Comprehensive REVIEWS in Food Science and Food Safety

Spoilage by *Alicyclobacillus* Bacteria in Juice and Beverage Products: Chemical, Physical, and Combined Control Methods

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Abstract: *Alicyclobacillus* is a genus of spoilage bacteria causing contamination of juices and other beverage products that cannot easily be contaminated by other microbes because of their high acid contents. During the last 4 decades since the first species of *Alicyclobacillus* was isolated in 1967, *Alicyclobacillus* has become a major concern to the global juice and beverage industries, and many promising methods have been developed and applied to control them. After introducing the history and general characteristics of *Alicyclobacillus*, as well as their heat resistance and spoilage, this review focuses on the control methods against *Alicyclobacillus*, including chemical and physical methods and combined methods. All these control methods show inhibitory or killing effects against *Alicyclobacillus* to some extent and, moreover, some of them have been put to use in the juice and beverage industries for decades and shown to be quite effective, although further developments can be achieved and new methods are constantly being established and investigated. Although it is difficult to compare the effects with one another among these methods because of the different experimental conditions in different reports, some of them, such as the treatments of nisin and high hydrostatic pressure, are well studied and proved to have extensive application prospect. The inhibitory factors, test strains and media, and especially the detailed experimental conditions and main results of these control methods are summarized here. The limitations of some methods mainly relating to the changes of products' sensory qualities are also presented.

Keywords: alicyclobacillus, contaminants, food safety

Historical Perspective of *Alicyclobacillus*

Juice and beverage products are not easily contaminated by microbes due to their low pH (usually <5.0, in some products even <4.0), but there are still several kinds of microbes that can survive this acid environment and cause spoilage, including some aerobes such as *Bacillus coagulans* and *Bacillus megaterium* (causing flat-sour type spoilage), some anaerobic spore-forming bacteria such as *Clostridium butyricum* and *Clostridium pasteurianum* (producing gas and butyric odors) (Silva and Gibbs 2004), some lactic acid bacteria such as *Lactobacillus brevis* and *Leuconostoc mesenteroides* (causing vinegary, buttermilk off-odors) and some heat-resistant mycelial fungi such as *Byssochlamys nivea* and *Talaromyces flavus* (Steyn and others 2011). Nevertheless, a large-scale apple juice spoilage incident in 1982 in Germany made people realize that besides the microbes mentioned above, a new type of aerobic spore-forming bacterium can also spoil juice and beverage products (flat-sour spoilage and generating medicinal, antiseptic offensive off-odor). It can survive the commercially applied pasteurization procedures (88 to 96 °C for about 2 min, or 90 to 95 °C for about 30 to 60 s) and germinate in an acid environment. It has now become a new threat to juice and beverage processors (Cerny and others 1984). During the next 2 decades following this major incidence, more and more reports and surveys about spoilage caused by this kind of bacterium were published. In 2005, the European Fruit Juice Association (AIJN) conducted a survey, in which 68 fruit processing industrial participants were investigated. The results showed that 45% of the participants have experienced problems caused by the spoilage of this kind of bacterium in the past 3 y, and 33% have experienced such problems twice or more (Howard 2006). In a survey conducted in Argentina from 1996 to 2009, a total of 8556 samples of nearly 20 kinds of fruit and vegetable clarified/nonclarified juices and concentrated pulps from 7 provinces were checked and, except kiwi and orange, this kind of bacterium was found in all kinds of samples, with positive rates of 24% to 100% for different kinds samples (Oteiza and others 2011). Danyluk and others (2011) tested 180 tropical and subtropical fruit concentrates obtained from Florida's juice processing facilities and found that 6.1% were spoiled by this kind of bacterium. Till now this kind of bacterium has been isolated from many kinds of juice and beverage products and nearly all segments of juice and beverage production lines (see details in the part "Spoilage caused by *Alicyclobacillus*") (Splittstoesser and others 1994; Walls and Chuyate 1998; Eiroa and others 1999; Duong and Jensen 2000; Walker and Phillips 2008a). In 1992, this kind of bacterium was formally named as *Alicyclobacillus* by Wisotzkey and others (1992).

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These reports and surveys have indicated that the problems caused by *Alicyclobacillus* were of widespread microbial spoilage concern for the juice and beverage industries.

Today, the demands for safe and high-quality juice and beverage products continue to increase at a fast pace worldwide. However, with the development of juice and beverage manufacturers especially, *Alicyclobacillus* contamination has become a major concern and challenge. More and more research has been initiated to develop new technologies to control *Alicyclobacillus*, as well as to enhance the methods already in existence, and to study the characteristics and mechanism of their heat resistance and spoilage for the purpose of control. Therefore, this article's objective is to review general characteristics of *Alicyclobacillus* and to reflect on the limitations and effectiveness of most of the control methods reported, including chemical, physical, and the combination of 2, 3, or more thereof.

General Characteristics of *Alicyclobacillus*

Physiological characteristics

Alicyclobacillus are rod-shaped (usually <6 μm in length), thermoacidophilic, endospore-forming, nonpathogenic bacteria, which were isolated first from hot springs (pH 2 to 3, 75 to 80 °C) in the Tohoku district in Japan in 1967 and categorized as new strains of *B. coagulans*, although they were more aerobic and acidophilic than *B. coagulans* (Uchino and Doi 1967). Darland and Brock (1971) isolated some microbes from acid thermal aqueous/terrestrial environments (isolation from alkaline or neutral environments failed), which were Gram-variable spore-forming rods and therefore should be classified into the genus *Bacillus*. However, many physiological and morphological characteristics of these isolates, such as pH and temperature range for growth, and especially the DNA G + C content (approximately 62 mol%, compared to 45 to 50 mol% of *Bacillus*), were more similar to the isolates of Uchino and Doi (1967) than to *B. coagulans*. Therefore, they proposed a new species, *Bacillus acidocaldarius*. In the same year, another important feature of *B. acidocaldarius* was found by De Rosa and others (1971): the principal components in the lipids of their cell membranes were ω-cyclohexane fatty acids (up to 65%). Other strains of these acidophilic, ω-cyclohexane fatty acid-containing spore-forming rods were isolated from neutral soils by Hippchen and others (1981) and also from the spoiled apple juice of the incident in Germany mentioned above (Cerny and others 1984). However, Deinhard and others (1987a) found that there were many differences between these newly isolated bacteria from those identified as *B. acidocaldarius*, including pH and temperature ranges, carbon sources, and DNA G + C content (51.0 to 53.3 mol%, compared to that of *B. acidocaldarius*, which is 57.3 to 61.1 mol%). Therefore, a new species, *Bacillus acidoterrestris* (acid-loving, isolated from soil), was proposed. In the same year, Deinhard and others (1987b) isolated another kind of *Bacillus*, and along with the similar strains isolated by Poralla and König (1983) classified them into a new species, *Bacillus cycloheptanicus*, for their content of ω-cycloheptane fatty acids instead of ω-cyclohexane fatty acids, as well as other difference from *B. acidocaldarius* and *B. acidoterrestris* (different nutrient requirements, narrow growth temperature range, and low values of DNA–DNA hybridization). Wisotzkey and others (1992) studied these 3 unique species of *Bacillus* and found that the similarity of their 16S rRNA gene sequences was very high (92.7% to 98.8%), whereas the similarity was relatively low between these 3 species and other species of *Bacillus*, such as *B. coagulans* (85.0% to 85.2%). Besides, the 16S rRNAs' secondary structures among these 3 species were very similar to one another, but differed from those of other *Bacillus* species. Therefore, a new genus, *Alicyclobacillus*, was proposed, and *B. acidocaldarius*, *B. acidoterrestris*, and *B. cycloheptanicus* were renamed as *A. acidocaldarius*, *A. acidoterrestris*, and *A. cycloheptanicus* accordingly (Wisotzkey and others 1992). During the following years, more and more new species of *Alicyclobacillus* were isolated and identified, including *Alicyclobacillus tolerans* and *Alicyclobacillus disulfidooxidans*, which were once classified into the genus *Sulfobacillus*; and *Alicyclobacillus pomorum* was the first species of *Alicyclobacillus* that does not contain ω-alicyclic fatty acids in its membrane.

Up to now 20 species, 2 subspecies, and 2 genomic species (a genomic species is a possible new species without a formal name, because the data were not sufficient to support the proposal of a new species or subspecies when it was published) of *Alicyclobacillus* have been identified, including 1 species newly reported in May 2013 (Glaeser and others 2013). The first isolating sources and some important physiological and biochemical characteristics of all species in the genus *Alicyclobacillus* are summarized in Table 1. Among the 24 published species, 16 species were first isolated from soil, which proved that *Alicyclobacillus* are soilborne bacteria (Deinhard and others 1987a). Although *Alicyclobacillus* are thermo-acidophilic bacteria, there were 2 species first isolated from cold Antarctica: *A. pohliae* (Imperio and others 2008) and *A. acidocaldarius* subsp. *rittmannii* (Nicolaus and others 1998). All these species are Gram-positive (or variable, usually in old cultures) and aerobic, except *A. sendaiensis* (Gram-negative) (Tsuruoka and others 2003) and *A. pohliae* (facultatively anaerobic sometimes). Many species cannot live under 30 or 40 °C, but *A. acidiphilus* (Matsubara and others 2002), *A. acidoterrestris* (Wisotzkey and others 1992), *A. consociatus* (Glaeser and others 2013), *A. disulfidooxidans* (Dufresne and others 1996), *A. fastidiosus* (Goto and others 2007), *A. ferrooxydans* (Jiang and others 2008), and *A. tolerans* (Karavaiko and others 2005) can grow under 30 °C, and *A. disulfidooxidans* can even grow at 4 °C. Most species can live above 50 °C (up to 70 °C), but *A. consociatus*, *A. disulfidooxidans*, and *A. ferrooxydans* cannot grow at temperatures above 50 °C. Most species' pH range for growth is 2.0 to 6.0, with the exception of *A. disulffidooxidans* and *A. tolerans*, which can grow at pH 0.5 and 1.5, respectively, and *A. consociatus* and *A. pohliae*, which can grow at pH 10.5 and 7.5, respectively. According to the reports on these species resulting from experiments regarding salt tolerance, most species cannot live at a NaCl concentration of 5% or higher, except *A. macrosporangiidus* and *A. shizuokensis* (Goto and others 2007), which can grow above 5% NaCl, but cannot grow at a concentration of 7%. Most species are motile, but some are not. All species form oval or ellipsoidal endospores in a terminal or subterminal position. The main quinone of all the species is menaquinone 7 (MK-7, up to 90% in some species), and the results of the Voges–Prokauer test showed that most species are negative, with the exception of *A. acidophilus* (weekly positive), genomic species 2 (Goto and others 2002a), and *A. sendaiensis* (positive). All species' DNA G + C content is between 47 and 64.3 mol%, with *A. acidocaldarius* subsp. *rittmannii* containing the highest DNA G + C content, and *A. consociatus*, *A. ferrooxydans*, and *A. tolerans* containing a DNA G + C content below 50 mol%, which might be related to their natural habitats. As thermoacidophilic bacteria, containing ω-alicyclic fatty acids (up to 80% in some species) in cell membranes is an important feature of *Alicyclobacillus* (14 contain ω-cyclohexane fatty acids predominantly, while 4 mainly possess ω-cycloheptane fatty acids, among all 24 known species). These ω-alicyclic fatty acids may contribute to the bacteria's heat resistance and protect them against acidic conditions and high

Table 1–Some physiological and biochemical characteristics of all species in the genus *Alicyclobacillus*.

Species	Source	*T* range (°C)	pH range	Growth in NaCl	Enzymes, metabolism, and other characteristics	Main fatty acids	Main menaquinone	Voges–Prokauer test	DNA G+C content	Type stain	References
A. acidiphilus	Acidic beverage	20 to 55	2.5 to 5.5	5% : −	Catalase: +; oxidase −; gelatin, starch, tyrosine, nitrate, indole: −	ω-Cyclohexane $C_{17:0}$ (82.6%)	MK-7	±	54.1 mol%	DSM 14558^T = IAM 14935^T = NRIC 6496^T	Matsubara and others (2002)
A. acidocaldarius	Acid thermal terrestrial and aqueous environments	45 to 70	2.0 to 6.0	5% : −	Galactose, glycerol, ammonia, catalase, gelatin: +; oxidase, acetate, sorbitol, citrate, nitrate, ethanol: −; Hopanoids and sulfonolipids are present	ω-Cyclohexyl-undecanoic and ω-cyclohexyl-tridecanoic acids	MK-7	n	61.2 to 62.5 mol%	DSM 446	Wisotzkey and others (1992); Goto and others (2006); Smit and others (2011)
A. acidocaldarius subsp. acidocaldarius	Same as above	45 to 70	2.0 to 6.0	5% : −	Starch, glycerol, arbutin, catalase, aesculin, gelatin: +; oxidase, nitrate, tyrosine, phenylalanine: −	Same as above	MK-7	n	61.9 mol%	ATCC 27009	Goto and others (2006)
A. acidocaldarius subsp. rittmannii	Geothermal soil from Mount Rittmann, Antarctica	45 to 70	2.5 to 5.0	<1% :+	Oxidase, catalase, urease: −; gelatin, starch, tyrosine: −; maltose, mannose: +; Antibiotics sensitivity: ampicillin AMP_{25} yes; bacitracin B_{10} no	ω-Cyclohexyl FAMEs (Fatty Acid Methyl Esters), iso-series	Hopanoid and MK-7	n	64.3 mol%	DSM 11297	Nicolaus and others (1998)
A. acidoterrestris	Soil and apple juice	20 to 70	2.2 to 5.8	Variable 5% : −	Dihydroxyacetate, indole−; Hopanoids and sulfonolipids are present	ω-Cyclohexane fatty acids	MK-7	− to variable	51.6 to 53.3 mol%	DSM 3922	Wisotzkey and others (1992); Smit and others (2011)
A. consociatus	Human blood	15 to 45	5.5 to 10.5	≤2%:+	Catalase, oxidase: ±; urease, gelatin, casein, citrate, nitrate: −; Gram positive but containing mesodiaminopimelic acid	Iso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{15:0}$; No ω-cyclic fatty acids	MK-7	—	47 mol%	CCM 8439^T	Glaeser and others (2013)
A. contaminans	Soil of a crop field in Fuji city	35 to 60	No growth at pH 3.0 and 6.0	≤2%:+ 5%:−	Catalase, oxidase, nitrate, indole, phenylalanine, starch, tyrosine: −; gelatin, aesculin: +	Anteiso-$C_{17:0}$, iso-$C_{17:0}$, iso-$C_{16:0}$; No ω-cyclic fatty acids	MK-7	−	60.1 to 60.6 mol%	DSM 17975^T = IAM 15224^T	Goto and others (2007)

(*Continued*)

Table 1–Continued.

