Food Control 99 (2019) 146–157

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Review

A review of innovative techniques for rapid detection and enrichment of *Alicyclobacillus* during industrial processing of fruit juices and concentrates

Babasola Adewunmi Osopale[a], Gbenga Adedeji Adewumi[b], Regina Corli Witthuhn[c], Oluwasegun Olalekan Kuloyo[c], Folarin Anthony Oguntoyinbo[b,d,*]

[a] Department of Microbiology, School of Science and Technology, Babcock University, Ilishan-Remo, Ogun State, Nigeria
[b] Department of Microbiology, Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria
[c] Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, Nelson Mandela Drive, Bloemfontein, South Africa
[d] A.R. Smith Department of Chemistry and Fermentation Sciences, Appalachian State University, Boone, NC, 28608, USA

ARTICLE INFO

Keywords:
Alicyclobacillus
Fruit juice
Spoilage
Detection
Rapid enrichment
Quality control

ABSTRACT

Alicyclobacillus genus includes thermo-acidophilic, endospore-forming bacteria that cause spoilage of pasteurized fruit juice through formation of phenolic off-flavours. Effective quality control during fruit juice processing is a prerequisite and early detection of *Alicyclobacillus* in raw materials and finished products can guarantee product quality and longer shelf life. Highly sensitive, cost-effective traditional microbiological methods for *Alicyclobacillus* detection are often too slow in achieving results, therefore alternative culture-independent approaches have been proposed for rapid detection of *Alicyclobacillus* contamination in industrial production line. In this review, the various innovative techniques which have been exploited for rapid detection of *Alicyclobacillus* in contaminated juices and concentrates were appraised. The techniques were discussed under three analytical approaches, including (i) cells/spores-based detection (spectroscopy and immunoassays), (ii) nucleic acid-based detection (amplification and non-amplification methods), and (iii) metabolites-based detection (detection of specific phenolic off-flavours, assessment of total volatile profile of sample or detection of non-volatile metabolites). The sensitivity and rapidity of individual technique were highlighted as well as their drawbacks and recommendations for optimization. The combination of these innovative detection techniques with immunomagnetic separation and DNA-aptamer binding, as to achieve better assay sensitivity and rapidity are also discussed.

1. Introduction

Commercial fruit juices have low pH that inhibits most pathogenic and spoilage-causing microorganisms, which are further inactivated by heat-processing of the final product. However, species of the bacterial genus *Alicyclobacillus* are capable of overcoming these hurdles by forming thermo-acidophilic endospores, which survive fruit juice pasteurization and subsequently germinate in acidic juice matrix (Cerny, Hennlich, & Porolla, 1984; Simpson, Amon, & Daw, 1986; Zhang, Yue, & Yuan, 2013). *Alicyclobacillus* are non-pathogenic, but due to their ability to form phenolic taints by metabolizing natural juice components, they cause flat-sour spoilage in fruit juices and other acidic beverages. This has led to significant research interest and need for industrial control and monitoring (Cerny et al., 1984; Walls & Chuyate, 2000). Historically, *Alicyclobacillus*-induced spoilage was first reported in Germany during summer of 1982, when flat-sour spoilage was observed in pasteurized shelf-stable apple juice products (Cerny et al., 1984). The spoiled products were described as having 'medicinal', 'phenolic' or 'antiseptic' off-odour and in subsequent years, similar spoilage cases were reported in several other fruit juice varieties in different parts of the world, leading to consumer rejection of products, poor reputation and economic loss for the fruit juice manufacturers (Chang & Kang, 2004). *Alicyclobacillus* species have wide pH (1.5–6.5) and soluble solid content (5.4–16.2 °Brix) growth ranges and this attribute makes them persist and metabolize in a variety of juice and beverages (Karavaiko et al., 2005; Splittstoesser, Churey, & Lee, 1994).

In most cases, *Alicyclobacillus*-induced fruit juice spoilage is difficult to detect visually due to minimal turbidity formation (Walls & Chuyate, 2000). Rather, spoilage is mostly recognized as off-flavours in the odour and taste of spoiled product. The chemical compounds responsible for the phenolic off-flavours have been identified as 2-methoxyphenol (guaiacol) and the halophenols: 2, 6-dibromophenol (2, 6-DBP) and 2,

* Corresponding author. A.R. Smith Department of Chemistry and Fermentation Sciences, Appalachian State University, Boone, NC, 28608, USA.
E-mail address: oguntoyinbofa@appstate.edu (F.A. Oguntoyinbo).

https://doi.org/10.1016/j.foodcont.2018.12.032
Received 7 September 2018; Received in revised form 20 December 2018; Accepted 22 December 2018

6-dichlorophenol (2, 6-DCP) (Baumgart, Husemann, & Schmidt, 1997; Borlinghaus & Engel, 1997; Pettipher, Osmundson, & Murphy, 1997; Whitfield, 1998). Guaiacol, which is currently regarded as the predominant taint in *Alicyclobacillus*-spoiled products, is a volatile organic compound (VOC) produced by the non-oxidative decarboxylation of vanillic acid and other natural fruit juice components (Jensen & Whitfield, 2003; Witthuhn, Smit, Caneron, & Venter, 2011).

