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REVIEW ARTICLE

Inactivation of *Alicyclobacillus acidoterrestris* by Non Thermal Processing Technologies - A Review

[1*]Kumar, R., [2]Bawa, A.S., [1]Kathiravan, T and [1]Nadanasabapathi, S.

1. Food Engineering & Packaging Division, Defence Food Research Laboratory, Mysore-570 011 Karnataka, India.
2. Former Director, Defence Food Research Laboratory.

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Corresponding author
kumardfrl@gmail.com

Abstract

Alicyclobacillus acidoterrestris is a spore-forming food spoilage bacterium. Its spore is problematic to the juice industry because of its ability to grow in low pH environments and survive pasteurization processes. Thermal processing has been considered as the only way to reduce the initial spore number of *Alicyclobacillus acidoterrestris* and prevent the spoilage of acidic beverage. Heat treatment could have negative effects of the quality of juices, including loss of nutrients, and change in flavor, color, and texture. Non-thermal treatments can be attractive alternatives to traditional heat treatments for producing high quality, convenient and safe food products. A number of non-thermal methods such as high hydrostatic pressure (HHP), irradiation, ultrasound, high pressure homogenization (HPH), microwaves and high pressure carbon dioxide (HPCD) have been discussed in this article for their effectiveness at controlling *A. acidoterrestris.*

Introduction

Alicyclobacillus spp. is gram-positive, non-pathogenic, obligately aerobic, rod shaped, thermophilc, and acidophilic spore-forming bacteria (Chang, *et al.,* 2004). *Alicyclobacillus* spp. has been isolated from soil, spoiled fruit juices, and several thermal environments. *Alicyclobacillus* are sometimes called acidophilic thermophilic bacteria (ATB) and grow well in acidic environments, surviving at pH levels as low as 2.5. This bacterium can grow in a pH range from 2.2-5.8 as well as at temperatures ranging from of 23-55°C (Baumgart, 1999). Its ability to survive at high temperatures and low pH levels has been attributed to the unique cellular membrane composition containing ω-cyclohexanoic fatty acids (Alpas, *et al.,* 2003). All *Alicyclobacillus spp.* metabolize sugars with acid production, but no gas production. Water activity greater than 0.9 is required for growth. Some species have been reported to grow in fruit juice with up to 18.2 °Brix (Splittstoesser, *et al.,* 1994). Of all species in the genus, *A. acidiphilus*, *A. acidoterrestris*, *A. herbarius* and *A. pomorum* have been reported to be associated with spoilage of fruit juices and beverages (Goto, *et al.*, 2003).

Alicyclobacillus acidoterrestris is of concern for the fruit juice industry due to the spoilage of commercially pasteurized fruit juices and has also been associated with bottled tea, isotonic drinks, and other low pH, shelf-stable products (Sapers, *et al.,* 2005). The spores can survive the pasteurization treatment given to most shelf-stable, glass-packaged fruit juices. The heat treatment induces germination of the spores (Pena, *et al.,* 2009). Since *A. acidoterrestris* spores have been shown to resist high temperatures and acidic environments, they have been suggested as the target microorganism for the design of a thermal process for fruit juices.

Until the mid-1980's, the occurrence of bacterial spore-formers in low pH foods was thought to be insignificant because it was assumed that gram-positive, spore-forming bacteria could not germinate and grow to any great degree at pH levels below 4.5. In 1982, contamination of pasteurized apple juice occurred in Germany on a large scale. This was the first reported incidence of spoilage by *Alicyclobacillus*. The cause was attributed to an organism related to *Bacillus acidocaldarius*. However, later studies showed that the cause was *Alicyclobacillus acidoterrestris* (Yokota, 2007). In 1989, an incident of deterioration of an acidic juice product was reported in Japan.

The causative agent was found to be similar to the bacterium identified in Germany. By the mid-1990's spoilage of acidic juice products by members of the recently named genus *Alicyclobacillus* was recognized and the seriousness of this situation began to be appreciated.

