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Biofilms in Beverage Industry

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Abstract

Over the years, numerous studies have been conducted into the possible links between biofilms in beverage industry and health safety. Consumers trust that the soft drinks they buy are safe and their quality is guaranteed. This chapter provides an overview of available scientific knowledge and cites numerous studies on various aspects of biofilms in drinking water technology and soft drinks industry and their implications for health safety. Particular attention is given to *Proteobacteria*, including two different genera: *Aeromonas*, which represents *Gammaproteobacteria*, and *Asaia*, a member of *Alphaproteobacteria*.

Keywords: biofilms, water, soft drinks, *Aeromonas*, *Asaia*

1. Drinking water systems

In water systems, both natural and industrial dominate *Proteobacteria*. This is the main group (phylum) of Gram-negative bacteria, taxonomically very diverse, consisting of more than 200 genera. Its membership includes both pathogenic bacteria of the genera *Escherichia*, *Salmonella*, *Vibrio*, *Helicobacter*, and many other types of free-living or symbiotic, motile or nonmotile, chemoautotrophic or heterotrophic bacteria from outstanding aerobes to obligatory anaerobes.

Although bacteria are physiologically and morphologically diverse, they constitute a coherent set of six main classes: *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, *Epsilonproteobacteria*, and *Zetaproteobacteria*. Taxonomy of the group is determined primarily on the basis of ribosomal RNA sequences [1]. Species belonging to the classes *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria* are very heterogeneous in their physiological characteristics. Each of the three classes includes aerobes and anaerobes, photosynthetic and nonphotosynthetic cells. They are distributed in both terrestrial and aquatic environments in very high abundance.

In natural systems, freshwater or potable water distribution networks, *Betaproteobacteria* dominate (87–99%), while *Alphaproteobacteria* are in marine waters [2]. *Proteobacteria* predominated in biofilms present in drinking water distribution systems, but the compositions of the dominant proteobacterial classes and genera and their proportions varied among biofilm samples [3]. The majority of strains isolated from biofilms in water distribution networks is *Alpha-* or *Gammaproteobacteria* [4]. Except *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Nitrospirae*, and *Cyanobacteria* are usually the major components of biofilm bacterial community.

One of the common features of *Proteobacteria* is the ability of biofilm formation and/or aggregation and formation of the so-called “flocs”. An important component of such structures, in addition to microbial cells, is water – it represents about 97%. Besides water, the biofilm or flocs matrix are extracellular polymeric substances (EPSs). The bacterial cells in biofilms are embedded in a heteropolymeric matrix containing humic substances, glycoproteins, polysaccharides, and nucleic acids [5].

A first step in the successional development of biofilms is the coating of uncolonized surfaces with many particles, organic or inorganic (conditioning film), which enhances attachment of initial colonizing bacteria. Anything that may be present within the bulk fluid can through gravitational force or movement of flow settle onto a surface and become part of a conditioning layer. Surface charge, potential, and tensions can be altered favorably by the interactions between the conditioning layer and the surface. Factors such as available energy, surface functionality, bacterial orientation, temperature and pressure conditions are local environmental variables which contribute to bacterial adhesion. Physical forces associated to bacterial adhesion include the van der Waals’ forces, steric interactions, and electrostatic (double layer) interactions, collectively known as the DVLO (Derjaguin, Verwey, Landau, and Overbeek) forces [6]. An extended DVLO theory takes into consideration hydrophobic/hydrophilic and osmotic interactions.

In real time, a number of the reversibly adsorbed cells remain immobilized and become irreversibly adsorbed. The physical appendages of bacteria (flagella, fimbriae, and pili) overcome the physical repulsive forces of the electrical double layer. Some evidence has shown that microbial adhesion strongly depends on the hydrophobic–hydrophilic properties of interacting surfaces. The first colonizers grow in surface-attached microcolonies and produce EPS. After an initial lag phase, a rapid increase in population is observed, which is described as the exponential growth phase. As the microcolonies develop, additional species, the so-called secondary colonizers, are recruited through coaggregation and nonspecific aggregation interactions, increasing the biofilm biomass and species complexity [7].