Several *Alicyclobacillus* species are of industrial significance either because they were isolated from tainted fruit juice or have demonstrated the ability for *in vitro* formation of guaiacol, however, *Alicyclobacillus acidoterrestris* is currently recognized as the target species for industrial quality control, due to its higher rate of isolation from spoiled products and its enhanced ability for quaiacol formation (Danyluk et al., 2011; Durak, Churey, Danyluk, & Worobo, 2010). *A. acidocaldarius* is equally common industrially and environmentally but it does not form guaiacol (Goto, Tanaka, Yamamoto, & Tokuda, 2007). *Alicyclobacillus* contamination may occur at any stage of juice processing, including raw materials and processing environment (Danyluk et al., 2011; Groenewald, Gouws, & Witthuhn, 2009). It is now recognized that *Alicyclobacillus* contamination is inevitable in juice products and elimination of these contaminants is a challenge for the industry, both in developed and developing countries (Osopale, Witthuhn, Albertyn, & Oguntoyinbo, 2016; Witthuhn et al., 2011).

The major challenge posed by *Alicyclobacillus* is the high heat resistance of their endospores, which is attributed to the ω-alicyclic fatty acids present in their cell wall membrane (Daron, 1970; Kannenberg, Blume, & Poralla, 1984). This thermo-tolerance enables the bacteria to survive commercial fruit juice pasteurization and hot-fill processes (i.e. heating of fruit juices at 90–95 °C for 15–20 s followed by immediate cooling to 82–84 °C before filling into package) (Spinelli, Sant'Ana, Rodrigues-Junior, & Massaguer, 2009). Rather than killing, commercial pasteurization induces germination and outgrowth of dormant *Alicyclobacillus* spores (Pettipher et al., 1997). Although more intensive heat processing such as the ultra temperature treatments can completely inactivate *Alicyclobacillus* spores, such treatments may adversely impact the organoleptic and nutritional attributes of products, while increasing energy costs for manufacturers, especially in resource limited countries (Palop, Alvarez, Razo, & Condon, 2000; Spinelli et al., 2009; Walls & Chuyate, 1998). Consequently, alternative non-thermal strategies such as inclusion of natural preservatives in fruit juice, application of chemical disinfectants for cleaning fruits and production surfaces and physical control measures such as irradiation and high hydrostatic pressure have been exploited for the inactivation of *Alicyclobacillus* (Bevilacqua, Corbo, & Sinigaglia, 2010, 2008; Danyluk et al., 2011; Grande et al., 2005; Komitopoulou, Boziaris, Davies, Delves-Broughton, & Adams, 1999; Lee, Gray, Dougherty, & Kang, 2004; Maldonado, Aban, & Navarro, 2013; Nakauma, Saito, Katayama, Tada, & Todoriki, 2004; Orr & Beuchat, 2000; Osopale, Witthuhn, Albertyn, & Oguntoyinbo, 2017; Pei, Yue, & Yuan, 2014; Torlak, 2014; Tremarin, Brand, & Silva, 2017; Walker & Phillips, 2005; Yamazaki, Murakami, Kawai, Inoue, & Matsuda, 2000).

For effective quality control against *Alicyclobacillus*, it is crucial for juice processors to adopt rapid, sensitive and accurate methods for early detection of contamination and prompt intervention in the production line. The traditional microbiological methods for *Alicyclobacillus* detection generally involve selective enrichment of samples in acidified broth medium for 2–5 days followed by plating on acidified agar medium (Bevilacqua, Sinigaglia, & Corbo, 2008; Deinhard, Blanz, Poralla, & Altan, 1987; Goto et al., 2002a; Hiraishi et al., 1997; Walls & Chuyate, 1998; Yamazaki, Teduka, & Shinano, 1996a). Heat-shock treatment may be applied to samples to activate dormant spores (Pettipher et al., 1997), while membrane filtration of samples is often recommended for improved sensitivity (Splittstoesser et al., 1994). These traditional plating methods are highly sensitive [detecting as low as one colony forming unit per milliliter (CFU/mL) of *Alicyclobacillus*], cost-effective and mostly less-technical to perform. However, they are generally too slow (2–7 days analysis time) in achieving conclusive results, thus are not very effective for routine monitoring of *Alicyclobacillus* contamination in the production line. Therefore, as consumers demand for safe and good quality food increases, rapid and equally sensitive *Alicyclobacillus* detection techniques are desirable in the industry.