Species	Source	*T* range (°C)	pH range	Growth in NaCl	Enzymes, metabolism, and other characteristics	Main fatty acids	Main menaquinone	Voges–Prokauer test	DNA G+C content	Type stain	References
A. cycloheptanicus	Soil	40 to 53	3.0 to 5.5	n	Aesculin: +; sulfonolipids are present; required growth factors: methionine or vitamin B_{12}, pantothenate and isoleucine	ω-Cycloheptyl-undecanoic, ω-cycloheptyl-tridecanoic and ω-cycloheptyl-α-hydroxyundecanoic acids	MK-7	n	54.0 to 56.9 mol%	DSM 4006	Wisotzkey and others (1992)
A. disulfidooxidans	Waste water sludge	4 to 40	0.5 to 6.0	n	Growth in S, Fe^{2+}, Fe_2S, cysteine, glycerol: +; $S_2O_3^-$, acetate: −; disulfide-oxidizing bacteria	ω-Cyclohexane fatty acids	n	n	53 mol%	ATCC 51911^T = DSM 12064^T.	Dufresne and others (1996); Karavaiko and others (2005)
A. fastidiosus	Apple juice	20 to 55	No growth at pH 2.0 and 5.5	≤2%:+ 5%:−	Oxidase, nitrate, indole, aesculin, phenylalanine, starch, tyrosine: −; gelatin, catalase,: +	ω-Cyclohexyl $C_{17:0}$ (61.1%), ω-cyclohexyl $C_{19:0}$	MK-7	−	53.9 mol%	DSM 17978^T = IAM 15229^T	Goto and others (2007)
A. ferrooxydans	Solfataric soil from Tengchong, Yunnan, China	17 to 40	2.0 to 6.0	≤3%:+ 4%:−	Nitrate, aesculin, H_2S, phenylalanine, gelatin: −; starch, casein, phosphatase, esterase, lipase, oxidase, indole, catalase, pyrite, Fe^{2+}, $K_2S_4O_6$: +	Anteiso-$C_{15:0}$, anteiso-$C_{17:0}$, iso-$C_{16:0}$, iso-$C_{15:0}$, no ω-cyclic fatty acids	MK-7	−	48.6 mol%	JCM 15090^T = CGMCC 1.6357^T	Jiang and others (2008)
Genomic species 1	Solfataric soil in the Furnas area of the Island of São Miguel, the Azores	40 to 70	2.0 to 6.0	3%:−	Catalase, oxidase, nitrate, elastin: −; starch, gelatin, citrate, arbutin, aesculin: +; unable to grow on most of the polyols, except glycerol and mannitol	ω-Cyclohexyl $C_{17:0}$ (51.8%), ω-cyclohexyl $C_{19:0}$	MK-7	n	60.3 mol%	DSM 11984^T	Albuquerque and others (2000)
Genomic species 2	Acid soil from Kirishima, Kagoshima, Japan	35 to 70	2.0 to 6.5	5%:−	Indole, phenylalanine, tyrosine: −; gelatin, aesculin, nitrate, arbutin, starch, catalase,: +	ω-Cyclohexyl $C_{17:0}$ (47.6%), ω-cyclohexyl $C_{19:0}$ (42.6%)	MK-7	+	62.5 mol%	IAM 14934 = DSM 14672	Goto and others (2002a)
A. herbarius	Herbal tea (dried flowers of hibiscus)	35 to 65	3.5 to 6.0	5%:+	Oxidase, indole, gelatin, starch: −; catalase, nitrate: +	ω-Cycloheptane $C_{18:0}$ (67.1%)	MK-7	−	56.2 mol%	DSM 13609^T = IAM 14883^T = NRIC 0477^T	Goto and others (2002b)

(*Continued*)

Table 1–Continued.

Species	Source	*T* range (°C)	pH range	Growth in NaCl	Enzymes, metabolism, and other characteristics	Main fatty acids	Main menaquinone	Voges–Prokauer test	DNA G+C content	Type stain	References
A. hesperidum	Solfataric soil in the Furnas area of the Island of São Miguel, the Azores	35 to 60	2.0 to 6.0	≤3%:+ 4%:−	Catalase, oxidase, nitrate, citrate, elastin: −; gelatin, arbutin, aesculin: +; starch: ±; unable to grow on most of the polyols, except glycerol and mannitol	ω-Cyclohexyl $C_{17:0}$ (56.8%), ω-cyclohexyl $C_{19:0}$	MK-7	n	53.3 mol%	DSM 12489^T	Albuquerque and others (2000)
A. kakegawensis	Soil of a crop field in Kakegawa city	40 to 60	No growth at pH 3.0 and 6.5	≤2%:+ 5%:−	Oxidase, nitrate, indole, starch, phenylalanine, tyrosine, gelatin: −; aesculin: +; catalase: ±	ω-Cycloheptyl $C_{18:0}$ (65.6%), ω-cycloheptyl $C_{18:0}$ 2-OH, ω-cycloheptyl $C_{20:0}$	MK-7	−	61.3 to 61.7 mol%	DSM 17979^T = IAM 15227^T	Goto and others (2007)
A. macrosporangiidus	Soil of a crop field in Fujieda city	35 to 60	No growth at pH 3.0 and 6.5	≤5%:+ 7%:−	Oxidase, nitrate, indole, starch, phenylalanine, tyrosine, gelatin: −; aesculin: +; catalase: ±	Iso-$C_{16:0}$ (44.2%), iso-$C_{17:0}$, anteiso-$C_{17:0}$; No ω-cyclic fatty acids	MK-7	−	62.5 mol%	DSM 17980^T = IAM 15370^T	Goto and others (2007)
A. pohliae	Geothermal soil from Mount Melbourne, Antarctica	42 to 60	4.5 to 7.5	5%:−	Oxidase, catalase: −; aesculin: +; arbutin: ±; Fe^{2+} is needed; sensitive to (mL^{-1}): ampicillin (25 mg), chloramphenicol (10 mg), tetracycline (50 mg), penicillin G (25 mg), bacitracin (10 mg), and streptomycin (25 mg)	Iso-branched fatty acids $C_{15:0}$ (45.56%) and $C_{17:0}$ (35.81%)	n	n	55.1 mol%	CIP 109385^T = NCIMB 14276^T	Imperio and others (2008)
A. pomorum	Mixed fruit juice	30 to 60	3.0 to 6.0	5%:−	Nitrate, phenylalanine, tyrosine, arbutin: −; oxidase, catalase starch, aesculin, gelatin: +	Iso-$C_{15:0}$ (45.56%), Iso-$C_{15:0}$ (19.9%), iso-$C_{16:0}$, iso-$C_{17:0}$, anteiso-$C_{15:0}$, anteiso-$C_{17:0}$ (34.2%); No ω-cyclic fatty acids	MK-7	n	53.1 mol%	DSM 14955^T = IAM 14988^T	Goto and others (2003)

(*Continued*)

Table 1–Continued.

Species	Source	*T* range (°C)	pH range	Growth in NaCl	Enzymes, metabolism, and other characteristics	Main fatty acids	Main menaquinone	Voges–Prokauer test	DNA G+C content	Type stain	References
A. sacchari	Liquid sugar	30 to 55	No growth at pH 2.0 and 6.0	≤2%:+ 5%:−	Oxidase, catalase, nitrate, indole, phenylalanine, tyrosine, aesculin: −; gelatin, starch: +	ω-Cyclohexyl $C_{17:0}$ (64.4%), ω-cyclohexyl $C_{19:0}$	MK-7	−	56.6 mol%	DSM 17974^T = IAM 15230^T	Goto and others (2007)
A. sendaiensis	Soil of Aoba-yama Park, Sendai, Japan	40 to 65	2.5 to 6.5	≤4%:+ 5%:−	Oxidase, catalase, H_2S, urease, gelatin, aesculin: −; arbutin, nitrate, glycerol: +	ω-Cyclohexane $C_{17:0}$ (44.1%), ω-cyclohexane $C_{19:0}$ (30.2%)	MK-7	+	62.3 mol%	JCM 11817^T = ATCCBAA-609^T	Tsuruoka and others (2003)
A. shizuokensis	Soil of a crop field in Shizuoka city	35 to 60	No growth at pH 3.0 and 6.5	≤5%:+ 7%:−	Oxidase, nitrate, indole, gelatin, phenylalanine, starch, tyrosine: −; catalase, aesculin: +	ω-Cycloheptyl $C_{18:0}$ (65.4%), ω-cycloheptyl $C_{18:0}$ 2-OH, ω-cycloheptyl $C_{20:0}$	MK-7	−	60.5 mol%	DSM 17981^T = IAM 15226^T	Goto and others (2007)
A. tolerans	Oxidizable lead-zinc ores	20 to 55	1.5 to 5.0	n	Fe^{2+}, S, sulfide minerals, starch, aesculin, reducing Fe^{3+}: +; arbutin, nitrate, gelatin: −; oxidase, catalase: ±; vesicular inclusions, polyphosphate and poly-β-hydroxybutyrate granules in cytoplasm	ω-Cyclohexane $C_{17:0}$, $C_{18:0}$, $C_{19:0}$, ω-cyclohexane $C_{17:0}$ 2OH	MK-7 (90%)	n	48.7± 0.6 mol%	VKM B-2304^T = DSM 16297^T	Karavaiko and others (2005)
A. vulcanalis	Hot spring in Coso Hot Springs in the Mojave Desert, U.S.A.	35 to 65	2.0 to 6.0	≤2%:+ 5%:−	Oxidase, catalase, aesculin: −; starch: +; arbutin: ±; resistant to 0.2 mM molybdic acid, 40 mM zinc chloride; sensitive to 10 mM sodium iodide, 0.8 mM cadmium chloride	ω-Cyclohexane $C_{17:0}$, (46.2), ω-cyclohexane $C_{19:0}$	n	n	62 mol%	ATCC BAA-915^T = DSM 16176^T	Simbahan and others (2004)

+: positive (oxidation, reduction, hydrolysis, degradation, and other reactions); −: negative; ±: weakly positive; n: not reported.

temperatures, by packing the membrane lipids more densely, which leads to a more stable membrane structure and lower membrane fluidity (Kannenberg and others 1984), but among the other 6 species, including *A. consociatus*, *A. contaminans* (Goto and others 2007), *A. ferrooxydans*, *A. macrosporangiidus* (Goto and others 2007), *A. pohliae*, and *A. pomorum* (Goto and others 2003), there were no *ω*-cyclic fatty acids detected.

A. consociatus, which was identified and reported in May 2013, has altered the description of the genus *Alicyclobacillus* further. Although it was isolated from human blood of a 51-y-old woman, it is still unclear and there is no evidence that proves it was pathogenic. Compared to most species of *Alicyclobacillus*, the temperature range of *A. consociatus* is low (15 to 45 °C on R2A agar), and its pH range is high (5.5 to 10.5); therefore, it cannot be classed as thermo-acidophilic, if the identification only relies on these general characteristics for growth. Even so, it was allocated to the genus *Alicyclobacillus* based on the 16S rRNA similarities (<95%) as with all other species of this genus, although it shows some unique characteristics: containing no *ω*-cyclic fatty acids in cell membranes, containing *meso*-diaminopimelic acid in peptidoglycan, similar to Gram-positive bacteria, and containing the lowest DNA G + C content (47%) of all species in the genus *Alicyclobacillus*.

Heat resistance

Among all the characteristics of *Alicyclobacillus*, heat resistance is definitely the most noteworthy one and has been intensively studied (Bevilacqua and Corbo 2011; Chmal-Fudali and Papiewska 2011; Jovetta and others 2011; López and others 2011; Groenewald and others 2013). The values of $D_{90°C}$ and $D_{95°C}$ of *Alicyclobacillus* endospores are usually between 5 to 20 and 1 to 10 min, respectively, depending on the species and the thermal conditions, which makes them able to survive the common pasteurization procedures applied in the juice and beverage industries (90 to 95 °C for 30 to 60 s) (Smit and others 2011). According to a review article by Merle and Montville (2014), the $D_{95°C}$ values of *A. acidoterrestris* spores range from 0.06 to 5.3 min in different reports.

The main factors that influence the heat resistance of *Alicyclobacillus* are displayed in Table 2. The factor with the greatest influence is temperature, which shows a considerable nonlinear negative correlation with the heat resistance (positive with *D* values, with the *z* values ranging from about 5 to 22 °C, depending on the species and the thermal conditions) of *Alicyclobacillus* (Splittstoesser and others 1994; Pontius and others 1998; Silva and others 1999; Bahçeci and Acar 2007a; Maldonado and others 2008). Temperature also affects the sensitivity of *D* values of *Alicyclobacillus* to pH and soluble solids content (SSC), which is higher at relatively low temperatures (Pontius and others 1998; Komitopoulou and others 1999; Silva and others 1999). Sporulation temperature also affects the heat resistance of *Alicyclobacillus*. According to the studies of Palop and others (2000), the heat resistance of endospores of *A. acidocaldarius* that sporulated and grew at 65 °C was much higher than when at 45 °C, for they had adapted to the lowered protoplast water content. pH is another important factor influencing the heat resistance, which has a linear positive correlation with *D* values (Silva and others 1999). However, Murakami and others (1998) found that *A. acidoterrestris* AB-1 endospores' *D* values were not significantly affected by the pH (3.0 to 8.0) in McIlvaine buffer at a certain temperature. Ceviz and others (2009) obtained similar results in their works. Similar to pH, the SSC of the media also has a linear positive correlation with *D* values (Silva and others 1999), resulting in higher heat re-

Table 2–Main factors influencing the heat resistance (*D* value) of *Alicyclobacillus* and examples.

Factors	Influence on *D* value	Example	Result	Reference
Temperature	Negative correlation	Strain, *Alicyclobacillus acidoterrestris* (supplied by CIATlac) spores; medium, clarified lemon juice concentrate; SSC, 50 °Brix; pH: 2.28; temperature, 82, 86, 92, and 95 °C	$D_{82°C} = 17.36$ min, $D_{86°C} = 18.06$ min, $D_{92°C} = 7.6$ min, $D_{95°C} = 6.2$ min	Maldonado and others (2008)
pH	Positive correlation	Strain, *Alicyclobacillus acidoterrestris* NCIMB 13137 spores; medium, malt extract broth (MEB); temperature, 91 °C; SSC, 5 °Brix; pH: 2.5 to 6	$D_{91°C} \rightarrow 0$ (pH 2.5); $D_{91°C} \approx 10$ min (pH 6.0)	Silva and others (1999)
SSC*	Positive correlation	Strain, *Alicyclobacillus acidoterrestris* NCIMB 13137 spores; medium, MEB; temperature, 91 °C; SSC, 5 to 60 °Brix; pH: 2.5	$D_{91°C} \rightarrow 0$ (5 °Brix); $D_{91°C} \approx 20$ min (60 °Brix)	Silva and others (1999)
Strain	Depending on the strain	Strain, *Alicyclobacillus acidoterrestris*: DSM 2498 and 3 isolates' spores; orange juice; temperature, 95 °C; SSC, 9 °Brix; pH: 3.15	$D_{95°C} = 2.5$ min (strain 46); $D_{95°C} = 8.7$ min (strain 70); $D_{95°C} = 3.8$ min (strain 145); $D_{95°C} = 2.7$ min (strain DSM 2498)	Eiroa and others (1999)
Medium	Depending on the medium	Strain, *Alicyclobacillus acidoterrestris* DSM2498 spores; medium: apple juice, orange juice, MEB; temperature, 95 °C; SSC, 10 °Brix; pH: 4.0	$D_{95°C} = 27.8 \pm 1.70$ min (apple juice); $D_{95°C} = 20.8 \pm 1.27$ min (orange juice); $D_{95°C} = 11.1 \pm 2.26$ (MEB)	Ceviz and others (2009)
Divalent cations	Negative correlation or no significant relationship	Strain, *Alicyclobacillus acidoterrestris* (isolated by the authors) spores; medium: YPGA medium; temperature, 89 °C; SSC, not reported; pH: 4.0; divalent cations (5 mM): $CaCl_2$, $MgCl_2$, $BaCl_2$, $MnCl_2$, $SrCl_2$;	$D_{89°C} \approx 13$ min ($CaCl_2$); $D_{89°C} \approx 10$ min (others)	Yamazaki and others (1997)

*Soluble solids content.

sistance of *Alicyclobacillus* endospores in juice concentrate than in single-strength juice, which may be related to the different water activity (Juven and others 1978).