Simultaneously, expression of a number of genes for the production of cell surface proteins and excretion products increases. Surface proteins (porins) such as Opr C and Opr E allow the transport of extracellular products into the cell and excretion materials out of the cell, e.g., polysaccharides. EPS molecules impart mechanical stability and are pivotal to biofilm adhesion and cohesion, and evasion from harsh dynamic environmental conditions. The differences in gene expression of planktonic and sessile cells were identified, and as many as 57 biofilm-associated proteins were not found in the planktonic profile [6].

Physicochemical nature of such consortia implies differentiation of the physiological condition of individuals forming them [8]. Creating consortium is an effective adaptation strategy, including cell protection against adverse environmental factors; increased nutrient availability; increased binding of water molecules, thereby reducing the risk of dehydration; and increased ability to transfer DNA.

Microbial consortia exhibit altered phenotypic characteristics compared to planktonic cells, particularly with respect to growth and gene expression. All these factors increase the survival of cells forming biofilms. As a result, the inactivation of bacterial cells by conventional methods such as the use of antibiotics and disinfectants is often ineffective [9]. Especially this exopolymer matrix confers resistant properties to the whole system via the limitation of the effectiveness of disinfection by consuming the oxidants used, such as chlorine and chloramines [10].

At high cell concentration, a series of cell signaling mechanisms are employed by the biofilm, and this is collectively termed *quorum sensing*. *Quorum sensing* describes a process where a number of autoinducers (chemical and peptide signals in high concentrations, e.g., homoserine lactones) are used to stimulate genetic expression of both mechanical and enzymatic processes. In mature biofilms, enzymes are produced by the community itself which breakdown polysaccharides holding the biofilm together, actively releasing surface bacteria for colonization of fresh substrates. For example, alginate lyase produced by *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*, N-acetyl-heparosan lyase by *Escherichia coli*, and hyaluronidase by *Streptococcus equi* are used in the breakdown of the biofilm matrix [6].

Biofilms are polymicrobial communities, therefore the potential for cell coaggregation plays an integral role in spatiotemporal biofilm development and the moderation of biofilm composition. Coaggregation is mediated by the interaction between specific macromolecules on the cell surface of one species and cognate macromolecules expressed on the cell surface of the partner species. Microbial cells may also come into contact through hydrophobic interactions or electrostatic forces, but these last associations are relatively weak. Coaggregation-mediating proteins are referred as adhesins. Coaggregation may occur between lectin-like protein adhesins and their polysaccharide receptors or by protein–protein (adhesin–adhesin) interactions. These interactions may be unimodal, but in some cases are bimodal, involving two different interacting pairs of macromolecules [7].

Cell aggregation, as well as biofilm formation may have both intrageneric and intergeneric character [11]. Consortia are very changeable and their components depend on the environmental conditions. The study conducted by Rickard et al. [12] revealed that intergeneric and intraspecies coaggregation between water bacteria are common phenomena, and expression of coaggregation is dependent on cells being in the optimum physiological state for coaggregation, which usually occurs in stationary phase. Therefore, it is possible that since cells grow very slowly in nutrient-limited biofilms, these biofilms would provide suitable conditions for expression of coaggregation.

Different materials such as cast iron galvanized steel, stainless steel, copper, and polyethylene are used to manufacture water distribution pipes. It is worth noting that these materials favor biofilm formation in the water distribution systems. The presence of biofilms in drinking

water distribution pipes usually leads to a number of undesirable effects on the quality of water that is supplied to consumers. For example, the development of biofilms in copper pipes facilitates cuprosolvency which increases the release of copper into the distribution system. What's more, increased carbon influences the growth of heterotrophic plate count bacteria which are also involved in the corrosion of copper [13]. Silhan et al. [14] showed that among drinking water pipe materials such as galvanized steel, cross-linked polyethylene, copper pipes, and medium-density polyethylene, the most dense biofilm of *E. coli* was formed on the steel surface.

