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MICROBIOLOGICAL SAFETY OF SOFT DRINKS

Abstract. For the past few decades, beverage manufacturers have spent on solving a global problem: to achieve products that are microbiologically safe and highly resistant. This goal was successfully achieved by improving production sanitary condition, as well as by expanding pest microorganisms' knowledges. However, significant changes are currently being observed in non-alcoholic drinks assortment: juice drinks, non-alcoholic malt drinks, tooth-friendly drinks, functional drinks are becoming increasingly popular. There is a tendency to create drinks of more complex composition, enriched with additional nutrients, dietary fiber, with low acidity and low carbonation level. The consumer wants to get more "natural" drinks, without adding chemical preservatives and without negative effects of pasteurization. This leads to loss of many of antimicrobial barriers that existed in traditional drinks. Due to these changes in drinks, number of cases of spoilage will increase. The main types of spoilage microorganisms, lactic acid bacteria and yeast are likely to remain in new drinks, but it is very likely that list of species will expand. It is assumed that role of bacteria in drinks spoilage will increase. New, recently discovered spoilage microorganisms appeared, among which are acid-resistant aerobic bacillus *Alicyclobacillus* in drinks, bottled in PET bottles, *Asaia* species in flavored mineral waters, *Propionibacterium cyclohexanicum* in drinks, enriched with juice, and spore-forming bacteria and enteric bacteria in low acidity drinks. New health risks in drinks production may also arise due to expansion list of countries importing ingredients, as well as due to use of juices with low acidity, in particular vegetable ones, as ingredients. Predictive microbiology will help in predicting and describing behavior of contaminant microorganisms in drinks and in optimizing preservative systems. New challenge, faced by drinks manufacturers is creation of safe products with high resistance and preservation of best taste and aromatic properties with minimal processing. Implementation of this task consists in qualified and scientifically sound combination of antimicrobial barriers. This review contains up-to-date information on microorganisms for spoiling soft drinks and microorganisms that pose food safety risks for consumers.

Key words: soft drinks, ingredients, spoilage microorganisms, pathogenic microorganisms, preservatives, pasteurization, barrier technologies, predictive microbiology.

Over the past twenty years, significant changes have taken place in global soft drinks market. Functional drinks and bottled water currently represent fastest growing sectors [1, 2]. Energy drinks, non-carbonated drinks, tooth-friendly drinks, and non-alcoholic malt drinks are gaining popularity. Alcohol-containing mixed drinks are becoming increasingly popular. These new drinks are often more complex in composition than traditional drinks and have fewer antimicrobial barriers compared to them due to their higher nutrient's concentration, lower acidity (pH > 3.5-4.0) and lower carbonation levels. Heat treatment and use of chemical preservatives are also being reduced in order to create more "natural" products. In addition, increasing number of consumers want minimal introduction of chemical and technological additives in food products made from natural ingredients in order to preserve their nutritional and taste advantages. Studies that have shown possible presence of carcinogenic benzene in soft drinks due to

interaction of benzoates (preservatives) with ascorbic acid, as well as possible allergic reactions to sulfites and benzoates, have contributed to development of this trend among consumers. Possible negative effect of benzoic acid has already forced many soft drinks manufacturers to abandon of this preservative use. Thus, traditional antimicrobial barriers that were present in previous non-alcoholic drinks are no longer applied, while duration of products transportation, shelf storage time, vastness of international relations, as well as new ingredients inclusion have increased significantly [3-5].

The purpose of this review is to summarize current knowledges about two sides of the problem in soft drinks production: microbiological spoilage and food safety risks for consumers.

Non-alcoholic drinks spoilage microorganisms. The main microorganisms' types causing modern drinks spoilage may remain the same as for traditional ones, but the list of species will inevitably expand. It is expected that bacteria will play an increasingly important role in food spoilage among other microorganisms. Among new, previously unknown and relatively recently identified causative spoilage agents (in English literature a special term has been created for them - **emergent**, from English emergence), acid-resistant aerobic bacteria *Alicyclobacillus* are found in drinks, bottled in PET bottles; acetic acid bacteria of genus *Asaia* in aromatic mineral waters; *Propionibacterium cyclohexanicum* in juice drinks; and spore-forming bacteria and enterobacteria in drinks with low acidity [3, 4].

When new soft drinks are being developed, it is very important to track all changes in recipe, packaging, and preservation methods in order to assess possible microbiological risks. Modern drinks usually contain several stimulants and inhibitors of microorganisms growth, and therefore it is very difficult to predict their microbiological resistance. **Predictive microbiology** can help in optimizing preservative systems and in predicting and describing behavior of contaminants in beverages [6, 7]. The task of the future in the field of drinks production is to create safe and durable products with minimal processing [2, 4, 8].

Soft drinks can be classified according to various criteria (table 1): sugar content (high-calorie or dietary), juice content, carbonation degree (carbonated and non-carbonated), and the main ingredient in addition to water (fruits, malt, tea, soy, milk, etc.) and functionality. Functional drinks are the trend of today. Detailed composition and characteristics analysis of all ingredients (sweeteners, acidity regulators, flavors, preservatives, etc.) in modern drinks of various categories can be found in review [4].