2. Innovative techniques for *Alicyclobacillus* detection in fruit juices and concentrates

Sensitivity (the ability to detect target microorganism at low contamination level), specificity (the ability to selectively detect target microorganism without cross-reaction with other species) and rapidity (the ability to give fast and reliable result), are some of the key attributes of a microbial detection system (Mandal, Biswas, Choi, & Pal, 2011). It is not uncommon for target bacteria to occur at low population in natural environments, including food. Thus, it is often necessary to subject analytical samples to pre-enrichment steps in order to increase the population of target bacteria before detection. Previous reviews highlighted various culture-dependent and independent methods for rapid detection of *Alicyclobacillus*, including flow-cytometry, polymerase chain reactions (PCRs), enzyme linked immunosorbent assay (ELISA) and metabolite-based detection (Chang & Kang, 2004; Huang, Yuana, Guoa, Gekasb, & Yuea, 2014; Steyn, Cameron, Venter, & Witthuhn, 2011; Walker & Phillips, 2008). However, none of these emphasized the role of innovative enrichment procedures in shortening analytical time, and improving assay sensitivity and specificity. Moreover, newer techniques have been reported since these reviews were published. Therefore, the current review aims to provide a different perspective on the previously reported innovative *Alicyclobacillus* detection techniques, appraise newer techniques and discuss the inclusion of innovative enrichment procedures for the improvement of sensitivity, specificity and rapidity of *Alicyclobcillus* detection methods.

The innovative techniques for *Alicyclobaciullus* detection have been applied majorly under three analytical strategies including; (i) cells/spores-based detection, (ii) nucleic acid analyses, and (iii) measurement of *Alicyclobacillus* metabolites. An overview of the analytical steps involved in the various innovative techniques is described in Fig. 1. The rapidity and sensitivity of each technique are presented in Table 1.

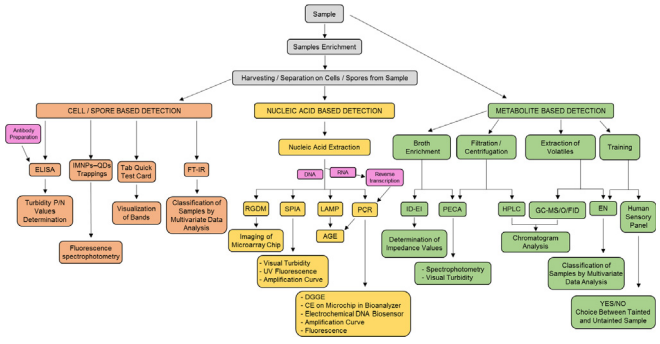

Fig. 1. Flow chart of innovative techniques for *Alicyclobacillus* detection in fruit juice and concentrate. ELISA-enzyme linked immunosorbent assay; IMNPs-QDs-immunomagnetic nanoparticles quantum dots; FT-IR-fourier transform infrared; RGDM-random genomic DNA microarray; SPIA-single primer isothermal amplification; LAMP- loop mediated isothermal amplification; AGE-agarose gel electrophoresis; DGGE-denaturing gradient gel electrophoresis; CE- Capillary electrophoresis; PCR-polymerase chain reaction; ID-EI- indirect electrical impedance; PECA-peroxide enzyme colorimetric assay; HPLC- high performance liquid chromatography; GC-MS/O/FID-gas chromatography-mass spectrometry/olfactometry/flame ionization detector; EN- electronic nose; P/N- positive/negative.

Table 1
Rapidity and detection limits of selected innovative techniques applied for *Alicyclobacillus* detection in fruit juices and related samples.

