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Alicyclobacillus spp. Control in the Fruit Juice Industry

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In the current competitive environment, safety in the Food and Beverage industry is a question of company credibility and stability.

The definition of food quality may differ for processors and consumers, but generally organoleptic characteristics, such as flavour, odour and appearance, as well as extended shelf life are among the most important of its attributes. Spoilage of food by microbial contamination may occur at any point during production, altering any one or all of these characteristics renders the product unusable and results in financial damage for the food manufacturer.

The fruit juice industry faces significant product spoilage challenges. Pasteurization is an effective microbial control method for common yeast, mould and lactic acid bacteria that tolerate or thrive in acidic fruit juices (pH < 4) due to their low heat resistance. Additionally, spores are common contaminants. However, most bacterial spores fail to germinate at pH levels below 4.1, thus product spoilage is limited to a small number of organisms able to grow at pH 3.8

For the past few years consumers are asking and purchasing “natural” products over those containing chemically synthesised preservatives. Maintaining safety, quality and extended shelf life of these “natural” products is critical. Concurrently, the emergence of novel spoilage heat-resistant spores forming microbes such as *Alicyclobacillus* spp, have further complicated product quality and safety challenges for the fruit juice industry.[1]

2. Impact of *Alicyclobacillus* species in the Juice Industry

2.1. Alicyclobacillus genus

The genus *Alicyclobacillus* spp., including identified 18 species, was first recognized in 1992. These spore-forming bacteria are aerobic or facultatively anaerobic, moderately thermophilic and acidophilic. *Alicyclobacillus* are able to grow within a pH range of 2.0 to 6.5 and at temperatures from 25 to 70 °C [1, 2, 3, 4, 5]. Optimum parameters for growth are: pH from 3.5 to 5.0 and temperatures between 45 to 65 °C [2, 6]. The rod-shaped cells range in size from 0.3 – 1.0 µm in width by 2.0 – 5 µm in length. Endospores are formed under adverse environmental (such as heat exposure) or nutritional conditions and sporulation may occur even in an aerobic environment [6].

2.2. Identification of Alicyclobacillus species along the fruit juice process

In early 1980s, in Germany, an acidophilic *“Bacillus”* strain was isolated from spoiled apple juice [1]. A summary of the main characteristics of *Alicyclobacillus* species most challenging to the beverage industry are presented in Table I.

Table 1: Species of the genus *Alicyclobacillus* identified in the beverage industry

Species	Source	pH	Temperature (°C)	Size (length by width)	References
A. acidocaldarius	Acid soil	2.0 - 6.0 (opt. 4 - 4.5)	25 – 70 (opt. 60)	Vegetative cell: 2.0 – 4.3 µm by 0.7 – 1.0 µm Spore: 1.5 – 1.8 µm by 0.9 – 1 µm	Goto *et al.*, 2006, Yokota *et al.*, 2007
A. acidoterrestris	Acid soil	2.0 - 7.5 (opt. 3.5 - 4.5)	20 – 60 (opt. 40 - 53)	Vegetative cell: 2.9 – 4.3 µm by 0.6 – 0.8 µm Spore: 1.5 – 1.8 µm by 0.9 – 1.0 µm	Goto *et al.*, 2006, Yokota *et al.*, 2007, Walker and Phillips, 2008, Bevilacqua *et al.*, 2008
A. acidiphilus	Tainted orange juice	2.5 - 5.5 (opt. 3.0)	20 - 55 (opt. 50)	Vegetative cell: 4.8 – 6.3 µm by 0.9 – 1.1 µm	Matsubara *et al.*, 2002, Yokota *et al.*, 2007
A. herbarius	Dried hibiscus flower (herbal tea)	3.5 - 6.0 (opt. 4.5 - 5.0)	35 - 65 (opt. 55 - 60)		Goto *et al.*, 2002 [36], Yokota *et al.*, 2007

2.3. Heat resistance of Alicyclobacillus genus

The real challenge coming from *Alicyclobacillus* species is the heat resistance of spores as more than 99% of the vegetative cells are killed at 80 °C for 20 minutes at a pH of 4.0 [7]. Additionally, typical pasteurization conditions for juice or storage of shelf-stable juice at elevated temperatures may stimulate spore germination leading to potential juice spoilage [1, 5, 8]. It has been reported that heat resistance of spores was physiologically lost during germination under acidic conditions but the mechanism of germination under these conditions requires further evaluation [7].