The ability of spores of *A. acidoterrestris* to grow under highly acidic conditions makes it a good candidate for spoilage of shelf stable fruit juices and beverages. Following the first spoilage incident caused by *A. acidoterrestris* reported in 1982, when aseptically packaged apple juice in Germany was contaminated (Cerny, *et al.*, 1984); several spoilage incidents have been reported over the past two decades. Several spoilage cases were reported in Japan, Europe, and the USA in the 1990's (Jensen, 2000). In addition, more food products have been reported spoiled by *A. acidoterrestris*, including isotonic water and lemonade (Yamaziki, *et al.*, 1996), carbonated fruit juice drinks (Pettipher and Osmundson, 2000), fruit pulps, shelf-stable iced tea containing berry juice (Duong and Jensen, 2000), and canned diced tomatoes (Walls and Chuyate, 1998).

In 1990 and 1995, an unpleasant odor was detected in 40% of apple juice samples in Australia. It was reported that this bad odor was not caused by any additives or preservatives added to the juice, but was due to microbial contamination. Splittstoesser et al., (1994) reported the strain isolated from pasteurized apple juice in 1990 was a thermo acidophilic bacterium. This was the first mention of the isolation of this organism from contaminated fruit juice in the United States. Though spoilage by *Alicyclobacillus spp*. was previously regarded as sporadic, the survey by the National Food Processors Association (NFPA) in 1998 showed the large scale of fruit juice spoilage associated with *Alicyclobacillus spp.* Results of the survey indicated that 35% of the fruit juice manufactures who responded experienced spoilage unconfirmed but consistent with growth of acidophilic spore formers.

Source

Soil is considered to be the major source of *A. acidoterrestris* and also the most important source of contamination of acidic products. Studies have suggested that contamination of fruit juices is most likely caused by fruit contaminated by soil during harvest or by unwashed or poorly washed raw fruit used in processing facilities (Chang and Kang, 2004). Another possibility is that soil can be carried into the manufacturing facilities by employees. Water has also been proposed to be another important source of contamination. McIntyre, *et al.*, (1995) isolated a strain of *Alicyclobacillus* from spoiled juice product and found the same strain in a sample of ingredient water from the processing facility.

Detection and identification of *Alicyclobacillus acidoterrestris*

The organism has the following characteristics: motile rod-shaped bacillus, gram-positive, aerobic, forming aerobically oval endospores, showing no growth in media containing 5% w/v NaCl. Generally, *A. acidoterrestris* growth characteristics in Bacillus acidocaldarius medium (also called BAM) is limited to temperatures ranging between 25 and 60°C and pH values between 2.5 and 5.5 (Yamazaki et al., 1996). *A. acidoterrestris* vegetative cells have a rapid growth cycle, reaching the plateau of exponential growth at optimum pH after 8–12 h incubation. *A. Acidoterrestris* cells do not grow on nutrient, trypticase soy, brain heart infusion and veal infusion agars and broths, even when adjusted to a pH of 3.5.

Detection and isolation from fruit products

The method followed by Previdi, *et al.*, (1997) for isolation was the following: juice samples and diluted concentrates were submitted to a heat treatment (80°C for 10 min) and then incubated at 37°C for 7 days to permit growth of *A. acidoterrestris*; the product was then spread on malt extract agar adjusted to pH 4.0. Pinhatti, *et al.*, (1997) brought the frozen concentrated orange juice to single strength and submitted this to a heat shock at 80°C for 10 min. Samples were incubated at 50°C for 24 and 48 h for enrichment. Detection of *A. acidoterrestris* was done by pour -plating in BAM and incubated at 50°C for 24 h in sealed plastic bags to avoid drying of the medium. *Alicyclobacillus spp*. were detected in several industrialized fruit concentrates and orange juices (>1.0-102 cfu/ml) that were not spoiled. It was concluded that spoilage of fruit juices by *Alicyclobacilli* was incidental, requiring a combination of adequate conditions for growth, such as low pH and high temperatures, for long periods of time.

Identification of biochemical profile

The identification of different *A. acidoterrestris* strains was carried out by several authors with the characterization of the biochemical profile. The biochemical profile of the *A. acidoterrestris* type strain used by Silva (2000) and Silva, *et al.*, (2000) was investigated using the API 50 CH strip. All the 13 *A. acidoterrestris*

strains tested by Deinhard, *et al.,* (1987) formed acid from the following carbohydrates sources: glycerol, erythritol, l-arabinose, ribose, d-xylose, galactose, glucose, fructose, mannose, rhamnose, mannitol, esculin and cellobiose. *A. acidocaldarius*, another thermo acidophilic microbe very similar to *A. acidoterrestris*, can be distinguished from the latter organism very easily, as it (8 strains tested) does not ferment erythritol as opposed to *A. Acidoterrestris* (13 strains tested)

Germination of spores in fruit products and growth

Spores of *A. acidoterrestris* can have a slow growth cycle (up to 5 days) but its growth is responsible for off flavors in commercial fruit products. The visual detection of spoilage is very difficult because *A. Acidoterrestris* does not produce gas during growth and incipient swelling of containers does not occur.

