*Original scientific paper*
*UDC 663.4:579.67*

# MICROBIOLOGICAL SAFETY AND QUALITY EVALUATION OF THE RAW MATERIALS USED IN BEER PRODUCTION

**Linda Luarasi[1*], Rozana Troja[2], Luljeta Pinguli[2]**

**[1]Department of Biotechnology, Faculty of Natural Sciences, University of Tirana, Bulevardi "Zogu I", 1016 Tirana, Albania**
**[2]Department of Industrial Chemistry, Faculty of Natural Sciences, University of Tirana, Bulevardi "Zogu I", 1016 Tirana, Albania**

**[*]e-mail: linda.luarasi@gmail.com**

## Abstract

The safety and quality of the final beer product depends not only on the fermentation process, but also on the quality of the raw materials used. Microbiological contamination of raw materials can lead to a premature spoilage of the beer, caused by spoilage bacteria, yeasts and molds, and gushing - an unhealthy strong beer foaming - caused by barley *Fusarium* spp. Water is an important raw material. It is used in malt steeping, boiling and even in the cleaning processes and beer containers, so it must meet the microbiological standard of drinking water. The ionic composition of water is also important for mashing, hop boiling, fermentation and contribution to beer flavor. The routine microbiological analysis is to ensure absence of pathogenic bacteria that are spread by contaminated water supplies. Hops or hop extracts have not actually been implicated in instances of contamination.

The experimental work involved a general microbiological evaluation of raw materials using morphological and physiological methods for the microorganisms' determination. Coliform counting in brewing water, as an indicator of the presence of enteric bacteria, was performed using McConkey culture medium, applying limited dilution method. Malt microbiological examination of *Fusarium* spp. and others, a potential risk caused by the heat stable mycotoxins or the polypeptide gushing factor, was performed using pour plate method in Czapek and Plate Count Agar medium for the identification of the superficial microorganisms in malt grain. Microbiological control of hop and hop extracts was considered a negligible risk because of the hop antimicrobial properties.

The gained results showed a microbiological purity of brewing water related with water pretreatments applied in the brewery. Some specific microbial populations were observed during malt microbiological control. These species include *Fusarium* spp. and some colonies of *Penicillium* and *Aspergillus*.

The brewing water was treated using reverse osmosis technique, so the respective microbiological charge was negative. The most problematic specie was a Fusarium sp. identified during the experimental work, deriving from the surrounding environment of the malt storage.

***Key words**: Microbiological, Raw material, Quality control, Malt, Water.*

## 1. Introduction

This research work involves the microbiological stability of beer in regard with the quality of its raw materials. Beer is generally regarded as safe (GRAS) in terms of food-borne illnesses, because there are evidences that pathogens cannot grow in beer [1]. Since the modern brewery products are designed in very good conditions, hygiene still remains an important aspect in the brewing industry.

The brewing process itself is exposed to the risk of microorganisms because of the wort nutrient-rich environment. The entire production process, from wort boiling to beer packaging, with batch fermentation of up to several weeks, gives rise for unwanted microorganisms to develop if they are given the opportunity [1].

The objective of the research work is the detection and identification of beer contaminants originating mainly from the raw materials, including malt, water and hops.

### 1.1 Sources of contamination of beer

A potential source of contamination is through the raw materials, particularly brewing water, malt and hops. These are natural products whose surface can be populated with different strains of living organisms [10].

Water is the most important raw material. It is widely used, in many processes of brewing such as malt steeping, wort boiling, high-gravity beer dilution water, as well as water used in cleaning processes. So it must meet the microbiological standards for drinking water. Any water supply intended for direct consumption or used in beverages must be clear, odorless, and free of dangerous chemical substances. The aim of the routine microbiological analysis is important to determine the presence of these pathogenic microorganisms which may be spread by water supply.

Although molds are not spoilers of beer, their presence in barley may negatively impact on the quality of the malt, wort and beer. The nature and magnitude of the barley-associated microflora will depend on both the field conditions under which the barley crop was grown and the post-harvest history of the grain [2]. The expected barley microflora consists of molds that contaminate and colonize the grain in the field, and molds that grow on the grains during storage. Mold contaminants may produce heat stable mycotoxins or the polypeptide gushing factor particularly associated with certain *Fusarium* spp. [3].

