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Use of a multivariate approach to assess the incidence of *Alicyclobacillus* spp. in concentrate fruit juices marketed in Argentina: Results of a 14-year survey

Juan Martìn Oteiza [a,*], Gastón Ares [b], Anderson S. Sant'Ana [c], Silvina Soto [a], Leda Giannuzzi [d]

[a] Centro de Investigación y Asistencia Técnica a la Industria Agroalimentaria (CIATI AC), Neuquén, Argentina
[b] Departamento de Ciencia y Tecnología de Alimentos, Facultad de Química, Universidad de la República (UdelaR), Montevideo, Uruguay
[c] Department of Food and Experimental Nutrition, Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo, SP, Brazil
[d] Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), CCT-La Plata, Facultad Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina

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A B S T R A C T

The purpose of this study was to determine the incidence of *Alicyclobacillus* spp. in fruit/vegetable juices (concentrated pulps and clarified and non-clarified juices) marketed in Argentina between 1996 and 2009. The presence of *Alicyclobacillus* was determined in a total of 8556 samples of fruit and vegetable juices (apple, pear, grape, peach, blend of juices, tangerine, pineapple, orange, mango, plum, guava, apricot, lemon, banana, kiwi, carrot, strawberry, grapefruit, and beetroot) collected in seven Argentinean provinces. Multiple factor analysis (MFA) was carried out on a data matrix that contained the percentage of positive samples, type of juice, raw material and production year.

Except for kiwi and orange, *Alicyclobacillus* was found in juices from all the evaluated raw materials. The highest percentage of positive samples was found for beetroot, strawberry, banana, peach, mango, carrot and plum juices. The percentage of positive samples for these juices ranged from 100% to 24%.

Furthermore, the application of multivariate techniques provided an insight on the relationship between the incidence of *Alicyclobacillus* and production variables. This approach enabled the identification of the most relevant variables that increased the percentage of positive samples among the juices, which could help in developing strategies to avoid the incidence of this bacterium.

By means of hierarchical cluster analysis seven groups (clusters) of juices which showed different percentages of positive samples for *Alicyclobacillus* spp. were identified. This analysis showed that pineapple, peach, strawberry, mango and beetroot juices had higher rates of positivity for *Alicyclobacillus* than the rest of the evaluated juices. MFA analysis also showed that some clear relationships could be highlighted between the percentage of samples positive for *Alicyclobacillus* and five types of fruit juices (strawberry, beetroot, grapefruit, pineapple and mango). It was observed that a large proportion of juices produced in 2000, 2005 and 2008 were located in clusters with higher incidence of *Alicyclobacillus* spp., whereas a larger proportion of clarified concentrate juice and concentrate pulp samples showed higher probability of incidence of *Alicyclobacillus* in these products. Data presented in this study brings a contribution to the ecology of *Alicyclobacillus* in fruit/vegetable juices marketed in Argentina. This information would be useful to enhance the microbiological stability of fruit juices regarding the presence of *Alicyclobacillus* spp.

1. Introduction

Alicyclobacillus is a major challenge for fruit juice industries (Tribst et al., 2009). The microorganisms within the *Alicyclobacillus* genus present as main characteristics high chemical resistance (Silva and Gibbs, 2001; Friedrich et al., 2009), acidothermophilic behavior (Spinelli et al., 2009), and high heat resistance (Peña and Massaguer, 2009; Bahçeci and Acar, 2007). These characteristics allow the survival of *Alicyclobacillus* to pasteurization treatments used for processing fruit juices (Spinelli et al., 2010) and its growth under storage conditions, which leads to the spoilage of fruit juices (Spinelli et al., 2009).

The spoilage caused by *Alicyclobacillus* is mainly characterized by smoky and medicinal off-flavors due to the generation of compounds like 2-methoxyphenol (guaiacol), 2,6-dibromophenol and 2,6-dichlorophenol (Siegmund and Pöllinger-Zierler, 2006; Siegmund and Pöllinger-Zierler, 2007; Concina et al., 2010). Currently, more than 15 species have been described within the *Alicyclobacillus* genus (Tribst et al., 2009). However, few of them (*A. acidoterrestris*, *A. pomorum*, *A. herbarius*, *A. acidiphillus*, *A. hesperidum*, *A. cycloheptanicus* and *A. acidocaldarius*) have been shown to spoil fruit juices (Cerny et al.