Heat resistance is characterized by the D-value which corresponds to the time required to reduce the microbial population to 1/10th of the initial number at a specific temperature [4]. Reported D-values for *A. acidoterrestris* spores are 16 to 23 minutes at 90 °C and 0.06 to 5.3 minutes at 95 °C [1, 5, 9]. It has also been reported that spores of *A. acidocaldarius* have relatively weak heat resistance (half time for death at 86 °C for 10 – 12 minutes) [10].

Despite temperature having the major impact on D-values, additional factors including pH, suspended solids, species and strain may have a significant impact on heat resistance of *Alicyclobacillus* spores in fruit products [4, 8, 11]. Heat resistance decreases when pH decreases and increases incrementally when Brix concentration increases.

It also has been reported when spores are produced at lower temperatures, heat resistance is also lower. For example, spores of *A. acidocaldarius* produced at 45 °C had a D-value at 110 °C of 0.48 minutes while those produced at 65 °C had a D-value of 3.9 minutes at the same temperature [4].

Under the thermal juice processing conditions, typically 86 to 96 °C for 15 seconds to up to 2 minutes, pasteurization may not sufficiently reduce *Alicyclobacillus* levels and may lead to juice spoilage [1, 7, 9]. Furthermore, juice concentrate will always be more difficult to treat due to the high heat resistance of *Alicyclobacillus* spores. As juice concentrate may be a raw material used in other beverage industry, it is also critical to propose immediate solutions for *Alicyclobacillus* contamination prevention.

2.4. Spoilage by Alicyclobacillus spp.

Since 1984 when the first case of a massive apple juice spoilage was reported in Germany involving *Alicyclobacillus* species, other spoilage incidents due to *Alicyclobacilli* increased in the last years involving different types of food such as juices (orange, apple, tomato, white grape, pear, mango, pineapple), juice blends, juice concentrates (orange, apricot, peach, pear, apple, mango, coconut), carbonated fruit drinks and shelf stable ice tea [1, 4, 5, 12, 13].

In a survey of 57 companies, Lee *et al.* [9] reported 60% of survey respondants experienced spoilage events and of these 35% were caused by *A. acidoterrestris*. Müller and Schulz-Schroeder [14] reported a study made on 53 shelf-stable fruit juices. In this study 17% of shelf-stable juices were found to contain *Alicyclobacilli*. A survey of the literature shows different levels of risk for contamination of different juices types: fruit juice for blending, 9 to 42.1%; apple juice concentrate: 30.5 to 75%; orange juice concentrate, 82.1%; fresh juice products, 32%; apple concentrate, 83.3%; orange juice, 14.7% and fruit concentrates (orange, mango, coconut, pineapple), 8% [1, 13, 14]. In addition, a study carried by Jensen [15] reported that 20 to 30% of orange and apple concentrates used in Australia are likely to contain taint producing *Alicyclobacilli*. Kang [16] reported also that *Alicyclobacillus* spoilage may be responsible for annual economic losses of at least 0.41 $ million in Washington state, USA.

In summary this data shows that spoilage of fruit products by *Alicyclobacillus* species is a critical and expensive industry reality.

The visual detection of spoilage is difficult as the organism does not produce gas during growth which could result in easily identified swelling of flexible containers. The spoiled juice appears normal or may have a light cloudiness or haze. The main spoilage characteristic is an off-odour described as a “medicinal”, “phenolic”, “disinfectant”, or a “smoky” offensive smell [1, 5, 12, 17].

The predominant metabolite associated with the off-odour has been identified as guaiacol but studies also report halophenolic compounds such as 2,6–dibromophenol and 2,6–dichlorophenol participate to the taint.

The best estimate of the recognition threshold for guaiacol in shelf-stable apple juice by an experienced sensory panel is about 2.23 ppb. There is not always a correlation between the levels of spores and cells present and the guaiacol levels detected. It has also been reported that spoiled carbonated soft drinks were tested positive for the presence of guaiacol at 43 ppb although low levels of *Alicyclobacilli* were detected in the raw material used to make the beverage. In this case the numbers of bacilli increased after dilution of the concentrate to levels that developed into a tainted product [1].

Specific strains of *A. acidoterrestris* have been implicated in the production of fruit juice taints, mainly due to high level of guaiacol and frequency reported [1, 6, 12, 16, 17, 18]. However, additional species, *A. acidiphilus, A. herbarius* and *A. hesperidum* subsp. *Aigle*, have also been identified in some cases of taint [2, 18].

Temperature has an effect on taint formation as *A. acidoterrestris* does not grow well at room temperatures suggesting that guaiacol formation is also limited at those temperatures [1].