Table 1 – Categories and typical properties of soft drinks [3, 4]

Category	Typical ingredients	Carbonization	pH	Monosaccharides
Cola and lemonade	Sweeteners, sugars, acids, including phosphoric (E 338), flavors, preservatives	From medium to high	2.4-3.2	0-10%
Health drinks	Plant extracts, soluble fiber, vitamins, minerals, preservatives	From low to medium	3.5-4.5	2-7%
Malt drinks	Fermented wort, organic flavor, sweeteners	From low to medium	–	–
Energy drinks	Caffeine, laurin, herbal extracts, L-carnitine, sugar, glucuronolactone, B vitamins, preservatives	From low to medium	2.5-3.2	1.4-14%
Sport drinks	Salts, monosaccharides, (caffeine, amino acids), preservatives	Weak or 0	3.2-4.0	5.5-8%
Tooth-friendly drinks	Low calorie carbohydrates, preservatives	Weak or 0	≥5.0	0%

In USA and EU countries, the following mandatory programs are used to ensure drinks food safety: Good Manufacturing Practice (GMP), Good Hygienic Practice (GHP) and HACCP (Hazard Analysis and Critical Control Points) [4].

In Russian Federation, the system for ensuring microbiological safety of food products consists of several positions: state policy; good manufacturing practice (technology, sanitary regime, production control) in production, storage, transportation, food sale; hygienic regulation and sanitary and epidemiological requirements for food products; sanitary requirements and examination of food raw materials; implementation of state supervision (control) in circulation; development, unification, standardization of analysis methods and ensuring adequate metrological parameters of laboratory control; foodborne disease surveillance [9].

Legal and methodological framework for food biosafety control in Russian Federation and EAEU for non-alcoholic drinks sector includes technical regulations of TR TS 021/2011 “On Food Safety”, TR TS 023/2011 “Technical Regulations for Juice Products”, TR TS 029/2012 “Requirements the safety of food additives, flavorings and technological aids” [9].

As a rule, microbiological contamination of soft drinks occurs during their preparation. Microbiological condition of raw materials, production facilities, equipment, violation of sanitary-hygienic regime - all this can cause infection. The source of infection can be containers - bottles and cans.

Microbiological drinks spoilage leads to changes in taste, aroma, to visible changes appearance in product - turbidity, precipitation, color change, sliming due to exopolysaccharides formation (EPS), etc. (table 2). According to Stratford [8], in order for drink spoilage, it is necessary to achieve a certain, critical microbial cells concentration (10^5 – 10^6 cells/ml), i.e. presence of microorganisms' reproduction process.

Table 2 – Spoilage microorganisms and defects in drinks caused by them [3, 4]

Spoilage microorganisms	Foreign tastes/smells	Visual defects	Metabolites
Yeast	Yeast, spoiled beer, vinegar, aldehyde, oil, pineapple shade	Blown, damaged pack, clouding, flakes, surface film	CO ₂ , ethanol, diacetyl, acetaldehyde, acetone, esters, 1,3-pentadiene, pectin degradation, extracellular polysaccharides (EPS)
Lactic acid bacteria (LAB)	Cheesy, sour, green apples	CO ₂ loss, sliming, clouding	lactic acid, CO ₂ , ethanol, diacetyl, formic acid, extracellular polysaccharides (EPS)
Acetic Acid Bacteria (AAB)	Sour, vinegar	Clouding, blown pack, sliming	Acetic acid, gluconic acid, CO ₂ , ethyl acetate, acetone, extracellular polysaccharides (EPS)
Spore bacillus <i>Alicyclobacillus</i> spp.	Antiseptic shades, smoke	Without defects	2,6- dibromophenol, guaiacol (from vanilla acid)
Mycelial fungi (mold)	Musty, stale	Cottony mycelium lumps in liquid thickness, films of mycelium on surface, discoloration, blown pack	Formic acid, gluconic acid pH increase due to acid metabolism, gas formation, pectin degradation

Since microorganisms differ in their nutritional needs, various drinks are populated by different groups of spoilage microorganisms [5, 8, 10]. Introduction of new ingredients or new use of traditional ingredients can contribute to emergence of new types of pests in drinks, thereby expanding list of pests.

Yeast is typical soft drinks contaminant. They are constantly present both in industrial premises, and in ingredients. Yeast takes first place in carbonated drinks spoilage, mainly due to their ability to withstand a high degree of carbonation, as well as increased acidity. Most species grow in pH range of 1.5–8.5 [11], and optimum for their reproduction is in range of pH 3.0–6.5 [1]. Yeast, forming heat-resistant ascospores, are the main pests in carbonated drinks that undergo heat treatment [1].

According to Davenport [3], yeast can be divided into four groups depending on their ability to spoil soft drinks (table 3). The most dangerous of these are fermentation yeasts that are resistant to preservatives. They can cause product spoilage at almost any stage of production. The second group consists of yeast, which cause spoilage due to violations in technology of washing and disinfection. Most cases of infection are caused precisely by these species, which under normal conditions should be destroyed by preservatives and disinfectants. Yeast of third group serves as indicators of poor sanitary conditions in workplace, but by themselves don't cause damage to the drink. Fourth group consists of species that aren't usually associated with conditions for soft drinks production, i.e. are random.

