were carefully examined and taken for microbiological analysis at University of Agricultural Sciences and Veterinary Medicine, Iasi. To accomplish the purpose of this study the brands were coded as A, B and C.

Cultivation and determination of bacteria and fungi in noodles and seasoning was done by serial dilution and plating into nutritive media. One gram of each brand of noodles and seasonings was mixed with 9 mL sterile water (dilution 10^{-1}) and then 1 mL of the dilution 10^{-1} was poured into 9 mL sterile water (dilution 10^{-2}). After a successive tenfold dilution series, 10^{-2} to 10^{-4} dilution were prepared. From one dilution to another, mixture was thoroughly stirred for 5 min for the cold noodles and cold seasonings samples. The hot samples were obtained by boiling each of the three brands at 100°C for 10 min. Aliquots (1 mL) of 10^{-2} to 10^{-4} dilution were spread on nutritive media for assessing the total number of bacteria and fungi (Lipsa F.D., Ulea E., 2018).

Average numbers of colony forming units in 1 g of noodles and seasoning (cfu·g^{-1}) was determined using the plate counting method (Bressan *et al*, 2015), on potato dextrose agar medium (PDA) in different compositions: classic, with streptomycin and rose-bengal stain. Streptomycin antibiotic (35 mg·L^{-1}) was used to control the reproduction of Gram negative bacteria and rose-bengal stain was used to limit the growth of fast-growing moulds (e.g. *Rhizopus* spp., *Trichoderma* spp.). Czapek-Dox agar media was used for filamentous fungi identification. Light microscopy (1000x magnification) was used to determine the colonial features and the morphological structures of the fungi. The determination of the morphological structures of fungi was carried out on fungal material mounted in lactophenol by slide culture technique. Fungi were identified to genus level based on morphological and physiological characteristics following the works provided by Ellis (1971, 1997), De Hoog *et al* (2000), Barnett and Hunter (1999).

The number of bacterial colonies was determined at 24 hours and the fungus colonies at 5 days (incubation temperature 28°C). The experiment was conducted with a threefold repetition for each microbiological determination and the counts obtained were averaged. Microbiological media plates were prepared using Masterclave 09 plate maker and an aliquot portion of 15mL of media was poured using APS 320 automated Petri plate filler (AES Laboratoire, France).

The data obtained in the experiments were statistically evaluated and the results with $p<0.05$ were considered statistically significant.

RESULTS AND DISCUSSIONS

The generally result obtained for microbial quality evaluation is presented in figure 1. Based on the results, the ratio between the main groups of microorganisms for all three brands of noodles was dominated by filamentous fungi, present as spores, with a value of 54.9%, followed by Gram-negative (G-) and Gram-positive bacteria (G+), with 34.7 and 10.4%, respectively.

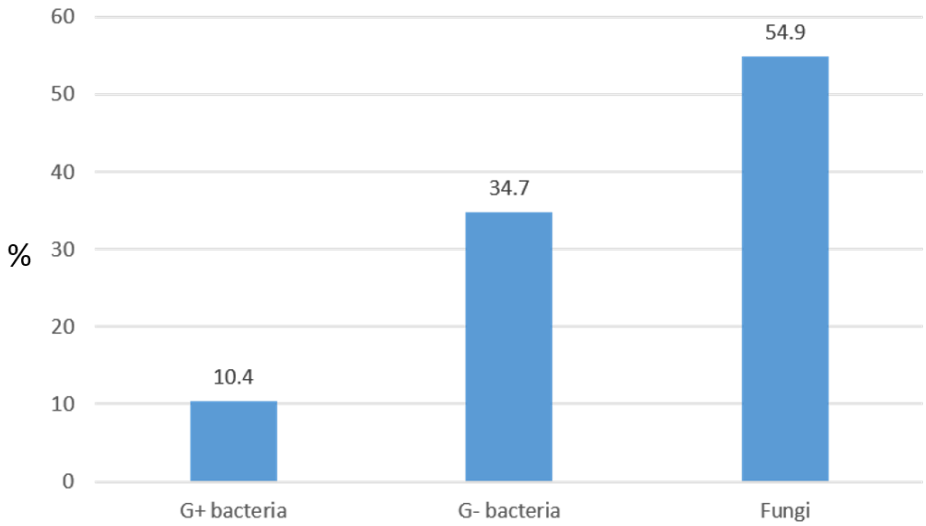

Figure 1 **Frequency of isolated microbiota from all 3 noodles samples (%)**

The categories of microorganisms in instant noodles primarily depend on wheat flour, condiments and the manufacturing and packaging methods, while microbial changes during storage depend on the composition of the substrate of the noodles and the conditions of storage. Under certain conditions, nearly all microorganisms can cause food spoilage, which is mainly based on nutrient composition and physicochemical parameters (Losio M.N. *et al*, 2017).

The microbial load varied with brands, and when considering both bacterial and fungal counts, the number of colony forming units per g of the product (cfu/g) was greater in cold samples than in hot samples (*tables 1 and 2*). In all the cases, the fungal counts were relatively smaller than the bacterial population.