Analytical approach	Technique	Detection Medium	Limit of Detection (CFU/mL)	Detection Time (rapidity)	Agreement with SPTs	Reference
Cells/spores based detection	FT-IR spectroscopy coupled with multivariate analysis	Apple juice	10^3–10^7	> 2 days	Not reported	Al-Qadiri et al., 2006; Wang et al., 2011
	ID-ELISA	Apple juice	10^5	6–7 h*	Yes	Wang et al. (2012)
	SPA-ELISA	Apple juice concentrate	10^5	> 12 h	Yes	Li et al. (2013)
	DAS-ELISA	Apple juice concentrate	10^3	> 12 h	Yes	Li et al. (2014)
	IMS-ELISA	Apple juice	10^3	3 h*	Yes	Wang et al., 2013a; Wang et al., 2013b
	IMS-QDS-fluorescence	Apple juice	10^4	1.5 h	Not reported	Wang et al. (2018)
	TAB quick test card	Apple juice	10^7	5–10 min*	Yes	Liu et al. (2017)
Nucleic acid based detection	RT-PCR-AGE	MBA medium	10^4	Not reported	Yes	Yamazaki et al. (1996b)
	TaqMan® Real-time PCR	Apple juice, orange juice, lemonade, sport drink	10^2	3–5 h*	Yes	Connor et al., 2005; Luo et al., 2004
	Aptamer enrichment coupled with Real-time PCR	Distilled water	2.8×10^{-1}	Not reported	Yes	Hünniger et al. (2015b)
	RT-PCR coupled with DNA Bio-analyzer	Orange juice	2	Not reported	Yes	Funes-Haucca et al., 2004
	Nested RT-PCR coupled with electrochemical conduction in a DNA Biosensor	Sterile acid buffer	2	Not reported	Not reported	Eguiluz et al. (2008)
	PCR–DGGE	Orange juice, pineapple juice, mixed fruit juice	Not reported	Not reported	Yes	Osopale et al. (2016)
	SPIA-SYBR Green II coupled with UV fluorescence detection	Apple juice	6.1×10^1	2–3 h*	Yes	Yang et al. (2017)
	SPIA-SYBR Green II coupled with amplicon detection by visual turbidity	Apple juice	6.1×10^2	2–3 h*	Yes	Yang et al. (2017)
	LAMP-AGE	Apple juice	2.25×10^1	2 h*	Yes	Chen et al. (2011)
	IMS enrichment coupled with PCR-AGE	Apple juice	2×10^1	3–4 h	Yes	Wang et al. (2013c)
	IMS enrichment coupled with TaqMan® PCR	Apple juice	< 10	2–3 h	Yes	Wang et al. (2014)
	IMS enrichment coupled with SYBR Green I real-time PCR	Kiwi juice	2.8×10^1	Not reported	Yes	Cai et al. (2015)
	Random genomic DNA Microarray (RGDM)	Orange juice	2×10^3	Not reported	Not reported	Jang et al. (2011)
Metabolite based detection	Human odour/aroma panel	Apple juice, Orange juice	$\geq 10^4$	Not applicable	Yes	Orr et al., 2000; Pettipher et al., 1997;
	HS-SPME-GC MS-SIM at m/z 109/124		$\geq 10^4$	Not reported	Yes	Bianchi et al., 2010; Pérez-Cacho et al., 2011;
	HPLC	Apple juice	$\geq 10^4$	Not reported	Yes	Witthuhn et al. (2012)
	Electronic Nose with Multivariate data analysis	Apple juice, Orange & pear juices	10^{2a} - 10^{3b}	30 min*	Yes	Huang et al., 2015b; Cagnasso et al., 2010
	Guaiacol detection kit based on Peroxidase enzyme colorimetric assay (PECA)	Not specified	10^1	5–24 h*	Not reported	Niwa (2003)
	Indirect electrical impedance (ID-EI)	Concentrated apple juice	10^0	28 h	Yes	Fernández et al. (2017)

*-Detection time excludes duration for sample pre-enrichment in broth medium.
SPTs- Standard plating techniques.
[a] Orange and peer juice.
[b] Apple juice.

2.1. Cells/spores-based detection

This strategy involves the analysis of intact *Alicyclobacillus* cell or spore contaminants in products. Two major classes of techniques have been employed under this detection strategy. They include spectroscopy and immunoassay.