* Corresponding author at: Calle Chile y Primeros Pobladores, Centenario (8309), Neuquén, Argentina. Tel./fax: +54 299 4899680.
E-mail address: juano@ciati.com.ar (J.M. Oteiza).

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1984; Matsubara et al. 2002; Goto et al. 2003; AIJN, 2007; Smit et al. 2010). Although these seven species may represent a risk for fruit juice spoilage (Smit et al. 2010), most published studies have been focused on the incidence of *A. acidoterrestris*, since this is the species most commonly involved in juice spoilage (AIJN, 2007; Tribst et al., 2009).

The incidence of *A. acidoterrestris* has been reported to be restricted to few types of fruit juices such as apples (Siegmund and Pöllinger-Zierler, 2006; Durak et al., 2010; Steyn et al., 2011), citrus (Durak et al., 2010), blueberry (Durak et al., 2010), mango (Durak et al., 2010), pear (Walls and Chuyate, 2000; Groenewald et al., 2009), tomato (Walls and Chuyate, 2000), passion fruit (McKnight et al., 2010), coconut cream, and grapefruit concentrate (Danyluk et al., 2011).

Argentina has an important international participation in producing, elaborating, and exporting concentrated lemon, grape, pear and apple juices. The country is the second largest exporter of concentrated apple and pear juices in the world and the leading producer and exporter of concentrated lemon and grape juices. Thus, the knowledge about the prevalence of *Alicyclobacillus* spp. in a wide variety of juices is of major importance to add more information on the ecology of the microorganism and on the susceptibility of juices to *Alicyclobacillus* spoilage. Additionally, this information might be useful to design pasteurization processes for new fruit products that guarantee products as shelf-stable as possible.

The worldwide demands with respect to the presence of *Alicyclobacillus* spp. in juices is variable and approximately 60% of importers require their absence for purchasing the product. The detection of *Alicyclobacillus* spp. in a container of fruit juice received in the destination country may result in economic losses, and leads to distrust by buyers to suppliers. Due to the time required for isolation and identification of *Alicyclobacillus* at species level and the limited availability of quick, simple and cost-effective methods for differentiating strains with spoilage potential, analysis during commercialization among industries is carried out mainly based on presence/absence approach of the microorganism at the genus level, i.e., *Alicyclobacillus*. Although it might be considered a conservative policy, presence/absence approach is necessary to overcome the previously mentioned issues in order to speed up the commercialization process. In this context, in this study the incidence of *Alicyclobacillus* spp. in fruit/vegetable juices (concentrated pulp, concentrate clarified and non-clarified juices) marketed in Argentina during 14 years was investigated using a multivariate approach.

2. Material and methods

2.1. Samples

A total of 8556 samples of different types of fruit or vegetable juices (concentrate pulps, clarified concentrate and non-clarified concentrate) were collected in different Argentinean provinces (n = 7) between 1996 and 2009. Clarified concentrate and non-clarified juices were: apple, pear, grape, peach, blend of juices (orange, banana, mango, kiwi and strawberry), tangerine, pineapple, orange, mango, plum, guava, apricot, lemon, banana, kiwi, carrot, strawberry, grapefruit, and beetroot. Concentrate fruit pulps were: plum, peach, pear and apricot. pH and ^{0}Brix values of samples were measured using pH-meter (Selecta, model pH-2005, Spain) and refractometer (Bellingham-Stanley Ltd, model RFM 330+, United Kingdom) and the ranges are presented as a supplementary material. The distribution of samples analyzed through the years is shown in Table 1.

2.2. Juice collection and detection of *Alicyclobacillus* spp.

Juices were collected under aseptic conditions, disposed in pouches or other plastic sterile flasks and transported to the lab under refrigeration. Samples were analyzed for the presence or absence of *Alicyclobacillus* spp. based on the method described by the International Federation of Fruit Juice Producers (IFU, 2007). A 10 ± 0.1 g portion of each fruit juice was aseptically diluted in 90 mL of either BAT (pH 4.0 ± 0.2) or YSG (pH 3.7 ± 0.1) enrichment broths and gently homogenized during 1 min. If necessary, the pH of the media containing the juices was adjusted with HCl/H_2SO_4 or NaOH 1N to the reference values (4.0 or 3.7).