Spore germination and growth to 10^6 cfu/ml in orange juice stored at 44°C for 24 h was detected and a level of 105–106 cells/ml in apple and orange juices formed enough guaiacol (ppb) to produce sensory taint (Pettipher et al., 1997). Also, taint was subjectively noticed in apple, orange and grapefruit juices after 4 days storage at 30°C when the count increased from 10^2 to 10^5 cfu/ml (Komitopoulou, *et al.,* 1999). The same report noted that *A. acidoterrestris* spores were sensitized to nisin with a 40% reduction in the D-values. Germination of *A. acidoterrestris* spores (VF and WAC strains) and growth in apple juice (pH=3.5, 11.4°Brix), tomato juice (pH=4.0, 7.0°Brix) and several varieties of white grape juice (pH=2.8–3.4, 7.8–10.8°Brix) was detected after 2 days storage at 43°C (Splittstoesser et al., 1994). The viable counts of the initial inoculated juice and the same juice kept for 2 days at 43°C were compared by plating on PDA (Splittstoesser, *et al.,* 1994). Spores of VF strain also grew in grapefruit (pH=3.1, 10.4° Brix), orange (pH=3.6, 12° Brix) and pineapple (pH=3.3, 13.4° Brix) juices. However, growth was inhibited in juices with a high content of total soluble solids.

In red grape juices (pH=3.1–3.8, 9.1–12.2° Brix) no growth was observed. The inhibitory effect of red grape juice suggested that certain phenolic compounds might affect the ability of the *A. acidoterrestris* spores to germinate and grow. After heat activation of type strain *A. acidoterrestris* spores in cupuacu (Amazonian fruit) pulp (pH=3.6, 11.3°Brix), no growth was observed during 1 month of storage under aerobic and anaerobic conditions at 25 and 43°C.

Heat resistance of *A. Acidoterrestris*

Spores of *A. acidoterrestris* can germinate and grow at pH <4 and exhibit high heat resistance. D-values of *A. acidoterrestris* in juices at 95°C are reported to range from 0.06 to 5.3 minutes and a z-value range from 7.2 to 12.9°C (Silva and Gibbs, 2001). Targets microorganisms in the fruit products industry are generally much less heat resistant than spores of *A. acidoterrestris*. The standard juice pasteurization treatment is 80 to 95°C for 45 to15 seconds, which is not sufficient to inactivate spores of *A. acidoterrestris*. This fact demonstrates the vulnerability of fruit juices and beverages to spoilage by *A. acidoterrestris.* Use of *A. acidoterrestris* spores as the target of pasteurization processes in high acid fruit products.

D-value decreased with an increase in temperature, which indicates decreased heat resistance. D-values decreased dramatically when temperature increased from 85°C to 90°C, and the highest D-values were recorded in black currant concentrate (24.1 min at 91°C) and lemon juice concentrate (12.63 min at 95°C). For most of the juices evaluated, D values were reduced to less than 4 min at 95°C. The higher the sugar content (°Brix), the greater the heat resistance recorded. This indicates that it is more difficult to destroy spores present in concentrated juices than in single strength juices. pH also has an effect on the heat resistance of spores, generally with lower heat sensitivity at higher pH. Other properties of juices may play a greater role in heat resistance than pH. As seen in the table, heat resistance in orange juice is greater than in grape juice, even though grape juice had a slightly higher pH. It was reported that pH affects heat resistance of the spores at lower temperatures. Moreover, the effects of soluble solids and pH were diminished when the temperature reached about 97°C (Silva, *et al.,* 1999). Studies also have shown that the type of organic acid (malic, citric, and tartaric acid) did not significantly affect D-value in the temperature range of 91-100°C. It was also found that bacterial spores were more heat resistant as water activity decreased (Murrel and Scott, 1996). Silva et al., (1999) suggested that water activity may be considered instead of soluble solids in future studies because different sugars generate different water activities at the same concentrations and could have different effects on D-value.