Alicyclobacillus species (mainly *acidoterrestris*) represent a real current threat to the entire juice industry, from concentrate manufacturer to juice bottlers as well as other beverage industries manufacturing juice-containing drinks such as carbonated soft drinks, ice tea or functional drinks and using juice concentrate or juice puree as an ingredient.

3. Juice Industry requirements

3.1. Identification of critical control points

As previously described, juice concentrates (raw material) and juice-containing products may be exposed to and contain *Alicyclobacillus* species. The typical process flow diagram is represented on Figures 1 A and B.

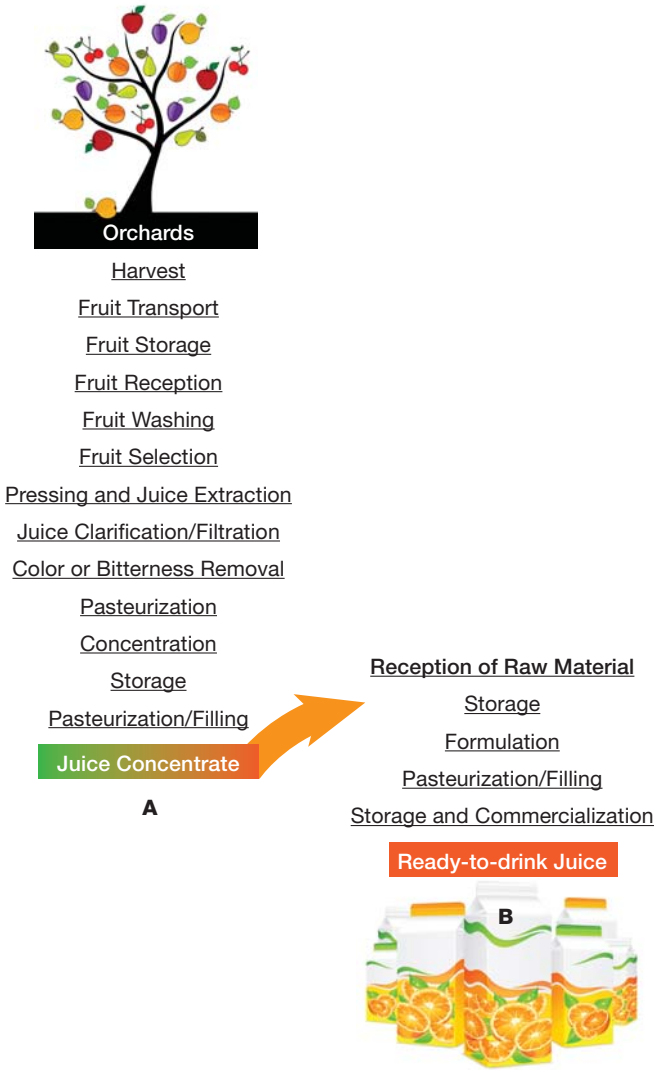

Figure 1: A – Juice concentrate production; **B –** Ready-to-drink juice production

The first main entry doors into the production process for *Alicyclobacillus* species, depending on the juice type, are soil contaminated fruit (fruit concentrates) and fruit concentrates or ingredients such as sugar syrup (ready-to-drink juices) [1, 5, 10, 12, 19].

Several publications reported cases of identification of *Alicyclobacillus* species at different steps of the production process. Chen *et al.* [3] isolated strains of *Alicyclobacillus* species from samples taken along the apple juice concentrate process including water after apples washing. More recently, Groenewald *et al.* [12] isolated strains of *A. acidoterrestris* or *A. acidocaldarius* from different samples taken from juice processing facilities including water from fruit washing, water from flume transportation and condensate water from evaporator. Walker and Phillips [21] showed that headspaces level of storage tanks or containers may have an impact on microbial growth.

Once introduced into the process by fruit contaminated from the soil, *Alicyclobacillus* may contaminate the entire fruit juice production line.

Due to the serious and increasing threat that *Alicyclobacillus* represent for the juice industry and other industries using fruit juice, concentrates or purees as raw materials, the European Juice Association (AIJN) has written a guideline listing the main critical control points along the juice processing for the attention of raw material producers and packers/bottlers of finished products [19]. Table II provides a summary of the recommended critical control points.