2.1.1. Spectroscopy

Fourier transform infrared (FT-IR) spectroscopy, a technique based on the measurement of infrared absorbance spectra of bacterial cell components has been explored for detection and identification of *Alicyclobacillus* in fruit juices. The attractions for FT-IR spectroscopy as an *Alicyclobacillus* detection technique lie in the simplicity of sample preparation and the ability to obtain chemical-based classification of bacteria without cell disruption. In the studies that reported FT-IR spectroscopy for *Alicyclobacillus* detection, bacterial cells were simply harvested from samples, washed in buffered solutions and applied to a membrane filter where the cells' FT-IR spectra were recorded directly in a spectrometer (Al-Qadiri, Lin, Cavinato, & Rasco, 2006; Lin et al., 2005; Wang et al., 2011). The obtained infrared spectra were then subjected to multivariate data analysis for comparison and classification of the bacterial species. Based on spectra variations of glycoproteins, extrapolysaccharides and peptidoglycan layer, FT-IR spectroscopy effectively separated closely-related *A. acidoterrestris* strains and differentiated *Alicyclobacillus* from *Escherichia coli* O157:H7 in apple juice (Al-Quadri et al., 2006; Grasso, Yousef, Castellvi, & Rodriguez-Saona, 2009; Lin et al., 2005). Factors such as cells concentration and distribution on membrane filters used for spectra measurement influenced FT-IR sensitivity, i.e. the larger the cell concentration and surface area covered on the filter unit, the lower the interference of the filter paper with the bacterial infrared spectra which results in better and reproducible spectra data. Al-Qadiri et al. (2006) and Wang et al. (2011) reported that the optimized FT-IR data was obtained when *Alicyclobacillus* concentrations ranged between 10^3 - 10^7 CFU/mL. To achieve these cell concentrations, Wang et al. (2013a) recommended a 24–48 h sample pre-enrichment. This enrichment duration coupled with 1–2 days data analysis suggest that FT-IR spectroscopy may not be rapid enough for analyzing samples containing low population of *Alicyclobacillus* contaminants. Other parameters for improved FT-IR analysis include harvesting cells from samples by membrane filtration rather than centrifugation (Al-Qadiri et al., 2006), and using hydrophobic grid membrane (HGM) filters for spectra measurement rather than nitrocellulose or aluminium-oxide membrane filters (Grasso et al., 2009). The advantage of HGM filter according to Grasso et al. (2009) is that it allows the collection of reproducible spectra from individual cell which are isolated in hydrophobic squares, rather than from cell aggregates as obtained with filter types, thus preventing colony-overlap that may interfere with cells' spectra data. Lin et al. (2005) and Wang et al. (2011) also suggested that the establishment of comprehensive infrared spectral reference database may shorten FT-IR analytical time for identification of unclassified *Alicyclobacillus* strains and also enable the prediction of strain characteristics such as taint production directly from spectral measurements.

2.1.2. Immunoassays

A variety of enzyme linked immunosorbent assay (ELISA)-based techniques have been exploited for the detection of *Alicyclobacillus* (Li et al. 2013, 2014; Wang et al., 2012). The parameters recommended for optimization of these techniques are presented in Table 2. Generally, the ELISA methods involve the conjugation of antigens (*Alicyclobacillus*) to polyclonal anti-*Alicyclobacillus* antibodies, which are in turn linked to an enzyme in micro-titer polystyrene plates, with intermittent washing steps to prevent non-specific binding. The addition of the enzyme's substrate to the reaction system produces colour or turbidity changes, which can be measured spectrophotometrically and computed as positive/negative (P/N) values. The rapidity of ELISA-based detection of

Table 2
Recommendations for optimization of ELISA-based techniques for specific detection of *Alicyclobacillus* in apple juice.

Technique	Antigen concentration (CFU/mL)	Antibody dilution	Enzyme	Enzyme dilution	Enzyme's substrate solution	Blocking agent	Blocking time (minutes)	Reaction incubation time (minutes)	Reaction temperature (°C)	Reference
ID- ELISA	10^6	1/40000	GAR-HRP	1/2000	Not reported	0.5% BSA in PBS	120	60	37	Wang et al. (2012)
SPA-ELISA	10^5–10^7	1/1600	Rabbit Anti-protein A/HRP (SPA)	1/2000	A-TMB (1 mg/mL) B- 0.03% H_2O_2	5% skimmed milk in PBS	120	60	37	Li et al. (2013)
DAS-ELISA	10^3	CA-1/25,600 DA-1/12,800-	IgG-HRP	1/2000	A-TMB (1 mg/mL) B- 0.03% H_2O_2	4% gelatine in PBS	120	60	37	Li et al. (2014)
IMS-ELISA	10^3	Not reported	GAR-HRP	1/2000	TMB	0.5% BSA in PBS	120	60	37	Wang et al. (2013b)

TMB- 3,3′,5,5′- tetramethylbenzidine; PBS- phosphate buffered saline; GAR-HRP-goat anti-rabbit IgG-horseradish peroxidise; BSA-bovine serum albumin; CA-capture antibody; DA-detection antibody; IMS-immunomagnetic separation; DAS-double antibody sandwich; SPA-staphylococcal protein A; ID-indirect; ELISA-enzyme-linked immunosorbent assay.