Spoilage incidents caused by *A. Acidoterrestris*

The ability of spores of *A. acidoterrestris* to grow under highly acidic conditions makes it a good candidate for spoilage of shelf stable fruit juices and beverages. Following the first spoilage incident caused by *A. acidoterrestris* reported in 1982, when aseptically packaged apple juice in Germany was contaminated, several spoilage incidents have been reported over the past two decades. Several spoilage cases were reported in Japan, Europe, and the USA in the 1990s. In addition, more food products have been reported spoiled by *A. acidoterrestris*, including isotonic water and lemonade, carbonated fruit juice drinks, fruit pulps, shelf stable iced tea containing berry juice and canned diced tomatoes.

Off flavors caused by spoilage

Spoilage of *A. acidoterrestris* is primarily manifested as off flavor or off odor. Visual detection of spoilage is very difficult since no gas is produced during growth and swelling of containers does not occur. The major compounds associated with off-flavors caused by *A. acidoterrestris* are guaiacol and halophenols, including 2, 6-dibromophenol (2, 6-DBP) and 2, 6- dichlorophenol (2, 6-DCP).

Guaiacol

Guaiacol (2-methoxyphenol) is a phenolic compound with the formula C_6 H_4 (OH) (OCH_3). Guaiacol is accepted to be the major metabolite associated with off odors in fruit juices and is detected in juices at concentrations about 1000 times higher than halophenols (Jensen, 2000). Guaiacol is usually derived from wood creosote or guaiacum and can be biosynthesized by a variety of organisms, such as *Bacillus magaterium*, *Pseudomonas acidovorans*, and *A. acidoterrestris*. It is recognized as a flavor compound and has been used as synthetic flavoring in processed foods with a description of "sweet", "smoky", "phenolic", and "medicinal" (Burduck, 2005). The characteristic odor of some roasted foods such as Arabica coffee and barley malt is attributed to the presence of guaiacol. However, it is better known as an off flavor/odor compound in many other foods such as wine, fruit juices, chocolate ice cream, chocolate milk and vanilla yogurt.

In fruit juices, guaiacol is formed from ferulic acid via vanillin (Bahceci, *et al.,* 2005). Ferulic acid is a major component in lignin and can be found abundantly in plant cell walls. It can be metabolized by bacteria and fungi and converted to vanillin, vanillic acid, and protocatechuic acid. Vanillic acid can be further converted to guaiacol. Crawford and Olson (1978) demonstrated that several strains of *B. megaterium* and a strain of *Streptomyces* convert vanillic acid to guaiacol and CO_2 by a non-oxidative decarboxylation mechanism. They also suggested that the ability to decarboxylate vanillic acid to guaiacol is quite common among soil bacilli.

The human sensory threshold for guaiacol is low, so it is easily detected. Wasserman (1966) reported that the threshold concentration of guaiacol in water is 0.021 ppm for odor and 0.013 ppm for taste; the odor threshold in oil is 0.07 ppm. The threshold for smelling guaiacol in 12% aqueous ethanol is reported as 0.03 ppm (Chang and Kang, 2004). Pettipher, *et al.,* (1997) used a GC-MS method and found that the odor threshold for guaiacol in orange, apple juice, and a non-carbonated fruit juice drink was about 2 ppb. Another study using a sensory panel and the forced-choice ascending concentration method of limits conducted by Orr, *et al.,* (2000) also showed similar results. They reported the best estimate threshold of guaiacol in apple juice is 2.23 ppb.

In the case of *A. acidoterrestris* spoilage, guaiacol is produced when cell numbers reach a critical level. Komitopoulou, *et al.*, (1999) reported that guaiacol was detected in apple juice, orange juice, and grape juice stored at 30°C when the population of *A. acidoterrestris* reached 105 CFU/ml; at 25°C, the same population was required to detect guaiacol in apple and orange juices, but only 10^4 CFU/ml were necessary to detect it in grape juice. Similarly, Pettipher et al., (1997) reported that 10^5 CFU/ml *A. acidoterrestris* was required before guaiacol was detectable level in orange juice and apple juice stored at 25, 35, and 44°C. Generally, it is proposed that the higher the incubation temperature, the faster guaiacol is produced.