Table 2: Summary of recommended critical control points at various stages of the juice processing (based on AIJN, 2008)

Process	Stage	Control Point
A	Harvest and fruit transportation	Fallen fruits Cleanliness of fruit containers, intermediate storage andtransport
	Fruit reception and handling	Excessively dirty fruit or fruit excessively contaminated with extraneous vegetal matter (*e.g.* leaves)
	Fruit storage	Buildings optimization
	Fruit cleaning and sorting	Water flume Washing and rinsing water
	Pressing and juice extraction	Water for juice extraction Water for equipment cleaning
	Juice clarification / filtration	Cleaning efficiency and membrane integrity
	Colour or bitterness removal	Cleaning effectiveness of resins
	Pasteurization	Temperature, holding time and procedure
	Concentration	Temperature conditions
	Filling and Storage	Storage temperature Temperature conditions Headspace management
	Aroma (from concentration)	Quality control
B	Storage of raw material	Storage temperature
	Formulation	Quality of ingredients used (*e.g.* sugar syrup) Guaiacol precursors monitoring
	Storage	Temperature conditions Headspace management
	Pasteurization	Temperature conditions
	Filling	Temperature conditions
	Storage and distribution	Temperature conditions

3.2. Prevention of Alicyclobacillus contamination

Literature reported different suggestions, mainly regarding fruit washing, juice preservation, juice stabilization and juice storage in order to propose solutions for *A. acidoterrestris* contamination in the fruit juice industry.

3.2.1 Use of oxidizing agents for fruit washing

The first source of *Alicyclobacillus* contamination in the fruit juice processing facilities is found at the surface of the fruit contaminated by soil during harvesting, transportation or storage. Thus, several studies have focused on the treatment of fruit to reduce contamination before it enters the processing plant.

Typically aqueous chlorine at concentrations between 50 to 200 ppm are used to wash fruits and vegetables resulting in a 2 to 3 log microbial reduction depending on the use or not of commercial cleansers. Orr and Beuchat [21] showed spores resistance to those disinfectant agents on apple fruit surface. In their study, less than 1 log reduction was obtained after contact to 500 ppm chlorine or 2% hydrogen peroxide for 1 minute. Higher levels of and exposure time to chlorine is required to achieve increased log reduction of spores. A reduction of 2.2 log was obtained with

200 ppm chlorine during 10 minutes at 23 °C on *A. acidoterrestris* spore mixtures and a more than 5 log reduction was achieved with 1000 ppm chlorine or 4% hydrogen peroxide.

Aqueous chlorine dioxide (ClO2), allowed by the Food and Drug Administration since 1998 to wash fruits and vegetables, is a strong oxidizing and sanitizing agent which may be used as an alternative sanitizer. As it has 3.5 times oxidation capacity than chlorine, studies have been done in order to check its ability to reduce spores from apple surfaces [22, 23]. Lee *et al. (24)* showed a 4 log reduction of spores in aqueous suspension was obtained with 40 ppm of chlorine dioxide after a 5 minutes exposure time. A reduction of more than 5 log was achieved after exposure to 40 ppm chlorine dioxide for 4 minutes on spore infested apples [1, 25]. However, even if treatment of fruits by aqueous chlorine dioxide is effective, it is still an inconvenient and relatively expensive treatment requiring some technical expertise. Furthermore, it has been reported that the oxidizing power of aqueous chlorine dioxide is diminished by contact with organic matter, such as fruits [23].

As gaseous chlorine dioxide offers more penetration and is more effective in reducing micro organisms on fruit, Lee *et al.* [22] tested 0.6 mg/L of gaseous chlorine dioxide for 3 hours resulting in a 4.5 log reduction without negatively affecting the visual quality of fruit. But this treatment presents some limits: the treatment must be conducted in a firmly and safely sealed chamber; high concentrations of gas are potentially explosive and numerous mechanical devices or steps are necessary to handle the gas as well as to provide precise concentration for sanitization [23].

3.2.2 Use of natural components for juice preservation

Some studies reported the use of natural components for juice preservation. Yokota *et al.* [4] reported that *Alicyclobacillus* species were not able to grow in red grape juice due to the presence of polyphenols such as trans-resveratrol or ferulic acid. Brodbeck *et al.* [26] showed the influence of polyphenols content on growth of *A. acidoterrestris* in iced tea. The phenol content in iced tea made from infusions of green and black tea had inhibitory influence on growth of *A. acidoterretris* and iced tea made from soluble extracts with lower levels of phenols did not possess antimicrobial properties.

Other authors proposed the use of essential oils to control the germination of *A. acidoterrestris* spores. Cinnamaldehyde was reported to be the most effective compound which can inhibit spore germination for 13 days at a concentration of 0.5 g/L. But the antimicrobial activity of some essential oils compounds against *Alicyclobacilli* spores is strain-dependent [5].

Calcium lactate, used in the fruit juice industry to fortify fruit juice, has been shown to effectively inhibit the growth of *A. acidoterrestris*. However, this inhibition is transitory and if a temperature abuse occurs the organism may grow and produce spoilage [1].