Heat resistance may differ among different species or even different strains of the same species. Eiroa and others (1999) found spores of 4 different strains of *A. acidoterrestris* having different *D* values under the same conditions (see detail in Table 2), and Palop and others (2000) found that *A. acidoterrestris* was less heat-resistant than *A. acidocaldarius*. The heat resistance of *Alicyclobacillus* endospores is usually higher in juice or beverage than in buffers, which has been observed in many studies in terms of *z* values ranging from about 6 to 22 °C in different juices and beverages, and from about 5 to 10 °C in buffers (Splittstoesser and others 1994; Silva and others 1999; Maldonado and others 2008; Smit an others 2011), whereas Maldonado and others (2008) found that *A. acidoterrestris* endospores' heat resistance is lower in nonclarified lemon concentrates than in clarified lemon concentrates. The heat resistance is also affected by the mineralization of divalent cations (especially Ca^{2+}) with dipicolinic acid (DPA) to form the Ca-DPA complex (Chang and Kang 2004). The depleted content of divalent cations of endospores (such as in an adverse pH environment) would cause the decrease of their heat resistance, while the demineralized endospores would regain the heat resistance (Bender and Marquis 1985), but Yamazaki and others (1997) found that the changes of heat resistance of *A. acidoterrestris* endospores were not significant when different divalent cations (including Ca^{2+}) were added into the medium, with better effects being observed when DPA and Ca^{2+} were both added. Other factors influencing the heat resistance of vegetative cells include cell age, which is reflected in that the heat resistance is usually lower during the logarithmic phase than during the stationary phase, and cell populations, with large cell populations always to increase the heat resistance by excreting thermoprotective, extracellular proteins (Heredia and others 2009).

Spoilage caused by *Alicyclobacillus*

After tests conducted on mice and guinea pigs with *A. acidoterrestris*, Walls and Chuyate (2000) concluded that *Alicyclobacillus* was not pathogenic at the tested levels. Nevertheless, the ability of *Alicyclobacillus* to spoil juice and beverages has been noticed since the spoilage incident in 1982 in Germany (Cerny and others 1984) and was taken into serious account as more and more such incidents happened (see above). Nowadays, although it is believed that the contamination source of *Alicyclobacillus* is soil (Takahashi and others 2007), *Alicyclobacillus* species have been isolated from juice and beverage products of many kinds of fruits, including apple (Splittstoesser and others 1994), pear (Wisse and Parish 1998; Groenewald and others 2009), orange (Goto and others 2006), banana (Baumgart and Menje 2000), watermelon (Goto and others 2006), mango (Gouws and others 2005), grapefruit and blueberry (Durak and others 2010), lemon (Pinhatti and others 1997), as well as canned tomatoes (Walls and Chuyate 1998), iced tea (Duong and Jensen 2000), and from nearly all segments of fruit juice in the fruit-processing environments, including fruits (Parish and Goodrich 2005), process waters (Groenewald and others 2009), finished products (Cerny and others 1984), and other environments such as material from orchards, walls, and floor of the processing shop, and so on (Groenewald and others 2009; Zhang and others 2013). There is no main loss of nutritional components in the spoilage caused by *Alicyclobacillus*. The only changes when a juice product is spoiled by *Alicyclobacillus* are offensive off-odor and the deterioration of taste without gas generation, although there are sediment, cloudiness, or discoloration occurred in some products.

The major off-flavors caused by *Alicyclobacillus* species have been shown to include 2 main chemicals: guaiacol (2-methoxyphenol) (Yamazaki and others 1996; Pettipher and others 1997) and the halophenols, including 2,6-dibromophenol (2,6-DBP) and 2,6-dichlorophenol (2,6-DCP) (Baumgart and others 1997; Borlinghaus and Engel 1997). Not all species of *Alicyclobacillus* can cause spoilage of juice and beverage products. The most commonly known species of *Alicyclobacillus* causing spoilage is *A. acidoterrestris*, and it has been demonstrated that *A. contaminans* can produce guaiacol in pH-adjusted kiwi fruit juice (Zhang and others 2013). Other spoilage-related *Alicyclobacillus* species include *A. acidiphilus* (Matsubara and others 2002; Goto and others 2008), *A. acidocaldarius* (Gouws and others 2005), *A. cycloheptanicus* (Gocmen and others 2005), *A. herbarius*, *A. hesperidum* (Goto and others 2008), and *A. pomorum* (Goto and others 2003). Guaiacol, which can be caused by heat decomposition or produced by many microbes, is a common compound in a variety of foods, including spoiled wines and fruit juices (Cerny and others 1984; Simpson and others 1986). The postulated pathway of guaiacol production is during ferulic acid (a common compound in fruit juices) metabolism: 1) ferulic acid is decarboxylated to 4-vinylguaiacol (Mathew and others 2007) or transformed to vanillic acid (Huang and others 1993) or vanillin (Peleg and others 1992); 2) 4-vinylguaiacol is oxidized to vanillin (Karmakar and others 2000); 3) vanillin is oxidized to vanillic acid (Ander and others 1980); 4) vanillic acid is decarboxylated to guaiacol (Crawford and Olson 1978). Another possible precursor of guaiacol was tyrosine, which has not been deeply studied (Jensen 1999). The metabolic pathways of the halophenols are still not clear.

Although influenced by the differences among the panel members, sensory methods for the detection of the off-flavor compound produced by *Alicyclobacillus* in spoiled juice and beverage products have been studied in many investigations, showing that taste and odor thresholds for guaiacol in water and juices or other media were usually between 0.01 and 40 ppb (Orr and others 2000; Jensen and others 2001; Eisele and Semon 2005). In recent years, chemical methods have been developed and used to detect this off-flavor compound through detection kits (Doerge and others 1997; Niwa and Kawamoto 2003). Nevertheless, analytical methods have always been the most widely applied methods to detect the off-flavor compound of *Alicyclobacillus*, including extraction/sample preparation methods, such as liquid–liquid extraction (LLE) (Pettipher and others 1997; Gocmen and others 2005), solid-phase extraction (SPE) (López and others 2002), solid-phase microextraction (SPME) (Wardencki and others 2004), and the most popular one, headspace solid-phase extraction (HS-SPME) (Zierler and others 2004), coupled with separation and detection methods, such as high-performance liquid chromatography (HPLC) (Bahçeci and Acar 2007b), gas chromatography with flame-ionization detection (GC-FID) (Bieniek 2003), gas chromatography-olfactometry (GC-O) (Gocmen and others 2005), and the most often used, gas chromatography-mass spectrometry (GC-MS) (Zierler and others 2004; Gocmen and others 2005; Zhang and others 2013).

The production of odor by *Alicyclobacillus* is affected by several factors, including *Alicyclobacillus* species, juice type (*A. acidoterrestris* and *A. acidocaldarius* are the most prevalent species, while apple juice and orange juice are the most frequent spoiled juices, and some juices are unable to support the growth of *Alicyclobacillus*) (Eiroa and others 1999; Walls and Chuyate 2000; Jensen 2005),

Table 3–Chemical methods to control *Alicyclobacillus* and their effectiveness.

Method/ inhibitory factor	Target microbe	Test media or environment	Experimental conditions	Main results	References
Essential oils					
Cinnamaldehyde, eugenol, limonene	A (2 isolated strains)	Acidified MEA medium	Concentration of sodium benzoate: 10 to 500 ppm; time: 13 d; temperature: 44 °C; detection: OD_{420}; inoculum: 10^3 spore/mL	500 ppm of eugenol: inhibited spore germination for 13 d; 100 ppm of cinnamaldehyde: slowed the germination; limonene: not effective	Bevilacqua and others (2008a)
Bacteriocins					
Nisin	A (4 isolated strains, ATCC 49025^T, 49026, 49027)	mYPGA plates; commercial clear apple, orange and fruit-mixed drinks	MIC test: Nisin stock solutions (<0.78 to 100 IU/mL) were filter-sterilized and added to mYPGA (pH 3.4 or 4.2) at 55 °C and incubated at 46 °C for 48 h. Thermal resistance test: nisin concentration: 0, 50, 100, and 200 IU/mL; temperature: 90 °C; strain: AB-5 spores; medium: commercial clear apple and orange drinks; detection: mYPGA. Survival test: nisin concentration: 6.25, 12.5, 25, 50, 100, 200, 400, and 600 IU/mL; temperature: 40 °C; strain: AB-5 spores; medium: commercial clear apple, orange and fruit-mixed drinks; time: 12 d; detection: mYPGA.	MIC test: spores: <0.78 to 12.5 IU/mL (pH 3.4), 25 to 100 IU/mL (pH 4.2); cells: 1.56 to 50 IU/mL (pH 3.4), 25 to 100 IU/mL (pH 4.2), higher than the concentrations against spores for each strain. Thermal resistance test: The $D_{90°C}$ values reduced to about 70%. Survival test: Spores were inhibited by 25 to 50 IU/mL nisin in both orange and fruit-mixed drinks, but was not inhibited by 600 IU/mL nisin addition in a clear-apple drink.	Yamazaki and others (2000)
Nisin	A (CRA 7152)	Orange juice	pH (3 to 5.8), temperature (20 to 54 °C), soluble solids concentration (11 to 19 °Brix), and nisin concentration (0 to 70 IU/mL), detection: K agar. Three models were used.	The Baranyi and Roberts model was better than the modified Gompertz model, and the adaptation time has a minimum value without nisin added (soluble solids 13.5%, pH 5.0, temperature 43.8 °C).	Peña and Massaguer (2006)
Nisin	A (Z CRA 7182)	Orange juice, grapefruit juice and apple juice	The *D* values at the temperature of 80, 90, and 95 °C were tested.	The presence of nisin decreased the *D* value by up to 40%. The MIC for nisin was 5 IU/mL at 25 °C.	Komitopoulou and others (1999)
Bovicin HC5	A (DSM 2498)	AAM broth or in mango pulp	Growth test: medium, AAM; temperature, 40 °C; inoculum, 10^6 CFU/mL; bovicin HC5, 40, 80, and 160 AU/mL; time, 40 h. Spore germination test: detection, AAM plates; temperature, room temperature; inoculum, 10^6 spore /mL; bovicin HC5, 100 AU/mL; time, 7 d. Thermal sensitivity test: same as above, except: bovicin HC5 concentration, 80 AU/mL; temperature, 80, 85, 90, 95 °C.	Growth test: growth was completely inhibited at any concentration. Spore germination test: no visible spores after 1.5 d (confirmed by another test in pH 6.5 phosphate buffer with 20 AU/mL bovicin HC5). Thermal sensitivity test: *D* values decreased 77% to 95% at different temperatures.	Carvalho and others (2008)
Enterocin AS-48	A (LMG 16906 DSM 2498 DSM 3922) B (CECT 4328)	Nature orange and apple juices; commercial fruit juices	Detection, YPDA plates; temperature, 37, 15, and 4 °C; concentration of enterocin AS-485, 2.5 μg/mL; time, 14 d (nature juices) or 90 d (commercial juices)	Nature juices: no growth was observed; commercial juices: apple, peach and grapefruit juices (37 °C): protection up to 60 d; others: no viable cells were detected	Grande and others (2005)
Warnericin RB4	A (ATCC 49025^T)	OSA medium	Method: the agar well diffusion method; detection, after incubation at 45 °C for 24 h.	The diameter of inhibition was about 16 to 19 mm, and there was no significant difference between the 2 strains.	Minamikawa and others (2005)
Bificin C6165	A (DSM 3922^T; 10 strains got from Japan; 9 isolated strains)	AAM and diluted apple juice (DAJ)	Survival test: medium, DAJ, commercial apple juice, orange juice, peach juice, and grape fruit juice; temperature, 45 °C; inoculum, 10^4 to 10^5 CFU/mL vegetative cells; concentrations, 10, 20, 40 μg/mL; time, 12 d. Thermal resistance test: strains, DSM 3922^T, CFD1; concentrations, 10, 20, 40, 80, and 160 μg/mL; temperature, 90 °C.	Survival test: from day 1 to the end of all tests, the survival cells with 40 μg/mL bificin C6165 were significantly lower than those in other tests (about 6 to 8 log CFU/mL reductions). Thermal resistance test: along with the increase of concentration, $D_{90°C}$: DSM 3922^T, about 24 to 18 min, CFD1, about 21 to 13 min.	Pei and others (2014)

(*Continued*)

Table 3–Continued.