Halophenols

Although guaiacol is considered the predominant off-odor compound, researchers also detect halophenols, 2, 6-DBP and 2, 6-DCP (Jensen and Whitfield, 2001) produced by *A. acidoterrestris* in spoiled juices. The odor/flavor is often described as "medicinal" and "disinfectant." Halophenols are well known for causing off-flavors in foods. Their occurrence in food can be either from chemical contamination or microbial synthesis. The taste

threshold in water of 2, 6-DCP is 6.2 ppt (Young et al., 1996) and 0.5 ppt for 2, 6-DBP. In juices, the taste threshold is reported to be 0.5 ppt for 2, 6-DBP and 30 ppt for 2, 6-DCP.

Design of pasteurization processes

In commercial canning a pasteurization value (P value) resulting in 2D and 3D (decimal reduction) in the target microorganism is recommended. The target microorganism is often the most heat resistant or the most common spoilage microorganism, which in many cases are the same. However, the microbial targets normally used in the fruit products industry are, in general, much less heat resistant than the spores of A. acidoterrestris. The severity of pasteurization treatments applied in the fruit industry is subjective. Companies make this decision based on empirical experience and problems they might have experienced in the past. Temperatures between 80 and 100°C are normally used. In the design of pasteurization processes, the establishment of a P-value, which is the minimum heat required (time-temperature exposure to heat) to result in a product retaining quality during storage, should be based on the following experiments performed with the product to be pasteurized:

1. determination of D-value and z-value of *A. Acidoterrestris* spores;
2. potential for *A. acidoterrestris* spore growth during product storage for at least 1 month at 25 and 43°C;
3. monitor product quality during storage following pasteurization treatments of different severity

Non-thermal methods

Traditional thermal pasteurization is effective for inactivating vegetative cells of bacterial food borne pathogens, but, as stated before, the current juice pasteurization treatment is not adequate to destroy spores of *A. acidoterrestris*. Moreover, heat treatment could have negative effects of the quality of juices, including loss of nutrients, and change in flavor, color, and texture. Non-thermal treatments can be attractive alternatives to traditional heat treatments for producing high quality, convenient and safe food products. A number of non-thermal methods have been studied for their effectiveness at controlling *A. Acidoterrestris.*

High hydrostatic pressure (HHP)

It is suggested that HPP can preserve certain foods better than heat by extending shelf life and inactivating microorganisms while retaining the inherent color, flavor, nutrients, and texture of the food. Pressure is transmitted instantaneously and is independent of mass, so the treatment throughout the food is uniform (Zimmerman and Bergman, 1993). The application of HPP in ensuring food safety and quality has been widely studied. HPP inactivate vegetative cells of microorganisms by breaking non-covalent bonds and causing damage to the cell membrane (Morris, *et al.,* 2007). The mechanism of inactivation of bacterial spores through high pressure was suggested to have two steps: high pressure will first induce spore germination and then inactivate the germinated spores.

The application of HHP for inactivating *A. acidoterrestris* has been studied by many researchers over a wide range of pressure treatments. Alpas and Bozoglu (2003) studied the effect of HHP on inactivation of *A. acidoterrestris* vegetative cells in BAM broth and in orange, apple, and tomato juices. After treating with 350 MPa at 50°C for 20 min, a 4.7-log reduction of cells was achieved in BAM broth while in all juices, over a 4-log reduction was achieved. Apple juices (17.5, 35 and 70 °Brix) inoculated with *A. acidoterrestris* spores were subjected to three pressure treatments (207, 414 and 621 MPa) at 22, 45, 71 and 90°C. Results showed that the effectiveness of treatment increased as pressure and temperature increased. At room temperature, there was no significant reduction of spores in all juice samples for all three pressures, which indicates that in order to use high pressure for spore inactivation; other treatments such as mild heat are required. They also found that the effectiveness of HHP was affected by soluble solids content, with reduction in inhibition observed when the concentration of juice was increased. Over 5- and 4-log reduction was found in juice of 17.5 and 35 °Brix, respectively at 90°C; however, there was no significant reduction of spores at the highest concentration (70 °Brix).