The use of the enzyme lysozyme has been reported to reduce heat resistance and prevent spore germination. However no evidence is available in the literature to explain the action against spores and further investigation is needed [4, 5].

Emulsifiers such as monolaurin and some related compounds (*i.e.* sucrose fatty acid esters) may also be used to prevent spores germination *(4, 5)*.

3.2.3 Use of preservatives for juice preservation

Natural bacteriocins such as nisin, enterocin AS-48 or warnericin produced or extracted respectively from *Lactococcus lactis* supsp. *lactis, Enterococcus faecalis* A-48-32 and *Staphylococcus warneri* have proved to have an ability to inhibit growth of spores and vegetative cells of *A. acidoterrestris* [1, 4, 5].

Nisin, an antibacterial food preservative, non-toxic, considered as natural because strains producing are food-grade (GRAS, generally recognized as safe) is sold since 1953 and approved over 50 countries in Europe and America but not in Japan for use in different food processing. Its

use against *A. acidoterrestris* is well documented. Its action is predominantly sporostatic rather than sporocidal. The effectiveness against *A. acidoterrestris* is strain, pH and juice dependent. The presence of nisin during the heat treatment can decrease the D-value of spores by up to 40 % at 25 °C at a concentration of 5 IU/mL in apple, orange and grapefruit juices [1, 5, 27, 28]. But nisin is not yet an allowed preservative in fruit juice or juice products.

Enterocin AS-48 may be an alternative to nisin in food biopreservation. Concentrations of 2.5 µg/mL promote immediate effects that are sustained for 60 to 90 days depending on juice, temperature of storage and number of cells and spores in the juice [1].

Although the industry trends are moving away from the use of preservatives, particular attention is required to find other means to prevent spoilage by heat resistance of spores of *A. Acidoterrestris* during the shelf-life of a product.

In the soft drinks industry, the preservatives most commonly used are sodium benzoate and potassium sorbate which aid in preventing or delaying the spoilage by inhibiting microbial growth. It has been reported that sorbic acid and benzoic acid alone and in combination and the use of carbonation in unpreserved soft drink prevent spoilage by *A. acidoterrestris*.

Sodium benzoate, with its broad antibacterial range is widely used as a fruit beverage preservative. Walker and Phillips [29] showed that sodium benzoate and potassium sorbate used respectively at 0.5 to 1.0 mg/mL and 1.0 mg/mL are effective against *A. acidoterrestris* but the levels for inhibition of each preservative are higher for vegetative cells than spores. Then, if a temperature abuse occurs, these may multiply and cause spoilage.

Nisin (10 IU/mL) and sodium benzoate added to fruit juice have an additive effect compared with sodium benzoate alone but not compared with nisin alone, showing the strong effectiveness of nisin in decreasing heat resistance of spores.

3.2.4 Use of non conventional methods for juice stabilization and storage

The optimization of thermal processing has been considered for a long time as the only way to control *A. acidoterrestris* in acidic drinks and inhibit spore germination. However new techniques have been studied in order to reduce the loss of sensorial and nutritional quality due to a thermal treatment [5].

Ultraviolet radiation involving the use of UV-C (200 – 280 nm) radiation is considered to be germicidal against micro organisms. A wavelength of 254 nm is commonly used for the disinfection of surfaces, water and various liquid food products including fruit juices. In fact, ultraviolet treatment requires very little energy compared to thermal pasteurization while protecting the final product quality. But, the high suspended and soluble solids content reduces the effectiveness of ultraviolet treatment of juice due to low UV transmittance.

Keyser *et al.* [30] showed that it was not possible to achieve 5 log reduction in all juices due to the juice characteristics. For example, a clear juice (apple juice) required a lower UV dose to achieve effective reduction than juices with higher levels of suspended matter like orange juice with cells or tropical juices which required higher UV doses to achieve the same level of reduction. Franz *et al.* [32] showed also the effectiveness of ultraviolet treatment on clear apple juice in achieving the 5 log reduction required by the Food and Drug Administration in the finished juice but failed using freshly squeezed apple juice due to high amount of solids content.

Ultraviolet treatment may be effective in reducing the micro organisms content of juices but due to the soluble and suspended solids content of juices, the treatment may be combined with other heating treatment for more efficiency.

Bevilacqua *et al.* [5] and Walker and Phillips [1] reported also studies done on electron beam and gamma ray irradiation showing the effectiveness of spores inactivation in combination with a heat treatment. But, for the moment, in Europe, this kind of treatment is not well accepted by consumers.