Soft drinks yeast infection often manifests itself in change in taste and aroma caused by yeast fermentation products, as well as in cloudiness [1]. Accumulation of CO₂ can cause deformation and even package rupture. By destroying preservatives such as weak acids, yeast can thereby create conditions for development of other pests in drinks. Yeast forms ethanol as final fermentation product, which concentration in soft drink may exceed acceptable levels.

Table 3 – Pest yeast in soft drinks production [3, 4]

Group 1 Fermenter yeast, resistant to preservatives	Group 2 Pests and hygiene indicators	Group 3 Hygiene indicators	Group 4 Random
<i>Dekkera anomala</i> , <i>D. bruxellensis</i> , <i>D. naardenensis</i> <i>Saccharomyces cerevisiae</i> (atypical), <i>Sacch. exiguus</i> <i>Schizosaccharomyces pombe</i> <i>Zygosaccharomyces bailii</i> , <i>Z. bisporus</i> , <i>Z. lentus</i> , <i>Z. rouxii</i>	<i>Candida davenportii</i> , <i>C. parapsilosis</i> <i>Debaryomyces hansenii</i> <i>Galactomyces geotrichum</i> / <i>Geotrichum candidum</i> <i>Hanseniaspora uvarum</i> <i>Issatchenkia orientalis</i> <i>Lodderomyces alongisporus</i> <i>Pichia anomala</i> , <i>P. membranifaciens</i> <i>Saccharomyces bayanus</i> , <i>Sacch.</i> <i>cerevisiae</i>	<i>Aureobasidium pullulans</i> <i>Candida sake</i> , <i>C. solani</i> , <i>C. tropicalis</i> <i>Clavispora lusitaniae</i> <i>Cryptococcus albidus</i> , <i>C. laurentii</i> <i>Debaryomyces etchellsii</i> <i>Exophiala dermatitidis</i> <i>Rhodotorula glutinis</i> <i>Sporobolomyces salmonicolor</i> / <i>Sporidiobolus salmonicolor</i>	<i>Kluyveromyces lactis</i> , <i>K. marxianus</i>

Saccharomyces cerevisiae is the most common pest of soft drinks and fruit juices [10]. This species has a high fermentation activity and forms large CO₂ amount. A number of strains are also resistant to benzoates, sorbates and sulfates.

Zygosaccharomyces bailii is an organism widely known for its extreme resistance to weak organic acids, including common preservatives, high osmotolerance and ability to ferment sugars energetically, including fructose. *Z. bailii* is often found in fruit concentrates and syrups [12, 13]. Just few cells of this type in bottle are enough to spoil drink.

Ascospore-forming *Dekkera* yeast, which is teleomorph of *Brettanomyces* yeast, is one of the most common types of non-alcoholic pest yeast in first group [3]. *Dekkera* species are slow-growing, and spoilage symptoms development can take several weeks. They are extremely resistant to carbonization, moderately resistant to sorbates and benzoates. Usually form dense turbidity and precipitate, can oxidize sugars to acetic acid.

Special group of weakly fermenting yeast causes spoilage when washing and disinfection processes are disturbed (group 2). These are *Candida davenportii*, *C. parapsilosis* or *Debaryomyces spp.* *Candida davenportii* is relatively new pest species. It grows well in both fruit and synthetic drinks and in drinks such as cola [14].

Yeast are indicators of hygiene disorders include also red aerobic yeast of genera *Rhodotorula* and *Sporidiobolus/Sporobolomyces* (teleomorph/anamorph, respectively) and black yeast *Aureobasidium pullulans* and *Exophiala dermatitidis* [3, 4]. These yeasts are found in fouling films on technological surfaces located in places that are difficult to access for washing and disinfection [3, 4].

Bacteria - pests of soft drinks. Lactic acid (LAB) and acetic acid (AAB) bacteria are most common pest bacteria. Propagation in soft drinks allows them their resistance to an acidic environment and low pH values. LAB - microaerophilic gram-positive bacillus or cocci. LAB usually go into production with raw materials, juice ingredients and packaging materials [1, 3, 4].

The most common species are *Lactobacillus paracasei* and *Leuconostoc mesenteroides* [1, 10]. In addition to them, *Lactobacillus brevis*, *Lb. buchneri*, *Lb. plantarum*, *Lb. perolens* and *Weissella confuse* are usually found in infected products. LAB ferment sugar primarily to lactate. Strains of *Leuc. mesenteroides* and *W. confuse* can synthesize extracellular polymers of fructose or glucose from sucrose, which cause drink sliming [10].

