GERF Bulletin of Biosciences 2017
ISSN: 2229-6433 December, 8(2):1-12

Review Article

Alicyclobacillus species in fruit products spoilage: Causes and Control Measures

H.K. Bhandarkar[1,2], K.S. Bhamare[1], V.A. Agnihotri[1], S.S. Bharrdwaj[1*]

[1]Microbiology Laboratory, Jain R&D, Jain Irrigation Systems Ltd, Agri park, Jain Hills, PO Box no. 72, Shirsholi Road, Jalgaon-425001, Maharashtra, India.

[2]North Maharashtra University, Jalgaon, Maharashtra, India.

Open Access

Received: 12 August 2017
Accepted: 14 December 2017
Published Online: 31 December 2017

Citation:

Bhandarkar HK, Bhamare KS, Agnihotri VA, Bharrdwaj SS. *Alicylclobacillus species* in fruit products spoilage: Causes and control measuresGERF Bull Biosci. December 2017; 8(2):1-12.

Conflict of Interests:

The authors declare no conflict of interests.

***Corresponding author:**

S.S. Bharrdwaj
bhardwaj.sudhanshu@jains.com

Abstract

Alicyclobacillus is gram positive, rod shaped, heterotrophic, thermoacidophilic and aerobic. It has ability to survive at high temperature and low pH, so it called Thermophilic Acidophilic Bacteria (TAB). The genus *Alicyclobacillus* is non-pathogenic but spoilage forming organism for fruit juice and juice concentrates. It is widely recognized to survive at pasteurization temperature, so it is not easily eliminated from fruit juice and fruit products. Spoilage by *Alicyclobacillus* is major concern for producers, as if not eliminated they are unaware of spoilage until product reaches consumers. This review focused on source of *Alicyclobacillus*, methods for isolation, causes of contamination of fruit juice and fruit juice concentrates by TAB, control measures including physical, chemical and biological treatments available till date for removal of *Alicyclobacillus* species. It is necessary to eliminate any traces of this spoilage organism from fruit juice and their products to improve the quality of product and to reduce economic loss, so new methods are constantly being established and investigated.

Key words: Thermophilic Acidophilic Bacteria, spoilage, elimination, pasteurization.

Introduction

Fruit juices are consumed widely around the world, mainly because they are considered a healthy natural source of nutrients. However, the high water activity and high carbohydrate content along with other nutrients of fruit juices favor microbial growth. Microbial contamination of fruit juice and juice products have caused several cases of food borne illnesses and spoilage incidents, which not only results in threats to human health but also leads to huge economic loss of producers (1). Juice is defined as "the aqueous liquid expressed or extracted from one or more fruits or vegetables, purees of the edible portions of one or more fruits or vegetables, or any concentrates of such liquid or puree" (2).

The production of pasteurized shelf-stable juices is an important part of the global beverage industry (3) and its requirements are a large quantity of the global juice market; therefore spoilage of these types of products may effect on product and manufacturer image. Consumption of juices has grown day by day due to health awareness, consumption of safe product, green food product and nutritional requirements of humans are increased (4). So, many companies have started producing new juice and juice concentrate containing fruit juice or juices. Fruit juice and fruit based products also form an important part of the rapidly growing functional foods market, because these products are considered to be healthy and nutritious (5), consumers have greater expectations with respects to their quality and safety from manufacturer.

The spoilage incident reported in Germany in 1982 involving pasteurized shelf-stable apple juice (4,6,7). The efficiency of pasteurization treatments applied on to fruit juices and fruit juice concentrates for the control of spoilage organisms is doubtful, because some microorganisms can stable in pasteurization temperature. Mostly, microorganisms are responsible for the spoilage of pasteurized self-stable juices and juice concentrate even after processing and that is *Alicyclobacillus* species, was identified as the causative organism in this spoilage incident (8,9).

Alicyclobacillus are gram positive, straight rod shape (0.3-1.1µm wide and 1.5-6.3µm long), heterotrophic, thermo acidophilic, aerobic or facultative aerobic, generally non-motile endospore forming microorganism (4,7,10,11,12). The spore generally forms terminal or sub-terminal. It is not a pathogenic, but a spoilage forming organism. *Alicyclobacillus* are growing in acidic condition, the optimum pH is 1.5 to 5.5 and growth pH range is 0.5 to 6.5 (13). The optimum temperature range is 35°C to 65°C and growth temperature range is 4°C to 70°C (7,9,13,14). All species metabolize sugars with acid production but no gas production. *Alicyclobacillus* shows no growth media containing 5% w/v NaCl. Water activity is greater than 0.9 is required for growth, and some species have been reported to grow in fruit juice with up to 18.2° brix (15). Its ability to survive vast condition i.e., at high temperatures and low pH level so it's called TAB. TAB ability to survive vast condition because to the unique cellular membrane composition containing ω-alicyclic fatty acids could be strong heat and acid resistance (11). The ω-alicyclic fatty acids packed tightly resulting in low diffusion at high temperature and acidic pH (7,16). It has been shown that as growth temperature increases, the content of the ω-alicyclic compound increase and hydrophobic bond might be stabilizing and reduce membrane permeability in extreme acidic and high-temperature environment (9,16,17,18,19).

Alicyclobacillus species are able to survive the pasteurization processes as well as the acidic condition of juices and the pasteurization treatment may act as a heat shock treatment that activates spores (18,20) and they favor the acidic environment they can germinate and grow to cell populations high enough to produce spoilage taints and at this condition prevents other microorganisms from growing (21). In case of *Alicyclobacillus acidoterrestris* spoilage is more concern, because producers are unaware of the spoilage until the product reaches the consumer (22), the problem can be resolved by successful study to prevent spoilage at initial stages. This will help to lessen the effect to customer dissatisfaction, loss of brand quality image.

The source for *Alicyclobacillus* species has been found the incoming the raw unwashed or poorly washed fruits (19), water used throughout process and finished products like fruit juices and juice concentrates. Unreported spoilage and difficulty in detection is primary cause for impact of the spoilage on the juice industry (23). It poses the main challenge for elimination of *Alicyclobacillus* species as it is ubiquitous and has ability to survive at high temperatures and low pH for a period of time. In addition, *Alicyclobacillus* species is highly resistant to many cleansers, sanitizers, and other physical treatments (24). Therefore, a change in processing is not sufficient to inhibit this microbe in all products and it is required excessive energy to inhibit or control and this reason behind that increase cost of products.

Prevention of spoilage can be achieved by using different natural, chemical and physical agents without any effect on product quality (25). Determination of the efficacy of antimicrobials is required to find effective agent for inhibiting vegetative cells and spores of *Alicyclobacillus* species for prevention of spoilage in juices.