Then, flasks containing juice and culture media were set in a thermostatic controlled water bath (Model Masson 1203-Vicking, Buenos Aires, Argentina) previously adjusted at 80 ± 1 °C. Come-up time was determined and considered in the heat shock. Heat shock was applied for 10 min and the flasks were promptly cooled down to 40–45 °C in an ice-water bath and incubated at 45 ± 1 °C for 5 days. Then, a loop of enriched samples was streaked either onto BAT (4.0 ± 0.2) (Merck, Darmstadt, Germany) or YSG (pH = 3.7 ± 0.1) agar plates. BAT/YSG agar plates were incubated at 45 ± 1 °C for 2–5 days and checked for the presence of typical colonies of *Alicyclobacillus* and at least 5 colonies were selected for further confirmation. Macroscopically the isolates were characterized according to their shape, size and color, while microscopically; they were characterized according to the shape of the cells, size and spore formation with an optical microscope (Leica DM750, Swiss).

After this, the isolated colonies were streaked onto a neutral pH medium (Plate Count Agar) (Merck, Darmstadt, Germany), incubated for 2 days at 45 °C and for 4 days at 25 °C.

Moreover, the isolates were further tested for growth in *Sulfobacillus* broth medium (pH 2.0) (DSMZ, 2007) to differentiate between *Alicyclobacillus* and *Sulfobacillus* (Parish and Goodrich, 2005). The presence of at least one colony of gram-positive (or gram variable), spore-forming rods on BAT/YSG incubated aerobically at 45 °C, coupled with the lack of growth on PCA at 45 and 25 °C and the lack of growth in *Sulfobacillus* medium was recorded as positive for *Alicyclobacillus* spp. In all the other cases the results were reported as negative for *Alicyclobacillus* spp.

2.3. Statistical analysis

The average percentage of positive samples was determined for each raw material.

Multiple factor analysis (MFA) was carried out in order to study the relationship between the incidence of *Alicyclobacillus* and juice characteristics. This analysis was performed on a data matrix that contained the percentage of positive samples, type of juice (qualitative variable indicating if clarified concentrate juice, non-clarified concentrate juice or concentrate pulp was considered), raw material (qualitative variable which indicated if the juice was from apple, pear, grape, peach, tangerine, pineapple, orange, mango, plum, guava, apricot, lemon, banana, kiwi, carrot, strawberry, grapefruit, beetroot, or blend of fruits) and production year (qualitative variable).

In order to identify groups of juices with similar characteristics and incidence of *Alicyclobacillus*, hierarchical cluster analysis was performed on sample coordinates in the first five dimensions of the MFA considering Euclidean distances and Ward agglomeration criterion. Statistical analyses were carried out in R language (R Development Core Team, 2007). Multiple factor analysis was performed using FactoMineR (Husson et al., 2007; Lê et al., 2008).

3. Results and discussion

This is the first throughout report on the incidence of *Alicyclobacillus* spp. in fruit and vegetable juices marketed in Argentina. Table 1 shows the number and types of juices collected during 14 years in Argentina and analyzed for presence of this bacterium.

As shown in Table 2, except for kiwi and orange, *Alicyclobacillus* spp. was found in juices from all the evaluated raw materials. Therefore, the increasing consumption of mixed beverages made of different types of vegetables and fruits reinforces the need to strengthen measures to ensure shelf-stable products regarding *Alicyclobacillus* (Tribst et al., 2009).

To our knowledge there is no data in the literature describing the presence of *Alicyclobacillus* in banana, apricot, peach, grape, guava and kiwi juices, which are becoming more popular and much appreciated among consumers. Therefore, this might be the first study reporting the contamination of these juices with *Alicyclobacillus*.

The highest percentage of positive samples was found for beetroot and strawberry juices, with 100% and 83% of positive samples respectively. The high incidence of *Alicyclobacillus* spp. in these juices may be related to the direct contact of these vegetable/fruit with soil during cultivation period. The role of soil as source of spores of *Alicyclobacillus* has been well described in the literature (Pinhatti et al., 1997; Groenewald et al., 2009). It is important to take into account that a low number of juice samples were analyzed for these raw materials taken (2 for beetroot and 6 for strawberry). However, taking into consideration the close contact of these materials with soil, it is reasonable to expect a high incidence of *Alicyclobacillus* in these juices even with an increase in the number of samples collected. Apart from beetroot and strawberry, there were other raw materials that showed a high percentage of positive samples throughout the 14 year survey. The percentage of positive samples for banana, peach, mango, carrot and plum juices ranged from 33% to 24% (Table 2). The incidence of *Alicyclobacillus* spp. in mango juices has been already reported in the literature (Gouws et al., 2005; Durak et al., 2010; Danyluk et al., 2011). Some practices during cultivation of mangoes may explain the large positivity rate for *Alicyclobacillus* spp. found in these juices. For example, harvest of mangoes when they fell to the ground (as an indication of the best point of maturation) may make these fruits more susceptible to contamination by *Alicyclobacillus* spp.