The most common **acetic acid bacteria (AAB)** belong to genera *Acetobacter* and *Gluconobacter*. In addition, types of *Gluconacetobacter* and *Asaia* are involved in soft drinks spoilege. The genus *Asaia* was described in 2000 and currently includes eight species [15, 16]. Species *Asaia* are pests of non-carbonated fruit drinks, iced teas and flavored bottled water [3]. AAB are aerobic gram-negative short or cocciform movable or fixed bacillus. They are widespread in nature, in particular, in media rich in sugars and ethanol [10, 16]. Their presence in large numbers in industrial premises indicates poor sanitation [10, 17]. Many species are able to form biofilms on production equipment surface [10, 118]. AAB are acid resistant bacteria. Most species grow at pH 3.6–3.8, and some even at pH 3.0 [1, 16, 17]. The optimum growth

temperature is 25-30 °C [10]. Development of AAB in non-alcoholic drinks can cause change in taste and aroma, blown pack, sliming, clouding and sediment [17, 18]. *Gluconobacter* species are most common spoilage microorganisms in soft drinks. AAB are strict aerobes and need oxygen for their growth. Many AAB are quite resistant to preservatives: benzoates, sorbates, dimethyldicarbonate (velkorin) [17, 18].

Propionibacterium cyclohexanicum species was isolated from spoiled pasteurized orange juice with an extraneous taste, but can grow in other juices even at refrigerator temperature. This is gram-positive pleomorphic bacillus that forms propionic acid as the main product of sugar fermentation. Acetic and lactic acids are also formed. Minimum pH for growth in juice is about 3.6. The organism is able to withstand heat treatment of 95 °C for 10 minutes, so it doesn't die under standard juice pasteurization procedures [3].

Enterobacteria (*Klebsiella*, *Citrobacter*, *Serratia*) are heterogeneous group of facultative anaerobic gram-negative bacteria that carry out mixed fermentation, which spoils aroma and taste, and also leads to gas formation. Extracellular polymers and sulfur compounds can form [1].

Spore-forming bacteria growth of genera *Bacillus* and *Clostridium* is usually inhibited in soft drinks due to low pH values. However, spores in product may remain viable. Species *Bacillus* and *Clostridium* are typical pests of vegetable juices that have lower acidity (pH > 4) compared to fruit juices [5, 10]. As the sector of mixed drinks containing cereal dietary fiber in combination with vegetable or fruit juices grows, their significance as drinks pests is expected to increase. Anaerobic butyrate-forming clostridia, *Clostridium butyricum* and *Clostridium sporogenes*, can infect sugar syrups used in drinks production during processing or storage, causing rancid taste in drink. These bacteria are active only in range of pH 3.6-3.8 [5]. Getting rid of spore-forming bacteria is very difficult task because of their resistance to many physical and chemical factors.

Genus *Alicyclobacillus* is mainly associated with fruit juices spoilage. Spoilage cases have also been found in carbonated fruit drinks, soft drinks, isotonic water and iced teas [3]. *Alicyclobacillus acidoterrestris* is the main pest, but at least 6 more are also involved in spoilage. Genus *Alicyclobacillus*, which currently contains 18 species, was first described in 1992 [19]. These are gram-positive spore-forming aerobic or facultative anaerobic bacillus, moderately thermophilic and acidophilic. They are bacillus 0.3-1.0x2.0-5.0 microns. They are able to grow in range of pH 2.0-6.5 and temperature 25-70 °C. Optimum conditions for growth: pH 3.5-5.0, temperature 45-65 °C. Endospores withstand usual procedure for drink pasteurizing and can germinate and multiply even at pH 2-3. Endospores can form under different conditions, including aerobic and at high temperatures [19].

According to Lee et al. [19], 35% of spoiled juice cases in American market were caused precisely by *A. acidoterrestris*; losses of juice industry from spoilage under influence of alicyclobacilli are very large and present real problem. Visually determining spoilage from alicyclobacilli is difficult, because organism doesn't form gas during growth. Spoiled juice looks outwardly normal or exhibits slight clouding. The main sign of spoilage is an extraneous odor, which is described as an unpleasant medical, phenolic or disinfectants odor, smoke. Source of this smell is guaiacol, as well as halofenol components.

Mycelial fungi (molds) and actinomycetes in soft drinks. When pasteurized apple juice is infected with *Streptomyces griseus* streptomycetes, musty, mold, ground smells appear. This is gram-positive actinomycete that forms branching mycelium and spores, often resistant to high temperatures. Responsible for smell (pungent ground smell) are several metabolites, including geosmin [5].

Raw materials, intermediates and final products can be contaminated by spores, conidia or fragments of **mold mycelium**. Like yeast, a number of molds are resistant to low pH values; therefore, insufficient acidity is considered most important factor in spoilage of fruit and berry products by mycelial fungi [5]. In contrast to many bacteria and yeast, fungi need oxygen to grow. However, some species can also grow under anaerobic conditions, carrying out fermentation [20]. In addition, some species of *Fusarium* and *Rhizopus* can grow at low oxygen concentrations (0.01% vol.) [20].

Fungi growth in raw materials, ingredients and in finished product can lead to different types of drink spoilage. Mold can produce a huge number of enzymes - lipases, proteases and carbohydrases, and uncontrolled fungal activity can lead to appearance of extraneous odors and tastes. Volatile compounds formation - dimethyl sulfide and geosmin, having ground smell, musty smell, can serve as an indicator of fungal activity. In addition, fungal infection can lead to product discoloration, allergens and toxigenic compounds formation [20].