Historical background

The study on hot water springs in Japan and isolated an aerobic, acidophilic, spore-forming bacterium and first

classified as *Bacillus coagulans* due to observed tolerance to low pH (26). Which is further identified by Darland and Brock in 1971 based on DNA-based identification tests and it is suggested that the organism was a new species which they named, *Bacillus acidocaldarius* (11,27). In 1981, Hippchen (28) described an acid and heat tolerant, spore-forming thermophilic isolate from garden soil that differed from *B. acidocaldarius* based upon a lower growth temperature, biochemical characterization, and DNA based composition (23,29,30). Another strain of an acidophilic and thermotolerant spore-former was isolated from garden soil, but it contains different ω-fatty acid profile. In 1987, Deinhard and others (31) used strains that were isolated by Poralla and Cerny who found two new species: *Bacillus cycloheptanicus* and *Bacillus acidoterrestris*. The genus *Alicyclobacillus* was established in 1992. Origin of new genus was supported by and based upon bacterial cellular membrane structure. The genus *Alicyclobacillus* was uniquely characterized by presence of ω-alicyclic acids and DNA sequence of the organism. In 1982, Cerny and others isolated *Alicyclobacillus* very first from spoiled pasteurized apple juice (4,6,18,21,32,33).

The genus *Alicyclobacillus* presently includes 20 species, 2 subspecies and 2 genomic species (33): *A. acidiphilus*, *A. acidocaldarius*, *A. acidoterrestris*, *A. cycloheptanicus*, *A. herbarius*, *A. hesperidium*, and *A. sendaiensis*. *A. disulfidooxidans*, *A. pomorum*, *A. tolerans*, *A. vulcanali*, *A. shizuokensis*, *A. contaminans*, *A. fastidiosus*, *A. pohliae*, *A. ferrooxydans*, *A. kakegawensis*, *A. macrosporangiidus*, *A. sacchari*, *A. aeris*, *A. acidocaldarius* subsp. *acidocaldarius*, *A. acidocaldarius* subsp. *Rittmannii* (34).

Sources

Alicyclobacillus species are ubiquitous in nature, it is originally isolated from soil or hot springs (4), the soil is considered to be the major sources of *Alicyclobacillus* species and also the most important source of contamination of acidic products. Studies have suggested that contamination of fruit juices is most likely caused by fruit contaminated by soil during harvest or through windfall (32) or by unwashed or poorly washed raw fruit used in processing facilities (11) and soil can be carried into the manufacturing area by employees. Various important sources for contamination include raw fruits, processing materials, water from recovery systems of thermal evaporators used in production of fruit juices (7,24). McIntyre et al. (35) isolated a strain of *Alicyclobacillus* from spoiled juice product and in a sample of ingredient water from the processing facility.

Heat resistance of *Alicyclobacillus* species

Spores of *Alicyclobacillus* species can germinate and grow at low pH (<4) and show high heat resistance. D-values of *A. acidoterrestris* spore in juices at 90°C and 95°C is reported to range from 16 to 23 minutes and 0.06 to 5.3 minutes respectively. Z-value range from 7.2 to 12.9°C reported (12,33). Targets microorganisms in the fruit juice and fruit juice concentrate industry are generally much less heat resistant than spores of *A. acidoterrestris*. The standard juice pasteurization treatment is 80°C to 95°C for 45 to 15 seconds, which is not enough to inactivate spores of *A. acidoterrestris*. This is the major problem to spoilage fruit juices and concentrates by *A. acidoterrestris* (7,36).

A summary of *A. acidoterrestris* D-values in different fruit juices is different. D-value decreased with an increase in temperature, which indicates decreased heat resistance. D-values decreased dramatically when temperature increased from 85°C to 90°C, and the highest D-values were recorded in black currant concentrate (24.1 min at 91°C) and lemon juice concentrate (12.63 min at 95°C). For most of the juices evaluated, D-values were reduced to less than 4 min at 95°C. The higher the sugar content (°Brix), the greater the heat resistance recorded. In black currant concentrate increase soluble solids from 26.1 to 58.5° brix, the D-value at 91°C increased from 3.8 to 24.1 minute. This indicates that the spores of *A. acidoterrestris* are more difficult to destroy in concentrated juices than in single strength juices. pH also has an effect on the heat resistance of spores, generally with lower heat sensitivity at higher pH (7,36).

Fruit juice pasteurized process involves heating the product at 90°-95°C for 15-20 seconds, followed by package filling while the product cools to 82°-84°C. The product is then held at this temperature for approximately 2 minutes before chilling (37). Due to its high heat resistance and involvement in several spoilage incidents, *Alicyclobacillus* species has been suggested the target organism in the design of pasteurization processes for acidic foods, fruit juices and juice concentrates (36,38,39,40).

Spoilage Compounds

Spoilage by *Alicyclobacillus* species is primarily manifested as off flavor and off odor. Visual detection of spoilage is very difficult since no gas is produced during growth and swelling of containers does not occur. The cause of observed off flavor or odor as “smoky”, “medicinal”, “antiseptic” was attributed to the formation of the chemical compounds, guaiacol (2-methoxyphenol) and halophenols(2, 6 dibromophenol) by *A. acidoterrestris* in the juices (7,12,16,21). The guaiacol is resulted from the decomposition of lignin, phenolic acid and ferulic acid found in fruits. *A. acidoterrestris* acts upon vanillin naturally found in juices to form guaiacol and 2, 6 dibromophenol (41,42).

Guaiacol

Guaiacol (2-methoxyphenol) is a phenolic compound with the formula C_6H_4 (OH)(OCH_3). Guaiacol concentration is 1000 times higher than halophenols (7,43). In fruit juices, guaiacol is formed from ferulic acid via vanillin (4,44). Ferulic acid is a major component in lignin and can be found abundantly in plant cell walls. It can be metabolized by bacteria and fungi (45) and converted to vanillin, vanillic acid, and protocatechuic acid. Vanillic acid can be further converted to guaiacol. The threshold detection level of guaiacolvaries product to product and about 2 ppb (parts per billion) in orange and apple juice that rose up to 100 ppb (42). Detection levels of guaiacol (1-100 ppb) with taint production were 5 log CFU/ml and higher (4,46).

The human sensory threshold level for guaiacol is low, so it is easily detected. Wasserman (1966) reported that the threshold concentration level of guaiacol in water is 0.021 ppm (parts per million) for odor and 0.013 ppm for taste; the odor threshold level in oil is 0.07 ppm and in12% aqueous ethanol is reported as 0.03 ppm (11). Pettipher et. al. (1997) (47) used a Gas-chromatography Mass-spectrophotometer (GC-MS) method and found that the odor threshold level for guaiacol in orange, apple juice, and a non-carbonated fruit juice drink was about 2 ppb (46). Another study using a sensory panel and the forced-choice ascending concentration method of limits conducted by Orr et. al. (2000) (48) also showed similar results. They reported the best estimate threshold level of guaiacol in apple juice is 2.23 ppb (7).

In the case of *A. acidoterrestris* spoilage, guaiacol is produced when cell numbers reach a critical level. The guaiacol was detected in apple juice, orange juice, and grape juice stored at 30°C when the population of *A. acidoterrestris* reached 10^5 CFU/ml; at 25°C, the same population was required to detect guaiacol in apple and orange juices, but only 10^4 CFU/ml were necessary to detect it in grape juice at 25°C (47).