Molecular analysis of microbial communities by Yu et al. [15] indicated the presence of *Alpha*- and *Betaproteobacteria*, *Actinobacteria*, and *Bacteroidetes* in biofilms on the pipe materials. Moreover, the DGGE profile of bacterial 16S rDNA fragments showed significant differences among different surfaces, suggesting that the pipe materials affect not only biofilm formation potential but also microbial diversity.

The development of biofilms inside water distribution pipes facilitates the propagation of mixed microbial populations and is considered the main source of planktonic bacteria in water supply systems. Among the heterotrophic bacteria in drinking water systems, the pathogenic bacteria or at least opportunistic pathogens often appear. Enteropathogenic *E. coli* or other members of *Enterobacteriaceae* may appear in water supply systems due to contamination as a result of flooding, water supply failure, or insufficient disinfection. Other opportunistic bacteria such as *P. aeruginosa*, *Burkholderia* spp., *Stenotrophomonas maltophilia*, and *Legionella* spp. were quite often detected [16]. They increase the health risks associated with the consumption of water [13].

In the last decade, a group of new, potentially dangerous pathogens forming biofilms were classified as *Aeromonas* spp. rods from class *Gammaproteobacteria* [17, 18] (**Figure 1**). The experimental data and clinical and epidemiological evidence show that *Aeromonas* spp. may be an etiological factor of bacterial gastroenteritis in children and people with reduced immunity.

Bacteria *Aeromonas* spp. are capable not only of survival, but also propagation in water at temperatures up to 10°C and show a greater ability to utilize different carbon compounds than other Gram-negative bacteria.

According to Sautour et al. [19], the genus *Aeromonas* shows the ability to use not only carbohydrates, amino acids, and carboxylic acids, but also fatty acids and saturated hydrocarbons. Growth of these bacteria in an aqueous medium follows in the presence of even a small amount of biodegradable dissolved organic carbon compounds.

It was noted that there was an intense increase in the number of heterotrophic bacteria in the summer months. The results obtained by Craveiro et al. [20] demonstrated that *Aeromonas* spp. strains were able to form biofilm at both room and refrigeration temperatures. The chlorine-based disinfectant demonstrated to be efficient in removing preformed biofilm, but both were unsuccessful in preventing biofilm formation, highlighting the importance of adequate cleaning and disinfection procedures, with emphasis on food processing surfaces.

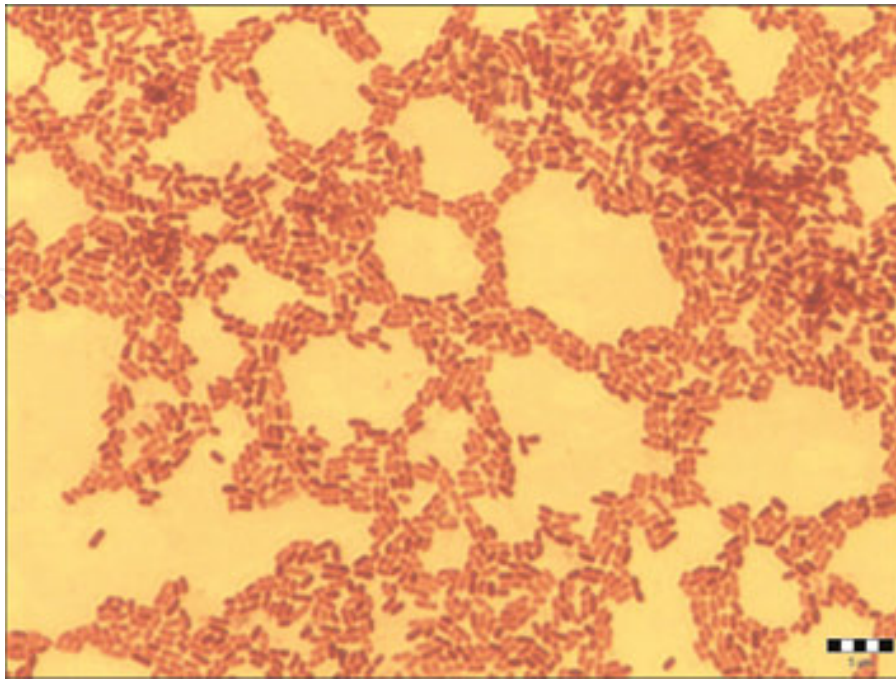


Figure 1. Gram-negative rods of *Aeromonas hydrophila*.