Hop and hops extracts, which serve as additives in the final steps of wort preparation, are also a theoretical microbiological risk, but have not been implicated in instances of contamination [4]. It is not usual practice to carry out a microbiological count on hop products. Studies of the roles of beer components such as dissolved carbon dioxide, phenolic compounds, undissociated hop compounds and undissociated sulphur dioxide have shown that these components have a positive impact under certain conditions on the biological stability of beer [2].

The standard methods for microbiological analyses in brewing and other related industries are described in Analytica Microbiologica EBC (European Standards), Recommended Methods of Analysis of the Institute of Brewing (Britain and Ireland Standards) and the Official Methods of American Society of Brewing Chemists (North and South America) [5]

## 2. Materials and Methods

### 2.1 Microbiological control of water

The main purpose of the bacteriological examination of water is to assure that water does not contain any harmful and pathogenic microorganisms. The indicator organisms are usually the group of total coliform bacteria [6].

Sterile 500 mL capped glass bottles were used for sampling, with the neck covered by aluminum foil to maintain sterility during transport. The samples were taken directly by holding the bottle in the tank. The initial procedure was the fermentation in multiple test tubes. And then the confirmation test was realized in solid medium. The coliforms were incubated at 37 $^0$C on a selective medium for enteric bacteria. The cultivation method was performed using Mac Conkey medium [7].

### 2.2 Microbiological control of malt

In the evaluation of fungal contamination of barley and malt, the extent of contamination is expressed as the percentage of kernels contaminated with the respective fungus [8]. Since the growth of fungi is slow, longer incubation times are necessary.

For a general evaluation of malt grain microorganisms, the whole kernels were plated directly on solid media. Five randomly selected kernels were placed in Petri dishes and incubated at 27 - 28 $^0$C for 7 days. The cultivation method was performed using PDA and Czapek media for the identification of both field and storage molds. Number and type of microorganisms were determined after the period of incubation in the appropriate temperature [8].

### 2.3 Microbiological control of hops

Although, hops and hop extracts are considered as theoretical microbiological risk, because of antimicrobial properties of their chemical substances, a formal examination was performed using the cultivation method on pour plates. The petri dishes with PCA and Czapek medium were incubated in 28 - 30 $^0$C for 7 days.

## 3. Results and Discussion

Considering the reference values for contaminated water in assessing the results, even one positive test at 44 $^0$C, indicating fecal pollution excludes the water for drinking or for food and beverages processing. Organisms in the 37 $^0$C samples are potentially of human and animal origin and even 10 colonies per mL is a warning of doubtful supply [3].

During the bacteriological assessment of the brewing water, there was no formation of colonies after the incubation (Figure 1). The obtained results showed a microbiological purity of the water related with water pretreatments applied in the brewery. The applied purification technology of water was reverse osmosis.

Regarding the barley malt test, some specific microbial populations were observed during malt microbiological control. These species include *Fusarium* sp. and some colonies of *Penicillium* and *Aspergillus genera*. *Fusarium* sp. was believed to originate from barley grains

grown on field. The other species observed during the kernel testing were *Aspergillus candidus*, *Penicillium* spp., and *Rhizopus nigricans* (Figure 2).

When kernels have been plated directly on to agar media without surface disinfection, small numbers have yielded penicillia and aspergilli. The aspergilli most frequently reported belong to the *A. glaucus* group (a reserach work carried out by Tuite and Christensen) [11].

Other related studies have shown that mycotoxin, fumonisin B1, produced by *Fusarium* molds was identified in beer products. The Lincoln study followed an earlier report of fumonisin contamination of beer in Canada that had included beers that were imported from the United States. These authors agreed that the most likely source of fumonisin in beer is the maize grits that are used as a brewing adjunct [9].

During the microbiological evaluation of hops, there was no growth of microorganisms after the incubation of Petri dishes. So according to Wiles study carried out in Tennant's Brewery, when hops of the 1948 crops were examined, out of 127 tests, growth within 1 week was only recorded in 7 cases [4].

Grain with a high moisture content must be dried to < 14% if it is to be stored for any period of time, and to < 12.5% to exclude the possibility of any mold growth in storage. The resulting delay in drying the harvested grain can allow microorganisms to develop and the grain to heat [5].