Microwaves also were used to inhibit spore-forming micro organisms. A reduction of 2 logs was achieved after a processing time of 5–7 minutes at 80 to 100 % of power (2450 MHz – 900 W) for spores of *A. acidoterrestris* inoculated in asparagus cream [5]. But this treatment needs further studies to check its effectiveness of spore removal in the fruit juice industry.

The combination of high temperature, time and high pressure can be used to inactivate spores using 207 to 621 MPa in a range from 45 to 71 °C for 10 minutes to achieve respectively 3.5 to 5.5 log reduction [9]. As expected, spores appeared to be more resistant than vegetative cells and a two steps treatment is needed to reduce their levels. High pressure was used to induce spore to germination and high hydrostatic pressure in a second step causes the inactivation of germinated spores. High hydrostatic pressure (HHP) treatment is influenced by sugar and solids contents of juices and no effect was observed in a juice concentrate (70° Brix) limiting the use of the technology to low brix products [5].

Walker and Phillips [21] showed that the amount of headspace available in juice containers or bottles makes a significant difference in growth of vegetative cells and sporulation of *A. acidoterrestris* and hence detection rates when apple juice is stored at 35 °C. Storage of juice products at low temperature and filling empty headspace with an inert gas such as nitrogen may be effective in slowing growth of *A. acidoterrestris*.

It has also been reported that the addition of ascorbic acid into some juices may prevent or inhibit the growth of *A. acidoterrestris* at concentration of 150 mg/L or higher [32].

Another path for the inhibition of *A. acidoterrestris* in fruit juices is by developing antimicrobial films for packaging of the final product. Such developments are lending themselves to an emerging area of research in the food industry [1].

3.3. Immediate solutions to control Alicyclobacillus along the juice processing

Even literature reported different working paths, juice producers or packers have limited immediate solutions in order to prevent *Alicyclobacillus* along the fruit juice processing covering the critical control points listed by AIJN.

3.3.1 Use of oxidizing agents for fruit washing

AIJN [19] suggests that all water used in the juice plant can contain *Alicyclobacillus*. Different water quality is used within a juice processing plant including incoming water, treated water (obtained after treatment of incoming water) and recovered water (mainly from evaporator condensates).

The water used for fruit transportation, cleaning and rinsing, equipment cleaning and/or rinsing, as well as the water incorporated during juice extraction, should not promote process contamination by *Alicyclobacillus*. Indeed, different studies listed above already reported the identification of *Alicyclobacillus* in water flumes, fruit washing water or condensates from evaporators. Water from fruit washing can easily be contaminated by fruit spoiled by soil and condensates from evaporator offer the perfect growth conditions for *Alicyclobacillus*. Water re-use from evaporator condensates represent a major risk.

Water used for fruit or equipment cleaning is generally treated by chemicals, mainly chlorine or hydrogen peroxide at determined quantities and contact time but spores resistance at chlorine concentration of up to 500 ppm or 2 % hydrogen peroxide during 10 minutes has been reported. For some fruit species, the use of chemicals may lead to formation of residual component on the surface of fruit skin that may use further in the juice processing. This is the typical case of citrus fruits for which chlorine residue can damaged the essential oil with chlorinated terpene. Young and Setlow [33] reported also some resistance of spore to ozone.

Chemical treatment of water may lead to an acceptable water quality but this is absolutely critical to select the right oxidizing agent type (peracetic acid, hydrogen peroxide, chlorine dioxide, hypochlorite or ozone) and determine the right concentration and contact time.

Ultraviolet can be also used to treat water but to ensure effectiveness of UV treatment, the exposure time and the light density must be constantly monitored as well as the water turbidity that may offers shade to bacteria. That's why generally ultraviolet treatment is used in combination with other treatment to increase the effectiveness of the water treatment.

As today the trend of the food industry is to re-use and recycle water as part of its sustainability program, the re-use of condensates for fruit washing as well as the re-use of water from flume are ways to optimize water usage and save water costs.

Membrane filtration systems such as Pall® Aria™ FB systems (Figure 2) are now used to obtain a consistent water quality production regardless to raw water quality while improving water costs. Membrane filtration systems consist of robust testable hollow fiber modules installed on a stainless steel frame. The system runs in an automatic mode through a simple and easy to handle program. The system offers all the required conditions to ensure the removal of spores from the water used:

- a simple single step water treatment
- high microbiological safety thanks to its stainless steel hygienic design and automatic disinfection
- typically, 6 log removal on *Cryptosporidium* and *Giardia* oocysts
- direct water treatment, no additional time required for chemical treatment
- low chemical consumption thanks to efficient mechanical backwash
- low energy consumption (typically 0.09 kWh per m^3 filtered water)
- minimal losses (up to 98 % water recovery)
- materials suitable for use in contact with food
- process traceability (performance record)
- modules testing

Figure 2: Pall Aria FB membrane system

The system in combination with a final membrane stage solution to ensure spores removal can be easily installed to treat difficult and critical water such as condensates ensuring effective water quality and preventing from spoilage by *Alicyclobacillus* spores.