Halophenols

Halophenols are well known for causing off-flavors in foods (4). Halophenols can be formed when weak halogen solutions are used in cleaning raw materials and food processing lines and are inadequately rinsed away. It can be present in food products either due to chemical contamination or microbial synthesis (46). If they are not completely removed, they can be present in the final product and cause off odors and flavors (11). Jensen and Whitfield (49) proposed that some strains of *Alicyclobacillus* species may contain certain enzymes that are capable of halogenations, which leads to theproduction of 2, 6- dibromophenol ($C_6H_4Br_2O$) and 2, 6-dichlorophenol ($C_6H_4Cl_2O$) (4,7,46).

The compound, 2, 6- dibromophenol and 2, 6-dichlorophenol can be detected at 0.5 ppt and 6.2 ppt in water and 0.5 ppt and 30 ppt in fruit juices respectively (7,50,51). Their occurrence in food can be either from chemical contamination or microbial synthesis (11). However in apple juice, the threshold detection level is 0.004 ppb (parts per billion) of 2, 6- dibromophenol(52). About 40 times higher the human detection level of 2, 6-dichlorophenol is demonstrated by researchers during inoculation studies (43,52), while 6.2 ppt in water (53) and 30 ppt in juices for 2, 6-dichlorophenol was reported (18).

Isolation and enumeration techniques

The *Alicyclobacillus* species is aerobic and require high temperature and low pH to grow. It cannot grow in neutral pH media, including Nutrient Agar (NA), Trypticase Soy Agar (TSA), Plate Count Agar (PCA) and Brain Heart Infusion (BHI) that support the growth of fastidious bacteria. No growth was observed even when these media were acidified to pH 3.5 with tartaric acid (7,15). However, a number of other media have been suggested to enumeration of *Alicyclobacillus* species, these include Orange Serum Agar (OSA), K agar, *Bacillus acidocaldarius* Medium (BAM), acidified Potato Dextrose Agar (aPDA), Yeast Extract Starch Glucose agar(YESG), *Alicyclobacillus* agar (ALIA), *Bacillus acidoterrestris* Thermophilic agar (BAT) (37).

In different research has been done to compare different isolation media. The BAM, OSA and aPDA all performed well at recovering *Alicyclobacillus* species from orange juice, with OSA recovering the highest numbers and compared to OSA (pH 5.0) and aPDA (pH 3.5), K agar (pH 3.7) showed the best recovery of chemically treated spores (47). Murray et al. (2007) (54) evaluated ten test media for their suitability to support *A. acidoterrestris* growth. Their results showed that K agar (pH 3.7), ALI agar (pH 4.0) and BAT agar (pH 4.0) recovered the highest number of spores than OSA (pH 3.5). They also found that surface plating shows good recovering of *A. acidoterrestris* then pour plate and that incubation temperatures of 43 to 50°C are significantly recover.

However, some researchers suggest that direct plating is not sufficient to detect very low numbers of *Alicyclobacillus* species. Membrane filtration was proposed as a more sensitive method because it can test large sample volumes while traditional plating methods are limited by the volume that can be plated on an agar plate. Before the use of plating method or filtration method for isolation procedures apply heat shock treatment in order to activate dormant spores and encourage germination and enumeration for better recovery of *Alicyclobacillus* species (11).

Membrane structure

The unique characteristic of *Alicyclobacillus* species is the presence of ω-alicyclic fatty acids is the major component of the membrane (46). Researchers suggest that ω-alicyclic fatty acids provide resistance to *Alicyclobacillus* species from acidic conditions and high temperatures because the ω-alicyclic fatty acids may form closely packed rings for a protective coating the cell membrane (11). These fatty acids contribute to the heat resistance of *Alicyclobacillus* species by forming a protective coating with strong hydrophobic bonds. These hydrophobic bonds stabilize and reduce membrane permeability in extreme acidic and high temperature environments (9,16,17,18).

Kannenberg et. al. (1984) (17) studied the properties of ω-cyclohexane fatty acids in model membranes and found that the presence of the cyclohexane ring increased the acyl chain density, leading to a denser packing of the lipids in the membrane core, structural stabilization of the membrane, lower membrane fluidity and reduced permeability.

Oshima and Ariga (1975) found that the total fatty acid content of strains of *A. acidocaldarius* isolated from Japanese thermal acid environments consisted of 74% to 93% ω-cyclohexane fatty acids. Investigations into the lipid content of the membranes of *A. acidoterrestris* showed that depending on the strain, ω-cyclohexane fatty acids comprised 15% to 91% of the total fatty acid content (46,55).

A. pomorum was found not to contain ω-alicyclic fatty acids in its membrane, it is classified into the genus *Alicyclobacillus* based on phylogenetic analyses of the 16S rRNA and DNA gyrase B subunit (gyr B) gene sequences. This led to an amendment of the description of the genus *Alicyclobacillus* to include organisms not containing ω-alicyclic fatty acids in their membranes (56). Four other *Alicyclobacillus* species, namely *A. contaminans*, *A. macrosporangiidusi* (57), *A. pohliae* (58) and *A. ferrooxydans* (59) have this fatty acid profile.

A number of species contain hopanoids in their membranes (6,50,60). The hopane ring is structurally similar to cholesterol, which is known to affect membrane lipid organization. It has been shown that the hopane glycolipids have a condensing effect on the membrane, which decreases the mobility of the acyl chains of the lipids and stabilizes the membrane. This condensing action is also advantageous at low pH, since it delays the passive diffusion of protons through the membrane, thereby facilitating the establishment of an approximately neutral cytoplasmic pH (16,54).

Control Measures

Many control measures have been established that are excessively costly to producers, resulting in increased production cost or if not used decreases quality of juice and juice concentrate products. To avoid Spoilage caused by *Alicyclobacillus* species the producers are interested to discover control measures (11).

Physical control measures

Temperature and Pressure

Since *Alicyclobacillus* species are spore-formers and thermal deactivation is very difficult procedures. Apply heat shock treatment to activate dormant spores and encourage germination and enumeration. Cell concentrations are often higher after a heat shock treatment if the bacteria are mostly present as spores.

One of the studies described that only high pressure at room temperature was not significantly reduced spore viability. According to study performed by Lee et al. (61) on commercial, pasteurized apple juice (pH 3.7) inoculated with a strain of *Alicyclobacillus* species (6 log CFU/ml), and treated at different temperature and pressure such as room temperature, 45°C,71°C and 90°C for 0, 207, 414, and 621 MPa (Mega Pascal).Viability of spore was not significantly reduced by high pressure alone at room temperature, but with high pressure and heat (45, 71, and 90°C) spores were significantly reduced (7). To reduce viable spore up to 3.5 log CFU/ml pressure of 207 MPa at 45°C for 10 minutes or at 71°C for 1 minute. To reduce viable spore up to 4 log CFU/ml pressure of 414 or 621 MPa at 71°C for 1 minute and reduced spores to undetectable level or >5.5 log CFU/ml pressure of 414 or 621 MPa at 71°C for 10 minutes. When heated at 90°C spores viability were reduced up to undetectable level or >5.5 log CFU/ml at pressure of 414 or 621 MPa for 1 minute, or 207 MPa for 5 minute, but only at 90°C without high pressure spores was not significantly reduced. The author concluded that increased temperature with high pressure was more effective to reduce *Alicyclobacillus acidoterrestris* (61).