Sample B had the highest bacterial counts of 16.0 and 6.6 x 10^3 cfu/g for both cold and hot noodles, respectively, while sample A recorded the lowest counts of 6.6 and 3.3 x 10^3 cfu/g, respectively. In case of accompanying seasoning packets, the most abundant bacterial community was recorded also in case of sample B (33.0 x 10^3 cfu/g), followed by tha samples C and A, both with 6.6 x 10^3 cfu/g (*table 1*).

These results show that the bacterial load is significantly reduced by heating the noodles. In the hot noodle samples, the least microbial load was found in all brands, while higher microbial load occurred in cold samples. The fact that all hot samples had a decrease in microbial load suggests that the use of hot water has an effect on the microbial quality compared to that of cold samples.

Table 1

Bacterial counts of three brands of noodles and accompanying seasonings (cfu/g)

Samples	Hot noodles	Cold noodles	Seasonings
A	3.3×10^3	6.6×10^3	6.6×10^3
B	6.6×10^3	16×10^3	33×10^3
C	3.3×10^3	16×10^3	6.6×10^3

Values as means of triplicate replication

The highest fungal load of 16.6 x 10^3 cfu/g were found in the accompanying seasoning packets of the samples B and C., while, in case of seasonings, the least occurred in sample A (*table 2*). In fact, the lowest count values of 3.3, 5.6 and 9.0 cfu/g were found in all hot noodles samples, respectively. Sample B had the highest fungal spore concentrations of 16.6 x 10^3 cfu/g for seasoning, 9.99 x 10^3 cfu/g for cold noodles and 9.0 x 10^3 cfu/g for hot noodles. Sample C recorded the lowest fungal load. Interesting was the fact, that in case of cold noodles from sample A, the concentration of filamentous fungi spores was higher (9.99 x 10^3 cfu/g) than the seasonings (6.6 x 10^3 cfu/g).

An explanation for this occurrence, could be that the peoples engaged in the production, packaging and retailing of these noodles do not take the required precautions or may indicate storage conditions, contamination from the seals of packets or from handlers before sampling.

Table 2

Fungal counts of three brands of noodles and accompanying seasonings (cfu/g)

Samples	Hot noodles	Cold noodles	Seasonings
A	5.6×10^3	9.99×10^3	6.6×10^3
B	9.0×10^3	9.99×10^3	16.6×10^3
C	3.3×10^3	6.6×10^3	16.6×10^3

Values as means of triplicate replication

The isolated species belonging to three micromycetes genera: *Penicillium*, *Aspergillus* and *Rhizopus*. Among the determined micromycetes in all the three different noodles samples, we pointed out *Penicillium* genus, which was isolated at a rate comprised between 68.9 (sample C) and 93.1% (sample A) of the total identified genera (*figure 2*). In smaller ratios were present *Aspergillus* and *Rhizopus* genera, with maximal values of 30.6 and 28.2% in case of hot noodles from sample C.

The presence of fungi from genera *Aspergillus* and *Penicillium* is of major health concern as they may produce mycotoxins (especially aflatoxins) in various foods (Barnett J.A. *et al*, 2000). Aflatoxins have been isolated from legumes, grains, fruits, meats, spices, cheeses, milk, rice or mais and have carcinogenic, hemorrhagic, hepatotoxic and neurotoxic properties (Akande O., Kuforiji O., 2013).

For some of the samples in this report, despite the high microbial counts obtained, it is important to note that these samples did not display any noticeable signs of spoilage. Therefore, external appearance may not be a good criterion for the microbial quality assessment of instant noodles.