Alicyclobacillus ranges between 6 and 7 h (Table 1), although a recently developed 'TAB quick test card', which is based on the principle of double antibody sandwich-ELISA significantly reduced *Alicyclobacillus* detection in apple juice to 5–10 min (Liu et al., 2017). On the other hand, ELISA standing alone is unable to detect *Alicyclobacillus* contamination at $< 10^3$ CFU/mL without a ≥12 h sample pre-enrichment in broth medium (Li et al. 2013, 2014; Wang et al., 2012). Other challenges identified with ELISA-based techniques include the high costs of producing antibodies from animals and the legal limitations of animal use for scientific purposes (Hünniger et al., 2015a).

2.2. Nucleic-acid based detection

Both nucleic acid amplification and non-amplification techniques have been exploited for rapid detection of *Alicyclobacillus* in fruit juices and concentrates.

2.2.1. Nucleic acid amplification techniques for Alicyclobacillus detection

The detection of *Alicyclobacillus* by nucleic acid amplification greatly depends on short oligonucleotide sequences or primers that specifically anneal to the nucleic acid of *Alicyclobacillus* species without recognising those of closely-related food-borne bacteria, due to the absence of complementary binding sites for such *Alicyclobacillus*-specific primers on the nucleic acid molecules of non-*Alicyclobacillus* bacteria. Several *Alicyclobacillus*-specific primers previously reported are listed in Table 3. The procedure for nucleic acid amplification-based detection generally involves the separation of *Alicyclobacillus* cells/spores from sample followed sequentially by nucleic acid extraction, amplification of specific gene locus on the extracted nucleic acid and analysis of amplicons (Fig. 1). Variations among nucleic acid amplification techniques majorly arise from; (i) the nature of nucleic acid and the target gene locus (ii) the method of amplification, and (iii) the method for amplicon analysis.

2.2.1.1. Nature of nucleic acid extracted and target gene locus. For nucleic acid analysis, either the DNA or RNA may be extracted from *Alicyclobacillus* cells or spore contaminants, with most studies targeting the 16S ribosomal RNA (rRNA) gene locus. Wisotzkey, Jurtshuk, Fox, Deinhart, and Poralla (1992) reported that the 16S rRNA gene was sufficiently divergent between *Alicyclobacillus* and their closest *Bacillus* relatives, indicating the suitability of this gene locus for specific detection of *Alicyclobacillus* without significant cross reactivity with other closely-related bacterial genera. In addition, the 5′-end hyper-variable region of the 16S rRNA gene is variable among *Alicyclobacillus* species; therefore suitable for intra-genus differentiation (Goto, Mochida, Asahara, Suzuki, & Yokota, 2002b). Other genes reported for *Alicyclobacillus* detection include the squalene-hopene cyclase (SHC) and vanillin decarboxylase (VDC) genes. By

Table 3
List of *Alicyclobacillus*-specific oligonucleotide primers and probes sequences.