Fungi, which are the main spoilage agents in fruit juice industry and cause millions of dollars in losses, include heat-resistant species *Byssoschlamys nivea* and *B. fulva*, *Talaromyces flavus* and *T. macrosporus*, *Neosartorya fischeri* and *Eupenicillium brefeldianum* [3, 4, 21]. These molds can withstand fruit products heat treatment, including fruits and fruit juices in aluminum cans, fruit purees (used as ingredients), flavored mineral waters, fruit jellies and baby fruit purees [3, 21].

These fungi are able to grow at low oxygen concentrations by fermenting. They are able to form a large number of different mycotoxins. So, *Paecilomyces variotii* (anamorph of fungus *Byssoschlamis spectabilis*) forms viriditoxin. *Byssoschlamys* species produce patulin [3].

Other mold fungi, often found in soft drinks and juice mills, belong to genera *Penicillium* and *Cladosporium*.

Strong infection of raw materials based on apples or grapes with molds can also lead to hydrophobins, proteins formation that provoke gushing (excessive foaming) in cider and carbonated wines. Wine and cider producers have confirmed this [3, 4].

Microbiological food safety risks in non-alcoholic drinks. Non-alcoholic drinks are traditionally considered safe and not causing food poisoning or disease. However, health risks cannot be completely ruled out. According to Parish [3], since 1922 there are 32 documented cases of intestinal diseases caused by drinks consumption, especially unpasteurized fruit juices. Most of these cases are associated with violations in technology and sanitary practices at factory or in retail outlets. Disease-related illnesses were caused by various intestinal pathogens, including bacteria, viruses and protozoa (table 4).

Mycotoxins are another group of health risks associated with drinks.

Table 4 – Health risks associated with microorganisms in drinks [1]

Microorganisms	Hazard types	Human exposure	Types of microorganisms	Source of infection
Mycelial fungi (molds)	Mycotoxins	Chronic and acute toxicosis	<i>Penicillium</i> , <i>Aspergillus</i> , <i>Byssoschlamys</i> <i>Fusarium</i>	Fruit juices, grain raw materials
Bacteria	Intestinal infections and intoxication, allergic reactions	Miscellanea	<i>Salmonella</i> , <i>Escherichia coli</i> O157:H7, <i>Listeria monocytogenes</i>	Fruit juices and concentrates, water
Viruses	Infections	Liver damage gastroenteritis	Hepatitis A virus Noroviruses, Rotaviruses	Fruit juices, water
Protozoa	Infections	Gastrointestinal Diseases	<i>Cryptosporidium parvum</i> , <i>Cr. hominis</i> , <i>Cyclospora cayatenensis</i>	Water, Fruit juices and concentrates
Yeast	Fermentation products	Emetic reactions	Unknown	Fruit juices

Pathogenic bacteria, viruses, protozoa. Some pathogenic bacteria can survive in acidic carbonated drinks, although they can't reproduce in them. *Escherichia coli* and *Salmonella*. for example, survive for 48 hours in soft drinks such as Coca-Cola (pH 2.7). *Yersinia enterocolitica* remained viable in commercial orange drink (pH 3.5) at 30 °C for 3 days [3].

In poisoning cases with fruit juices, cause is most often *E. coli*, which forms an enterohemorrhagic toxin or Shiga toxin, especially *E. coli* of serotype O157: H7, as well as several *Salmonella* serotypes [3]. Slightly acidic apple and orange juices are most common source of disease.

In modern drinks recipe, many exotic juices are used, for example, acai, melon, date-plum (persimmon), papaya, which have low acidity (pH 4.8-6.2). These juices provide conditions not only for survival, but also for propagation of pathogenic bacteria. Wort-based sweet drinks also create conditions for certain pathogenic bacteria propagation [3, 4].

Concentrates used to make drinks are also suitable for pathogenic bacteria survival. For example, it was found that *Listeria monocytogenes* and *Yersinia enterocolitica* are able to survive for a long time in various frozen juice concentrates and in freshly squeezed orange juice (pH 6.3) [3, 4].

Parasites and viruses can also cause intestinal diseases when consuming fruit juices. Protozoa don't breed in drinks, but they can remain viable for a long time, being in stage of suspended animation in form of oocysts.

Viruses can't multiply in food, as for this they need living cells. However, hepatitis A viruses, noroviruses and rotaviruses can be transmitted through drinks produced under unsanitary conditions. In

1960s, cases of hepatitis A virus transmission through orange juice were recorded [3]. Noroviruses were cause of diseases caused by raspberries, which were watered with infected sewage [3, 4].

Mycotoxins. The growth of mycelial fungi usually doesn't occur in drinks production. However, molds often infect raw materials - fruit and vegetable juices, extracts and cereal products. In such cases, presence of mycotoxins in feed can be expected. Being fairly stable compounds, mycotoxins pass through entire production chain and end up in finished product. Mycotoxins found in foods and drinks are synthesized mainly by representatives of genera *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria* and include aflatoxins, ochratoxin A (OTA), patulin, fusarium toxins from trichothecene group and zearalenone, alternaria toxins (alternariol and others) [22, 23].