Method/ inhibitory factor	Target microbe	Test media or environment	Experimental conditions	Main results	References
Lysozyme and immobilized silver ion					
Lysozyme (immobilized)	A (5 isolated strains)	MEB, commercial pasteurized apple juice	Cell test: type: (1) containing the lysozyme film, (2) containing the lysozyme-free film, and (3) control; detection: MEA plates; time: 200 h; temperature: 44 °C; inoculum: about 10^8 CFU/mL, 1 mL in 150, 300, and 600 mL MEB; Spore test: the same as above, except: inoculum: about 10^6 spore/mL.	The active film was equally effective against a single strain and the 5-strain culture cocktail (various medium volumes, about 2 $\log_{10}$ CFU/mL less than the control); Spores were better inhibited than cells in both MEB broth (about 3 log less than the control) and apple juice (about 2 log less).	Conte and others (2006)
Lysozyme (dissolved)	A (DSM 2498, 3922 and 3 isolated strains)	Acidified MEA medium	MIC test: strains, all 5 strains (vegetative cells and spores); medium: acidified MEA medium. Inhibitory test: time, 2 or 7 d; vegetative cells, 4 strains were tested; spores, 2 strains were tested. Spore viability test: time 80 h; strain, DSM 2498; concentration, 0.1 and 2 ppm. These 3 tests were detected at 420 nm. Spore germination test: time, 30 min; type, L-alanine, lysozyme, L-ala + Lys. This test was detected at 600 nm.	MIC test: vegetative cells, 0.1 to 6.0 ppm, spores, 0.1 to 3.0 ppm. Inhibitory test: for both 2 and 7 d: the Inhibition index reached 100% when the concentrations were beyond 0.4 to 6 ppm for different strains. Spore viability test: 2 ppm: after about 70 h, there were no viable spores detected. Spore germination test: after a heat shock, the decreases in absorbance of the treatments with lysozyme and with L-ala + Lys were reached about 80%.	Bevilacqua and others (2014)
Ag^+-based antimicrobial film	A (10 isolated strains)	MEB, commercial pasteurized apple juice	Test in MEB: type: (1) containing the Ag^+ film, (2) containing the Ag^+ free film, and (3) control; detection: MEA plates; time: 80 h; temperature: 44 °C; inoculum: about 10^5 CFU/mL, 1 mL into 20 mL MEB; test in apple juice: the same as above, except: time: 100 h.	Test in MEB: containing the Ag^+ film: about 2.5 to 3 $\log_{10}$ CFU/mL less than the control. Test in apple juice: containing the Ag^+ film: about 1 to 1.5 log less than the control	Nobile and others (2004)
Other natural compounds or extracts					
Herbs	A (DSM 2498)	Medium 402	Detection: 402 medium; time: 60 h; temperature: room temperature; inoculum: about 10^6 CFU/mL; types: herb extract: 1, 2, and 3 mL; final volume: 10 mL.	The extract of dog rose fruit and dwarf everlast flower were the most efficient, which reduced the number of *A. acidoterrestris* cells to 20% of the control at the end of test. Other results: nettle leaves, 30%; roots of liquorice and angelica, 50%.	Duda-Chodak and others (2009)
Piperaceae extracts	A (CBMAI 0244)	BAT medium	MIC test I: extracts sources, leaves of *Piper aduncum* and others 5 *Piper* plants; medium, BAT; inoculum, 5×10^4 CFU/mL; time, 24 h; temperature, 45 °C. MIC test II: tested inhibitor, 6 types o fractions from the chloroform extract of *Piper aduncum* and prenylated chromene; the rest conditions were the same as above. Survival test: prenylated chromene concentration, 0.25, 0.5, 1, 2, 4 ×MIC; inoculum, 10^3 to 10^4 CFU/mL; medium, BAT; time, 24 h; temperature, 45 °C.	MIC test I: 15.63 to >1000 μg/mL. MIC test II: 6 fractions, 7.81 to 125 μg/mL; prenylated chromene, 7.81 μg/mL. Survival test: the curves of 4, 2, 1 ×MIC reached the bottom of the *Y* axis (0 log CFU/mL) at 3, 6, and 9 h, respectively, while the curve of the control reached almost 7 log CFU/mL at the end of the test.	Ruiz and others (2013)
			Synthetic chemicals		
Chlorine dioxide and ozone					
Chlorine dioxide gas	A (2 isolated strains)	Apple surfaces	Spores were inoculated onto apple surfaces at room temperature in a chamber containing a chlorine dioxide generating sachet, releasing ClO_2 (low, medium, and high) for 30 min, 1 h, 2 h, or 3 h. Detection: K agar.	High and medium release (1 h): a 5 $\log_{10}$ CFU/mL reduction. Low release (1, 2, and 3 h): 2.7, 3.7, and 4.5 $\log_{10}$ reduction	Lee and others (2006a)

(*Continued*)

Table 3–Continued.

Method/ inhibitory factor	Target microbe	Test media or environment	Experimental conditions	Main results	References
Clorox (sodium hypochlorite); Carnebon 200 (stabilized chlorine dioxide); Vortexx (hydrogen peroxide, peroxyacetic acid, and octanoic acid)	A (1 isolated strain)	Apple juice (onto the surface of stainless steel chips)	Spore concentrations: approximately 10^4 CFU/cm^2; temperatures range: 40 to 90 °C; concentrations of disinfectants: Clorox: 200, 500, 1000, and 2000 ppm of total chlorine, Carnebon: 200: 50, 100, and 200 ppm of total chlorine, Vortexx: 1500, 2000, and 2600 ppm; Detection: ALIA plates	Vortexx at 2,600 ppm (90 °C) and Clorox at 2000 ppm (90 °C) were the most effective (2.55, 2.32 $\log_{10}$ CFU/cm^2 reductions after 2 min, respectively) treatments.	Podolak and others (2009)
Chlorine dioxide	A (ATCC 49025, ATCC 1013)	Aqueous suspension or apple surfaces (4 cultivars)	Aqueous suspension: 40 ppm, 80 ppm, 120 ppm for 0.5, 1, and 5 min; apple surfaces: 40 or 120 ppm, 1 to 5 min; Detection: K agar; temperature: room temperature	Aqueous suspension: 40 ppm for 5 min: 4 $\log_{10}$ CFU/mL reductions; 80 ppm for 1 min: 1.8 $\log_{10}$ reductions; 120 ppm for 0.5 min: 4.8 reductions. Apple surfaces: 40 ppm for 1, 2, 3, and 4 min: 1.5, 3.2, 4.5, >4.8 log reductions; 120 ppm for 1 min: >4.8 log reductions	Lee and others (2004)
ozone	A (1 isolated strain)	Aqueous suspension	Concentration of ozone in water: 6, 18, and 28 mg/mL; time: 30 to 150 s; temperature: room temperature; detection: K agar	Treatment at ozone concentration of 28 mg/mL and 60 s can cause approximately a 3 $\log_{10}$ CFU/mL reduction, while treatment at ozone concentration of 18 mg/mL and 90 s deactivate *A. acidoterrestris* spores to an undetectable level.	Qiu and Chen (2004); Chen and Qiu (2007)
Disinfectants					
Sodium benzoate; potassium sorbate	A (2 isolated strains)	Apple juice	Initial inoculum: low, 10 CFU/mL or high, 10^4 CFU/mL; test time: 12 d; concentrations of disinfectants: 0.1 to 0.5 mg/mL; temperature: 30 °C; detection: OSA plate	Low initial inoculum: can be inhibited by 0.1 mg/mL sodium benzoate or potassium. High initial inoculum: can be inhibited by 0.5 mg/mL sodium benzoate or potassium	Walker and Phillips (2008b)
Sodium benzoate	A (2 isolated strains)	Acidified MEA medium	Concentration of sodium benzoate: 10 to 500 ppm; time: 13 d; temperature: 44 °C; detection: OD_{420}; inoculum: 10^3 spore/mL	500 ppm: inhibited completely spore germination; 100 ppm: inhibited the outgrowth for 3 d, with a significant increase of absorbance after 6 d	Bevilacqua and others (2008a)
Chlorine; acidified sodium chlorite; H_2O_2; trisodium phosphate; peracetic acid	A (5-strain mixture)	Aqueous suspension or apple surfaces	Chlorine, 200 to 1000 ppm; acidified sodium chlorite, 500 to 1200 ppm; H_2O_2, 0.2%—4%; trisodium phosphate 8%; peracetic acid 80 ppm; time: 10 min (aqueous suspension) or 1 min (apple surfaces); temperature: 23 °C; detection: K agar or OSA plate or acid PDA plate	Aqueous suspension: 200, 1000 ppm chlorine: 2.2, 5 $\log_{10}$ spore/mL reduction; 500 ppm acidified sodium chlorite, 0.4 $\log_{10}$ spore/mL reduction; 0.2%, 4% H_2O_2: 0.1, >5 $\log_{10}$ spore/mL reduction. Apple surfaces: 500 ppm chlorine or 1200 acidified sodium chlorite: <1 $\log_{10}$ spore/mL reduction; 2% H_2O_2: ineffective	Orr and Beuchat (2000)
High-pressure and supercritical CO_2					
High-pressure CO_2	A (6 strains from the Spanish Type Culture Collection)	Sterile commercial apple cream	Pressures of CO_2: 0.1, 10, 15, and 35 MPa; temperature: 30 to 80 °C; time: 30 min; flow rate of CO_2: 4 g/min; agitation speed: 500 rpm; detection: modified BAM agar	Treatment at 10 MPa and 30 °C can deactivate *Alicyclobacillus* spores efficiently (a 4 $\log_{10}$ CFU/mL reduction).	Casas and others (2012)
Supercritical CO_2	A (ATCC 49025, DSM 2498, DSM 1016)	Apple juice	Supercritical carbon dioxide pressure: 80, 100, and 120 bar; temperature: 65 and 70 °C; time: 10 to 40 min; spore concentrations: 10^6 and 10^7 spores/mL; detection: OSA plate	Spores were completely inactivated by supercritical carbon dioxide to undetectable levels (about 6 $\log_{10}$ CFU/mL reduction) in above 65 °C, 100 bar for 40 min and 70 °C, 80 bar for 30 min.	Bae and others (2009)

(*Continued*)

Table 3–Continued.

Method/ inhibitory factor	Target microbe	Test media or environment	Experimental conditions	Main results	References
Acids					
Chlorous acid	A (ATCC 49025, ATCC 1013)	OSA medium and apple surfaces (4 cultivars)	Test in OSA: Chlorous acid concentration: 268 ppm; detection: OSA medium; time: 20 min; temperature: 22 ± 2 °C; spore concentration: 10^7 to 10^8 spore/mL; Test on apple surfaces: same as above, except: inoculum: 100 μL spore suspension, 10 locations on each apple's surface; amount of chlorous acid: 50 mL.	Test in OSA: the numbers of living spores decreased almost linearly (15 min, 7.0 $\log_{10}$ CFU/mL reductions). Test on apple surfaces: similar results (10 min, about 5 $\log_{10}$ CFU/mL reduction) were obtained, no significant difference among 4 cultivars; no synergistic effect was observed when chlorous acid treatment was combined with heat.	Lee and others (2010)
Micronized benzoic acid	A (CCT 4384; a isolated strain)	Diluted (1:10) concentrated orange juice (59.9 °Brix)	Micronized benzoic acid concentration: 50 mg/L; detection: OSA medium; time: 28 d; temperature: 45 °C; spore concentration: 10^3 to 10^4 spore/mL	A continuous bactericidal effect against 2 *Alicyclobacillus* strains (about 1 log less than the control at day 28) was observed, which was higher than that of commercial sodium benzoate and benzoic acid.	Kawase and others (2013)

A: *Alicyclobacillus acidoterrestris*; B: *Alicyclobacillus acidocaldarius*.

cell concentration (a certain concentration of 10^4 colony-forming units (CFU)/mL or 10^5 CFU/mL of cells must be reached before the production of an off-odor) (Pettipher and others 1997; Bahçeci and others 2005), temperature (larger amount of odor production at higher temperatures, within a certain range) (Pettipher and others 1997), heat-shock treatment (a heat-shock treatment is usually required to stimulate the endospores to germinate during incubation) (Terano and others 2005), and others.

Decontamination methods against *Alicyclobacillus*

The wide spectrum of chemical, physical, and combined methods developed in the last decade against *Alicyclobacillus* is summarized in Table 3 to 5 (including main experiments in each article, and conventional thermal methods are excluded). It is difficult to compare the differences in effectiveness among these intervention methods because the effectiveness is influenced by many factors, including tested strains, media, and experimental conditions, and even the effectiveness itself was displayed in different reports using different indexes (see details in Table 3 to 5). The target species of *Alicyclobacillus* in most studies was *A. acidoterrestris*, and apple juice was the most commonly used study material, while malt extract broth/agar (MEB/MEA) was the commonly used study medium. K agar, *B./A. acidocaldarius* medium (BAM/AAM), and orange serum agar (OSA) were the most frequently used recovery media for the enumeration of survival cells (when the target were spores, a heat-shock treatment usually was added before recovery incubation). The effectiveness, which depended on the methods and the conditions used, usually can be described with several $\log_{10}$ CFU(spore)/mL reductions or changes of *D* value. Although already applied on the detection of *Alicyclobacillus*, such as immunomagnetic nanoparticles (Wang and others 2013), biological control methods are still infrequently reported, because their cost makes them lack value for practical application.

Chemical Intervention Treatments

Naturalborne chemicals

Essential oils. Essential oils (EOs) are complex mixtures of aromatic oily liquids obtained by extraction from various parts of plants, or by distillation or fermentation (Bevilacqua and others 2011a). More than 15 kinds of EOs (such as lemon oil and clove oil) and active compounds (such as eugenol and cinnamaldehyde) have been used in the production of several kinds of juices against spoilage bacteria (such as *A. acidoterrestris*) or some pathogens, yeasts, and molds (Bevilacqua and others 2011b). Bevilacqua and others (2008a) found that 500 ppm of eugenol or cinnamaldehyde can inhibit the germination of *A. acidoterrestris* spores for at least 13 d, but limonene showed no effect. The mechanism might include the loss of ions, the permeabilization of cell membrane, the reduction of membrane potential (Dorman and Deans 2000), the coagulation of cytoplasm, and damage to lipids and proteins (Jerkovic and others 2001). During spore-to-cell transition, there was one or several processes inhibited (Bevilacqua and others 2008a). The effect of EOs depends on the strain (fatty acids' different distributions and/or compositions among different strains in cell membrane can cause different hydrophobic behaviors) (Bevilacqua and others 2011b) and the medium or juice pH (low pH can increase EOs' hydrophobicity) (Bevilacqua and others 2008a).

Bacteriocins. Bacteriocins are antimicrobial metabolites (mainly protein) against closely related species, but nisin, obtained from *Lactococcus lactis* subsp. *lactis*, shows a broad range of antimicrobial activity against Gram-positive bacteria, and its effect on

Alicyclobacillus has been well studied (Komitopoulou and others 1999; Yamazaki and others 2000; Peña and Massaguer 2006; Peña and others 2009; Walker and Phillips 2008b). The *D* values (80 to 95 °C) can be reduced up to about 20% to 40% (Komitopoulou and others 1999; Peña and Massaguer 2006; Peña and others 2009) and the germination/growth of *Alicyclobacillus* spores/cells can be slowed or completely inhibited in the presence of nisin (Yamazaki and others 2000; Walker and Phillips 2008b). The minimum inhibitory concentration (MIC) values ranged broadly from <1 to 100 IU/mL, and spores were usually more sensitive (Komitopoulou and others 1999; Yamazaki and others 2000). All these effects depend on nisin concentration, the character of juice or medium used, and the species. Yamazaki and others (2000) found that lower pH conditions can increase nisin's ability to inhibit *A. acidoterrestris* spores, and its inhibitory effect was similar to that of lysozyme. In this study, the researchers also found that nisin had no inhibitory effect in commercial clear apple juice, while in another 2 unclear commercial juices, it showed a certain inhibitory effect. The mechanism might be the binding of nisin to some apple particles, and the synergistic effect by polyphenols, according to the authors.