3.3.2 Juice processing

Ultrafiltration is widely used for juice clarification (*e.g.* apple juice). Theoretically, bacteria cells and spores should not be able to pass through the membranes of the system. However, experience has shown that product after ultrafiltration stage is not always free from *Alicyclobacillus* [19, 34]. Depending on the membrane type, the material of construction used and the age of the system, small lesions or leaks may happen allowing the bacteria and spores to pass through. It is also well known that retentate from ultrafiltration can be treated through a vacuum filter and re-incorporated in the process as clear filtered juice. If retentate is contaminated by *Alicyclobacillus*, the juice obtained after filtration through vacuum filter can also very easily contaminate all the downstream juice process. Conventional technologies using clarification aids and diatomaceous earth filter or vacuum filer can be also used. Bahçevi *et al.* [34] showed also the limit of those conventional technologies for *Alicyclobacillus* removal. The performance of technologies used for juice clarification should be particularly monitored due to these limits.

Typically, additional barriers are used to achieve juice stability, as measured by turbidity, after the clarification step. Depth filter sheets are widely used in the apple juice process before final concentration steps mainly for particle removal (*e.g.* trap filtration after diatomaceous filter), turbidity stability after ultrafiltration stage (*e.g.* polishing stage) and, under specific conditions, *Alicyclobacillus* removal.

Depth filter sheets combine the process mechanisms of surface filtration, depth filtration and adsorption to fulfil the specific requirements of the fruit juice industry. The flux to be used and then the number of sheets installed depend on the filter grade performance. Typical recommended operating parameters are given depending on filtration target:

- trap and polishing filtration: flow velocity up to 850 $L.h^{-1}.m^{-2}$ and maximum differential pressure before change out 300 kPa (3 bar).
- microorganism reduction: flow velocity from 150 to 500 $L.h^{-1}.m^{-2}$ and maximum differential pressure before change out 150 kPa (1.5 bar).

As an example, in the apple juice concentrate production, filter sheets may be used prior to concentration for *Alicyclobacillus* removal. The recommended flow velocity for concentrate (70°Brix) and half-concentrate (42°Brix) are respectively 200 to 250 $L.h^{-1}.m^{-2}$ and 400 to 500 $L.h^{-1}.m^{-2}$.

An internal study (data not shown) carried on clear apple juice inoculated with cells of *A. acidoterrestris* (DSM 3922, ATCC 49025) filtered through different filter sheets at 500 $L.h^{-1}.m^{-2}$ showed that all the filtered apple juice obtained were free of cells representing a log reduction range from 6 to 8 when challenged with 10^5 to 10^6 cells per cm^2 of surface area. These data showed the ability of depth filter sheets in reducing the level of *Alicyclobacillus* in fruit juices under specific conditions.

However, in order to propose a reliable and efficient solution that will fit the requirements of AIJN regarding filter performance and integrity monitoring, an internal study [36] has been carried out on 100 % clear apple juice (pH 3.5, 10.2°Brix) and high fructose corn syrup (55 % fructose, pH 4.5, 75°Brix) inoculated respectively with 10^4 – 10^5 and 10^4 CFU/mL of a spore suspension of *Alicyclobacillus acidoterretris* (DSM2498) prepared by the Research Laboratory of the Japanese Canners Association. The two medium used were filtered through a Pall Supor® 0.45 µm membrane, used in Pall Oenopure™ cartridges. At a challenge level between 106 and 107 CFU/cm^2 surface area, filtered medium obtained gave spore free filtrate. Controls (1/15 mol phosphate buffer) demonstrated that the method used was valid. We therefore conclude that the membrane tested and used in the construction of different Pall cartridges is capable to retain thermo-acidophilic bacterial spores (Figure 3).

These cartridges offer a very high level of safety to the juice manufacturer. Furthermore, an integrity test can be performed in order to monitor the performance of the filtration membrane, as recommended by the AIJN.

The study showed that cartridges solution, when suitable for juices (free of pulp or cells) and combined with appropriate disinfection procedures offer the highest level of safety against *A. acidoterretris*.

Figure 3: Cartridges for *Alicyclobacillus* removal

The juice aroma coming from the evaporator may be also a source of *Alicyclobacillus* contamination under certain conditions [19]. Cartridges solution as previously described may be used in order to prevent the juice to be contaminated through aroma when reintroduced before the final pasteurization step.