The soluble solid of fruit juice interferes with spore reduction. Increase the concentration of solid in fruit juices the reduction is inhibited over 4 and 5 log reductions found in juice of 35 and 17.5° brix at 90°C (7).

Ultraviolet (UV) radiation

Ultraviolet (UV) energy is a non-ionizing radiation with germicidal properties at wavelengths in the range of 200-280 nm (62,63). A commonly used wavelength is 254 nm for the disinfection of surfaces, water and liquid juices. The soluble solids content reduces effectiveness of UV

due to low UV transmittance (12).

UV radiation damages microbial DNA and the UV light initiates a reaction between two molecules of thymine, resulting in the formation of extra bonds between adjacent pyrimidines (specifically thymine) in DNA. When two pyrimidines are bound together in this way, it is called a pyrimidine dimer (thymine-thymine (T-T) dimer). These T-T dimers often change the shape of the DNA in the cell and can cause problems during replication. The cell often tries to repair T-T dimers before replication, but the repair mechanism can also lead to mutations and are unable to repair their radiation damaged DNA and cell die.

UV radiation has a high potential to inactivate a wide variety of microorganisms and its application can be used in food industry to decontaminate foods, equipment, building and the environment (64). However, spores are more resistant than growing cells to UV radiation at 254 nm, which is the most efficient wavelength for spore killing (65,66). The major reason for spore resistance to 254 nm radiation is the photochemistry of the DNA in spores. The major DNA products generated by 254 nm irradiation of growing cells are cyclobutane dimers and (6-4)-photoproducts formed between adjacent pyrimidines in the same DNA strand (67). UV radiation at wavelengths longer than 254 nm will also kill spores but is less effective.

Membrane filtration

Isolation of *Alicyclobacillus* species performed using plating media and membrane filtration (0.45 µm) (11,15,67). Some researchers have also suggested that membrane filtration be used to remove *Alicyclobacillus* species from beverages as part of quality control measures (11) but membrane filtration is not suitable for all products like concentrated or high soluble solid content, this products cannot be filtered (18).

Lee et al. (2007) (68) investigated the ability of different membrane filters todetect *Alicyclobacillus* spores in apple juice. Membrane filters with two different pore sizes (0.22 and 0.45 µm) from five different manufacturers were evaluated and compared to conventional spread plating on K agar. Results were varied, with spore recovery differing among filters and isolates. In some cases membrane filtration resulted in higher counts than spread plating on K agar and in other cases membranes failed to recover any spores. Absence of growth when filtrates were plated onto K agar suggested that all *Alicyclobacillus* spores had been retained on the membranes, but that the membranes had not supported growth of the spores. Membranes with a smaller pore size did not result in higher recoveries. Because of the varied results it was recommended that juice manufacturers test the efficacy of their preferred membrane filter before using it in quality control processes (68).

Chemical antimicrobial control measures

Lysozyme

Lysozyme has endogenous antimicrobial property in bacteria, fungi, plants and almost all animal tissues. Predominantly found in secretions like milk, mucus, saliva, tears and eggs (4,69).

Antimicrobial activity of lysozyme is due to its ability to hydrolyze β-1-4 glycosidic linkages between N-acetyl glucosamine and N-acetyl muramic acid in bacterial cell walls, which results in cell lysis so it is significant inhibition against visible cells of *A. acidoterrestris* (4).

The antimicrobial and preservative properties of lysozyme make it widely applicable to foods for storage including pickles, dairy products, meats, fresh vegetables, tofu, fresh fruit, fish surfaces, wine, sausage, and seafood (69,70). Lysozyme is effective against spoilage and pathogenic bacteria in meat, rice, winery and fermented products (71). Lysozyme is effective against *Micrococcus luteus*, as well as nonpathogenic strains of *Clostridium*, *Lactobacillus*, *Bacillus*, *Bifidobacterium*, *Corynebacterium*, and *Streptococcus*. Bevilaqua et al. (2007) (72) reported lysozyme (250-2000 ppm) was effective at inactivating *A. acidoterrestris*. Conte et al. (2006) (73) studied the antimicrobial effectiveness of lysozyme immobilized on a polyvinyl alcohol based polymeric film in apple juice and in laboratory media against *A. acidoterrestris*. They showed that *A. acidoterrestris* germinated and grew in the samples without film and with lysozyme-free film but populations decreased up to 2 log CFU/ml in the presence of film with lysozyme.

Lysozyme is approved as “Generally Regarded as Safe” (GRAS) from the FDA in March 1998 and used in cheese production (74). A maximum of 400 ppm (parts per million) lysozyme was obtained in cheese manufactured with egg white lysozyme. Up to 500 ppm in wine has been approved by International Office of Vine and Wine. It is currently used in foods such as cheese, cooked meat, poultry products, and frankfurters (71). The dose used is usually 20 to 400 ppm (75). Lysozyme has heat resistance (65 to 80°C) at low pH if boiled for 1 to 2 minutes. Optimum activity of lysozyme occurs at pH 6.0 at 55 to 60°C (76). Lysozyme can withstand boiling for 1 to 2 minutes at acidic pH and no loss at pH 5.0 when heated at 65°C for 1 hour (69).

Sodium Benzoate

Benzoic acid is natural ingredient of cranberries, plums, prunes, cinnamon, cloves, blackberries (77) and fresh tomatoes (78). Due to wide availability of sodium benzoate it is used as preservatives in the cosmetic, food, and drug industries (79,80). FDA has approved sodium

benzoate for use up to maximum concentration of 1000 ppm and is now commonly used in foods because it is inexpensive, easy to apply food products, a lack of synthetic color, low toxicity and the mechanism of its antimicrobial activity is most likely the same as other organic acids (69,79,80,81). Bevilacqua et. al. (2008)(82) found that the *A. acidoterrestris* spores could be inhibited by sodium benzoate at the concentration of 0.1 to 0.5 ppm.

Mechanism of antimicrobial activity of benzoic acid is by inhibiting cellular uptake of substrate molecules. In the undissociated form; they facilitate proton leakage into cells and increased energy directed out of cells in an effort for the cells to maintain an optimum internal pH. With this disruption of membrane activity, amino acid transport is adversely affected (25). Inhibition of transport in turn results from the destruction of the proton-motive force caused by the continuous shuttle of protons into cells by benzoic acid. Benzoic acid removes the negative charge of ions within the interior of cells and thus increases membrane mobility.