Table 2
Number of analyzed samples from 1996 to 2009 for each raw material and percentage of positive samples for *Alicyclobacillus* spp.

Raw material	Number of samples	Percentage of positive samples (%)
Apple	5287	11.4
Pear	1624	18.4
Grape	643	15.1
Blend	127	22.0
Peach	309	29.4
Orange	102	0.0
Tangerine	89	1.1
Lemon	109	7.3
Pineapple	34	14.7
Mango	31	29.0
Grapefruit	31	9.7
Plum	34	23.5
Apricot	98	9.2
Guava	15	20.0
Carrot	8	25.0
Strawberry	6	83.3
Banana	6	33.3
Beetroot	2	100.0
Kiwi	1	0.0

When thinking on the microbial stability of fruit juices and the presence of *Alicylobacillus*, it can be clearly noticed that concerns with the presence of this bacterium have been mainly focused on orange and apple juices. As these juices account for most of shipped juices in the world, countless losses to the producers may result due to spoilage caused by *Alicylobacillus*. However, in this study only 11.4% of the apple juice samples were positive, whereas the microorganism was not found in any of the 102 samples of orange juice collected. Moreover,

Table 1
Fruit/vegetable juices analyzed for the presence of *Alicyclobacillus* spp. in Argentina (1996–2009).

Year	Concentrated juices											
	Apple	Pear	Grape	Blend	Peach	Lemon	Orange	Tangerine	Pineapple	Mango	Grapefruit	Plum
1996	45	9	–	–	–	–	–	–	–	–	–	–
1997	58	6	–	–	–	–	–	–	–	–	–	–
1998	98	59	–	–	–	–	–	–	–	–	–	–
1999	74	34	1	–	–	–	–	–	–	–	–	–
2000	156	78	19	–	–	–	–	–	–	–	–	–
2001	586	99	8	–	–	24	61	–	–	–	–	–
2002	391	107	17	–	–	–	–	–	–	–	–	–
2003	514	116	9	–	–	–	–	–	–	–	–	–
2004	443	118	32	–	–	14	–	9	–	–	–	–
2005	874	321	85	–	13	2	4	12	1	–	2	7
2006	640	193	50	3	25	3	–	5	–	2	6	7
2007	523	104	95	25	32	5	4	23	18	16	23	6
2008	266	122	250	67	20	–	28	26	7	9	–	3
2009	427	146	77	32	17	56	5	14	8	4	–	2
Subtotal	5095	1512	643	127	107	104	102	89	34	31	31	25

Year	Concentrated juices							Concentrated pulps					
	Apricot	Guava	Carrot	Strawberry	Banana	Beetroot	Kiwi	Peach	Apple	Pear	Apricot	Plum	Lemon
1996	–	–	–	–	–	–	–	–	–	–	–	–	–
1997	–	–	–	–	–	–	–	–	–	–	–	–	–
1998	–	–	–	–	–	–	–	–	–	–	–	–	–
1999	–	–	–	–	–	–	–	–	–	–	–	–	–
2000	–	–	1	–	–	2	–	7	–	–	1	–	–
2001	–	–	–	–	–	–	–	–	–	–	–	–	–
2002	–	–	–	–	–	–	–	4	–	–	–	–	–
2003	–	–	1	–	–	–	–	31	–	3	35	–	1
2004	2	–	–	–	–	–	–	23	–	21	19	5	4
2005	5	–	3	–	–	–	–	35	7	40	14	2	–
2006	2	4	2	–	2	–	–	16	5	10	2	2	–
2007	1	6	–	4	3	–	–	36	180	6	–	–	–
2008	4	3	–	2	1	–	1	24	–	13	1	–	–
2009	2	2	1	–	–	–	–	26	–	19	10	–	–
Subtotal	16	15	8	6	6	2	1	202	192	112	82	9	5
Total number of analyzed samples = 8556													

in spite of the fact, that incidence *Alicyclobacillus* spp. has also been reported in pineapple juices (Gouws et al., 2005; Durak et al., 2010; Danyluk et al., 2011), mainly attributed to cultivation of these fruits close to soil and difficulties in washing and disinfection steps due to their rough surface. However, in the present study only 14.7% of the analyzed pineapple juice samples were positive.