Irradiation

Irradiation of food is the process by which food is exposed to sufficient radiation energy to cause ionization, thereby leading to microbial death due to genetic damage (FDA, 2001). Irradiation is regulated as a food additive in the U.S and requires approval by the FDA for each new application. Dose ranges of <1 to 3 kGy have

proven effective for reducing or eliminating populations of food borne pathogens and postharvest spoilage microorganisms on produce. Radiation is suitable for inactivation of spores in foods with low moisture content such as powders. The application of radiation to destroy bacterial spores has been widely studied. It was suggested that ionizing radiation is an effective means to destroy bacterial spores, especially when combined with heat, and has been applied to the sterilization of several kinds of foods that are contaminated primarily with bacterial spores. Pre treatment of *Clostridium sporogenes* spores with gamma rays enhanced their thermal sensitivity and combined treatment can efficiently inactivate the spores. Nakauma, *et al.,* (2004) studied the influence of radiation (electron-beam and gamma rays) combined with heat to inactivate *A. acidoterrestris* spores. Their results showed that the dose of electron-beam and gamma-ray irradiation required for 90% reduction of spores in dextrin was 1.72 and 1.79 kGy, respectively. The required dose was lowered by using irradiation in combination with thermal treatment. Also, it was shown that radiation accelerated the effect of subsequent thermal treatment: 4 log CFU/ml spores were completely inactivated by heating at 95°C for 188 min, and the heating time was reduced to 23 min when combined with a 2.0 kGy electron beam or gamma-ray treatment.

Ultrasound

Ultrasound refers to sound pressure with a frequency of greater than 20 kHz (upper limit of human hearing). It has been applied in the food industry in a wide range of applications including measurement of chocolate layers, fat lean tissues in meat, detection of contaminants like metal, glass or wood, and measurement of particle size. Ultrasound as a potential antimicrobial treatment has been studied for about two decades. Research of its inactivation effect on several food borne pathogens, *Listeria monocytogenes*, *Salmonella*, *Escherichia coli*, *Staphylococcus aureus*, and others, has been conducted. The mechanism of microbial inhibition is suggested to be due to thinning of cell membranes, localized heating, and production of free radicals (Butz and Tauscher, 2002). The advantages of ultrasound over heat pasteurization are minimizing flavor loss, greater homogeneity, and significant energy savings (Piyasena, *et al.,* 2003). It is also suggested that the combination of heat and ultrasound is more efficient at microbial inactivation than either treatment alone. Yuan, *et al.,* (2009) studied the effect of ultrasound treatment on *A. acidoterrestris* in apple juice and observed that 60% of cells were inactivated after 23 kHz ultrasound treatment at 300 W for 30 min. The inactivation rate increased as treatment time increased, with more than 80% reduction reported after 60 min of treatment. However, the loss of quality of apple juice was observed after treatment. Total sugar content, transmittance, and color (juice browning) were reduced with increased ultrasound processing time and power.

High pressure homogenization (HPH)

Homogenization was invented by August Gaulin and developed for emulsion stabilization and has then been used by the food industry in a wide variety of areas, mostly known for use in milk processing. Homogenization improved the texture, taste, flavor, and shelf-life characteristics for food emulsions and dairy products. A new generation of homogenization - high pressure homogenization (HPH) was developed in the early 1990s due to the demand of higher quality food. HPH can reach pressures as high as 300-500 MPa while the traditional homogenizer reaches less than 50 MPa. HPH enables a more stable food emulsion production with droplets small enough to ensure a longer shelf-life. HPH is now being used in the pharmaceutical, cosmetic, and food industries for the preparation or stabilization of emulsions and suspensions. It has also used to disrupt microbial cells, leading to the release of intracellular material.

Inactivation of bacteria using HPH has been investigated by a number of researchers. Gram-positive bacteria are more resistant to HPH than Gram-negative bacteria (Vachon, *et al.,* 2002). It is suggested that the composition in cell wall determines resistance to HPH. The cell wall is necessary for maintaining the structure and shape of the cell and protecting it from osmotic forces. Gram-positive bacteria have a thicker peptidoglycan layer in the cell wall than Gram-negative bacteria, which contributes to their greater resistance to HPH. It is also suggested that the growth rate of cells affects resistance to HPH. Cells are more easily disrupted when they are growing rapidly (e.g., exponential phase) than cells growing at a slower rate on the same medium. Wuytack, *et al.,* (2002) compared bacterial inactivation by HPH and HHP. Of all five Gram positive and six Gram-negative bacteria they studied, large differences in resistance to HHP but not HPH were observed. They concluded that the inactivation mechanisms for both techniques are different due to the different response of test bacteria. High pressure level is not a major factor during HPH, since bacteria are only exposed to high pressure for a second or less while the exposure time for HHP is much longer. Yeasts are generally easier to be destroyed than bacteria.