The vast majority of bacteria isolated from biofilms belonged to *Aeromonas hydrophila*. They showed the major virulence factors such as surface polysaccharides (capsule, lipopolysaccharide, and glucan), S-layers, iron-binding systems, exotoxins and extracellular enzymes, secretion systems, fimbriae, and other nonfilamentous adhesins, motility, and flagella [21, 22]. Despite the demonstration of the enterotoxic potential of some *Aeromonas* spp. strains, there is still a debate on its consideration as an etiological agent, as there were no big epidemical outbreaks described and no adequate animal model is available to reproduce the gastroenteritis caused by *Aeromonas*.

In clinical and environmental isolates of *Aeromonas* species, two distinct types of fimbriae have been found based on their morphology: short, rigid fimbriae (0,6–2 μm) that can be found in high numbers on the bacterial cell and long, wavy fimbriae (4–7 μm) found in smaller numbers. The short fimbriae are able to cause autoaggregation, and large ones considered hemagglutinins. Amino-acid sequence analysis indicates that they correspond to type IV pili, known as important structures for adhesion to epithelial cells and involved in biofilm formation. Some of them exhibit highest homology with the type IV pili of *Pseudomonas* and *Neisseria* species [22].

In studies conducted by Kregiel et al., *A. hydrophila* isolated from water distribution system, adhered to different abiotic surfaces such as glass, polystyrene, polyvinyl chloride, and gumosil, commonly used as packaging and installation materials [23–25]. After 3 weeks in an aqueous environment with a small amount of organic matter, bacteria formed numerous microcolonies surrounded extracellular mucilaginous substance (**Figure 2**). The results of microscopic examination demonstrated the strong adhesion properties of *A. hydrophila* and they were confirmed by luminometric measurements.

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Figure 2. Biofilm of *Aeromonas hydrophila* on a glass surface.

The studies have found that both due to the strong adhesive properties of *A. hydrophila*, and the possibility of the virulence factors determining its pathogenicity, it should be considered the inclusion of *Aeromonas* rods for routine microbiological water analysis, especially for monitoring water or beverage distribution systems.

2. Soft drinks

When a change in the chemical nature of a fluid occurred, there is usually a qualitative shift created microbial consortia [8]. While the succession is a well-known process in classical ecology, in the case of biofilms or cell aggregates it is not fully understood. Despite many researches, the full knowledge on the formation of microbial consortia is still lacking. However, succession processes seem to be rather stochastic (reproduction and death) [26]. During growth of consortia, competition for resources makes that weak individuals are eliminated, and stronger competitors become dominant. Finally, in the mature consortia, cells are becoming more diverse by individual differences and “internal recycling.”

Environmental factors may also shape the succession in microbial consortia. Changes in pH, the presence of carbon sources in the form of saccharides, and other additional substances cause significant qualitative changes in biofilms [27].

For example, the flavored drinking water samples with sucrose and natural fruit flavors showing signs of turbidity and the characteristic "flocs" formed by heterotrophic bacteria [28]. The developed specific methods allowed for the isolation of bacteria belonging to the *Asaia* spp. – a new, previously unknown in Poland, microbial contamination of mineral water and flavored beverages. Isolated bacteria were Gram-negative, aerobic rods with dimensions of $0.4\text{--}1.0 \times 0.8\text{--}2.5 \mu\text{m}$. These bacteria formed characteristic small (1–3 mm in diameter), pale pink or pink colonies in agar plates. The isolates were identified based on 16S rRNA gene sequences. It is worth noting that the same morphotypes and genotypes were isolated from fruit concentrates, which were previously used for production of flavored waters.