Bovicin HC5, which can be extracted from *Streptococcus bovis* HC5, was another bacteriocin reported by Carvalho and others (2008) to be very effective against *A. acidoterrestris* in the medium of AAM or in mango pulp (Table 3). In the study, it was demonstrated that bovicin HC5 could kill vegetative cells, as well as spores of *A. acidoterrestris*, and, although in different juices (this study used mango pulp), bovicin HC5 could reduce the *D* values of *A. acidoterrestris* spores remarkably compared with nisin, if the meaning of the activity unit in Carvalho's report (AU) was close to other nisin-concerning reports (IU), through the growth test, the spore germination test, and the thermal sensitivity test. The *D* values usually decreased to 70% at a concentration of nisin about 100 IU/mL, while bovicin HC5 can cause the *D* value to decrease up to 95% at 80 AU/mL (also see Table 3). It was thought that because bovicin HC5 resembles the lantibiotics, the inhibitory mechanism could be similar to that of nisin.

Grande and others (2005) studied another bacteriocin, enterocin AS-48, and found that enterocin AS-48 will decrease the amount of spores as well as living vegetative cells of *A. acidoterrestris* during a very short time of incubation in all tested juices (including natural orange and apple juices, and commercial fruit juices of orange, apple, pineapple, peach, and grapefruit). It was imagined that the bacteriocin molecules were adsorbed rapidly and the cell or spore structures were destroyed subsequently to explain the mechanism, which has been confirmed by electron microscopy. Minamikawa and others (2005) found Warnericin RB4 had a certain inhibitory effect against *A. acidoterrestris* through agar-well diffusion assay and concluded that it might be useful for the juice industries. Oita (2002) extracted α- and β-hordothionins from barley seeds and α-purothionin from wheat by 0.2 M citric and malic acids, and found that 20 μg/mL of α-purothionin could inhibit *A. acidoterrestris* from growing in satsuma mandarin juice and mixed fruit-vegetable juice, but the inhibitory effect was weak in a 30% dilution of apple juice.

Pei and others (2014) systemically studied the inhibitory effects of a newly discovered bacteriocin: bificin C6165, and found that 16 of 20 tested *A. acidoterrestris* strains were sensitive to it in diluted apple juice (DAJ), and the inhibitory effects also existed in commercial apple juice, orange juice, peach juice, and grape fruit juice for the strain DSM 3922^T. The results of survival tests and heat resistance tests (Table 3) showed that bificin C6165 was a strong bacteriocin for both vegetative cells and spores, and the electron microscopy photos showed the damage of cell structure and the loss of cytoplasmic content of the bacteriocin-treated cells. The authors also found bificin C6165 worked better when the pH values were slightly lower (through MIC tests), or when it was encapsulated by sodium alginate and $CaCl_2$, in DAJ against DSM 3922^T. Although all these results showed that bificin C6165 was a promising bacteriocin against *A. acidoterrestris*, the tests of sensory qualities showed that it might damage the color and clarity of DAJ.

Lysozyme and immobilized silver ion. Bevilacqua and others (2007a) studied the antimicrobial effectiveness of lysozyme incorporated in water-soluble polyvinyl alcohol (PVOH) film and found that *A. acidoterrestris* cells/spores showed great susceptibility to enzyme activity both in a model system and fruit juice. In a similar study, Conte and others (2006) also fabricated films with polyvinyl alcohol immobilizing lysozyme onto it and found that they could inhibit the growth of *A. acidoterrestris* during a 200-h test. A 1st-order kinetic equation was constructed to fit the experimental data. In a recent report, Bevilacqua and others (2014) showed that dissolved lysozyme also had the bioactivity toward *A. acidoterrestris* using MIC, spore viability, and spore germination tests. Besides lysozyme, other inhibitory materials can also be immobilized onto polymeric films. Del Nobile and others (2004) utilized a reactor and produced Ag/polyethylenoxide (PEO)-like films with it. These showed good inhibitory effect against *A. acidoterrestris* in both MEB and apple juice. The Ag^+ release tests showed that the different inhibitory effects in different media might lie in the different released amounts of Ag^+ (0.25 ppm in apple juice after 5 d). But the release of Ag^+ may also cause potential problems of food safety.

Other natural compounds or extracts. Many natural compounds or extracts have an inhibitory effect against *A. acidoterrestris* just like EOs. Altieri and others (2006) found that monolaurin could inhibit the growth of *A. acidoterrestris* vegetative cells, and by calculating some indices, 2 nonlinear models, Gompertz and Baranyi models were shown to be sufficient models to describe the effect of monolaurin. It was found that an optimal concentration (1.4 g/L) of low-molecular-weight chitosan (50 to 190 kDa, deacetylation degree: 75% to 85%) could enhance the effect of heat against *A. acidoterrestris* spores (Bevilacqua and others 2008c). Duda-Chodak and others (2009) tested 15 different herbs, and found that their extracts had certain inhibitory effects against *A. acidoterrestris*. But compared with other methods, the effect of these herb extracts was too low (compared to the control, the highest effect did not show even a 1 $\log_{10}$ CFU/mL reduction at the concentration of 10%) to have practical application. The reason might be the contents of active compounds in the extracts were too low. Ruiz and others (2013) tested crude extracts from the leaves of 6 species in the genus *Piper* and 6 types of fractions from the chloroform extract of *Piper aduncum* and found that some of them were very useful to inhibit the growth of *A. acidoterrestris*, especially a purified compound: prenylated chromene with a MIC value of 7.81 μg/mL in *Bacillus acidoterrestris* medium (BAT). A synergistic effect was also observed between nisin and the extrat of *P. aduncum* against *A. acidoterrestris* (fractional inhibitory concentration, FIC = 0.24). Takahashi and others (2004) tested the antimicrobial effect (MICs) of leaf extracts from 26 species of *eucalyptus* against 9 different species of microbes, including *A. acidoterrestris* (7.8- >250 mg/L, depending on the species of *eucalyptus*), and even isolated 3 compounds from these extracts and identified them as 2′,6′-dihydroxy-3′-methyl-4′-methoxy-dihydrochalcone,

eucalyptin, and 8-desmethyl-eucalyptin, respectively, by U.V., IR, and ^{1}H- and ^{13}C-NMR spectra.

Synthetic chemicals

Chlorine dioxide and ozone. Chlorine dioxide (ClO_2) is a strong sanitizing and oxidizing agent and has been much studied against *Alicyclobacillus* (Lee and others 2004, 2006a; Podolak and others 2009). It has been allowed for use in washing vegetables and fruits in the form of aqueous ClO_2 by the U.S. Food and Drug Administration (FDA) (1998). Antimicrobial chemicals like ClO_2 are commonly used for the surface disinfection of raw materials (fruits) and containers or processing equipments of juice and beverage products. This can decrease the extent of *Alicyclobacillus* vegetative cells or spores in the final products. ClO_2 can be prepared by gas sachet (in a small chamber with a fan) (Lee and others 2006a) or commercial disinfectant (Podolak and others 2009) or by adding Oxine sanitizer to citric acid crystals (the level of free ClO_2 can be measured by a commercial test kit) (Lee and others 2004). The effectiveness is about 2 to 5 $\log_{10}$ CFU(spore)/mL reduction, depending on the method. The mechanism of inactivation of *Alicyclobacillus* spores by chlorine dioxide might lie in damage to the inner membrane, resulting in the inhibition of spore germination and outgrowth (Podolak and others 2009). When applied on the surfaces of apples, ClO_2 gas can cause an adverse effect on visual quality. Lee and others (2006a) found that medium- and high-release sachets can cause small black spots on the surface of apples within 3 d, but low-release sachets did not affect apple visual quality over a 7-d period. Microbial spores can often adhere to a food contact surface, and Podolak and others (2009) proved that *A. acidoterrestris* spores attached to the surface of stainless steel were more difficult to kill compared to the results in other similar reports (Table 3), which may be because the attachment formation on steel surfaces protects the spores against disinfectants. Lee and others (2004) found that the effectiveness of ClO_2 against *A. acidoterrestris* spores was not significantly different among 4 apple cultivars, and in the same study, the researchers also concluded that there exsits no synergistic effect of ClO_2 and heat. Friedrich and others (2009) examined the killing effects of aqueous ClO_2 and hypochlorite on food contact surfaces (rubber, stainless steel, and wood) and found that the most effective treatment was 100 ppm ClO_2 for 10 min, resulting in 0 to 4.5 log CFU/mL reductions *in situ*, while hypochlorite was not effective in all tests.

Compared to ClO_2, treatment with another oxidizing agent, ozone, had a relatively moderate effect against *A. acidoterrestris* (Qiu and Chen 2004; Chen and Qiu 2007), which showed better inhibitory effect on apple surfaces than in aqueous suspensions of *A. acidoterrestris* cells, and, besides, its half-life had an adverse effect on its inhibitory effectiveness. In a recent study, Torlak (2014) found that bubbling ozonation at a ozone concentration of 2.8 mg/L at 4 °C for 40 min could reduce the population of *A. acidoterrestris* spores by 2 logs, without significant total phenolic content decreases in the tested apple juices.

Disinfectants. Walker and Phillips (2008b) concluded that the commonly used disinfectants of potassium sorbate and sodium benzoate were effective against *A. acidoterrestris* (sporulation was inhibited even when vegetative cells survived) in apple juice, but the concentrations for inhibition were higher for vegetative cells than spores. Orr and Beuchat (2000) studied 5 chemicals, including 4 oxidizing agents and 1 kind of inorganic salt (see Table 3), and the results showed 0.1 to >5 $\log_{10}$ spore/mL reduction, depending on the chemicals and the conditions used, and there was no significant difference of the resistance against 1 same disinfectant among 5 different *A. acidoterrestris* strains. They also proved that K agar was the best for recovering chemically treated spores, compared to Potato dextrose agar (PDA) and OSA medium.

High-pressure and supercritical CO_2. High-pressure carbon dioxide (HPCD) treatment is an effective method (4 to 5 $\log_{10}$ spore/mL reduction under a certain condition in apple cream), which can be promoted in a low-pH medium without affecting the most important rheological and sensory properties, such as apparent viscosity, except a little darkening related to Maillard reactions and a reduction in vitamin C (200 to 100 mg/L), which may be related mainly to heat (Casas and others 2012). Supercritical CO_2 (SC-CO_2) was a strong disinfectant against *A. acidoterrestris*. It can cause up to 6 $\log_{10}$ spore/mL reduction without affecting the physical–chemical properties (pH and °Brix) of the apple juice (Bae and others 2009). Similar to the results of treatment with HPCD, *A.* acidoter*restris* spores were more susceptible to SC-CO_2 in a relative low-pH medium such as apple juice. Different results were shown in a study by Garcia-Gonzalez and others (2007), which focused on the effect of high-pressure CO_2 on vegetative bacterial cells. This explained it mainly as cell membrane modification, and the energy-filtering transmission electron microscopy (EF-TEM) and scanning electron microscopy (SEM) photos showed that the SC-CO_2 directly affected the *A. acidoterrestris* spores (spores burst and deformed, with leakage and extraction of cytoplasm).

Acids. Chlorous acid is a strong oxidizing acid, which has a similar effect and mechanism to chlorine and chlorine dioxide discussed above (Orr and Beuchat 2000; Lee and others 2004). Lee and others (2010) found that no matter in laboratory media or on apple surfaces, it was very effective for chlorous acid to reduce an *A. acidoterrestris* population (Table 3). Because *Alicyclobacillus* spores can survive pasteurization, chemical treatments like chlorous acid hold great promise. Especially the results of the test on apple surfaces are very valuable, because the direct use of chlorous acid in the juice would have a negative effect on the juice's sensory properties. The solubility of benzoic acid is not good in water; therefore, it is less effective. Benzoate is more popular as a food preservative, while Kawase and others (2013) produced micronized benzoic acid, using an experimental unit, and found that the smaller shape (10 to 200 μm by SEM, regular shape) was more effective against *A. acidoterrestris* compared with common commercial sodium benzoate and benzoic acid (up to 500 μm by SEM, irregular shape), and the concentration of which was doubled. The bactericidal effect of micronized benzoic acid mainly included the lysis of cells/spores and intracellular material's exposure. Hsiao and Siebert (1999) found that the inhibitory effects among organic acids against microbes vary considerably, and after testing the MIC values for 8 organic acids with 6 microbes, including *A. acidoterrestris*, they found this effectiveness: benzoic acid > caprylic/butyric acids > acetic acid > tartaric/lactic/malic/citric acids. A mathematical model for predicting the effectiveness of organic acids was also constructed using principal components analysis (PCA).

Physical Intervention Treatments

Nonthermal technologies

High-pressure homogenization. High-pressure homogenization (HPH) processing can be used to prepare and stabilize food products as well as alter their physical properties, and its antimicrobial effectiveness against foodborne pathogens has been well

Table 4–Nonthermal physical methods to control *Alicyclobacillus* and their effectiveness.

