

Methods for staining microbial capsules and spores in the microbiological study of Rkatsiteli wine materials and fruit juices

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Abstract

The paper discusses the methods of staining microbial capsules and spores in Rkatsiteli wine material and fruit juices. It has been established that the correct selection of staining methods and the membrane filtration process ensure the stability, safety and quality of the product, which is of paramount importance in technological processes.

The paper discusses the methods of staining microbial capsules and spores in Rkatsiteli wine materials and fruit juices. It is established that the correct selection of staining methods and the membrane filtration process ensure the stability, safety and quality of the product, which is of paramount importance in technological processes.

Keywords: wine material, fruit juice, capsules, spores, staining methods, membrane filtration

Introduction

Microbiological safety of beverages is one of the main challenges of modern food technology. During the production of wine and fruit juices, microorganisms introduced from raw materials are often present in them, which affect the fermentation, organoleptic and stability properties of the product [1,2,5]. A diverse microbiological spectrum is of particular importance during the maturation of the traditional Georgian variety Rkatsiteli wine [6]. Similarly, fruit juices are easily colonized by yeasts, bacteria and sometimes spore-forming forms, which requires accurate identification of the microbes.

Literature Review

One of the main methods for studying the morphological features of microbes is the staining technique. Common stains (methylene blue, fuchsin) allow for the observation of cell shape [3]. The Gram method classifies according to the structure of the cell wall, which is essential for controlling the microflora of wine (*Lactobacillus*, *Oenococcus*, *Acetobacter*). Spore staining (Schaeffer–Fulton) provides for the detection of the genera *Bacillus* and *Clostridium*, which are often found in environmental sources. Capsule staining (India ink, nigrosine) is especially important for the identification of capsular yeasts and bacteria in fruit juices [4,5,6].

Analysis and Methods

The samples were taken for the study of Rkatsiteli wine material, pasteurized and raw fruit juice, which were prepared using the production method.

The samples were processed before and after filtration with membranes with a pore size of 0.2 μm for Rkatsiteli wine material and 0.45 μm for fruit juices. Microscopic smears were prepared from the precipitate remaining on the filter, which were fixed and stained using the following methods:

1. General staining – methylene blue, fuchsin.
2. Gram method – Crystal violet, Lugol's solution, Decolorizer, Safranin.
3. Spore staining (Schaeffer–Fulton) – malachite green and safranin.
4. Capsule staining – India ink, nigrosine.
5. Additional methods – Ziehl-Neelsen (acid-fast bacteria), Acridine Orange (fluorescent).

Results and Conclusions

Gram-positive lactobacilli and *Oenococcus oeni*, as well as gram-negative acetic acid bacteria and *Bacillus* spores, which are related to the microflora of the grape surface, were detected in the Rkatsiteli wine material.

Yeasts (*Saccharomyces*, *Candida*) dominated in the fruit juice. When staining with Indian ink, capsular forms of *Candida* were detected, which is related to the storage conditions of the product.

The results show that general staining is not sufficient for accurate identification of microbes. Specific staining of spores and capsules is necessary to differentiate pathogenic and technologically important microorganisms.

Using the Gram rule, a method of differential staining of bacteria, we were able to establish the systematics of bacteria and microbiological diagnostics of diseases. Spore staining is especially necessary for the detection of *Bacillus* and *Clostridium*. Coloring of capsules is necessary to control yeasts and Enterobacteriaceae in fruit juices.

Conclusion

It was determined that the methods of staining microbial capsules and spores are a reliable diagnostic tool, which is important for assessing the microbiological quality of both Rkatsiteli wine and fruit juice. The Gram method determines the main groups of bacteria. The use of membrane filtration ensures the stability, safety and quality of the product, which is of paramount importance in technological processes.

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**მიკრობთა კაფსულებისა და სპორების შეღებვის მეთოდები
რქაწითელის ღვინომასალისა და ხილის წვენების მიკრობიოლოგიურ
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ინსტიტუტი

რეზიუმე:

ნაშრომში განხილულია რქაწითელის ღვინომასალისა და ხილის წვენებში მიკრობთა კაფსულებისა და სპორების შეღებვის მეთოდები. დადგენილია, რომ შეღებვის მეთოდების სწორი შერჩევა და მემბრანული ფილტრაციის პროცესი უზრუნველყოფს პროდუქციის სტაბილურობას, უსაფრთხოებას და ხარისხს, რაც ტექნოლოგიურ პროცესებში უმნიშვნელოვანესია

საკვანძო სიტყვები: ღვინომასალა, ხილის წვენი, კაფსულები, სპორები, შეღებვის მეთოდები, მემბრანული ფილტრაცია