It is known that mycotoxins, in addition to their main toxic effects on consumer, can disrupt drinks preparation, affecting yeast metabolism during fermentation. The presence of mycotoxins may be cause of incomplete fermentation [3, 4].

Close attention is drawn to patulin, which is most dangerous and most common mycotoxin in fruit and berry juices production and mashed potatoes and is especially associated with apple and pear juices and apple cider [3, 4, 23]. *Penicillium expansum* is main patulin producer and causative agent of blue mold rot of fruits during storage. In addition to *P. expansum*, patulin is synthesized by number of species of penicilli and aspergillus, as well as *Byssoschlamys* spp. (Anamorph - *Paecilomyces* spp.), ascospores of which have high heat resistance and are preserved during pasteurization, and therefore this fungus is frequent contaminant and pest of heat-treated products. Patulin is considered as mycotoxin with potential carcinogenic effects. Other DNA damaging mycotoxins found in drinks are, according to Paterson and Lima [22], aflatoxins, sterigmatocystin, OTA, zearalenone, citrinin, luteoscin and penicillic acid.

U.S. Food and Drug Administration and European Commission (EC) have legislated maximum allowable concentrations of mycotoxins in foods and drinks. According to these documents (EC Regulation 1831/2003), maximum permissible level for patulin in apple products is 50 mg/kg; for baby food - 10 mg/kg [3, 4].

In Russian Federation, there are regulations on permissible levels of number of mycotoxins (DON, T-2 toxin, zearalenone, aflatoxin B1, OTA) in grain and raw materials, patulin in fruits and vegetables, juices and fruit purees (TR TS 021/2011 "On food safety"). The requirements for non-alcoholic products and mineral waters, established by current technical regulation, are largely harmonized with requirements of Codex Alimentarius and European directives, in particular, for patulin permissible level is identical to European one.

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АЛКОГОЛЬСІЗ СУСЫНДАРДЫҢ МИКРОБИОЛОГИЯЛЫҚ ҚАУІПСІЗДІГІ

Аннотация. Соңғы бірнеше ондаған жылдар ішінде сусын өндірушілер ғаламдық мәселені шешуге: микробиологиялық қауіпсіз және жоғары төзімді өнімдерді алуға қол жеткізуге жұмсады. Бұл мақсатқа өндірістің санитарлық жағдайын жақсарту, сонымен қатар зиянды микроорганизмдер туралы білімді кеңейту арқылы сәтті қол жеткізілді. Сонымен бірге, қазіргі уақытта алкогольсіз сусындар ассортиментінде айтарлықтай өзгерістер байқалады: шырын сусындары, алкогольсіз уыт сусындары, функционалды сусындар танымал бола бастады. Қосымша коректік заттармен, диеталық талшықтармен, қышқылдығы және карбонизация деңгейі төмен сусындар жасау үрдісі байқалады. Тұтынушы химиялық консерванттарсыз және пастерлеудің жағымсыз әсерлерінсіз табиғи «сусындарды» алғысы келеді. Бұл дәстүрлі сусындарда болған көптеген микробқа қарсы тосқауылдардың жоғалуына әкеледі. Сусындардың өзгеруіне байланысты бұзылу жағдайлары көбейеді. Бүлінетін микроорганизмдердің негізгі түрлері, сүт қышқылы бактериялары мен ашытқы жаңа сусындарда қалуы мүмкін, бірақ түрлердің кеңею ықтималдығы жоғары болады. Сусындардың

бұзылуында бактериялардың рөлі артады деген болжам бар. Жақында жаңадан ашылған бүлінген микроорганизмдер пайда болды, олардың қатарына ПЭТ бөтелкелеріне құйылған сусындардағы қышқылға төзімді аэробты бациллалар *Alicyclobacillus*, хош иісті минералды сулардағы *Asaia* түрлері, шырындармен байытылған сусындардағы *Propionibacterium cyclohexanicum* және спора түзетін бактериялар мен қышқылдығы төмен ішек бактериялары кіреді. Сусындар өндірісіндегі денсаулыққа жаңа қауіптер ингредиенттерді импорттайтын елдер тізбесінің кеңеюіне байланысты, сондай-ақ төмен қышқылдықты шырындарды, атап айтқанда, көкөніс қоспаларын ингредиенттер ретінде қолдануға байланысты туындауы мүмкін. Болжамды микробиология сусындардағы ластаушы микроорганизмдердің әрекетін болжауға, сипаттауға және консервант жүйелерін оңтайландыруға көмектеседі. Сусындар өндірушілері алдында тұрған жаңа міндет – жоғары қарсылыққа ие қауіпсіз өнімдерді құру және ең аз өңдеуде жақсы дәмі мен хош иісті қасиеттерін сақтау. Бұл міндетті орындау антимикробтық кедергілерді білікті және ғылыми негізделген үйлестіруден тұрады. Бұл шолуда осы мәселелерді шешетін алкогольсіз сусындарды бұзатын микроорганизмдер мен тұтынушылар үшін азық-түлік қауіпсіздігіне қатер төндіретін микроорганизмдер туралы ақпараттар бар.