Asaia sp. was established in 2000 as the fifth genera of acetic acid bacteria of the class *Alphaproteobacteria*. Bacteria *Asaia* sp. were first isolated from the orchid tree flower (*Bauhinia purpurea*) and flowers of *Pueraria* (*Plumbago*), growing in tropical climates. Currently, Genus *Asaia* contains eight species named as: *As. bogorensis*, *As. siamensis*, *As. krungthepensis*, *As. lannensis*, *As. spathodeae*, *As. astilbis*, *As. platycodi* and *As. prunellae*. It is distinguished from other types of acetic acid bacteria not only by genetic features, but also by biochemical properties. The optimum pH and temperature of these bacteria are 5.5 and 30°C, respectively. Nevertheless, the strains belonging to *Asaia* sp. isolated from environments in tropical Indonesia, Thailand, and Japan have optimum growth even at 37°C [29].

Asaia spp. belongs to the risk 1 group, which means that it is a group of saprophytic microorganisms without causing diseases in humans. However, according to the literature, these bacteria can cause opportunistic infections when they get into the bloodstream of a man with weakened immune systems. Several cases of bacteremia caused by *Asaia* spp. were documented, especially in chronically ill adults and pediatric patients with cardiomyopathy or cancer. The first documented case of bacteremia caused by *As. bogorensis* was reported in a young patient with a history of intravenous-drug abuse. *As. bogorensis* was identified by sequencing the 16S rRNA gene. The isolate was resistant to almost all antibiotics routinely tested for Gram-negative rods, but was susceptible to gentamicin and doxycycline [30]. One of the last reports describes transient bacteremia due to *As. lannensis* in a patient with a psychiatric disorder and compulsive self-injection of different substances. Only restriction fragment length polymorphism of PCR-amplified 16S rRNA gene allowed for proper identification of isolate. The strain was also highly resistant to most antibiotics [31].

Asaia spp. show strong ability to aggregate and form characteristic "flocs" and to create biofilms on selected surfaces commonly used in the food industry: glass, polyethylene terephthalate, and polypropylene [32] (**Figure 3**).

It was found that the hydrophobicity of the cells decreased with increasing the age of the population. The higher hydrophobicity of young cells stimulates the process of aggregation and formation of flocs. The studies proved that the adhesive abilities of *As. lannensis* depend on the carbon source, nutrient availability, and physicochemical properties of abiotic surface. The strongest adhesion properties were characterized by cells in the minimal medium with sucrose.

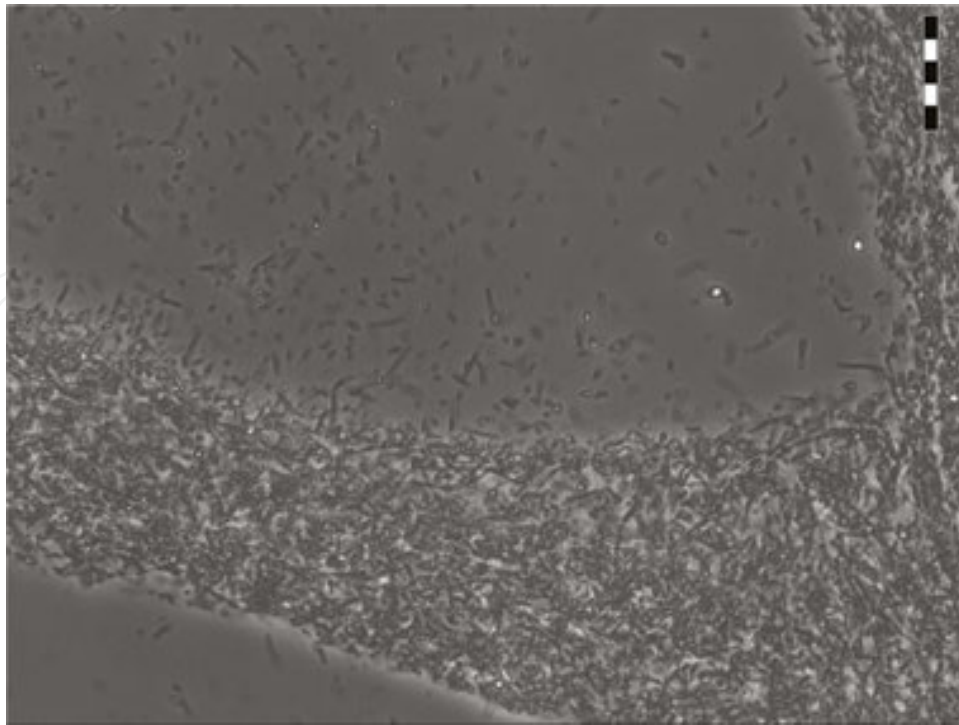


Figure 3. “Flocs” formed by cells of *Asaia* spp.