Method/inhibitory factor	Target microbe	Test media or environment	Experimental conditions	Main results	References
High-pressure homogenization (HPH)					
High-pressure homogenization	A (3 strains: Γ4, c8, DSM 2498)	MEB	HPH: 500 to 1700 bar, duration, 2 ms; detection: MEA; temperature: room temperature, <55 °C after 1700 bar treatment.	Cells: DSM 2498: about 2 $\log_{10}$ CFU/mL reduction after 1400 bar treatment; Γ4: about 1 $\log_{10}$ CFU/mL reduction at 1700 bar; c8: less than 0.5 log reduction at 1700 bar. Spores: Γ4: about 0.7 $\log_{10}$ CFU/mL reduction at 1400 and 1700 bar; DSM 2498: about 0.4 log reduction at 1400 bar; c8: less than 0.4 log reduction at 1700 bar.	Bevilacqua and others (2007b)
High hydrostatic pressure (HHP)					
High hydrostatic pressure	A (DSM 2492)	BAM broth and orange, apple, tomato juices	Survival test: pressure: 350, 450 MPa; temperature: 35, 45, 50, and 60 °C; time: 5, 10, and 20 min; initial numbers: 5.6 to 7.3 $\log_{10}$ CFU/mL; detection: commercial nutrient agar; Growth test: 350 MPa, 50 °C, 20 min, 3 kinds of juices, initial numbers: 5.7 to 7.15 $\log_{10}$ CFU/mL; time: 3 wk; detection: commercial nutrient agar.	Survival test: 350 MPa, 50 °C, 20 min: 4.7 $\log_{10}$ CFU/mL reduction; 450 MPa, 50 °C, 20 min: about 4.9 $\log_{10}$ CFU/mL reduction; Growth teat: about 5 log reductions in all juices after pressurization, and after 3 wk the growth in orange juice was the best (about 1 log increase).	Alpas and others (2003)
High hydrostatic pressure	A (LMG 16906)	Citric acid buffer, tomato sauce	Types: High pressure (HP) only (40 °C, 10 min) or HP + heat (80 °C, 10 min); medium: citric acid buffer, tomato sauce; pressure: 0.1 to 600 MPa; spore concentration: 10^5 to 10^6 spore/mL; pH: 4.0, 5.0, and 7.0 (for buffer test); temperature: 25, 40, and 60 °C (for tomato test); detection: BAT agar.	Buffer test: no significant inactivation for HP only, about 1 log reduction for HP+ heat at 100 to 300 MPa (PH 7.0 was the most effective); tomato test: HP only: germination was accelerated at 25 and 40 °C, but reduced at 60 °C (about 2 log down); HP+ heat: the best pressure was 200 MPa (about 2.5 log reductions) for 25 and 40 °C, but for 60 °C, the best pressure was 600 MPa (about 5 log reduction); the temperature of 25 °C accelerated germination at higher pressure (300 to 600 MPa).	Vercammen and others (2011)
High hydrostatic pressure	A (NZRM 4098)	Orange juice	Pressure: 200, 600 MPa; temperature: 45, 55, and 65 °C; time: 10 min; initial numbers: 6.5 $\log_{10}$ CFU/mL; detection: acidified PDA plates.	$D_{65°C}$ (200 MPa) = 5.0; $D_{65°C}$ (600 MPa) = 3.4; $\log_{10}$ CFU reductions (65 °C, 200 MPa) ≈ 2; $\log_{10}$ CFU reductions (65 °C, 600 MPa) ≈ 2.5.	Silva and others (2012)
High hydrostatic pressure	A (2 isolated strains)	Apple juice concentrate	Conditions: 200 MPa, 50 °C, detection by BAT agar, initial numbers: 6 $\log_{10}$ CFU/mL; test 1: concentrations of soluble solids: 11.2 to 71.1 °Brix, 45 min; test 2: spore ages: 11 d, 11 mo, 16 mo, 30 min; test 3: numbers of subsequent HHP treatments: 1, 2, 3, and 30 min.	At the end of the tests: test 1: 11.2 °Brix: 3 to 4 log reductions; 71.1 °Brix: almost no reduction; test 2: similar among different ages (3 to 4 log reductions); test 3: 1 HHP treatment: 2.5 to 3.5 log reductions; 3 HHP treatments: only 1 to 1.5 log reductions.	Sokołowska and others (2013)
High hydrostatic pressure	A (DSM 2498)	commercial pasteurized apple and orange juice	Temperature, 20, 50, and 60 °C; pressure, 0, 200, 400, and 600 MPa, 10 min; detection, 402 medium; inoculum, 10^4 to 10^6 CFU/mL vegetative cells or spores; media, apple and orange juice; time, 28 d.	At 20 °C: 0 d, spore, about 1 log reduction compared to the control; vegetative cells, about 2 log reductions; 28 d, the curves of all the 4 tests reached 1 $\log_{10}$ CFU/mL (the curve of the control reached 3 to 4 $\log_{10}$ CFU/mL), except the test of spores in orange juice. At 50 °C: 0 d, 2 to 4 log reductions from the control; 28 d, all reached 1 log, except spores in apple juice. At 60 °C: the same as the results at 50 °C, and at 28 d all curves reached 1 log (became straight lines for vegetative cells).	Hartyáni and others (2013)
Microwaves and ultrasonic waves					
Microwaves	A (1 isolated strain)	Commercial asparagus cream	Power: 540 to 900 W (60% to 100%), 2450 MHz; irradiation time: 20 to 30 s, total 180 to 420 s; interval time: 0 to 10 s, total 144 to 336 s; energy density: 1192 to 3128 W/g; spore concentration: 10^5 CFU/g; detection: MEA.	Compared to the control, the treatments caused 0.33 to 2.08 (100% power, 5min) $\log_{10}$ CFU/g reductions.	Giuliani and others (2010)

(*Continued*)

Table 4–Continued.

Method/inhibitory factor	Target microbe	Test media or environment	Experimental conditions	Main results	References
Ultrasonic waves	A (DSM 14558^T, 3922^T)	Apple juice	Power: 200, 400, and 600 W (25 kHz); time: 0 to 30 min; work/intermittent time: 3 s; temperature: < 50 °C at the end of the test; vegetable cell concentration: 1.91 to 5.82×10^5 CFU/mL; detection: BAM plates.	The effectiveness was improved as the power and exposure time increased. At the end of the tests: DSM 14558^T: about 2.5 to 4.5 log reductions; DSM 3922^T: about 3.5 to 4.5 log reductions.	Wang and others (2010b)
Ultrasonic waves	A (DSM 3922^T and 4 isolated strains)	AAM broth and apple juice	Time test: power: 300 W 23 kHz; time: 0 to 60 min; power test: power: 200 to 700 W at 100 W intervals; time: 30 min; *D* value test: power: 400, 500, and 600 W, vegetable cell concentration: 10^6 CFU/mL; detection: AAM plates	The effectiveness was improved as the power and exposure time increased. At the end of the tests: time test: >80% mortality; power test: >80% mortality; *D* value test: 400 W: about 51 to 93 (apple juice), 54 to 91 (AAM); 500 W: about 40 to 55 (apple juice), 42 to 57 (AAM); 600 W: about 36 to 46 (apple juice), 37 to 47 (AAM).	Yuan and others (2009)
Ohmic heating					
Ohmic heating	A (DSM 3922)	Apple juice	Vegetable cell concentration: 10^6 to 10^7 CFU/mL; detection: K agar; temperature: 50 to 90 °C; pH test: pH: 3.5, 3.7, and 3.9; voltage test: 150, 220, 250 V; time test: 0 to 10 min, 60, 70, and 80 °C; volume test: 2.5, 5.0, and 10.0 cm.	pH test: similar results, mortality→ 100% (>70 °C); voltage test: max mortality for 150 V was about 94% (>70 °C), others: mortality→ 100% (>70 °C); time test: for all time points tested, the result of 60 °C was about 95%, others: about 100%; volume test: >70 °C: 2.5 cm: 65%, 5.0 cm: 95%, 10.0 cm: 100%.	Geng and Qiu (2007)
Ohmic heating	A (DSM 3922)	Orange juice	Power: 10 kW; survival test: voltage gradient: 30, 40, and 50 V/cm; temperature: 70, 80, and 90 °C; time: 0 to 30 min; spore concentration: 10^5 to 10^6 CFU/mL; detection: BAM plates. *D* value test: the same as above.	Survival test: The effectiveness was improved as the power and exposure time increased. At the end of the tests: 30 V/cm, about 0.5 to 5 log reductions; 40 V/cm, about 1 to 4 log reductions; 50 V/cm, about 0.5 to 7 log reductions. *D* value test: 30 V/cm, about 5 to 58; 40 V/cm, about 6 to 51; 50 V/cm, about 4 to 40.	Baysal and Icier (2010)
Ohmic heating	A (JCM 21546)	Orange juice	Media flow rate: 100 L/h; conditions: maximum power voltage: 2000 V, 20 kHz, 25 kW, electrode length, 32 mm (passage time: 13.8 ms); spore concentration: 10^4 CFU/mL; detection: nutrient agar; temperature: 110 to 125 °C.	0.5 to 2.5 log reductions	Uemura and others (2009)
Gamma irradiation and UV-C light					
Gamma irradiation	A (ATCC 49025)	Unpasteurized apple and orange juice concentrates	Irradiation dose, 1, 3, 5, 7, and 10 kGy; SSC (apple): 72 °Brix, 36 °Brix, 18 °Brix; SSC (orange): 66 °Brix, 33 °Brix, 11 °Brix; detection, acidified PDA; dose rate, 10 kGy/h; inoculum, 10^6 to 10^7 CFU/mL.	Apple juice: 10 kGy, at all SSCs, about 1 $\log_{10}$CFU/mL; orange juice: 10 kGy, about 0.5 to 1.5 $\log_{10}$CFU/mL, killing effects increased with decreasing SSCs.	Lee and others (2014)
UV-C light	A (DSM 3922)	Commercial pasteurized white grape and apple juices	UV intensity: 1.31, 0.71, and 0.38 mW/cm^2; UV dose: 0 to 500 mJ/cm^2; inoculum, 10^6 CFU/mL; detection, BAM agar; exposure times, 0, 3, 5, 7, 10, 12, and 15 min; peak radiation, 254 nm; depth, 15 cm.	Grape juice: about 3.5 to 5.5 log reductions, depending on the UV doses; apple juice: about 0.8 to 2.2 log reductions, depending on the UV doses	Baysal and others (2013)

A: *Alicyclobacillus acidoterrestris*; C: *Alicyclobacillus cycloheptanicus*.

studied (Briñez and others 2006; Diels and Michiels 2006). Bevilacqua and others (2007b) studied HPH's antimicrobial effectiveness on controlling *A. acidoterrestris* and found that the inhibitory effect was strain-dependent, which might be because of fatty acids' different distribution in cell membrane, causing membrane proteins' different interactions and exposure to HPH, and the different isolation source. In the study, the 2 tested strains isolated from different sources (one from soil, and the other from fruit juice) displayed different susceptibilities to HPH. Similar strain-dependent susceptibility was also found in their subsequent study (Bevilacqua and others 2008a). It was also found that the vegetative cells were more sensitive than spores (Table 4). The authors also inferred that HPH did not result in any sublethal injury according to the storage test.

High hydrostatic pressure. Compared with high hydrostatic pressure (HPH) (<200 MPa, <10 ms, <1 $\log_{10}$ CFU/mL reductions after a certain treatment), HHP usually applies higher pressure (>200 MPa), takes longer time (>10 min), and obtains better bactericidal effect (3 to 5 $\log_{10}$ CFU/mL reductions after a certain treatment). But these 2 treatments share 1 common advantage: no qualities (such as sensory properties) declined, compared to thermal treatment such as a pasteurization. After testing in BAM broth and orange, apple, and tomato juices, Alpas and others (2003) stated that *A. acidoterrestris* vegetative cells can be killed by HHP at a certain condition (such as 350 MPa, 50 °C, 20 min) and the HHP treatment had an inhibitory effect through several weeks. The effect of HHP on spores is limited and higher pressure (>700 MPa) or longer time of treatment (>40 min) will be needed, which might alter some properties of the food. In a subsequent study, Buzrul and others (2005) proved the Weibull model, which does not follow 1st-order kinetics, can be used to extrapolate the inactivation kinetics of *A. acidoterrestris* vegetative cells with the treatment of heat (35, 45, and 50 °C) and pressure (350 and 450 MPa). They also obtained 2 linear empirical equations for scale factors under the experimental conditions. After testing different pressures (0.1 to 600 MPa), pH (for citric acid buffer), and temperatures, Vercammen and others (2011) found that HHP can induce germination of *A. acidoterrestris* spores under acidic experimental conditions (such as in pH 4.0 citric acid buffer for any pressures tested, or in tomato sauce at 25 °C). But when HHP was conducted at moderate temperature (such as 60 °C) or was followed by a moderate heating, the result turned to the killing of the spores (see details in Table 4). These results might be explained as the spore germination under different (low or high) pressures proceeds via different mechanisms, and the pressure-induced germinated spores can be killed by the following heating. Similar results were obtained in another study (Silva and others 2012) in orange juice (about 2.5 log reductions at 600 MPa and 65 °C for 10 min), in which the D values and z values under the experimental conditions were also obtained. In another study (Sokołowska and others 2013), the results showed that *A. acidoterrestris* spores (2 isolated strains) were more susceptible (about 3 to 4 log reductions), compared to the above, at relatively lower pressure (only 200 MPa and 50 °C) but longer treatment time (30 to 45 min) in apple juice. In this report, the author also proved that the treatment effects had no strong relations with spore ages, but had a certain relation with the SSC, and the repeat of pressurization treatments decreased the effects instead, which can be explained by a reduction of the number of germinating spores after each pressurization cycle. In another report, Hartyáni and others (2013) showed that there were almost no changes in sensory qualities (pH and color attributes) of the tested juice after HHP treatments (200 to 600 MPa, 10 min), and what is interesting is that the authors also applied an electronic nose instrument to track the differences during the 4 wk storage time after HHP treatments and found that it was useful.

Microwaves and ultrasonic waves. Microwaves (MWs) are electromagnetic waves (wavelength: 1 mm to 1 m, frequency: 300 MHz to 300 GHz), which can usually be generated by a MW oven. Giuliani and others (2010) reported that a MW treatment (900 W, 2450 MHz, 5 min) can cause about 2 log reductions of *A. acidoterrestris* spores in a commercial asparagus cream, and through building a polynomial equation of the dependent variable (reduction of spore number), it was found that the inactivation effect depended on the time and power of the treatment. In addition, in this study, some primary and secondary oxidation indexes of the lipid fraction of the cream (peroxide value, K232, K270, and induction time) were tested and the result was that the lipid fraction was weakly affected by the MW treatment, especially at low powers. Although there existed a dispute about whether the inactivation effect of MW against microbes rooted in its thermal character (Fujikawa and others 1992) or resulted from its nonthermal effect (mainly damaged cell membrane) (Woo and others 2000). Wang and others (2010a) believed that the nonthermal effect was more important, because in their study (see the section of combined methods), the temperature of the samples was not beyond 50 °C at the end of all tests.

Ultrasound or ultrasonic waves (UWs) were defined as electromagnetic waves with frequency beyond 20 kHz. In a subsequent study, Wang and others (2010b) tested the inhibitory effect of UW against *A. acidoterrestris* vegetable cells in apple juice and found that the effect was a little lower than that in their previous study (in AAM), and the Weibull distribution function and biphasic linear model described the characteristic of ultrasonic inactivation the best among 4 tested models, for strain DSM 14558^T and DSM 3922^T, respectively. The inactivation mechanism of UW might lie in intracellular cavitation and these subsequent effects: localized heating, cell membranes being thinner, and free radical production (Piyasena and others 2003). Yuan and others (2009) tested the influence of treatment time and power of UW on the inactivation effect against *A. acidoterrestris* vegetative cells in apple juice, but obtained relatively lower results (mortality usually <80%). The D values of 5 strains were also tested and relatively higher results were obtained (usually 30 to 40).

Ohmic heating. Ohmic heating is an electric heating system that generates heat instantly inside the food to kill microbes by electrical current through a foodstuff that provides electrical resistance. Geng and Qiu (2007) developed an ohmic heating system and studied the influence of 4 factors (pH, voltage, time, and volume) on *A. acidoterrestris* vegetative cell mortality in apple juice and found that when the temperature was beyond 70 °C, the mortality was usually close to 100%. The photos from environmental scanning electron microscopy showed some depressions and traces of destruction on the cell surfaces, through which, combined with the results of the increase of the values of electroconductibility and OD_{260} and OD_{280}, it was inferred by the authors that the mechanism of ohmic heating was causing electroporation of cell membranes and the concomitant exudation of intracellular contents. Baysal and Icier (2010) studied the influence of voltage gradient and treatment time on *A. acidoterrestris* spore inactivation in orange juice with a static ohmic heating system and concluded the effect of ohmic heating was better than conventional heating (about 4 log gap at 50 V/cm, 90 °C, 30 min), which was supported by their D value tests too. They explained these results

as the additional killing effect caused by the electric current of the treatment of ohmic heating, compared with the treatment of conventional heating, which shared the common thermal killing effect with the treatment of ohmic heating. Uemura and others (2009) studied the inactivation effect against *A. acidoterrestris* spores using a high-electric-field alternating current (HEF-AC) system in orange juice, the killing mechanism of which was mainly ohmic heating, and they found that the effect of HEF-AC (or replacing the electrode unit with a tubular heat exchanger, resulting in longer holding time but similar effect) was better than conventional heating using 2 oil baths (30 times faster during the holding time).