Turbulence, stress, shear, or turbulence caused by impingement of a high velocity jet of suspended cells on a stationary surface and cavitation (Save, *et al.,* 1994), which is the process of gas cavity growth and collapse in a liquid when the liquid is subjected to rapid pressure change, have all been suggested as responsible for microbial inactivation by HPH. Inactivation of spores of three *A. acidoterrestris* strains in laboratory media by HPH in a pressure range 500-1700 bar was investigated by Bevilacqua, *et al.,* (2007). Their results revealed that up to a 2-log and 0.8-log reduction of vegetative cells and spores, respectively, was achieved, and that resistance of *A. acidoterrestris* to HPH is strain dependent

High Pressure Carbon-di-oxide (HPCD)

One possibility is the application of CO_2 at high pressure (HPCD): the HPCD conservation method provides several advantages because CO_2 is inert, non-toxic, accessible, and affordable. Under ambient conditions it is a gas so it leaves no residue in the treated product, and also it is considered a GRAS solvent. Carbon dioxide is commonly used at supercritical conditions (pressure above 7.4 MPa), where it shows excellent mass transfer properties

Recent studies have shown that this fluid can deactivate most microorganisms, bacteria, yeasts, and molds that cause food spoilage (Garcia-Gonzalez, *et al.,* 2009; Perrut, 2011). Moreover, HPCD has also been proven to deactivate certain enzymes, such as polyphenol oxidase and peroxidase, which cause browning in fruits, vegetables, and juices. Some authors have managed to deactivate endospores of microorganisms of the family *Bacillaceae*, although they had to combine the HPCD treatment with temperatures of around 75–90 °C. In particular, Watanabe et al., (2003) succeeded in deactivating *Geobacillus stearothermophilus* with HPCD at 95 °C and 30 MPa for 120 min. Spilimbergo, *et al.,* (2003) deactivated spores of Bacillus subtilis at 75 °C and 7 MPa for 120 min, and Ballestra and Cuq (1998) deactivated spores of B. subtilis when CO2 was supplied at 90 °C and 5 MPa for 60 min.

However, there are factors in HPCD that could hinder the deactivation of microorganisms in food. First, the matrices are protective environments that could hold back bacterial inactivation. Studies in vegetative forms have shown that higher proportions of lipids and fats decrease the penetration of CO_2. Also, the buffering capacity of the environment affects microbial inactivation (Garcia-Gonzalez et al., 2007). Furukawa et al., (2009) demonstrated the influence of salts and sugars on the inactivation of *G. Stearothermophilus* spores, which both exerted a protective effect in proportion to their concentrations in the solute. Moreover, the feasibility of this method of food preservation depends on the effects of CO_2 on the treated product. Specifically, foods processed using HPCD can suffer acidification, the extraction of volatile compounds or other adverse effects such as loss of vitamin C or changes in color and odor.

Currently, there are few reports in the literature describing the deactivation of A. acidoterrestris spores by HPCD. Bae, *et al.,* (2009) investigated the lethal effect of CO2 on this microorganism suspended in apple juice. Sims and Estigarribia (2002) investigated the effect of CO2 on the deactivation of bacterial flora and A. acidoterrestris in orange juice.

The HPCD treatment is effective in deactivating spores of A. Acidoterrestris in apple cream. This application does not require the use of either a high temperature or pressure since these parameters did not significantly affect the treatment. Therefore, CO_2 can be applied at near ambient temperatures and very moderate pressures (≤10 MPa), i.e., at much milder conditions than required for pasteurization or high hydrostatic pressures, respectively. In this way, the concentration of ***A. Acidoterrestris*** can be reduced by up to 4 logs, a reduction that could be enough since the pollutant load of these endospores does not usually exceed 1000 CFU/g in creams that are used as raw materials in industrial practices. At the same time, CO_2 could kill other spoilage microorganisms and inactivate enzymes that can cause deterioration of the cream.

Conclusion

The use of non thermal approaches to reduce the contamination due *to Alicyclobacilli* could be considered a promising way for the juice industry; however, data refer to laboratory media and/or to experiments performed in a lab scale. A future trend would be the scale up of these results to the industry level, in order to verify if the proposed approaches could be used successfully in real systems and conditions. Another field of great concern is the interaction among the different treatments and with the ingredients of food, an interesting approach for future

researches could be the study of the combinations of some natural compounds with one or two non thermal methods and their effects on food quality, as well as on the real shelf life of the product.

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