Definitely, the level of cell adhesion decreased in media that is rich in nutrients. Biofilm creation in a specific medium which was the commercial mineral flavored water had a dynamic character [32].

It is difficult to determine the origin of the contamination of soft drinks with the *Asaia* spp. However, the fruits and fruit concentrates are regarded as the source of the contamination [28, 33]. Most strains were isolated from the reclaimed fruit beverages and flavored mineral waters. This spoilage often occurs in the acid products preserved by the benzoate, sorbate, and dimethyldicarbonate. Horsakova et al. [34] found that these bacteria occur in the processing equipment in the form of biofilm, which is persistent and hardly removable by the common sanitation. The isolated bacteria *Asaia* spp. exhibit the polysaccharide encapsulation. The presence of preservatives is almost no effect on the *Asaia* spp. growth. The minimum inhibition concentration for sorbic and benzoic acid under the conditions of the model fruit drink (pH 3.45; Rf 10 Brix) were between 250 and 500 mg/l, while the concentration 250 mg/l is used for the stabilization of similar fruit beverage production.

The resistance of *Asaia* spp. to common preservatives limits the available possibilities to prevent spoilage of similar drinks. Additionally, the contamination of the technological equipment always brings the serious problem. The common sanitation procedures used in the beverage production may be insufficient to eliminate the very rigid biofilm, which is formed by *Asaia* spp. in the equipment. According to Horsakova et al. [34], the reliable elimination of such biofilm may require more forcing condition (e.g., hot sodium hydroxide and detergent and enzyme solutions) and in any hardly accessible points (pipe bends, branches, connections, and valves) mechanical treatment is the only possibility.

3. New antiadhesion strategy: organosilanes

It is known that it is best to prevent than to fight against biofilm formed on the internal surface of a distribution system. For drinking waters and soft drinks, reduction or elimination of the formation of cell consortia can be obtained only by changing the physicochemical properties of abiotic surfaces or bioactive properties of consumption waters.

Compounds of the biocidal and/or antiadhesive properties applied in potable water systems have to inhibit effectively the growth of microorganisms without releasing toxic compounds with low molecular weight into aquatic environment. Such compounds may be organosilanes containing at least one bond between the carbon and silicon atom Si-CH₃. A carbon-silicon bond is very durable, and the presence of an alkyl group causes a change in surface tension. Additionally, organosilanes can contain other functional groups with antimicrobial properties, for example, methoxy, ethoxy, amino, methacrylic, and sulfide [35].

Organofunctional silanes are hybrid compounds that combine the functionality of a reactive organic group and the inorganic functionality of an alkyl silicate in a single molecule. This special property means they can be used as 'molecular bridges' between organic substrates and inorganic materials (**Figure 4**).

Figure 4. Model structure of organosilanes.

These compounds are relatively environmentally friendly, improve adhesion, and provide better protection against corrosion. Surfaces on which they can be used include metal, plastic, glass, rubber, ceramic, porcelain, marble, cement, granite, tile, silica, sand, appliances that have been enameled, polyester, polyurethane, polyacrylic, resins that are melamine or phenolic, siliceous, polycarbonate and wood, as well as painted surfaces.

The growth of many microorganisms can be reduced on surfaces treated with alkylsilanes. In general, the reactivity of hydroxylated surfaces with organofunctional silanes decreases in the following order: Si-NR₂ > Si-Cl > Si-NH-Si > Si-O₂CCH₃ > Si-OCH₃ > Si-OCH₂CH₃. The methoxy and ethoxysilanes are the most widely used organofunctional silanes for surface modification. The methoxysilanes are capable of reacting with substrates under dry, protic

conditions, while the less reactive ethoxysilanes require catalysis. The low toxicity of ethanol, a byproduct of the reaction, favors the use of ethoxysilanes in many commercial applications [35].