The low incidence of *Alicyclobacillus* spp. in apple, orange and pineapple juices may indicate the adoption of good agricultural and manufacturing practices by Argentinean juice producers. Due to the amount of data, a multivariate approach has been used to study the relationship between the presence of *Alicyclobacillus* and characteristics of the juices. Multiple factor analysis (MFA) is a factor analysis technique that enables dealing with a mixture of different types of data sets, including quantitative variables, frequency tables and qualitative data (Bécue-Bertaut and Pagès, 2008; Bécue-Bertaut et al., 2008). This analysis provides an overview of the relationship between the evaluated variables and between the variables and the samples, and can also be used as a preprocessing step for clustering procedures.

Fig. 1 shows the representation of the variables in the first two dimensions of the MFA, which enables the identification of relationships between the incidence of *Alicyclobacillus* and the juice samples characteristics. The first two dimensions of the MFA explained 13.3% of the variance of the experimental data. Despite this percentage might seem low it is important to take into account that in factor analysis techniques, such as correspondence analysis, applied to large sets of qualitative variables necessarily provide axis that explain a small proportion of the inertia due to coding (for categorical variables) and to specificity of textual data (in the case of frequency variables) (Lebart et al., 2000).

MFA analysis has shown that some clear relationships could be highlighted between the percentage of samples positive for *Alicyclobacillus* and samples characteristics. First of all, the percentage of samples positive for *Alicyclobacillus* seemed to be correlated to four types of raw materials, i.e., strawberry, beetroot, pineapple and mango (Fig. 1), in agreement with results from Table 2.

In addition to raw material, through MFA relationships between production year and percentage of contamination by *Alicyclobacillus* were also found. It was clearly observed that the qualitative variable indicating production year 2005 was positively correlated to the percentage of positive samples. This suggests that in 2005 the highest incidence of *Alicyclobacillus* was registered, reaching the percentage of positive samples among the analyzed juices 26.6%.

In 2005 several juices were found to have high prevalence of *Alicyclobacillus* spp., i.e., pear (46%), peach (54%) and plum (86%). It is known that except for blend of juices (which are mixed and not pasteurized as they were previously pasteurized) and lemon, orange, grapefruit and tangerine juices (which were pasteurized in temperature conditions varying between 79 and 98 °C/20–45 s), all the other juices are submitted to higher time/temperature conditions (95–132 °C/5–207 s). This heat treatment should be enough to destroy spores of *Alicyclobacillus* spp., according to data available in the literature (Bahçeci and Acar, 2007; Spinelli et al., 2009; Bevilacqua et al., 2010). However, variations in pH and °Brix content (see supplementary material) are known to affect the heat resistance of this bacterium (Silva et al., 1999; Bahçeci and Acar, 2007; Maldonado et al., 2008; Ceviz et al., 2009), as the increase in Brix makes the spores more resistant, while decreases in pH results in reduction of thermal resistance. It is known that pH and °Brix together with variations in the field and common variations occurring during pasteurization process (Sant'Ana et al., 2009) may explain differences among different years.

Regarding the type of juice, no clear relationship was found between this variable and the percentage of positive samples for *Alicyclobacillus* (Fig. 1). This suggests that this production variable did not clearly affect the incidence of *Alicyclobacillus*. In order to identify groups of juices with similar characteristics a hierarchical cluster analysis was carried out on the samples coordinates for the first five dimensions of the MFA. This analysis enabled to simultaneously consider most of the original information from qualitative and quantitative variables. The dendrogram (not shown) indicated that the

