Identification of Microbial Contamination Sources in Distilled Spirits

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Abstract. Due to their high alcohol content, distilled spirits are not susceptible to microbial contamination. Because moulds were found in samples of vodka and spirit drinks, the present study was conducted to identify the sources of microbial contamination during the manufacturing process. Total bacterial count (TBC), total yeast and mould count (TYMC) and total coliform count (TCC) were determined in water and from different processing area surfaces, TBC and TYMC in the air of processing areas and TYMC in distilled spirits samples. The source of microbial contamination of distilled spirits was microaeroflora from processing areas.

Keywords: Vodka, spirit drink, contamination source, moulds.

INTRODUCTION

Ethyl alcohol, generally obtained by biotechnology, has applications in food, chemical and pharmaceutical industry (Banu, 2009).

In the context of quality control of alcoholic beverages in distilleries and breweries or in official food control, a range of different analytical methods has to be used (Lachenmeier et al., 2007).

Many studies classified and verified the adulteration of alcoholic beverages (Da Costa et al., 2004; Lachenmeier et al., 2003; Pontes et al., 2006; Sarvarova et al., 2011; Savchuk et al., 2001; Savchuk et al., 2007). The most evaluated compounds present in spirit drinks and other alcoholic beverages are phenolic and volatile compounds (Castro et al., 2008; Duran Guerrero et al., 2011; Korhonova et al., 2007; Madrera et al., 2005; Mangas et al., 1996; Schwarz et al., 2011; Versini et al., 2009).

All spirit drinks must have a minimum alcoholic strength of 15% vol. (Regulation (EC) No 110/2008). Vodka represents a spirit drink produced from ethyl alcohol of agricultural origin. It has a minimum alcohol content of 37.5% v/v. The most important raw materials used are potatoes, different kind of cereals or molasses. The flavourings added in spirit drinks can be allowed only if the type of spirit permitted it (is not allowed in the case of vodka) and must be specified on the label.

The main aim of this investigation was to identify the sources of microbial contamination of distilled spirits from a local distillery during manufacturing process.

MATERIALS AND METHODS

Sampling and experimental design

Samples of vodka and spirit drinks, with different alcohol content, were aseptically collected from a local distillery (Table 1) and tested for TYMC. Sanitation samples from various production area surfaces (hose, stainless steel table and chopper) were collected using sterile cotton swabs and air samples from different processing area surfaces and zones were collected using Petri dishes containing the growing medium (Table 2).
Sealed bottles from the warehouse were used for distilled spirits sampling. Sterile cotton swabs were used for sampling. After rubbing the surface to be sampled, the cotton swabs was inserted into the test tube. The sampled surface was 100 cm². Microbiological examination of the microaeroflora was done in the preparation room of distilled spirits and in the area of manual and automatic bottling.

Drinking water used in the manufacturing process was also tested. Sampling of drinking water was done in aseptic containers with ground-glass stoppers from the distribution pipeline network.

**The microbiological quality of spirit drinks and drinking water samples**

**TYMC test.** The reference method (SR ISO 21527-1:2009) was used for enumeration of yeasts and moulds in distilled spirits samples. Media used were Dichloran Rose Bengal Chloramphenicol Agar, DRBC (Oxoid, Basingstoke, Hampshire, UK) and Dichloran 18% Glycerol Agar, DG-18 (Oxoid, Basingstoke, Hampshire, UK). Chloramphenicol (Oxoid Ltd., Basingstoke, Hampshire, UK) was added (100 mg/L) to the media as a selective agent to inhibit the growth of bacteria.

Preparation and dilutions of distilled spirits and drinking water samples was in accordance with ISO 7218:2007, SR EN ISO 6887:1:2002 standards. For each sample was prepared two successive dilutions. Three Petri dishes was used for each dilution. An aliquot of 0.1 mL of the diluted sample was inoculated using a sterile pipette into each Petri dish containing DRBC and DG-18 media. Then, the Petri dishes were sealed in plastic bags (to avoid contamination) and incubated at 25 ± 1°C for 5 days.

After five days of incubation, the colonies are counted. The total number of yeasts and moulds is calculated using the formula specified in the standard:

\[
N = \frac{\Sigma c}{(V(n_1 + 0.1 \times n_2)) \times d} \quad [\text{cfu/mL}]
\]

where:

- \(\Sigma c\) - colonies sum;
- \(V\) - inoculum volume (mL);
- \(n_1\) - Petri dishes number for first dilution;
- \(n_2\) - Petri dishes number for second dilution;
- \(d\) - dilution.