Түйін сөздер: алкогольсіз сусындар, ингредиенттер, бүлінген микроорганизмдер, патогендік микроорганизмдер, консерванттар, пастеризация, тосқауыл технологиялары, болжамды микробиология.

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МИКРОБИОЛОГИЧЕСКАЯ БЕЗОПАСНОСТЬ БЕЗАЛКОГОЛЬНЫХ НАПИТКОВ

Аннотация. Производители напитков несколько последних десятилетий потратили на решение глобальной задачи: добиться получения продуктов, безопасных в микробиологическом отношении и обладающих высокой стойкостью. Эта цель была успешно достигнута за счёт улучшения санитарного состояния производства, а также за счёт расширения знаний о микроорганизмах-вредителях. Однако в настоящее время наблюдаются значительные изменения в ассортименте безалкогольных напитков: всё большую популярность приобретают соковые напитки, безалкогольные солодовые напитки, напитки, щадящие зубы, функциональные напитки. Имеется тенденция к созданию напитков более сложного состава, обогащённых дополнительными питательными веществами, пищевыми волокнами, с пониженной кислотностью и низким уровнем карбонизации. Потребитель хочет получать напитки более «натуральные», без добавления химических консервантов и без отрицательного воздействия пастеризации. Это приводит к потере многих антимикробных барьеров, которые существовали в традиционных напитках. Вследствие этих изменений в напитках будет возрастать число случаев их порчи. Основные типы микроорганизмов порчи, молочнокислые бактерии и дрожжи, по-видимому, останутся и в новых напитках, но весьма вероятно, что список видов будет расширяться. Предполагается, что в порче напитков возрастёт роль бактерий. Появились новые, недавно выявленные микроорганизмы порчи, среди которых отмечают кислотоустойчивые аэробные бациллы *Alicyclobacillus* в напитках, разлитых в ПЭТ-бутылки, виды *Asaia* в ароматизированных минеральных водах, *Propionibacterium cyclohexanicum* в напитках, обогащённых соком, и спорообразующие бактерии и энтеробактерии в напитках с пониженной кислотностью. Новые риски для здоровья при производстве напитков могут также возникать из-за расширения списка стран-импортёров ингредиентов, а также из-за использования в качестве ингредиентов соков с незначительной кислотностью, в частности, овощных. Предиктивная микробиология поможет в предсказании и описании поведения микроорганизмов-контаминантов в напитках и в оптимизации консервирующих систем. Новая задача, возникшая перед производителями напитков, - создание безопасных продуктов с высокой стойкостью и сохранением наилучших вкусо-ароматических свойств при минимальной их обработке. Осуществление этой задачи состоит в квалифицированной и научно обоснованной комбинации антимикробных барьеров. В настоящем обзоре содержатся необходимые для решения этой задачи современные сведения о микроорганизмах порчи безалкогольных напитков и о микроорганизмах, представляющих риски пищевой безопасности для потребителя.

Ключевые слова: безалкогольные напитки, ингредиенты, микроорганизмы порчи, патогенные микроорганизмы, консерванты, пастеризация, барьерные технологии, предсказательная микробиология.

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REFERENCES

- [1] Lawlor K.A., Schuman J.D., Simpson P.G., Taormina P.J. (2009) Microbiological Spoilage of Beverages // In: Sperber W., Doyle M. (eds). Compendium of the Microbiological Spoilage of Foods and Beverages. Food Microbiology and Food Safety. Springer, New York, NY. P. 246-284. DOI https://doi.org/10.1007/978-1-4419-0826-1_9
- [2] Gernet M.V., Gribkova I.N., Kobelev K.V., Nurmukhanbetova D.E., Assembayeva E.K. Biotechnological aspects of fermented drinks production on vegetable raw materials // News of the National academy of sciences of the Republic of Kazakhstan. Series of Geology and Technical Sciences. 433 (2019), 223-230. <https://doi.org/10.32014/2019.2518-170X.27>
- [3] Juvonen R., Virkajärvi V., Priha O., Laitila A. (2011). Microbiological spoilage and safety risks in non-beer beverages produced in a brewery environment // Espoo 2011. VTT Research Notes 2599. 107 p. + app. 4 p. URL: <http://www.vtt.fi/publications/index.jsp>
- [4] Kregiel D. (2015). Health Safety of Soft Drinks: Contents, Containers, and Microorganisms // Hindawi Publishing Corporation, BioMed Research International. Vol. 2015. Article ID 128697. 15 p. <http://dx.doi.org/10.1155/2015/128697>
- [5] Tribst A.A., Sant'Ana Ade S., de Massaguer P.R. (2009). Review: Microbiological quality and safety of fruit juices - past, present and future perspective // Crit. Rev. Microbiol. 35:310-339. doi: 10.3109/10408410903241428.
- [6] Jay J.M., Loessner M.J., Golden D.A. (2017). Modern Food Microbiology. M.: Binom. Laboratory of knowledge. 888 p. (in Russ.).
- [7] Buchanan R.L. (1998). The role of Predictive Microbiology in Microbial Risk Assessment // U.S. DHHS Food and Drug Administration Center for Food Safety and Applied Nutrition.
- [8] Stratford M. (2006). Food and Beverage Spoilage Yeasts // In: Querol H., Fleet G. (eds.) Yeasts in Food and Beverages. Berlin, Germany: Springer-Verlag. Chapter 11:335-379. DOI 10.1007/978-3-540-28398-0. ISBN 978-3-540-28388-1
- [9] Sheveleva S.A. (2019). Microbiological safety: problems and solutions // III All-Russian scientific-practical conference with international participation "Actual problems of diseases common to humans and animals", 24-25 April 2019. Stavropol (in Russ.).
- [10] Back W. (2005). Colour Atlas and Handbook of Beverage Biology // W. Back (ed.). Verlag Hans Carl: Nürnberg, Germany. P. 317.