Benzoic acid commonly used as a preservative in fruit products, beverages, bakery products, fruit juices and drinks, fruit salads and cocktails, salads and salad relishes, jams and jellies, dressings, olives, pickles, sauerkraut, dried fruits and preserves, and margarine (67) and in carbonated and beverages (200 to 500 ppm), syrups (1000 ppm), cider (500 to 1000 ppm), fruit juices and concentrates (500 ppm). Benzoic acid above 1000 ppm exerts negative impact imparting flavor change to burning taste. However to avoid flavor change, benzoic acid is used in combination with sorbic acid or another preservative to synergistically increase bacteriostatic effect at 1000 to 3000 ppm (79).

Benzoic acid concentrations of 1000 to 3000 ppm have a bacteriostatic effect but are generally not bactericidal. While pathogenic and spore-forming bacteria may be inhibited by 100 to 200 ppm undissociated acid, a larger amount of undissociated acid is needed to inhibit spoilage bacteria (79).

Several studies of the effects of benzoic acid against *A. acidoterrestris* have been conducted. Walker and Philips (2008) (83) reported that 100 ppm sodium benzoate can inhibit 1 log CFU/ml *A. acidoterrestris* growth in apple juice at 30°C while 500 ppm can inhibit growth of 4 log CFU/ml cell. Bevilacqua et. al. (2007) (72) reported 51-62% inhibition of *A. acidoterrestris* in malt extract broth with 100 ppm sodium benzoate after 13 days. However, the inhibition index decreased as the time increased. The Benzoate antimicrobial activity depends on food pH, water activity and microorganism's presents. Benzoate is most effective against Yeasts than against Bacteria and Molds (69).

Dimethyl Dicarbonate

Dimethyl dicarbonate was synthesized firstly by Kovalenko in 1952 (84). It was a colorless, fruity smelling liquid. Its solubility in water is very low, and it is more soluble in organic solvents (85).

Dimethyl dicarbonate functions as microbial control agent against a wide range of microbes, Yeast and Mold. Dimethyl dicarbonate acts on microbial enzymes (86). Its acts on active site of enzyme and block the site and form conformational changes on active site completely inactivate lactate dehydrogenase by reaction with the histidyl groups of the enzyme.

The acute toxicity of dimethyl dicarbonate is 330-900 mg/kg body weight. Toxicity testing was performed on mice after feeding juice and alcoholic beverages with 400 ppm of dimethyl dicarbonate for 3 months (86), resulted in no any adverse effect shown. Dimethyl dicarbonate is approved (87) as an inhibitor of yeast in wine. The concentration limit is 250 ppm for carbonated or noncarbonated, non-juice containing, flavored or unflavored beverages containing added electrolytes and in carbonated, dilute beverages containing juice, fruit flavor, or both, in which the juice content does not exceed 50%. For wine products, the limit is 200 ppm (73). It is marketed and sold for application to foods under the registered trademark, Velcorin®. The advantage of using Dimethyl dicarbonate is that no reactions occur with sugar, sugar alcohols, or artificial sweeteners such as saccharin or cyclamate (88).

Bacteriocin

Nisin

Nisin is odorless, tasteless and derived from "N inhibitory substance" by Matak and Hirsch [84]. *Lactococcus lactis* produces a polypeptide called Nisin (4). It is isolated and characterized in 1928 by Rogers and Whittier (89,90). The Nisin has antimicrobial activity and occurs as a polypeptide including three unusual amino acids including dehydroalanine, lanthionine, and β-methyl lanthionine and exists as a dimer with a molecular weight of 7,000 Da (89,90). Nisin causes disruption of cytoplasmic membrane of vegetative cells by acting as a surfactant that strongly absorbed on plasma membrane deactivating cellular structures and enzymes such as co-enzyme A (69,70,92). Absorption is followed by cell death with cell lysis and release of cytoplasmic materials and leakage of ATP (89,90,92).

Food and Drug Administration in 1988, has approved Nisin as a GRAS (12). Generally a maximum concentration of 250 ppm is recommended as per Good Manufacturing Practices (93). A wide range of applications is available for use of Nisin in a variety of food products including pasteurized, flavored, and long life milk, aged and

processed cheeses, frozen desserts, liquid egg products, canned vegetables, alcohol, meats, fish, bakery products, soups (91).

Nisin is effective on Gram positive microorganisms (spore formers), but less or no effect on yeasts and molds. *Micrococcus*, *Lactobacillus*, *Listeria monocytogenes*, *Staphylococcus*, *Streptococcus* and the majority of spore forming species of *Bacillus* and *Clostridium* are susceptible to nisin (89).

The Minimum Inhibitory Concentration (MIC) of nisin ranges from 2 to 4 IU/ml for *Bacillus* vegetative cells and 0.25 to 80 IU/ml for *Clostridia* spores (89). Komitopoulou in 1999 (94) suggested that 100 IU/ml of nisin is sufficient to completely inhibit the vegetative cell growth of *Alicyclobacillus* in grape, apple and orange fruit juices. Yamakazi in 2000 (95), focused on inhibition of vegetative cells by 1 to 100 IU/ml nisin as well as spore outgrowth of <0.78 to 100 IU/ml was needed to inhibit. *A. acidoterrestris* was sensitive to 1.25 x 10^{-4} µg nisin when applied directly to an inoculated agar plate (96).

Factors affecting antimicrobial activity of nisin include pH, sodium chloride concentration, and storage temp (69). Nisin is relatively heat stable at acidic pH and prevents the outgrowth of surviving spores (94).

Conclusion

Alicyclobacillus species are spoilage bacteria and cannot be destroyed by pasteurization techniques applied to fruit juices and juice concentrates. Spoilage incidents can be very costly for the manufacturer and risk of financial losses. Numerous isolation, identification and control methods for *Alicyclobacillus* species have been investigated and established. The use of different control methods, including physical method such as temperature with high pressure, UV, membrane filtration or chemical methods, such as lysozyme, sodium benzoate, dimethyl dicarbonate, and bacteriocins such as nisin these methods have been widely investigated and most of them were proved to be effective against *Alicyclobacillus* species in fruit juice and juice concentrate products. An interesting approach for future researchers could be the study of the combinations of some natural compounds and their effects of the ingredients of food and food quality, as well as on the real shelf life of the product.

Author's contributions

Dr Bharrdwaj S.S. conceived of the presented idea and supervised the findings of this work. Bhandarkar H.K., Bhamare K.S., Agnihotri V.A. writing of the manuscript.

Acknowledgements

Authors are thankful to Mr. Ashok Jain, Chairman of Jain Irrigation Systems Ltd, for providing necessary facilities.