Obtained microbiological parameters of malt grains are performed as follows (Tables 1, 2 and 3):

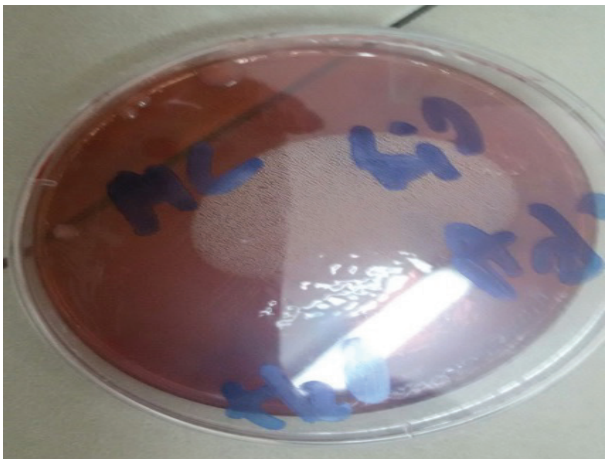

**Figure 1. Total count of microorganisms of brewing water in MacConkey agar**

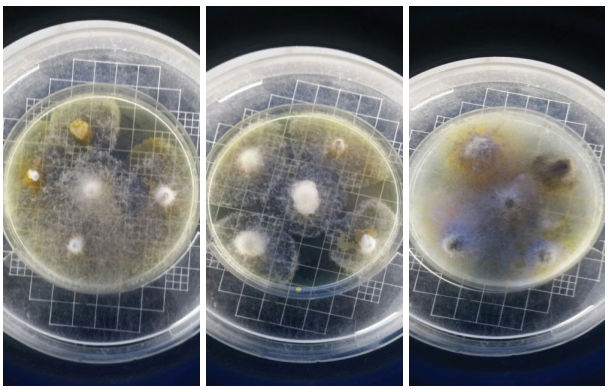

**Figure 2. Total count of microorganisms of malted barley in PDA**

**Table 1. Microbiological parameters of malt grains**

| Parameter | Units | Results | Limits | Compliant | Method |
|---|---|---|---|---|---|
| **Total aer. count** | CFU/g | < 10 | $10^5$ | Yes | HRN ISO 4833:2008 |
| **Enterobactericeae** | CFU/g | < 10 | $10^4$ | Yes | HRN ISO 21528-2:2008 |
| **Yeasts and molds** | CFU/g | < 10 | $10^4$ | Yes | HRN ISO 21527-1:2008 |

**Table 2. Mycotoxins LC-MS/MS**

| Parameter | Units | Results | Limits | Compliant | Method |
|---|---|---|---|---|---|
| **Total aflatoxin** | µg/kg | < 2 | 4 | Yes | RU-MET-84 |
| **Aflatoxin B1** | µg/kg | < 0.5 | 2 | Yes | RU-MET-84 |

**Table 3. Mycotoxin ELISA**

| Parameter | Units | Results | Limits | Compliant | Method |
|---|---|---|---|---|---|
| **Deoxynivalenone** | µg/kg | < 50 | 750 | Yes | RU-MET-60 |
| **Ochratoxin A** | µg/kg | < 2 | 3 | Yes | RU-MET-80 |
| **Total aflatoxin** | µg/kg | < 2 | 4 | Yes | RU-MET-104 |
| **Zearalenon** | µg/kg | < 3 | 75 | Yes | RU-MET-98 |

## 4. Conclusions

- Considering the obtained results during the evaluation of microbial charge in the raw materials for beer production, it is noted that the fungal charge in malt grains is inconsiderable. There is no evidence of spoilage bacteria and yeast which means that there is no symbiotic, metabiotic and antagonistic activity of these microorganisms against the fermenting yeast.

- The mold colonies observed during the microbiological control include species of *Aspergillus*, *Penicillium* and *Rhizopus*. The *Fusarium* sp. requires a periodical monitoring for the detection of the grain molds. It is important to emphasize that there is no evidence of *Aspergillus flavus* and *Aspergillus glaucus*.

- Since the microbial charge of malt, during the microbiological control of the raw materials, is relatively small, all the contaminants in the final product originate from the surrounding environment as well as from the production technology.

- In the further studies concerning the contaminants, it is suggested a periodical control of the production areas. In the brewing environment it is important the application of the hygienic practices by setting the critical control points in the technological line.

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