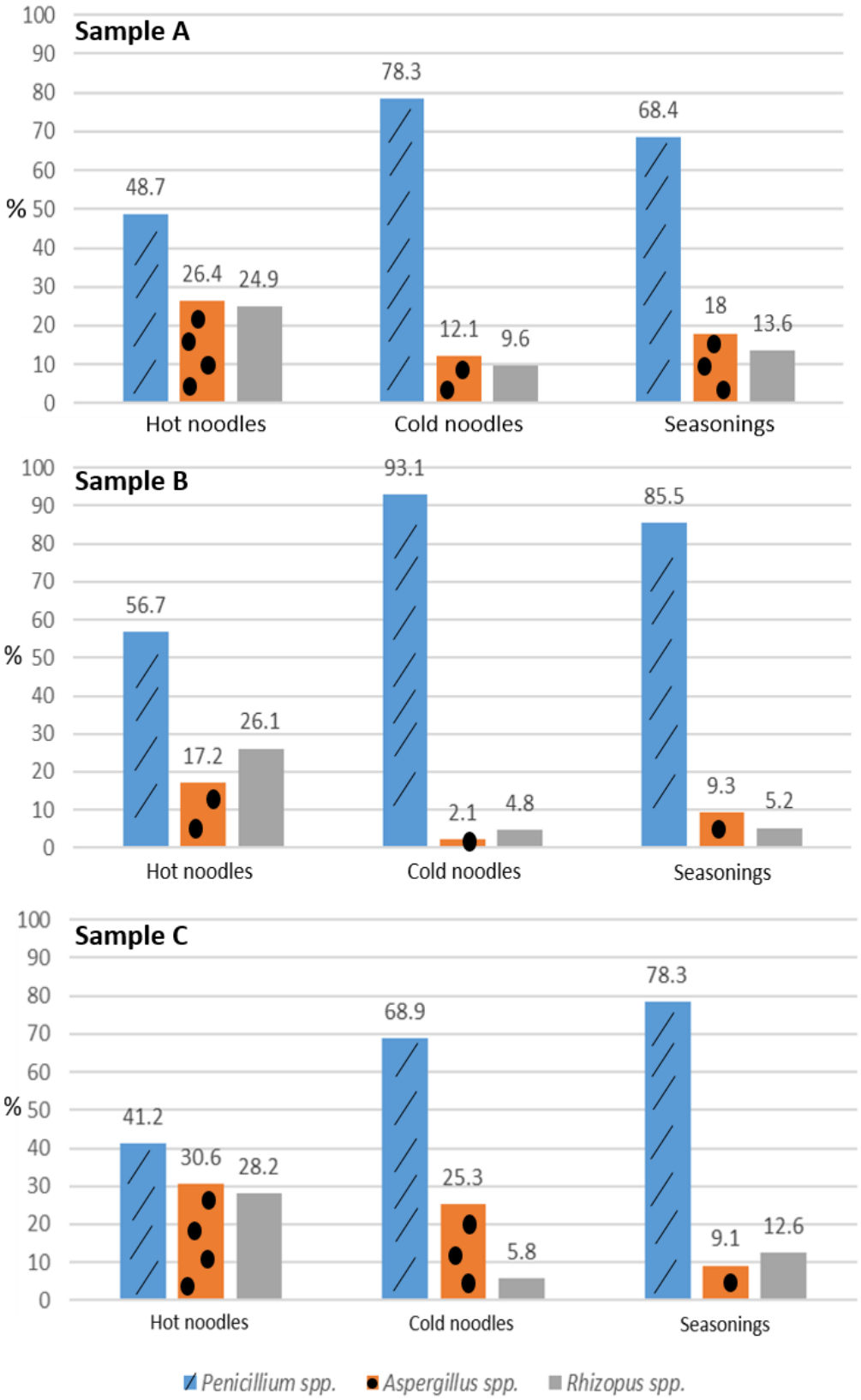

Figure 2 **Frequency of isolated filamentous fungi from samples A, B and C (%)**

The reduction in microbial load for the hot samples is in line with ICMSF (2011), which indicated that pH, water activity, and temperature control can be modified to control the production of fungal toxins. However, temperature does not protect against all toxigenic molds, since many can expand at cooling temperatures. Multiple toxigenic species have been found to be capable of growth and toxin production takes place at temperatures as low as 10°C (Akhigbemidu W. *et al*, 2015).

Because of the fact that some strains may be more toxigenic at low temperatures than at optimum growth temperatures, proper cooking of noodles and its seasonings is the best means of controlling growth of microorganisms in foods

The results underline the importance of performing microbiological control in order to ensure safe and uncontaminated food, but they may also indicate the existence or absence of a nutrient substrate necessary for the proliferation of microorganisms.

In all three instant noodles samples, a maximum concentration of about 15.6 cfu/g of bacterial and fungal spores in the hot samples was in agreement with the value recommended by ICMSF (ICMSF, 2011) for unfilled pasta. Similar results were reported by Akhigbemidu W. *et al* (2015) and Akineden *et al* (2015) in instant noodles and, respectively, in raw dried pasta.

The quality and safety of the finished goods depend directly on the quality and safety of the raw materials. Flour, water and various ingredients are raw materials for noodles and related items that are added to enrich the nutritional value of the final product (Bejarovic G., 2001).

CONCLUSIONS

The presence of microorganisms in noodles may be unavoidable due to the production, storage and handling, but they can drastically be reduced to a minimum level by good packaging and storing in a suitable environment to ensure good quality and safe noodles.

Highest microbial load is found in accompanying seasoning packets.

Rehydration with hot water reduce the concentration of microorganisms significantly.

Sample B had the highest bacterial count of 16×10^3 cfu/g for cold noodles, and also the highest count of 6.6×10^3 cfu/g for hot noodles.

For the accompanying seasoning, the total bacterial count varied from 6×10^3 cfu/g (sample A) to 33×10^3 cfu/g (sample B). The total fungal count of all samples was slightly higher than that of the bacterial counts.

Microbial analysis showed the presence of Gram negative bacteria as predominant bacteria type, while *Aspergillus, Rhizopus* and *Penicillum* were the three isolated genera of fungi. *Penicillum* was the most frequently isolated genera of fungi in case of all brands of noodles.

In all three instant noodles samples the maximum concentration of bacterial and fungal spores was in agreement with the recommended value.

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