**TBC test.** ISO 4833:2003 method was used for enumeration of total bacteria in drinking water. Drinking water sample was inoculated using a sterile pipette into two Petri dishes (1 mL in each Petri dish). Fifteen mL of Nutrient Agar (Oxoid, Basingstoke, Hampshire, UK) were added and mixed in order to homogenized and allowed to solidify. Then, the plates were incubated at 30 ± 1°C for 2 days. The total number of bacteria is calculated using the formula:

\[
N = \frac{\Sigma c}{n} \quad [\text{cfu/mL}]
\]

where:

- \(\Sigma c\) - colonies sum;
- \(n\) - Petri dishes number;

**The microbiological quality of material and equipment surfaces from processing areas**

**TBC test.** ISO 4833:2003 method was used for enumeration of total bacteria from the surfaces. The cotton swabs were suspended in 10 mL of sterile saline solution and vortexed for 1.0 min. An aliquot of 1.0 mL is inoculated using a sterile pipette in two Petri dishes (1 mL in each Petri dish). Fifteen mL of Nutrient Agar (Oxoid, Basingstoke, Hampshire, UK) were added.
and mixed in order to homogenize and allowed to solidify. Then, the plates were incubated at 35 ± 1°C for 2 days. The total number of bacteria is calculated using the formula:

\[ N = \frac{\Sigma c}{10} \quad \text{[cfu/cm}^2] \]

where:
\( \Sigma c \)-colonies sum;
10-to express the results in cm\(^2\);

**TCC test.** SR ISO 4832:2009 method was used for detection of coliform bacteria from the surfaces. The cotton swabs were suspended in 10 mL of sterile saline solution and vortexed for 1.0 min. An aliquot of 1.0 mL is inoculated in 10 mL brilliant green lactose bile broth with fermentation tube and incubated at 35 ± 2°C for 48 hr. Gas production in 48 ± 2 hours or less is a positive reaction. The result is expressed as positive or negative/100 cm\(^2\).

**The microbiological air quality at processing areas**

**TBC test.** ISO 4833:2003 method was used for enumeration of total bacteria in air. Two Petri dishes with Nutrient Agar are left uncovered in the targeted environment (one on the floor and one at 0.8-1.0 m above the floor). After 10 min of sampling, the plates are covered and incubated at 35 ± 1°C for two days. After two days of incubation, the colonies are counted. The total number of bacteria is calculated using the above formula. The result is expressed as cfu/m\(^3\).

**TYMC test.** The same reference method (SR ISO 21527-1:2009) was used for enumeration of yeasts and moulds in air. Two Petri dishes with DRBC and DG-18 are left uncovered in the targeted environment (one on the floor and one at 0.8-1.0 m above the floor). After 10 min of sampling, the plates are covered and incubated at 25 ± 1°C for five days. After five days of incubation, the colonies are counted. The total number of yeasts and moulds is calculated using the above formula. The result is expressed as cfu/m\(^3\).

**RESULTS AND DISCUSSIONS**

From the microbiological point of view, distilled spirits must be sterile, free of microorganisms and without risk to consumer health. Distilled spirits contains alcohol, water and different flavors, sugar or other sweetening products (Regulation (EC) No 110/2008). The Spirit Drinks Regulation 2008 set out clearly defined criteria for the production, description, presentation and labelling of spirit drinks, as well as on the protection of geographical indications.

In distilled spirits, the ethyl alcohol is obtained by distilling from agricultural origin raw. The alcohol has the ability to destroy microorganisms and prevent their multiplication, so, is supposed to be a limiting factor for microbial growth. In the samples taken in study, TYMC ranged between 0.35 and 0.70 x 10\(^1\) cfu/ml (Table 1) and only moulds were found (Figure 1). The highest TYMC level was found in distilled spirits with the biggest alcohol content. Thus, we concluded that samples contamination could occur during processing through drinking water used in distilled spirits production, materials and equipments or the air from the production areas.
Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>TYMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vodka 1, 40% v/v alcohol</td>
<td>0.70 x 10^3 cfu/mL</td>
</tr>
<tr>
<td>Vodka 2, 40% v/v alcohol</td>
<td>0.66 x 10^3 cfu/mL</td>
</tr>
<tr>
<td>Vodka 3, 37.5 % v/v alcohol</td>
<td>0.40 x 10^3 cfu/mL</td>
</tr>
<tr>
<td>Vodka 4, 37.5 % v/v alcohol</td>
<td>0.35 x 10^3 cfu/mL</td>
</tr>
<tr>
<td>Spirit drink, 26 % v/v alcohol</td>
<td>0.41 x 10^3 cfu/mL</td>
</tr>
<tr>
<td>Spirit drink, 26 % v/v alcohol</td>
<td>0.45 x 10^3 cfu/mL</td>
</tr>
</tbody>
</table>

Figure 1. Moulds in vodka with 40% and 37.5% v/v alcohol

The water used in the preparation of distilled spirits must have the quality of water for human consumption (Council Directive 98/83/EC) and may be distilled, demineralised, permuted or softened. According to this Directive, TBA at 37°C should be ≤ 20 cfu/mL, TBA at 22°C ≤ 100 cfu/mL, *Escherichia coli* (*E. coli*) absent/250 mL, Enterococci absent/250 mL, *Pseudomonas aeruginosa* absent/250 mL. TYMC in drinking water is not regulated. Yeasts and moulds were absent in distilled spirits (Table 2), suggesting that the drinking water was not the source of contamination.