Primer/probe Name	Sequence (5′–3′)	Target	Amplicon size (base pairs)	Specificity	Reference
Ba190F/Ba490R	ACRGGTAGGCATCTCTTGT/ AGGAGCTTTCCACTCTCCTTGT	16S rRNA gene	294	AT	Yamazaki et al. (1996b)
Shc primers-and-probe	ATGCAGAGYTCGAACG/ AAGCTGCCGAARCACTC/ Probe- TCRGARGACGTCACCGC	SHC gene	149	AT & AC	Luo et al. (2004)
CC16S−F/CC16S-R	CGTAGTTCGGATTGCAGGC/ GTGTTGCCGACTCTCGTG/ Probe- CGGAATTGCTAGTAATCGC	16S rRNA gene	134	ACB	Connor et al. (2005)
NAtb-F/NAtb-R	Biotin-GATGCGTAGCCGAGCCGACCTGAGAG TTGCCACTCTCCTTGTGTCGCTC	16S rRNA gene	191	AT	Eguiluz et al., 2008
AliSqualfw/AliSqualrev	TACTGGTGGGGGCCGCTWYTG CCGCCCTSGYTCTGAATGAA	SHC gene	275	ACB	Huch et al. (2010)
Ali16-SqRTfw/Ali16-SqRTrev	CTC GGGGAGAGCGRYAAGGAGA CTT TACGCCCAG TGATTCCG	16S rRNA gene	134	ACB	Huch et al. (2010)
F3 B3 FIP BIP LoopF LoopB	CGGCGCATTAGCTAGTTGG ACTCTCCTTGTCGCTCTCC GTGTCTCAGTCCCAGTGTGGCGAGGTAACGGCTCACCAAG TAGGGAATCTTCCGCAATGGGCAGAGCTTTACAACCCGAAGG CCTCTCAGGTCGGCTACGCA AAGCCTGACGGAGCAACGC	16S - 23S rRNA ITS	LAMP	AT	Chen et al. (2011)
U	TGAGTAACACGTGGGCAATCTG/ CTACCCGTGTATTATCCGGCAT/ Probe- CTTTCAGACTGGAATAAC	16S rRNA gene	81	AT	Wang et al. (2014)
16S rDNA Fw/16S rDNA Rv	CGAAGGAAACCCAATAAGCAC GGATGTCAAGCCCTGGTAAG	16S rRNA gene	143	ACB	Hünniger et al. (2015b)
AliApt1	CATCCGTCACACCTGCTCCATCCGTCACACCTGCTCACGTCAT CCGTCACACCTGCTCGGTGTTCGGTCCCGTATC	ACB spore coat	na	ACB	Hünniger et al. (2015a)
AliApt3	CATCCGTCACACCTGCTCCAGCGTGCGTCGACCCCGGACCCT GTCAGCCCCCCTCGCGGGTGTTCGGTCCCGTATC	ACB spore coat	na	ACB	Hünniger et al. (2015a)
AliApt5	CATCCGTCACACCTGCTCCCAGCGTGGCGTCGACCCGGACCC TGTCAGCCCCCTCGCGGGTGTTCGGTCCCGTATC	ACB spore coat	na	ACB	Hünniger et al. (2015a)
AliApt9	CATCCGTCACACCTGCTCCACCCGTCACACCCATCCGTCACAC CTGCTCCCCCCACTGGGTGTTCGGTCCCGTATC	ACB spore coat	na	ACB	Hünniger et al. (2015a)
U	ATGCGTAGATATGTGGAGGA/ CAGGCGGAGTGCTTATTG	16S rRNA gene	188	ACB	Cai et al. (2015)
Upper 16S/Lower 16S	GCTTGACATCCCTCTGACCG/ CGCCTTCCTCCGACTTAC	16S rRNA gene	200	AT	Yang et al. (2017)
ST-49025/ST- blocker	GCUUGACATCCCTCTGACCG/ CGCCTTCCTCCGACTTAC-biotin	16S rRNA gene	200	AT	Yang et al. (2017)
Ali-F/Ali-R	GCGAAGAAGGCCTTCGGGTTG/ TTATTGGGTTTCCTTCGGCACTG	16S rRNA gene	474	ACB	Osopale et al. (2016)

U–unnamed by author; na-not applicable; AT- *A. acidoterrestris*; AC- *A. acidocaldarius*; ACB- *Alicyclobacillus* species; SHC-squalene-hopene cyclase.

coding for hopanoids, the SHC gene plays a crucial role in the maintenance of cell membrane fluidity and stability of *Alicyclobacillus* under adverse environmental conditions (Kannenberg et al., 1999; Luo, Yousef, & Wang, 2004). On the other hand, the VDC is involved in the non-oxidative decarboxylation of vanillin to guaiacol, therefore plays key role in *Alicyclobacillus* spoilage potential. Thus, targeting the VDC gene for *Alicyclobacillus* detection has the additional advantage of providing information on the spoilage potential of the contaminant (Niwa, 2003). Although highly specific and sensitive, amplifying bacterial DNA may not reliably indicate the viability of contaminants. This is because the DNA of both viable and dead cells can be exponentially amplified by PCR. In contrast, RNA-based amplification methods have proven to be reliable for the detection and quantification of viable *Alicyclobacillus* contaminants in samples. By targeting RNA, Funes-Haucca et al. (2004) successfully monitored population decline of viable *A. acidoterrestris* cells in orange juice spiked with a bacterial growth inhibitor. The suitability of RNA for measuring cell viability is due to its short half-life of about 2 min after cell death (Funes-Haucca et al., 2004). However, when *Alicyclobacillus* contaminants are mostly endospores, RNA may not be readily produced. Therefore heat-shocking of samples, followed by broth enrichment is recommended to activate dormant spores and increase RNA formation.