One of the most established and successful uses of the application of organosilanes is prevention against biofilm formation. The use of the proper quaternary amine-based organosilane can provide durable antimicrobial protection against a wide variety of microorganisms [36].

Adhesion abilities of *A. hydrophila* to the glass surface modified by coating with four different organosilanes with active functional groups were described by Kregiel [23]. The presence of active functional groups had an impact on a significant reduction in the surface tension of the test surfaces due to reduced participation polar forces – one of the components of surface forces. Among the modifiers, organosilanes containing methoxy groups and quaternary ammonium salts showed the best antiadhesive and antibacterial properties. Organosilanes were stable in an aqueous environment. Interesting results from the modification of the surface of the glass gave impulsion to extend the study on modification of plastic materials commonly used as pipe materials in water systems [24, 25]. The modified PVC surfaces were made by silane coupling on the native material. Modifications of silicone elastomer were carried by cocrosslinking organosilane with silicone. Almost all of the modified surfaces were characterized by antimicrobial and antiadhesive features. Among the modifications, especially polydimethylsiloxane with a quaternary ammonium salt and a methoxy group in the silicone elastomer showed the greatest antiadhesive and antibacterial properties against *A. hydrophila*.

4. New antiadhesion strategy: proanthocyanidins

Scientific studies showed that natural compounds from different fruits have potential health benefits against cancer, aging and neurological diseases, inflammation, diabetes, and bacterial infections. For example, cranberry juice was recognized for benefits of maintenance of a healthy urinary tract. Cranberry is a term derived from the contraction of “crane berry.” This name is derived from the nickname of the bilberry flower, and the sand crane, a bird that often feeds on the berries of this plant. The cranberry is part of the *Ericaceae* family and naturally grows in acidic swamps full of peat moss in humid forests [37].

Bacterial adhesion is accomplished by the binding of lectins exposed on the cell surfaces of pili and fimbriae to complementary carbohydrates on the host tissues. Pili are small filaments that can be either mannose-resistant or mannose-sensitive. The mannose-sensitive pili, called type 1 pili, permit bacterial adhesion to the urothelium. The fimbriae (p-fimbriae) are inhibited by fructose, present in cranberries. The more virulent strains of *E. coli*, isolated from patients with urinary tract infections, have other types of these structures that bind to glycosphingolipids of the lipid double membrane of renal cells, which precedes renal parenchymal invasion.

The current hypothesis is that cranberries work principally by preventing the adhesion of type 1 and p-fimbriae *E. coli* strains to the urothelium. Without adhesion, the bacteria cannot infect the mucosal surface. In vitro, this adhesion is mediated by two components of cranber-

ries: fructose, which inhibits the adherence of type 1 fimbriae, and proanthocyanidins, which inhibits the adherence of p-fimbriae. The binding of the proteinaceous bacterial fimbrial tips to mucosal surfaces on the uroepithelium occurs as a specific receptor-ligand association favored by hydrophobic interactions. This possible mechanism is that the cranberry compounds, acting as receptor analogs, competitively inhibit the adhesion of *E. coli* to host cells by binding to the fimbrial tips. Another mechanism of cranberry activity is the in vitro reduction in the expression of p-fimbriae in *E. coli* by changing the conformation of surface molecules [38]. Zafriri et al. were the first to postulate that compounds in cranberry could affect p and type 1 fimbriae of *E. coli* [39]. In 1998, Howell et al. [40] identified specific proanthocyanidin compounds in cranberry responsible to antiadhesive properties.

Proanthocyanidins are one of many plant phenols, which are aromatic secondary metabolites found in the plant kingdom. They are mainly found in *Vaccinium* berries such as cranberries and blueberries. They are dimers or oligomers of catechin and epicatechin and their gallic acid esters. Proanthocyanidins are in the first place very strong antioxidants. Studies have shown that proanthocyanidins act as anticancer and antiallergic agents, and that they improve heart health. These flavonoids have several potential clinical effects, including antiatherosclerotic, anti-inflammatory, antitumor, antithrombogenic, antiosteoporotic, and antiviral. Some of these effects, such as antitumor, are still up for discussion, and the role of flavonoids in different effects is not fully known.