The working conditions should be clean, safe and hygienic. According to Ordin 976/1998, TBC on the working surfaces from the production chain that are exposed to food should be ≤ 2 cfu/cm² if coliform bacteria are absent/10 cm². TYMC in drinking water is not regulated. Coliform bacteria were absent, and TBC (Table 2) meet the regulatory limit. TYMC was very low (1.0-1.3 cfu/cm²) suggesting that the working surfaces were not the source of contamination.

The requirements for microbial air quality of processing areas are: TBC ≤ 600 cfu/m³ and TYMC ≤ 300 cfu/m³. The level of microbial air pollution was considerable high (Table 2). Air samples from the bottling line rooms had the higher microbial loads (Figure 2). 62.5% of air samples exceeded the regulatory limits for TYMC which shows that air was the microbial contamination source for distilled spirits.

Studying the chemical composition of distilled spirits was noticed that these alcoholic beverages contain glycerol. The presence of moulds in distilled spirits may be due to the fact that glycerol is a nutritional substrate for moulds.
Table 2

Microbial load of working surfaces, air and drinking water

<table>
<thead>
<tr>
<th>Sample</th>
<th>TBC</th>
<th>TCC</th>
<th>TYMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hose</td>
<td>1.0 cfu/cm²</td>
<td>absent/cm²</td>
<td>1.0 cfu/cm²</td>
</tr>
<tr>
<td>Stainless steel table-production room 1</td>
<td>1.3 cfu/cm²</td>
<td>absent/cm²</td>
<td>1.3 cfu/cm²</td>
</tr>
<tr>
<td>Chopper</td>
<td>1.3 cfu/cm²</td>
<td>absent/cm²</td>
<td>1.3 cfu/cm²</td>
</tr>
<tr>
<td>Air - production room 2-zone 1</td>
<td>0.30 x 10³ cfu/m³</td>
<td>-</td>
<td>0.375 x 10³ cfu/m³</td>
</tr>
<tr>
<td>Air - production room 2-zone 2</td>
<td>0.25 x 10³ cfu/m³</td>
<td>-</td>
<td>0.375 x 10³ cfu/m³</td>
</tr>
<tr>
<td>Air - production room 2-zone 3</td>
<td>1.50 x 10³ cfu/m³</td>
<td>-</td>
<td>0.320 x 10³ cfu/m³</td>
</tr>
<tr>
<td>Air - bottling line 2-zone 1</td>
<td>0.20 x 10³ cfu/m³</td>
<td>-</td>
<td>0.950 x 10³ cfu/m³</td>
</tr>
<tr>
<td>Air - bottling line 2-zone 2</td>
<td>0.25 x 10³ cfu/m³</td>
<td>-</td>
<td>1.225 x 10³ cfu/m³</td>
</tr>
<tr>
<td>Air - bottling line 2-zone 3</td>
<td>0.35 x 10⁵/m³</td>
<td>-</td>
<td>1.100 x 10⁵ cfu/m³</td>
</tr>
<tr>
<td>Air - bottling line 4-zone 1</td>
<td>0.20 x 10³/m³</td>
<td>-</td>
<td>0.625 x 10³ cfu/m³</td>
</tr>
<tr>
<td>Air - bottling line 4-zone 2</td>
<td>0.10 x 10⁵/m³</td>
<td>-</td>
<td>0.675 x 10⁵ cfu/m³</td>
</tr>
<tr>
<td>Air - bottling line 4-zone 3</td>
<td>0.25 x 10⁵/m³</td>
<td>-</td>
<td>0.500 x 10⁵ cfu/m³</td>
</tr>
<tr>
<td>Drinking water</td>
<td>absent/mL</td>
<td>-</td>
<td>absent/mL</td>
</tr>
</tbody>
</table>

Figure 2. TYMC in air (bottling line)

CONCLUSIONS

Air microflora was the source of moulds contamination of distilled spirits. Microbiological quality of distilled spirits is closely related to the implementation of GHP (Good Hygiene Practices). Further measures for reducing microaeroflora from bottling line rooms are mandatory.

ACKNOWLEDGMENTS

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