References

1. Silva FVM, Tan EK, and Farid M. Bacterial spore inactivation at 45°-65°C using high pressure processing: Study of *Alicyclobacillus acidoterrestris* in orange juice. Food Microbiol. 2012; 32:206-211.
2. FDA. 2001. Hazard analysis and critical control point (HACCP); procedures for the safe and sanitary processing and importing of juice: final rule (21 CFR Part120) *Federal Register*.66:6137-6202.
3. Roethenbaugh G. Trends in beverage markets. In: Chemistry and Technology of Soft Drinks and Fruit Juices. Oxford, UK: Blackwell Publishing Ltd. 2nd ed. (Edited by P.R. Ashurst); 2005:15-34.
4. Huang XC, Yuan YH, Guo CF, Gekas V, and Yue TL. Alicyclobacillus in the Fruit Juice Industry: Spoilage, Detection, and Prevention/Control. Food Rev Int. 2015; 31(2):91-124.
5. Gordon DT, and Kubomura K. Beverages as delivery systems for nutraceuticals. In: Beverage Quality and Safety (Edited by T Foster and PC Vasavada).CRC Press LLC, Florida; 2003:15-72.
6. Cerny G, Hennlich W, and Poralla K. Spoilage of fruit juice by bacilli: isolation and characterization of the spoiling microorganism.Z Lebensm Unters Forsch.1984; 179: 224–227.
7. Kumar R, Bawa AS, Kathiravan T, and Nadanasabapathi S. Inactivation of *Alicyclobacillus acidoterrestris* by Non Thermal Processing Technologies - A Review. Int J Adv Res. 2013 Oct; 1(8):386-395.
8. Deinhard G, Blanz P, Poralla K, and Altan E. *Bacillus acidoterrestris* sp. nov., a new thermotolerant acidophile isolated from different soils. Syst Appl Microbiol, 1987; 10: 47-53.
9. Wisotzkey JD, Jurtshuk P, Fox GE, Deinhard G, and Poralla K. Comparative sequence analyses on the 16S rRNA (rDNA) of *Bacillus acidocaldarius*, *Bacillus acidoterrestris*, and *Bacillus cycloheptenicus* and proposal for creation of a new genus, *Alicyclobacillus* gen. nov. Int J Syst Bacteriol. 1992; 42: 263-269.
10. Baumgart J. Media for the detection and enumeration of *Alicyclobacillus acidoterrestris* and *Alicyclobacillus acidocaldarius* in foods. Handbook of Culture Media for Food Microbiology. 1999; 34: 161-164.

11. Chang SS, and Kang DH. *Alicyclobacillus* species in the fruit juice industry: history, characteristics, and current isolation/detection procedures. CritRev Microbiol. 2004; 30(2): 55–74.
12. Clotteau MS. *Alicyclobacillus* species control in the fruit juice industry. Technical Bulletin [internet]; 2014:1-17. Available from: http://ru.pall.com/pdfs/Food-and-Beverage/FBTBTABFJEN.pdf.
13. Karavaiko GI, Bogdanova TI, Tourova TP, Kondrat'eva TF, Tsaplina IA, and Egorova MA, *et al*. Reclassification of '*Sulfobacillus thermosulfidooxidans* subsp. *thermotolerans*' strain K1 as *Alicyclobacillus toleran ssp*. nov. and *Sulfobacillus disulfidooxidans*, Dufresne*et al*. 1996 as *Alicyclobacillus disulfidooxidans* comb. nov., and emended description of the genus *Alicyclobacillus*. Int J Syst and Evol Microbiol. 2005; 55:941-947.
14. Albuquerque L, Rainey FA, Chung AP, Sunna A, Nobre MF, and Grote R, *et al*. *Alicyclobacillus hesperidum*. nov. and a related genomic species from sulfataric soil of Sao Miguel in the Azores, Int J Syst Evol Microbiol 2000; 50: 451-457.
15. Splittstoesser DF, Churey JJ, and Lee CY. Growth characteristics of aciduric spore forming bacilli isolated from fruit juices. J Food Prot.1994; 57:1080-1083.
16. Ciuffreda E, Bevilacqua S, Sinigaglia M, and Corbo MR. *Alicyclobacillus* spp.: New insights on ecology and preserving food quality through new approaches. MDPI. 2015; 3(4):625-640.
17. Kannenberg E, Blume A, and Poralla K. Properties of ù-cyclohexane fatty acids in membranes. FEBS Lett. 1984; 172: 331-334.
18. Jensen N. Alicyclobacillus - a new challenge for the food industry. Food Australia. 1999; 51: 33-36.
19. Akhbariyoon HR, Mirbagheri M, and Emtiazi G. Isolation and identification of *Alicyclobacillus* with high dipicolinic acid and heat resistant proteins from mango juice. App Food Biotechnol, 2016; 3(4):270-274.
20. Gouws PA, Gie L, Pretorius A, and Dhansay N. Isolation and identification of *Alicyclobacillus acidocaldarius* by 16S rDNA from mango juice and concentrate. Int J Food SciTechnol, 2005; 40: 789-792.
21. Eguchi SY, Manfio GP, Pinhatti ME, Azuma, and Variane SF. Acidothermophilic spore forming bacteria (ATSB) in orange juices: detection methods, ecology, and involvement in the deterioration of fruit juices. RibeiraoPreto, Brazil: Abecitrus. 1999; www.abecitrus.com.br/pesq_us.html.
22. Gouws PA, Gie L, Pretorius A, and Dhansay N. Isolation and identification of *Alicyclobacillus acidocaldarius* by 16S rDNA from mango juice and concentrate. Int J Food Sci Technol. 2005; 40:789–792.
23. Walls I, and Chuyate R. Spoilage of fruit juices by *Alicyclobacillus acidoterrestris*. Food Aust. 2000; 52(7):286-288.
24. Parrish M. *Alicyclobacillus* in citrus juice processing. Institute of Food Technologists annual meeting. 2000.
25. Jay JM. Modern food microbiology. 6th ed. Aspen Publishers Inc. Gaithersburg, MD. 2000.
26. Zhang J, Yue T, and Yuan Y. *Alicyclobacillus* Contamination in the Production Line of Kiwi Products in China. PLoS ONE. 2013; 8(7): e67704. doi:10.1371/journal.pone.0067704.
27. Wisotzkey JD, Jurtshuk P, Fox GE, Deinhard G, and Poralla K. Comparative sequence analyses on the 16S rRNA (rDNA) of *Bacillus acidocaldarius*, *Bacillus acidoterrestris*, and *Bacillus cycloheptanicus* and proposal for creation of a new genus, *Alicyclobacillus* gen. nov. Int J Syst Bacteriol. 1992; 42, 263–269.
28. Hippchen B, Roll A, and Poralla K. Occurrence in soil of thermophilic Bacilli possessing ù-cyclohexane fatty acids and hopanoids. Arch Microbiol. 1981; 129:53-5.
29. Jensen N. *Alicyclobacillus* - a source of flavor taints in acidic foods. Food Safety and Hygiene. 1999; Available at: www.dfst.csiro.au/fshbull/fshbull18.htm
30. Jensen N, Nonio E, and Goldberg D. "What do we really know about *alicyclobacilli*?" presentations and panel discussion. International Federation of Fruit Juice Producers. Sydney, Australia; 2001.
31. Deinhard P, Blanz K, Poralla K, and Altan E. *Bacillus acidoterrestris species nov*., a new thermotolerant acidophile isolated from different soils. Syst Appl Microbiol. 1987; 10:47-53.
32. Brown KL. Control of bacterial spores. Br Med Bull.2000; 56(1):158-171.
33. Tianli Y, Jiangbo Z. and Yahong Y. Spoilage by *Alicyclobacillus* Bacteria in Juice and Beverage Products: Chemical, Physical, and Combined Control Methods. Compr Rev Food Sci Food Saf. 2014; 13: 771–797. doi: 10.1111/1541-4337.12093
34. Walker M, and Phillips CA. The effects of preservatives on *Alicyclobacillus acidoterrestris* and *Propionibacterium cyclohexanicum* in fruit juice. Food Control. 2007; 19:974-981.