- [11] Sperber W.H. **(2009)** Introduction to the Microbiological Spoilage of Foods and Beverages // In: Sperber W., Doyle M. (eds) Compendium of the Microbiological Spoilage of Foods and Beverages. Food Microbiology and Food Safety. Springer, New York, NY. P. 1-40. https://doi.org/10.1007/978-1-4419-0826-1_1
- [12] Steels H., James S., Bond C., Roberts I. and Stratford M. **(2002)**. *Zygosaccharomyces kombuchaensis*: The physiology of a new species related to the spoilage yeasts *Zygosaccharomyces lentus* and *Zygosaccharomyces bailii* // FEMS Yeast Research, 2: 113-121. DOI:10.1111/j.1567-1364.2002.tb00076.x
- [13] Martorell P., Stratford M., Steels H., Fernández-Espinar M.T., Querol A. **(2007)**. Physiological characterization of spoilage strains of *Zygosaccharomyces bailii* and *Zygosaccharomyces rouxii* isolated from high sugar environments // Intern. J. Food Microbiol. 114: 234-242. doi: 10.1016/j.ijfoodmicro.2006.09.014.
- [14] Stratford M., Bond C.J., James S.A., Roberts I.N., Steels H. **(2002)**. *Candida davenportii* sp. nov., a potential soft drinks spoilage yeast isolated from a wasp // Intern. J. Systematic and Evolutionary Microbiology. 52: 1369-1375. DOI: 10.1099/ijs.0.02088-0
- [15] Yamada Y., Yukphan P. **(2008)**. Genera and species in acetic acid bacteria // Intern. J. Food Microbiol. 125(1): 15-24. DOI:10.1016/j.ijfoodmicro.2007.11.077
- [16] Suzuki R., Zhang Y., Iino T., Kosako Y., Komagata K., Uchimura T. **(2010)**. *Asaia astilbes* sp.nov., *Asaia platycodi* sp.nov., and *Asaia prunellae* sp.nov. novel acetic acid bacteria isolated from flowers in Japan // J. Gen. Appl. Microbiol. 56: 339-346. DOI:10.2323/jgam.56.339
- [17] Raspor P., Goranović D. **(2008)**. Biotechnological applications of acetic acid bacteria // Critical Reviews in Biotechnology, 28: 101-124. DOI: 10.1080/07388550802046749
- [18] Horsák I., Voldřich M., Hromádka M., Sedlář P., Jbicerová P., Ulbrich P. **(2009)**. *Asaia* sp. as a Bacterium Decaying the Packaged Still Fruit Beverages // Czech Journal of Food Science, Special Issue, 27: S362-S365.
- [19] Clotteau M. **(2014)**. *Alicyclobacillus* spp. Control in the Fruit Juice Industry // Pall Food and Beverage Technical Bulletin. 1:1-15.
- [20] Filtenborg O., Frisvad J., Samson R. **(2004)**. Specific association of fungi to foods and influence of physical environmental factors. In: Introduction to food- and airborne fungi. 7th ed. Samson, R., Hoekstra, E., Frisvad, J. (eds). Centraalbureau voor schimmelcultures, Utrecht, The Netherlands. P. 306-320. ISBN-10: 9070351528
- [21] Houburken J., Varga J., Rico-Munoz E., Johnson Sh., Samson R.A. **(2008)**. Sexual Reproduction as the Cause of Heat Resistance in the Food Spoilage Fungus *Byssosclamyces spectabilis* (Anamorph *Paecilomyces variotii*) // Appl. Environ. Microbiol. 74(5):1613-1619. DOI: 10.1128/AEM.01761-07
- [22] Paterson R.R.M., Lima N. **(2010)**. Toxicology of Mycotoxins // In: Lunch, A., Ed., Molecular, Clinical and Environmental Toxicology. Vol. 2. Springer, Basel, 31-63. http://dx.doi.org/10.1007/978-3-7643-8338-1_2
- [23] Laitila A. Toxigenic fungi and mycotoxins in the barley-to-beer chane. **(2015)**. // In: Brewing Microbiology. Managing Microbes, Ensuring Quality and Valorising Waste. Ed.: Annie E. Hill, Woodhead Publishing, Elsevier, 107-139. ISBN 978-1-78242-349-2