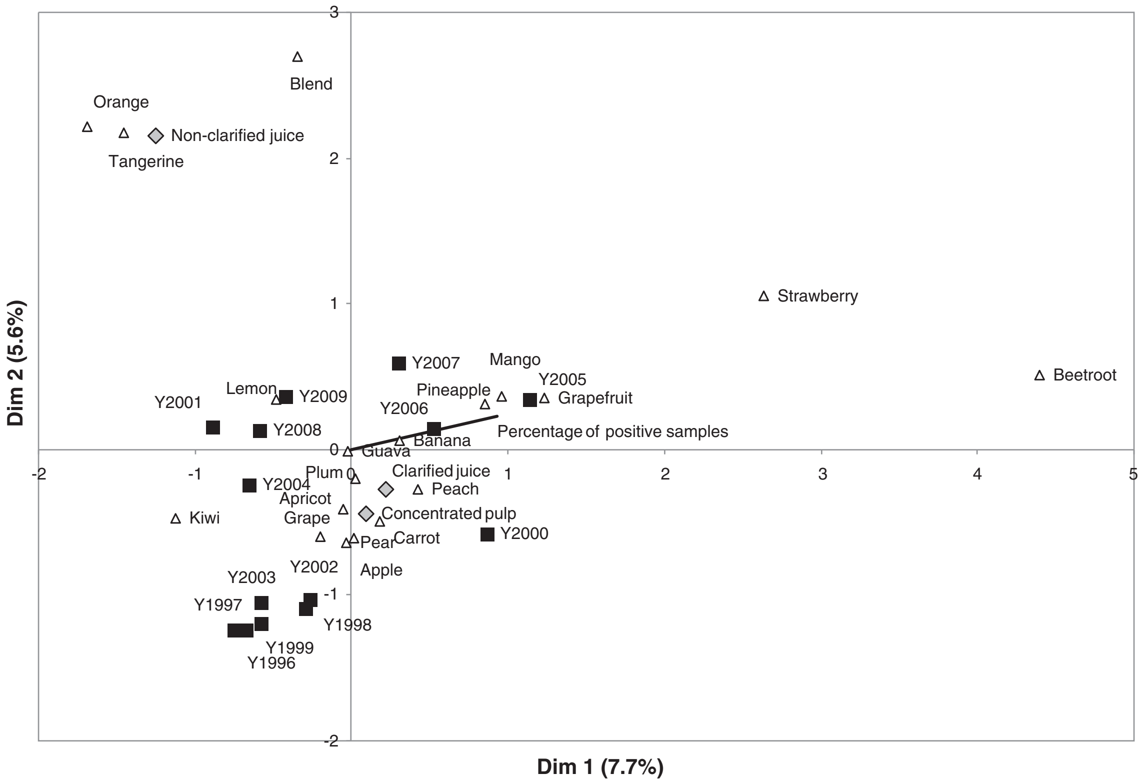

Fig. 1. Representation of the percentage of positive samples and juice characteristics in the first two dimensions of the Multiple Factor Analysis.

Table 3
Percentage of juices in each cluster and average percentage of positive samples for *Alicyclobacillus* spp.

Cluster	Percentage of juices (%)	Percentage of positive samples (%)
1	28.7	13.2
2	9.4	5.7
3	15.1	63.5
4	5.0	27.0
5	12.9	2.6
6	12.9	7.5
7	16.0	17.4

juices evaluated for the presence of *Alicyclobacillus* spp. could be separated into seven groups or clusters based on average percentage of positive samples. As shown in Table 3, the evaluated juices were homogeneously distributed among the seven clusters. Clusters 1 and 4 were the largest and smallest respectively. Samples within cluster 3 showed the highest average positivity for *Alicyclobacillus* (63.5%).

The analysis of the groups provided an insight on the relationship between the percentage of positive samples for *Alicyclobacillus* spp. and juice characteristics. Results from this analysis were similar to those obtained from MFA. As shown in Table 4, a large proportion of pineapple, peach, strawberry, mango and beetroot juices belonged to clusters 3, 4 and 7, suggesting that these juices had higher rates of positivity for *Alicyclobacillus* than the rest of the evaluated juices. These results are similar to those obtained from Table 1 and Fig. 1. It was observed that 66.7, 50 and 38.1% of grape, apple and pear juices collected, respectively were grouped in cluster 1. These three juices represented together approximately 87% of clarified concentrate samples analyzed in this study (Table 1). The average percentage of contamination by *Alicyclobacillus* spp. for these juices could be considered low (approximately 13%) (Table 3).

Furthermore, a large proportion of juices produced in 2005 were located in cluster 3, indicating a higher incidence of *Alicyclobacillus* spp. (Table 5), indicating a high prevalence of this bacterium in different types of juices during those years. During this period of time (between 2004 and 2006), fruit processors focused their attention on calibrating pasteurization conditions to neutralize the presence of *Alicyclobacillus* spp., which culminated with the increase of pasteurization temperature from 100 °C, 15 s to 132 °C, 5 s, depending on other variables such as

Table 4
Distribution (%) of juices in the clusters according to type of fruits.