35. McIntyre S, Ikawa JY, Parkinson N, Haglund J, and Lee J. Characteristics of an acidophilic *Bacillus* strain isolated from shelf-stable juices. J Food Prot. 1995; 58:319-321.
36. Silva FVM, and Gibbs P. *Alicyclobacillus acidoterrestris* spores in fruit products and design of pasteurization processes. Trends Food Sci Technol. 2001; 12:68-74.
37. Solberg P, Castberg HB, and Osmundsen JI. Packaging systems for fruit juices and non-carbonated beverages. In: Production and Packaging of Noncarbonated Fruit Juices and Fruit Beverages. London: Blackie and Son. 1st ed. (edited by D. Hicks); 1990:330-351.
38. Silva FMS, Gibbs P, Vieira MC, and Silva CLM. Thermal inactivation of *Alicyclobacillus acidoterrestris* spores under different temperature, soluble solids and pH conditions for the design of fruit processes. Int J Food Microbiol. 1999; 51:95-103.
39. Silva FVM, Gibbs P, and Silva CLM. Establishing a new pasteurization criterion based on *Alicyclobacillus acidoterrestris* spores for shelf-stable high acidic fruit products. Fruit Processing. 2000; 4:138-141.
40. Silva FVM, and Gibbs P. Target selection in designing pasteurization processes for shelf-stable high-acid fruit products. Crit Rev Food Sci Nutr. 2004; 44:353-360.
41. Krueger Food Laboratories, Inc. *Alicyclobacillus acidoterrestris*: a new spoilage organism in fruit juices. 2001[cited 2017 Oct 15]; Available at: www.kfl.com/atb.html.
42. Pettipher GL, and Osmundson ME. Methods for the detection, enumeration and identification of *Alicyclobacillus acidoterrestris*. Food Aust. 2000; 52(7):293-295.
43. Jensen N. *Alicyclobacillus* in Australia. Food Aust. 2000; 52:282.
44. Bahceci KS, Gokmen V, and Acar A. Formation of guaiacol from vanillin by *Alicyclobacillus acidoterrestris* in apple juice: a model study. Eur Food Res Technol. 2005; 220:196-199.
45. Rosazza JPN, Huang Z, Dostal L, Volm T, and Rousseau B. Review: Biocatalytic transformations of ferulic acid: an abundant aromatic natural product. J Ind Microbiol.1995; 15:457-471.
46. Smit Y, Cameron M, and Venter P, Witthuhn CR. Alicyclobacillus spoilage and isolation-A review. Food Microbiol. 2011; 28: 331-349. doi:10.1016/j.fm.2010.11.008.
47. Pettipher GL, Osmundson ME, and Murphy JM. Methods for detection and enumeration of *Alicyclobacillus acidoterrestris* and investigation of growth and production of taint in fruit juice and fruit juice-containing drinks. Lett Appl Microbiol. 1997; 24:185-189.
48. Orr RV, Shewfelt RL, Huang CJ, Tefera S, and Beuchat LR. Detection of guaiacol produced by *Alicyclobacillus acidoterrestris* in apple juice by sensory and chromatographic analyses and comparison with spore and vegetative cell populations. J Food Prot. 2000; 63(11):1517-1522.
49. Jensen N, and Whitfield FB. Role of *Alicyclobacillus acidoterrestris* in the development of a disinfectant taint in shelf-stable fruit juice. Lett Appl Microbiol. 2003; 36:9-14.
50. Borlinghaus A, and Engel R. *Alicyclobacillus* incidence in commercial apple juice concentrates supplies-method development and validation. Fruit Processing. 1997; 7(9):262-266.
51. Lusardi C, Previdi PM, Colla F, Barbieri G, and Bolzoni L. Ability of *Alicyclobacillus* strains to spoil fruit juices and nectars. Indust Conserve. 2000; 75(2):151-161.
52. Leatherhead Food. Taints: how leatherhead tracked down a rare species. RA Food News. 2000; 34(8). Available at: www.saafost.org.za/sn63.html.
53. Young WF, Horth H, Crane R, Ogden T, and Arnott M. Taste and odour threshold concentrations of potential potable water contaminants. Water Res. 1996; 30:331-340.
54. Murray MB, Gurtler JB, Ryu J, Harrison MA, and Beuchat LR. Evaluation of direct plating methods to enumerate *Alicyclobacillus* in beverages. Int J Food Microbiol. 2007; 115:59-69.
55. Hippchen B, Roll A, and Poralla K. Occurrence in soil of thermo-acidophilic bacilli possessing R-cyclohexane fatty acids and hopanoids. Arch Microbiol. 1981; 129:53-55.
56. Goto K, Mochida K, Asahara M, Suzuki M, Kasai H, and Yokota A. *Alicyclobacillus pomorum* sp. nov., a novel thermo-acidophilic, endospore forming bacterium that does not possess R-alicyclic fatty acids, and emended description of the genus *Alicyclobacillus*. Int J Syst Evol Microbiol. 2003; 53:1537-1544.
57. Goto K, Mochida K, Kato Y, Asahara M, Fujita R, An SY,*et al*. Proposal of six species of moderately thermophilic, acidophilic, endospore-forming bacteria: *Alicyclobacillus contaminans* sp. nov., *Alicyclobacillus fastidiosus* sp. nov., *Alicyclobacillus kakegawensis* sp. nov., *Alicyclobacillus macrosporangiidus* sp. nov., *Alicyclobacillus sacchari* sp. nov. and *Alicyclobacillus shizuokensis* sp. nov. Int J Syst Evol Microbiol. 2007; 57:1276-1285.
58. Imperio T, Viti C, and Marri L. *Alicyclobacillus pohliae* sp. nov., a thermophilic, endospore-