Gamma irradiation and UV-C light. These are energy-conserving new decontamination methods just studied in the recent 2 y. Lee and others (2014) studied the inhibitory effects of gamma irradiation against *A. acidoterrestris* spores in apple and orange juices and found that the effects were dose-dependent, and an approximate 4-log reduction was obtained in all tests at 10 kGy. The water activity and pH of the tested juices were not changed after the treatments ($P > 0.05$), and after measuring the Hunter color values for *L* (lightness), *a* (redness), and *b* (yellowness), a slight increase in the *L* and *b* values (except 1 sample) and decrease in the *a* values were observed. UV-C light is UV light with a relative short wave length (200 to 280 nm). Baysal and others (2013) found that UV-C light was quite effective against *A. acidoterrestris* spores in white grape juice (maximum to 5.5 log reductions), but the effects were unsatisfactory when applied on apple juice. Through analyzing the survival data, the authors also showed that the log-linear plus tail model was more fitted than the Weibull model when estimating reductions of spores, because the former's root mean square error (RMSE) values were smaller than the latter's.

Thermal technologies

Instead of studying new chemical and physical methods to inhibit the spores or live cells of *Alicyclobacillus*, many researchers focused on analyzing the limit of the inhibitory methods, which are mostly thermal methods and used in practical juice industrial production at present, or optimizing the conditions of some steps of them. Bahçeci and others (2003) studied 2 technologies applied in today's clear apple juice industrial production: the process of ultrafiltration and the conventional process (using bentonite and gelatin for clarification), and found that there was almost no difference in the results (*A. acidoterrestris* spore counts) after an intermediate step (hot or cold depectinization) between the 2 technologies, and the results after the final step (pasteurization for the conventional process and permeation for ultrafiltration) showed some difference (about 1 log reduction for the latter, no matter if 20 or 50 kDa) at the higher inoculum level (1.0×10^6 CFU/mL), although in the finished product (which was supposed to be sterile), spores were still detected, which may result from some spores penetrating through the ultrafiltration membranes. Steyn and others (2011) studied the viable aerobic counts of 4 production stages (during a 108-h continuous process) of apple concentrate (single-strength apple juice, intermediate apple concentrate, final apple concentrate, and the condensate water). The results showed an altering pattern in single-strength juice (indicating raw fruit's varying quality), low and stable counts ranged from nondetectable levels to <2 CFU/mL in the final product (indicating the microbiological stability of the product) and a significant accumulation in condensate water (about a 2 log increase, indicating that manufacturing facilities' condensate water should be a critical control point). The authors also studied the counts of *Alicyclobacillus* vegetative cells and spores of the 4 stages and found that they both accumulated (about 0 to 2 log increase), indicating the continuous process was insufficient to completely eliminate this microbe.

Spinelli and others (2010) studied the decimal reductions and several other growth parameters of an isolated *A. acidoterrestris* strain in orange juice, and after continuous pasteurization, a hot-fill water-spray-cooling process was apllied. They found that the pasteurization processes were not enough to inactivate *A. acidoterrestris* (not even a 1 decimal reduction), and the test strain could not be inactivated during the hot-fill process and spoiled the orange juice in 5 to 6 d stored at 35 °C. Vieira and others (2002) studied some kinetic parameters of the thermal processing applied to a tropical fruit nectar and found that the spores at 8 mo of frozen storage were easier to kill than those at 4 mo and just after rehydration ($D_{95°C}$ were about 3.8, 6.0, and 5.3, respectively), which was interpreted by the authors as different aging degrees under frozen storage. Through the comparison between the isothermal method (IM) and the paired equivalent isothermal exposures (PEIEs) method by the analysis of some parameters, the authors also concluded that *A. acidoterrestris*'s thermal inactivation kinetic parameters can be estimated more realistically using the PEIE method. Although most studies are concerned with the process of inactivation of *Alicyclobacillus* (thermal or nonthermal), Spinelli and others (2009) believed that the process of storage was also important to inhibit spoilage. After testing 6 different storage conditions with hot-filled orange juice after a certain thermal inactivation process (92 °C for 10 s, 85 °C for 150 s, cooling to 35 °C in about 30 min), they found that only under 1 condition, storing at 20 °C, did *A. acidoterrestris* remain inhibited during the 6-mo storage period, and spoilage under other storage conditions was significantly influenced by cooling and storage conditions or inoculum level, and they suggested that the required time to accumulate a *Alicyclobacillus* population of 10^4 CFU/mL in a juice product could be an adequate parameter to indicate the corresponding spoilage.

Combined Intervention Treatments

Combination of 2 inhibitory factors

Chemical methods and heat. Recently, as many chemical and physical inhibitory methods against *Alicyclobacillus* had been studied, some researchers began to study the combined effect of 2 or more inhibitory factors. Huertas and others (2014) studied the combined effect of nisin, citral, limonene, and heat against *A. acidoterrestris* spores in McIlvaine buffer or PDA, and they found that the lower concentrations of nisin (0.3 mg/L) and citral (0.34 mM) led to almost the same inhibitory effect as the higher concentrations, while limonene showed no inhibitory effect in both test media, which was in agreement with data by Bevilacqua and others (2008a). A high synergistic effect between nisin, citral, and heat was also observed. Peña and others (2009) established a quadratic polynomial model to analyze the effects of the factors and their interactions during a thermal treatment with nisin added and found that nisin can reduce the heat resistance of *Alicyclobacillus* spores. The lower the temperatures were, the bigger the enhancement of effects. Bevilacqua and others (2013) studied the separate and combined effects of citrus extracts (lemon and biocitro extract) and mild heating and found that these extracts did have an inhibitory effect against *A. acidoterrestris* spores, which was dose-dependent, but the susceptibility was not strictly linear, like some other microbes showed toward biocitro in another report of Bevilacqua and

others (2010a). They also found a different susceptibility between their 2 tested strains, and explained this by their hydrophobic behavior: the more hydrophobic, the more sensitive to hydrophobic agents (to avoid the strains' effect, they used a mixture of the 2 strains in the subsequent assays). It was inferred by the authors that there might exist 2 mechanisms explaining the inhibitory effect of the extracts: disrupting spore structure and interrupting the action of the nutrient receptors that would lead the spores to germinate, according to some previous studies (Cortezzo and others 2004; Hayley and Palombo 2009).

Similar explanations can be found in some reports concerning EOs discussed above (Dorman and Deans 2000; Jerkovic and others 2001; Bevilacqua and others 2008a, 2011b). The combination of lemon or biocitro extract with heating increased their effect both under *in vitro* conditions and in apple juice, and the mechanisms might be that the spores were injured by heat and those damages hampered the repair mechanisms of the spores in the presence of such extracts, according to Periago and others (2006), or that the spores were induced to germinate by a mild heat-shock and then inhibited by these extracts, according to Setlow (2006). Alberice and others (2012) found saponins extracted from *Sapindus saponaria*, whose antibacterial effect in food has seldom been studied, only by Tsuzuki and others (2007) who investigated their antifungal effect. They were advantageous inhibitors because their effects were considerable (200 mg/L, 5 d, 6 to 7 log reductions, compared to the control; the effect was enhanced when combined with a thermal treatment) and similar to commercial extracts, and they were easy to get from an abundant source, and they had no obvious influence on the juice odor or appearance (only generating some foam), although taste analysis and other properties of juices were not represented. The authors explained *A. acidoterrestris* spores' slightly higher heat resistance in concentrated orange juices than reconstituted juices as the dehydration induced by osmotic pressure difference generated by sucrose.

Physical methods and heat. Besides these chemical inhibitors combined with heat, some researchers studied the combined effects of heat with physical inhibitory factors. Lee and others (2002) found that HPH alone had no inhibitory effect against *A. acidoterrestris* spores in apple juice (22 °C), but a synergystic inhibitory effect was observed when combined with heat. Similar results were obtained in studies with HHP (or combined with mild heating) discussed above (Alpas and others 2003; Buzrul and others 2005; Vercammen and others 2011; Silva and others 2012; Sokołowska and others 2013). The authors explained the synergystic inhibitory effect of the combination of HHP and heat as that the spores were induced to germinate by pressure and destroyed subsequently by heat, which was in agreement with the sources mentioned above. Vercammen and others (2011) even proposed that high (500 to 800 MPa) and low (100 to 300 MPa) pressure germination proceeds via distinct mechanisms (different results obtained from different microbes). The authors also indicated that high-pressure technology is economically available for industrial use at reasonable cost today. In a subsequent report, Lee and others (2006b) studied the effect of the SSC (or water activity, osmotic pressure) on the inactivation of spores of *A. acidoterrestris* by combining heat and HHP, and they drew the same conclusion as Sokołowska and others (2013) that there was hardly any inactivation effect at high SSC, such as 70 °Brix. Nakauma and others (2004) found that irradiation (electron beam) at a certain dose can enhance the effect of heating, and by testing the inactivation of *A. acidoterrestris* spores in dextrin, the authors indicated that the irradiation of powdered ingredients in combination with thermal treatment of the juice may be a new and advantageous method to control *Alicyclobacillus*.

Combinations without heat. Combinations without heat were also studied by some researchers. Walker and Phillips (2008b) found that nisin had a synergistic effect with potassium sorbate or sodium benzoate. Bevilacqua and others (2010b) found that the combination of cinnamaldehyde and eugenol at lower concentrations (Table 5) can lead to good inhibitory effect (no growth for 7 d) against *A. acidoterrestris* spores, compared with using cinnamaldehyde alone (500 ppm, 13 d), as reported in 2 of their previous articles (Bevilacqua and others 2008a,b). Cinnamaldehyde was the key-element to inhibit the growth, but its organoleptic impact was strong (20 to 40 ppm, not revealed; <100 to 120 ppm, not typical of apple juice); therefore, the combination of cinnamaldehyde and eugenol was a good solution and may hold value in practical applications (the required concentrations were even lower in apple juice than in the laboratory medium). In a recent report, Bevilacqua and others (2012) also studied the combination effect of HPH and benzoate and found that a similar or even better inhibitory effect can be obtained at lower concentrations of benzoate (80 ppm) compared to previous reports using it alone (Bevilacqua and others 2008a; Walker and Phillips 2008b); and they compared it with using HPH alone (Bevilacqua and others 2007b), which had hardly any inhibitory effect. This combination enhanced the effect greatly. Like the mechanism of the combination of HHP and heat, the authors believed that the synergystic effect may lie in the damage induced by HPH on spores, and that made benzoate act more easily. Sokołowska and others (2012) found that oscillatory HHP was more effective than continuous HHP, and the combination of them was better than each alone. They also found that a synergystic inhibitory effect existed between HHP and nisin, like some other researchers had reported (Chaibi and others 1996; Shearer and others 2000), but the combination of HHP and lysozyme had hardly any effect, which was in agreement with the report by Conte and others (2006). Wang and others (2010a) studied the effects of UW and MW on killing *A. acidoterrestris* vegetable cells and found that the former (600 W, 20 to 24 kHz, 30 min) was more effective (2 to 3 log gap) than the latter (900 W, 2450 MHz, 30 min), and with the same treatment time, combined UW and MW was less than separate UW, but better than MW, and UW followed by MW (about 3 log reductions) was significantly better than the contrary (about 2 log reductions). The difference among the 3 test strains may result from the different distribution of the fatty acids, different composition of cell membrane, and different DNA G + C content.

Combination of 3 and more inhibitory factors

Shearer and others (2000) studied the combined effect of HHP, mild heat, and some sucrose esters (monolaurin, sucrose laurates, sucrose stearates, and sucrose palmitate) against some spore-forming bacteria (*Bacillus* sp., *Clostridium sporogenes* PA3679, and *Alicyclobacillus* sp). As for *Alicyclobacillus*, they found that the combination of mild heat (45 °C) and HHP (392 MPa) for 10 min can cause about 2 $\log_{10}$CFU(spore)/mL reductions, which was in agreement with findings by Lee and others (2002), but when a certain concentration of sucrose laurate (0.005 and 0.045 for the 2 strains tested, respectively) was added, a significant inhibitory effect was observed (about 3.0 and 5.5 $\log_{10}$CFU(spore)/mL reductions for the 2 strains, respectively), which proved that there existed a synergystic effect among these 3 inhibitory factors. Although the inhibitory mechanism of the fatty acid antimicrobial sucrose laurate is still not clear, it was inferred that the mechanism should

Table 5–Combined methods to control *Alicyclobacillus* and their effectiveness.