Type of fruit	Cluster						
	1	2	3	4	5	6	7
Pineapple	0	0	25.0	0	0	0	75.0
Banana	0	0	0	0	0	0	100
Plum	37.5	0	12.5	0	37.5	0	12.5
Apricot	7.7	0	30.8	7.7	46.2	0	7.7
Peach	14.3	0	42.9	7.1	28.6	0	7.1
Strawberry	0	0	50.0	0	0	0	50.0
Guava	0	0	0	0	0	0	100
Kiwi	0	0	0	0	0	0	100
Lemon	27.3	9.1	18.2	0	18.2	27.3	0
Tangerine	0	0	0	0	0	100	0
Mango	0	0	0	0	0	0	100
Apple	50.0	27.8	11.1	5.6	0	0	5.6
Blend	0	0	0	0	0	100	0
Orange	0	0	0	0	0	100	0
Pear	38.1	23.8	14.3	4.76	14.3	0	4.7
Grapefruit	66.7	0	33.3	0	0	0	0
Beetroot	0	0	0	100	0	0	0
Grape	66.7	16.7	0	8.3	0	0	8.3
Carrot	80.0	0	0	20.0	0	0	0

Table 5
Distribution (%) of the juices in the clusters according to their production year.

Year	Clusters						
	1	2	3	4	5	6	7
Y1996	0	100	0	0	0	0	0
Y1997	0	100	0	0	0	0	0
Y1998	0	100	0	0	0	0	0
Y1999	0	100	0	0	0	0	0
Y2000	0	0	0	100	0	0	0
Y2001	0	80	0	0	0	20	0
Y2002	75.0	0	0	0	25.0	0	0
Y2003	50	0	0	0	50	0	0
Y2004	30.8	0	15.4	0	38.5	15.4	0
Y2005	27.8	0	55.6	0	5.6	11.1	0
Y2006	42.1	0	21.1	0	10.5	10.5	15.8
Y2007	40	0	20	0	0	20.0	20.0
Y2008	5.6	0	0	0	11.1	16.7	66.7
Y2009	38.9	0	5.6	0	16.7	22.2	16.7

pH, fiber content, acidity, sugar concentration and where the fruit come from.

Table 6 shows no clear relationship between proportion of clarified concentrate juice and concentrate pulp samples located in clusters 3 and 4, indicating higher probability of incidence of *Alicyclobacillus* in these products. However, as previously mentioned no clear relationship between type of juice and the incidence of *Alicyclobacillus* could be concluded from the present study.

4. Conclusions

Data presented in this study brings a contribution to the ecology of *Alicyclobacillus* in fruit/vegetable juices marketed in Argentina. It is known that the simple presence of *Alicyclobacillus* in juice may not result in spoilage since not all strains have potential to spoil juices (Tribst et al., 2009; Chang and Kang, 2004). However, with the development of beverages mixing fruits and vegetables (Tribst et al., 2009) and with the introduction of new types of fruits or vegetables in the market, knowledge of prevalence of *Alicyclobacillus* in these matrixes can be considered the first step to develop methods to ensure their microbial stability during shelf-life. Further research is needed to study the behavior (growth, survival or inactivation) of *Alicyclobacillus* in the juices which showed the highest prevalence of this bacterium. This information will be useful to enhance the microbiological status of fruit juices regarding *Alicyclobacillus* and to ensure shelf-stable products.

Furthermore, the application of multivariate techniques, such as multiple factor analysis and hierarchical cluster analysis, provided an insight on the relationship between the incidence of *Alicyclobacillus* and production variables. This approach enabled the identification of the most relevant variables that increased the percentage of positive samples among the juices, which could help developing strategies to avoid the incidence of this bacterium.

Table 6
Distribution (%) of the juices in the clusters according to types of juice.

Type of juice	Clusters						
	1	2	3	4	5	6	7
Clarified concentrate juice	42.2	14.4	12.2	5.6	1.1	0	24.4
Non-clarified juice	0	0	0	0	0	100	0
Concentrated pulp	6.5	0	32.3	6.5	54.8	0	0

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.ijfoodmicro.2011.09.004.

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