They are also known as oligoflavanoids, and consist of monomer flavan-3-ol units. When linked through either C4 to C8 or C4 to C6 bonds, the linkages are called B-linked. When the linkages were through a C2 and C7 compound, they are called A type [41]. While B-linked proanthocyanidins can be found in different fruit products including apple juice, purple grape juice, green tea, and dark chocolate, A-linked ones are found in cranberries and it is a linkage with unique antiadhesion properties associated with them [42] (**Figure 5**).

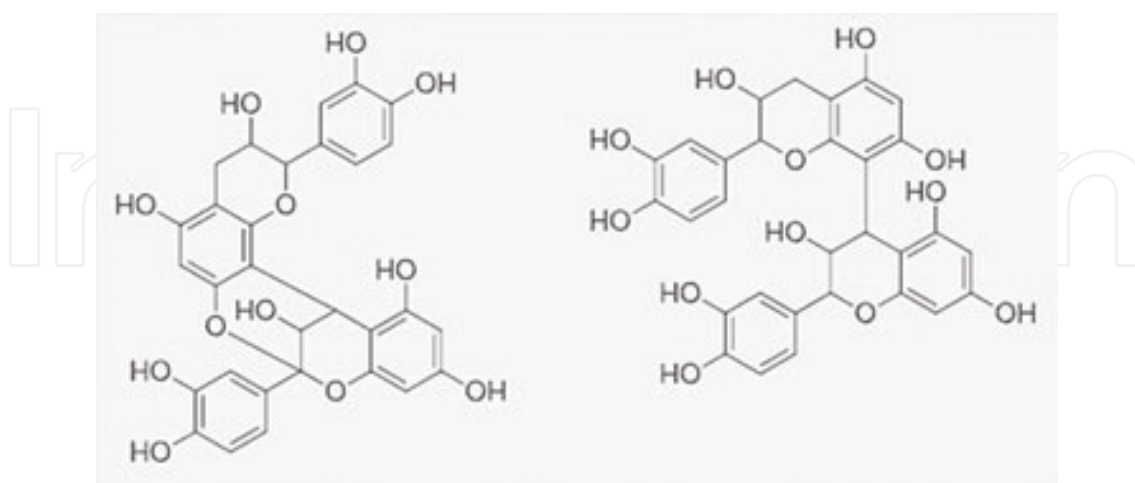


Figure 5. Proanthocyanidins: type A (left) and type B (right).

The antiadhesive properties of cranberry were demonstrated against different microorganisms: *E. coli*, *Proteus mirabilis*, or *Helicobacter pylori*, responsible for urinary tract infections and

gastritis, as well as other pathogenic Gram-negative and Gram-positive bacteria: *P. aeruginosa*, *Staphylococcus aureus*, or *Listeria monocytogenes* [43–45].

It was also noted that the adhesion of *Asaia* spp. cells in the presence of cranberry juice was much lower, especially for the packaging material – polystyrene [37]. In the presence of 10% cranberry juice, attachment of bacterial cells was three times lower. The obtained results suggested that compounds of cranberry inhibit both biofilm formation and coaggregation of microbial cells. This fact would help to utilize antioxidant-rich cranberry juice as a natural antiadhesive protectant and microbiological stability enhancing agent for functional soft drinks.

5. Conclusion

Problems related to microbial contamination in the beverage industry have been studied for more than a century. However, most of the knowledge acquired over the years relates to single-cells, but today it is generally accepted that microorganisms grow and survive in organized communities where their physiology is very different. This paper has given an overview of the most widely used research on the controlled attachment of specific bacteria present in drinking water or soft drinks. Both surfaces modified by organosilanes and cranberry juice supplementation are the latest developments in this area. Particularly, cranberry juice and cranberry extracts may be investigated as a natural solution for food industry by creating an additional barrier to inhibit the growth of spoilage bacteria and providing additional health benefits.

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