In the literature mentioned above, spores of *A. acidoterrestris* showed incredible heat resistance. Temperature conditions in evaporators are very conducive to the growth of *Alicyclobacillus*. The concentration step is critical. If the fruit juice quality allows, AIJN [19] recommends increasing the temperature up to 125 °C for a suitable holding time to destroy spores of *Alicyclobacillus*. As previously written, water condensates must be also appropriately treated before reuse in the juice process.

The intermediate storage of juice in tank or containers is also a critical control point as storage temperature may be a signal for spores to germinate. As previously mentioned, Walker and Phillips [20] showed that headspace volume in storage tanks or containers plays a role on the growth of *Alicyclobacillus* as well as the storage temperature. The restriction of oxygen availability may reduce the production of guaiacol and hence spoilage. That's why storage temperatures below 25 °C are recommended.

Furthermore, the use of inert gas such as nitrogen has then been suggested as well as the use of carbon dioxide on top of concentrate storage tanks. Those gases may be appropriately filtered in order to avoid additional contamination from airborne microbes. A vent filter installed on top of tanks or containers is generally recommended.

When considering juice bottler, the different ingredients used in the final product formulation are additional critical control point. AIJN [19] recommends testing of each raw material. As water may represent an important percentage of the final juice-containing beverage, it is absolutely critical to ensure *Alicyclobacillus* removal from the water used for beverage formulation. Previous literature mentioned above also reported the identification of *Alicyclobacillus* in sugar syrup that may be used in beverage formulation. In order to avoid any contamination of *Alicyclobacillus* from the sugar syrup, it may be filtered through membrane filters (Figure 3).

Different studies [8, 11] suggested to choose *Alicyclobacillus acidoterrestris* as the target micro organism and to define heat treatment conditions to achieve the 5 log reduction required by the Food and Drug Administration for fruit juice based on D-value reported as well as the potential for *Alicyclobacillus* spores to grow during the product storage for at least one month at 25 and 43 °C. Furthermore, Vieira *et al.* [11] showed that the use of high temperature – short time principle (HTST) treatment — offers a way to ensure the required log reduction of *Alicyclobacillus* spores while minimizing the impact on organoleptic quality of juices. Temperatures of 98, 115 and 135 °C for 9 minutes, 8 and 36 seconds showed respectively 55, 98.5 and 65 % retention of ascorbic acid.

3.3.3 Juice bottling

Final UHT treatment (up to 120 °C when possible) combined with aseptic filling is recommended as an effective heat step to destroy *Alicyclobacillus* spores [19]. If hot filling is preferred, the time for heating should be chosen appropriately to ensure the right protection against *Alicyclobacillus* spores as well as the right management of the cooling period. The cooling step must be done as quickly as possible. Furthermore, the product may be fortified with ascorbic acid or packed with a minimum headspace to limit the availability of oxygen and prevent from *Alicyclobacillus* growth during storage [11].

3.3.4 Detection methods

We already reported in this article that not all strains of *Alicyclobacillus* are able to produce guaiacol and cause juice spoilage. The International Federation of Fruit Juice Producers (IFU) undertook to develop internationally acceptable methods for the detection of *Alicyclobacillus* in concentrates and juices and has published a general method for *Alicyclobacillus* detection (Standard IFU Method 12 – Microbiology of *Alicyclobacillus* in fruit juices, 2004). But the method does not specifically detect the guaiacol producing strains.

4. Future trends

It is now very well documented that *Alicyclobacillus* spp. represent an important and increasing threat causing financial damages to the fruit juice or juice-containing beverage industry. Due to the ability of the micro organism to grow at low pH in combination with high heat resistance of its spores, juice pasteurization can no longer be seen as the final safety barrier to ensure protection against spoilage.

The use of non-conventional approaches to reduce the contamination by *Alicyclobacilli* could be considered as a promising way for the juice industry. However, as these approaches are currently successful at lab scale, these techniques require scale up to a production level before they will be commercially accepted.

AIJN [19] promotes a multiple barrier approach as a solution for contamination prevention at critical points. Solutions are available in the market for the juice producer to ensure *Alicyclobacillus* removal at all the stages of the process from water used within the process, to juice extraction, bottling and storage.

Not all strains of *A. acidoterrestris* are able to spoil the final product by producing guaiacol. In parallel to a multiple barrier approach, strain identification methods need further developments to reduce detection time of spores and cells responsible for guaiacol taint, to increase detection sensitivity and to find a suitable method for cloudy products [19].

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