forming bacterium isolated from geothermal soil of the north-west slope of Mount Melbourne (Antarctica). Int J Syst Evol Microbiol. 2008; 58:221-225.
59. Jiang CY, Liu Y, Liu YY, You XY, Guo X, and Liu SJ. *Alicyclobacillus ferrooxydans* sp. nov., a ferrous-oxidizing bacterium from solfataric soil. Int J Syst Evol Microbiol. 2008; 58:2898-2903.
60. Poralla K, Kannenberg E, and Blume A. A glycolipid containing hopane isolated from the acidophilic, thermophilic *Bacillus acidocaldarius*, has a cholesterol-like function in membranes. FEBS Letters, 1980; 113:107-110.
61. Lee SY, Dougherty RH, and Kang DH. Inhibition of *Alicyclobacillus acidoterrestris* by combination treatment (high pressure and high temperature) in apple juices. App Environ Microbiol. 2002; 68:4158-4161.
62. Bintsis T, Litopoulou-Tzanetaki E, and Robinson RK. Existing and potential applications of ultraviolet light in the food industry- a critical review. J Sci Food Agric. 2000; 80:637-645.
63. Lado BH, and Yousef AE. Alternative food-preservation technologies: efficacy and mechanisms. Microb Infect. 2002; 4:433-440.
64. Rice JK, and Ewell M. Examination of Peak Power Dependence in the UV Inactivation of Bacterial Spores. Appl Environ Microbiol. 2001; 67:830-5832.
65. Setlow P. Spores of *Bacillus subtilis*: their resistance to and killing by radiation, heat and chemicals. J Appl Microbiol. 2006; 101:514-525.
66. Nicholson WL, Munakata N, Horneck G, Melosh HJ, and Setlow P. Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. Microbiol Mol Biol Rev. 2000; 64:548-572.
67. Goto K, Matsubara H, Mochida K, Matsumura T, Hara Y, Niwa M, et al. *Alicyclobacillus herbarius* species nov., a novel bacterium containing R-cycloheptane fatty acids, isolated from herbal tea. Int J SystEvol Microbiol. 2002; 52:109-113.
68. Lee SY, Chang SS, Shin JH, and Kang DH. Membrane filtration method for enumeration and isolation of *Alicyclobacillus* species from apple juice. Lett Appl Microbiol. 2007; 45:540-546.
69. Sofos JN, Beuchat LR, Davidson PM, and Johnson EA. Naturally occurring antimicrobials in Food. Regul Toxicol Pharmacol. 1998; 28(2):71-72.
70. Daeschel M, McGuire J, and Bower C. Natural antimicrobials that inhibit food surface contamination. NRi research highlights. Cooperative State Research, Educations, and Extension Service. Oregon State University; 1999(Jul).
71. Davidson PM, and Harrison MA. Resistance and adaptation to food antimicrobials, sanitizers and other process controls. Food Technol. 2002; 56:69-78.
2. Bevilacqua A, Corbo MR, Buonocore GG, Del 7Nobile MA, and Sinigaglia M. Antimicrobial effectiveness of lysozyme against *Alicyclobacillus acidoterrestris*. AdvFood Sci. 2007; 29:47-52.
73. Conte A, Sinigaglia M, and Del Nobile MA. Antimicrobial effectiveness of lysozyme immobilized on polyvinyl alcohol-based film against *Alicyclobacillus acidoterrestris*. J Food Prot. 2006; 69:861-865.
74. Hazard analysis and critical control point (HACCP); procedures for the safe and sanitary processing and importing of juice: final rule (21 CFR Part 120) Fed. Reg. FDA. 2001; 66:6137-6202.
75. Gould GW. Control with naturally occurring antimicrobial systems including bacterilytic enzymes; 2002:281-302. In Juneja VK, Sofos JN (ed.). Control of food borne microorganisms. Marcel Dekker Inc, New York.
76. Lagarde G. The use of lysozyme: a natural and efficient solution to prevent the butyric late blowing in cheese. Marschall Italian and specialty cheese seminars. 1997 [cited 2017 Oct 19]. Available from: www.rhodiadiary.com/marschall/proceed/pdf/97_07.pdf.
77. Humpf JU, and Schreier P. Bound aroma compounds from the fruit and the leaves of blackberry (Rubuslaciniata L.). J Agric Food Chem. 1991; 39:1830-1832.
78. Marlatt C, Hoct C, and Chien MJ. Studies of aroma constituents bound as glycosides in tomato. J Agr Food Chem.1992; 40(2):249-252.
79. Chipley JR. Sodium Benzoate and Benzoic Acid. In Brannen AL, Davidson PM (ed.), Antimicrobials in foods 2nd ed. Marcel Dekker, Inc; 1993.
80. Davidson PM, and Juneja VK. Antimicrobial agents. In Brannen LA, Davidson PM, Salinen S (ed.), Food Additives. Marcel Dekker Inc., NY; 1990.
81. Code of federal regulations. 21 CFR 184.1021. FDA; 2001.
82. Bevilacqua A, Corbo MR, and Sinigaglia M. Inhibition of *Alicyclobacillus acidoterrestris* spores by natural compounds. Int. J Food Sci Technol. 2008; 43:1271–1275.
83. Walker M, and Phillips CA. The effect of preservatives on *Alicyclobacillus acidoterrestris* and *Propionibacterium cyclohexanicum* in fruit juice. Food Control. 2008; 19:974-981.

84. Ough CS. Dimethyl dicarbonate and diethyl dicarbonate. In Davidson PM, Brannen AL (ed.). Antimicrobials in foods. Marcel Dekker Inc., NY; 1983.
85. Fisher TL, and Golden DG. Survival of *Escherichia coli 0157:H7* in apple cider as affected by dimethyl dicarbonate, sodium bisulfite, and sodium benzoate. J Food Sci. 1998; 63(5): 904-906.
86. Ough CS. Dimethyl and diethyl dicarbonate. In Davidson PM, Brannen AL (ed.). Antimicrobials in foods 2nd ed. Marcel Dekker Inc., NY; 1993.
87. Code of federal regulations. 21 CFR 172.133. FDA; 2002.
88. Golden DA, Worobo RW, and Ough CS. Dimethyl dicarbonate and diethyl dicarbonate; 2005:305-326. InDavidson PM, Sofos JN, Brannen AL (ed.), Antimicrobials in foods. 3rd ed. CRC press, Boca Raton, FL.
89. Hoover DG, and Steenson LR. Bacteriocins with potential for use in foods. In Davidson PM, Brannen AL (ed.), Antimicrobials in foods. 2nd ed. Marcel Dekker Inc. New York; 1993.
90. Delves-Broughton J. Nisin and its uses as a food preservative. Food Techno; 1990 Nov: 100-112.
91. Davidson PM, and Juneja VK. Antimicrobial agents. In Brannen LA, Davidson PM, Salinen S (ed.), Food Additives. Marcel Dekker Inc., New York; 1990.
92. Ray B, and Daeschel M. Nisin of *Lactococcus lactis* as a bio preservative. Food Bio preservatives of Microbial Origin. CRC Press Inc., FL; 2000.
93. Code of federal regulations. 21 CFR 170.3. FDA; 2001.
94. Komitopoulou E, Boziaris IS, Davies EA, and Delves-Broughton J. *Alicyclobacillus acidoterrestris* in fruit juices and its control by nisin. Int J Food Sci. and Tech. 1999; 34:81-85.
95. Yamakazi, K., M. Murakami, Y. Kawai, N. Inoue, and T. Matsuda. Use of nisin for inhibition of *Alicyclobacillus acidoterrestris* in acidic drinks. Food Microbiol. 2000; 17(3):315-320.
96. Prittjarvi TSM, Wahlstrom G, Rainey FA, Saris PEJ, and Salinoja-Salonen MS. Inhibition of bacilli in industrial starches by nisin. J Indust Micro Biotech. 2001; 26:107-114.