Method/inhibitory factor	Target microbe	Test media or environment	Experimental conditions	Main results	References
			Combination of 2 inhibitory factors		
Chemical methods + heat					
Nisin/citral/limonene + heat	A (DSM 3922)	McIlvaine buffer of pH 3.5, PDA	Spore concentration: 5×10^5 spore/mL; detection: PDA; temperature: 95 °C; time: 3 s, 5 min; concentrations: nisin: 0 to 1.5 mg/L, citral, 0.34 or 0.69 mM, limonene: 0.52 mM	1.5 mg/L nisin + 3 s heat: about 2 $\log_{10}$ CFU/mL reductions; $D_{95°C}$ (nisin added): about 2 to 4 min; antimicrobial ability in buffer: 0.52 mM limonene (0.5 log reduction in 5 min)< 0.69 mM citral (1.3 log) < control/1.5 mg/L nisin/1.5 mg/L nisin + 0.69 mM citral (2.5 log); in PDA: control (1 log) < 0.69 mM citral (2 log) < 0.3/1.5 mg/L nisin (3 log) < 0.3/1.5 mg/L nisin + 0.34/0.69 mM citral (>4 log).	Huertas and others (2014)
Nisin +heat	A (CRA 7152)	Concentrated orange juice (64 °Brix)	Concentrations of nisin: 0, 50, 75, 100, and 150 IU/mL, temperature: 92, 95, 98, and 102 °C, detection: K agar.	$D_{90°C}$: 12.9 (no nisin), 12.34 (30 IU/mL), 11.38 (50 IU/mL), 10.49 (75 IU/mL), 9.49 (100 IU/mL), and 9.42 (150 IU/mL). Reduction: up to 27%. Results of other *D* values were similar.	Peña and others (2009)
Biocitro/lemon extract + heat	A (DSM 2498, and 1 isolated strain)	Apple juice, MEB	Separate test (in MEB): spore concentration: 10^5 to 10^7 spore/mL; detection: MEA; temperature: 44 °C; time: 2, 4 d; concentrations: 0 to 500 ppm; combined test (cocktail of 2 strains in apple juice): spore concentration: 10^5 spore/mL; detection: MEA; temperature: 45 °C; thermal treatment: 70 °C, 80 °C, 6 min; time: 16 d; concentrations: both 80 ppm	Separate test: isolated strain: 100 to 250 ppm 5 log reductions; DSM 2498: 500 ppm, 6 to 7 log reductions; combined test: biocitro/lemon extract + heat: 1.5 to 2 log reductions, biocitro + lemon extract + heat: 2 to 2.5 log reductions.	Bevilacqua and others (2013)
Saponin extracts + heat	A (CCT 49028)	Concentrated orange juice, reconstituted orange juice	*D* value test: spore concentration: 10^4 spore/mL; temperature: 87, 90, 95, and 99 °C; time: 50 min; detection: YSG plates; separated test: spore concentration: 10^4 spore/mL; detection: YSG plates; temperature: 45 °C; time: 250 h; concentrations: 100 to 500 ppm; combined test: the same as above, except, + thermal treatment: 99 °C, 1 min.	*D* value test: in both 2 kinds of juices: $D_{99°C}$: about 1.5 min, $D_{95°C}$: about 10- 11 min; separated test: both commercial saponin and purified extracts of saponin, in both 2 kinds of juices: 6 to 7 log reductions, compared with the control; combined test: in both 2 kinds of juices: 4.0 log reductions.	Alberice and others (2102)
Physical methods + heat					
High hydrostatic pressure + heat	A (ATCC 49025, NFPA 1013)	Apple juice	Spore concentration: 10^6 spore/mL (2 strains' cocktail); temperature: 22, 45, 71, and 90 °C; time: 10 min; detection: OSA plates; pressure: 0, 207, 414, and 621 MPa.	At the end of the tests: 22 °C: all samples: almost no reductions; 45 °C: all samples except treated with 0 MPa: about 4 log reductions; 71 °C: all samples except treated with 0 MPa: about 6 log reductions; 90 °C: 0 MPa: about 4 log reductions, others: about 6 log reductions before 5 min.	Lee and others (2002)
High hydrostatic pressure + heat	A (2 isolated strains)	Apple juice concentrate	Spore concentration: 10^6 spore/mL; temperature: 45, 71, and 90 °C; time: 10 min; detection: OSA plates; pressure: 207, 414, and 621 MPa; soluble solid contents: 17.5, 35, and 70 °Brix.	At the end of the tests: 45 °C: juice of 17.5 °Brix at all 3 test pressures: about 2 log reductions, others: almost no reductions; 71 °C: juice of 70 °Brix at all 3 test pressures: almost no reductions; others: about 4 to 6 log reductions; 90 °C: juice of 70 °Brix: the same with above; others: about 5 to 6 log reductions;	Lee and others (2006b)

(*Continued*)

Table 5–Continued.

Method/inhibitory factor	Target microbe	Test media or environment	Experimental conditions	Main results	References
Radiation + heat	A (NCIMB 13137)	Citrus juice, citrate buffer, dextrin powder (dissolved in a citrate buffer)	Thermal test (citrate buffer): temperature and time: 85 °C, 20 to 360 min, 90 °C, 10 to 180 min, 95 °C, 5 to 90 min; detection: 300AA medium; irradiation test 1 (followed by heating in citrate buffer): spore concentration: 10^3 to 10^7 spore/mL; temperature: 95 °C; time: 60 min; dose: 0.5, 1.0, and 2.0 kGy; detection: 300AA medium; irradiation test 2 (electron beam at 1.0 kGy, in 20% dextrin powder): spore concentration: 10^5 spore/mL; temperature: 85, 90, and 95 °C; time: 300 min; detection: 300AA medium; recovery test (citrus juice): spore concentration: 10^3 spore/mL; temperature: 95 °C, 20 min; time: 7 d (25, 45 °C); dose: 0.5, 1.0, and 2.0 kGy; detection: 300AA medium;	Thermal test: 95 °C, 90 min: 3 log reductions; irradiation test 1 (followed by heating in citrate buffer): electron beam: 95 °C, 20 min: about 4 log reductions; gamma ray: the same as above; irradiation test 2 (followed by heating in 20% dextrin powder): 95 °C, 20 min: about 3 log reductions; recovery test (citrus juice): electron beam at 1.0 kGy, 95 °C, 20 min, 45 °C, 7 d: no recovery detected.	Nakauma and others (2004)
Combinations without heat					
Nisin + sodium benzoate or potassium sorbate	A (2 isolated strains)	Apple juice	Initial inoculum: 3.45 log (for viable cell counts) or 3.82 log (for viable spore counts); test time: 29 d; concentrations of nisin: 2.5 to 10 IU/mL, combined disinfectants: 0.5 or 1 mg/mL; temperature: 30 °C; detection: OSA plate	Nisin along: about 2 to 3 log less than the control at day 29; combined with sodium benzoate or potassium sorbate: about 1 to 4 log less than the control	Walker and Phillips (2008b)
Eugenol + cinnamaldehyde	A (6 isolated strains)	Apple juice, MEB	Spore concentration: 10^3 spore/mL (6 strains' cocktail); temperature: room temperature; time: 7 d; detection: MEB plates; concentrations: cinnamaldehyde, 0 to 80 ppm, eugenol, 0 to 160 ppm; pH: 3.5 to 5.5.	Absorbance: pH 3.5 and 80 ppm cinnamaldehyde: OD_{420}: −1.5/d; lag days: 80 ppm cinnamaldehyde + >20 ppm eugenol: 12 d (pH 3.5), 5 d (pH 4.5).	Bevilacqua and others (2010b)
High-pressure homogenization + benzoate	A (DSM 2498, and 1 isolated strain)	Apple juice, MEB	Spore concentration: 10^5 and 10^3 spore/mL; temperature: room temperature; time: 10 to 12 d; detection: MEB plates; concentrations: Na benzoate, 80 mg/L, eugenol, 150 μL/L; pressure: 140 MPa.	Test in MEB: reductions after treatment: HPH + Na benzoate / eugenol, both 2 strains: about 0.75 log reductions; recovery at day 12: HPH + Na benzoate: about 1.2 log reductions; eugenol: no reductions in all samples; test in apple juice: reductions after treatment: HPH + Na benzoate, about 0.7 log reductions for both 2 strains (high inoculum); 2.43 log reductions for DSM 2498 (low inoculum); recovery (DSM 2498, low inoculum): no CFU detected after day 1.	Bevilacqua and others (2012)
High hydrostatic pressure + nisin or lysozyme	A (3 isolated strains)	Apple juice	Spore concentration: 10^6 spore/mL; temperature: 50 °C; detection: BAT plates; 1) continuous HHP: 200, 300, and 500 MPa, 30 min; 2) oscillatory HHP: holding time, 5 min, pause time at atmospheric pressure, 5 min, 6 cycles; 3) continuous + oscillatory HHP: 100 MPa 6 cycles, 200 MPa 6 cycles, 200 MPa 4 cycles, 60 min incubation, 30 min continuous HHP, 500 MPa; 4) continuous HHP + lysozyme: 300 MPa, 30 min, 0.05 and 0.1 mg/mL; 5) continuous HHP + nisin: 200 and 300 MPa, 45 min, 250, 500, 750, and 1000 IU/mL.	Best results, at the end of the tests: 1) 200 MPa, 2.5 log reductions; 2) 200 MPa, 4 log reductions; 3) combined treatment, 5.5 log reductions; 4) both 2 treatments, 2 log reductions; 5) 200 MPa, 250 IU/mL, 6 log reductions.	Sokołowska and others (2012)

(*Continued*)

Table 5–Continued.

Method/inhibitory factor	Target microbe	Test media or environment	Experimental conditions	Main results	References
Microwaves + ultrasonic waves	A (DSM 14558^T, 3922^T); C (DSM 4006^T)	AAM broth	MW Power: 300, 500, 700, and 900 W (2450 MHz), UW power: 200, 400, and 600 W (20 to 24 kHz); time: 0 to 30 min; temperature: <50 °C at the end of the test; vegetable cell concentration: 2 to 6 $\times 10^6$ CFU/mL; detection: AAM plates.	The effectiveness was improved as the power and exposure time increased. At the end of the tests: DSM 14558^T: about 1.0 to 1.5 (MW), 4 to 5 (UW) log reductions; DSM 3922^T: about 2.0 to 2.5 (MW), 4 to 5.5 (UW) log reductions; DSM 4006^T: about 1.0 to 2.0 (MW), 2.5 to 4.5 (UW) log reductions.	Wang and others (2010a)
Combination of 3 or more inhibitory factors					
Sucrose laurate + High hydrostatic pressure + mild heat	*Alicyclobacillus* sp. (2 isolated strains)	Apple juice, tomato juice	Spore concentration: 10^6 spore/mL; temperature: 45 °C; time: 10 to 15 min; pressure: 392 MPa; 1) test in tomato juice: detection: tomato juice agar; sucrose laurate concentration: 0.005%; 2) test in apple juice: detection: K agar; sucrose laurate concentration: 0.045%.	1) Pressure + heat, sucrose laurate + heat, sucrose laurate alone: <2 log reductions; sucrose laurate + pressure + heat: 3 log reductions; 2) combination of 3 factors: 5 log reductions; others: <2 log reductions.	Shearer and others (2000)
Heat + pH + cinnamaldehyde	A (1 isolated strains)	MEB	Spore concentration: 10^3 spore/mL; temperature: 80, 84, 88, 92, and 96 °C; time: 10 min; cinnamaldehyde concentrations: 0, 40, 80, 120, and 160 ppm; pH: 3.5, 4.0, 4.5, 5.0, and 5.5; detection: MEA plates.	Survival test: pH 4.5, 80/160 ppm, 88 °C: inhibit growth for 10 d, 4 log reductions compared to the control.	Bevilacqua and others (2009)
pH + heat + ascorbic acid	A (DSM 2498)	McIlvaine buffer, apple nectar, and apple juice	Spore concentration: 10^6 spore/mL; temperature: 90 to 100 °C; time: 50 min; ascorbic acid concentrations: 0, 125, and 250 mg/L; pH: 2.5 to 4.5; detection: BAM plates.	$D_{96°C}$: McIlvaine buffer (pH 3.0 to 4.0): 1.1 to 1.7 min; apple juice (pH 3.68): 2.1 min; apple nectar (pH 2.97): 3.3 min, apple nectar (pH 2.95, ascorbic acid 250 mg/L): 3.1 min.	Bahçeci and Acar (2007a)
pH + soluble solids content + mild heat + nisin	A (CRA 7152)	Apple juice	Spore concentration: 2×10^5 spore/mL; temperature: 25 to 50 °C; time: 10 d min; nisin concentrations: 0 to 70 UI/mL; pH: 3.5 to 5.5; soluble solids content: 11 to 19 °Brix; detection: BAM plates.	pH 4.0, 43 °C, 11 °Brix, 10 d, nisin concentration 50 IU/mL: about 2 log reductions compared to the initial inoculum, and about 0.5 log reductions compared to the control.	Peña and others (2011)

A: *Alicyclobacillus acidoterrestris*.

be inhibitory rather than lethal and related to perturbation of the membrane, and the treatment of HHP might help sucrose laurate deposit on the spore coat thus changing the surface hydrophobicity and water permeability, according to some reports (Hayakawa and others 1994; Chaibi and others 1996). Besides, sucrose laurate addition had no obvious influence on the odor or appearance of the tested tomato juice or apple juice (only foaming when mixing); therefore, the authors believed that this combination might be a promising method for the food industry. Sinigaglia and others (2003) studied the combined effects of pH, water activity, and temperature on the germination and growth of spores of *A. acidoterrestris* and established a response surface model. Although not directly concerning control, their data are useful to studies about the effect of multiple inhibitory factors.

Bevilacqua and others (2009) studied the combined inhibitory effect of cinnamaldehyde, heat, and pH against *A. acidoterrestris* in MEB through a 3-variables and 5 levels central composite design, and found that the pH and temperature actually reduced the initial number of *A. acidoterrestris* spores (0.7 log reductions) and 40 to 50 ppm of cinnamaldehyde inhibited the germination of the spores during storage (up to 10 d). Compared with using cinnamaldehyde alone (Bevilacqua and others 2008a), this work proved that cinnamaldehyde was a good inhibitor against *A. acidoterrestris* spores at relatively low concentrations, when combined with another inhibitory factor, which was in agreement with their other report (Bevilacqua and others 2010b).

Bahçeci and Acar (2007a) established a predictive model (a 2nd-order polynomial equation) for the determination of *A. acidoterrestris* spores' heat resistance (*D* values) in apple juice and other media as a function of ascorbic acid concentration, temperature, and pH. The *D* values obtained in this work were close to those of some others studies (Vieira and others 2002; Alberice and others 2012; Huertas and others 2014) concerning *A. acidoterrestris* spores' heat resistance mentioned above, and were influenced by pH at low temperatures (such as 90 °C) significantly, but not at high temperatures (such as 96 °C), which had been observed by other researchers (Pontius and others 1998; Komitopoulou and others 1999). This was interpreted by the authors, according to Wisotzkey and others (1992) and Chang and Kang (2004), as dense packing of the ω-cyclohexane fatty-acid-containing lipids in *Alicyclobacillus*'s cell membrane, leading to low diffusion at high temperatures and formation of a protective coating to protect the microbes under high temperatures and acidic conditions. In this study, the authors also found that the influence of ascorbic acid on *A. acidoterrestris* spores' heat resistance was not significant within the concentration studied (0 to 250 mg/L), which had some differences compared to the study of Cerny and others (2000). Peña and others (2011) studied the combined effect of pH, brix, temperature, and nisin against *A. acidoterrestris* spores in apple juice using the logistic regression model, and they found that under a certain condition (Table 5), there was only less than 1 log reduction (nisin concentration: 50 IU/mL).

Conclusions

The use of different control methods, including chemical methods, such as oxidizing agents, disinfectants, EOs, and bacteriocins, or physical methods, such as high pressure, MW, ultrasound, and ohmic heating, and a combination of these methods have been widely investigated and most of them were proved to be effective against *Alicyclobacillus*, a group of thermoacidophilic, nonpathogenic spoilage bacteria of juice and beverage products. In recent years, the most studied chemical method has been bacteriocin use, because of its biological safety and good effect, and the most studied physical method is HHP, for it does not cause changes of many sensory qualities of the juice, such as color (especially for some kinds of fruit juice whose color is easy to lose, such as the green color of kiwi fruit juice), which is inevitable to all physical methods with a thermal effect. HHP has been started to take into practical industrial use by many. Today some conventional methods, especially steam pasteurization, are still the predo minant approaches to reduce the contamination due to *Alicyclobacillus*, but more and more nonconventional, energy-conserving, promising new methods are designed and studied, although there still exist some unsolved problems preventing them from wide industrial application. Future research should focus on exploring the synergistic effect of 2 or more methods and continually establish novel approaches to control the contamination of *Alicyclobacillus*, without reducing the sensory qualities of juice and beverage products as far as possible.

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