2.2.1.2. Methods of nucleic acid amplification. In studies that reported *Alicyclobacillus* detection by DNA amplification-based techniques, DNA amplification was achieved by polymerase chain reaction (PCR) in conventional thermo-cyclers or in thermo-cyclers equipped with an optical module that enabled real time detection of PCR signals (real-time PCR) (Connor, Luo, McSpadden Gardener, & Wang, 2005; Luo et al., 2004). Alternatively, DNA amplification was achieved isothermally without a thermo-cycler, as obtained with the loop mediated isothermal amplification (LAMP) or single primer isothermal amplification (SPIA) techniques (Chen, Ma, Yuan, & Zhang, 2011; Yang et al., 2017). In LAMP and SPIA reactions, DNA polymerase enzyme having strong strand displacement activity [e.g. *Bacillus stearothermophilus* (*Bst*) or *B. caldotenax* (*Bca*) DNA polymerases] separates newly synthesized DNA strands from the complementary template DNA, thereby eliminating the need for repeated thermal denaturation steps in conventional PCR. Consequently, the amplification reaction can be performed at constant temperature in a water bath or metal heating block, thus eliminating the cost for expensive thermo-cycling machine and making the technique easily adaptable in resource-limited countries. In a LAMP technique reported by Chen et al. (2011), three primer pairs were used to amplify the 16S−23S internally transcribed spacer region of *Alicyclobacillus* rRNA gene (Table 3). As a result of these multiple primer binding activity, the LAMP assay was highly specific for *A. acidoterrestris*, although it was noted that false positive amplifications may arise from cross-reactivity among the multiple primers involved. In contrast, the SPIA technique reported by Yang et al. (2017) involved a single DNA/RNA chimeric primer (Primer ST-49025 in Table 3), *Bca* DNA polymerase, RNase H and a blocker oligonucleotide sequence. The schematic of the SPIA process is described in Yang et al. (2017). At the end of SPIA amplification, the RNase H cleaves the 5′-end RNA portion of the primer region of the newly synthesized single stranded DNA, rendering the amplicons non-amplifiable under the prevailing SPIA condition and reducing the risk of false positive amplifications (Gill & Ghaemi, 2008; Yang et al., 2017). In amplification reactions involving RNA as the nucleic acid template, the process of reverse-transcription PCR (RT-PCR) is applied, in which the RNA template is first transcribed to complementary DNA (cDNA) in a reverse transcription step, followed by the amplification of the cDNA by conventional PCR or real-time PCR (Eguiluz et al., 2008; Niwa, 2003; Yamazaki, Teduka, Inoue, & Shinano, 1996b).

2.2.1.3. Methods of amplicon analysis. Different methods of amplicon analysis have been reported for nucleic acid amplification-based detection of *Alicyclobacillus*. These include agarose gel electrophoresis which separates PCR amplicons based on their base-pair sizes (Chen et al., 2011; Wang et al., 2013c; Yamazaki et al., 1996b). In contrast, denaturing gradient gel electrophoresis (DGGE) separates and identifies nucleic acids according to their nucleotide sequences, even when the base pairs lengths are the same (Muyzer, De Waal, & Uitterlinden, 1993). Enhanced by a 40-base-pairs guanine-cytosine clamp attached to the 5′- end of the amplification primers, 16S rRNA gene amplicons of *Alicyclobacillus* contaminants in orange, pineapple and mixed fruit juices, were effectively separated by DGGE (Osopale et al., 2016). Furthermore, the inclusion of an *Alicyclobacillus* DNA sequence ladder mix on the DGGE gel enabled direct differentiation of guaiacol-producing and non-producing *Alicyclobacillus* species on DGGE gels without need for post-DGGE analysis (Osopale et al., 2016). More recently, other metagenomic approaches of direct sequencing of amplicons have become available for analyzing complex microbial communities and identification of bacterial populations. Although not yet reported for *Alicyclobacillus* detection in fruit juices or concentrates, technologies such as Illumina or PacBio sequencing can enhance detection rapidity because the bacterial nucleic acid molecule is obtained and sequenced directly from samples without prior isolation of cells from the samples (Rhoads & Au, 2015; Van Dijk, Auger, Jaszczyszyn, & Thermes, 2014).

Real-time quantification of DNA amplicons during *Alicyclobacillus* detection was also possible with the application of the Taqman® real-time PCR techniques, which involved the inclusion of flourogenic *Alicyclobacillus*-specific oligonucleotide probes or DNA-intercalating fluorescent dyes such as SYBR Green I and SYBR Green II in PCRs (Cai et al., 2015; Connor et al., 2005; Hünniger et al., 2015b; Luo et al., 2004; Wang et al., 2014). Application of real time PCR techniques eliminates the need for elaborate post-amplification analyses and limits cross contamination or exposure to hazardous chemicals such as ethidium bromide. In addition, signal detection in the Taqman® system depends on a two-step specificity barrier of primers annealing to complementary target DNA to produce amplicons followed by flourogenic probes annealing complementarily to the amplicons, therefore the risk of false positive results is significantly reduced (Luo et al., 2004). By including SYBR green II in single primer isothermal amplification assay, *Alicyclobacillus* amplicons were simply detected by visualization of turbidity in day light or by fluorescence under UV light, thus eliminating the costs for real-time PCR machine (Yang et al., 2017). Other methods of PCR amplicon analysis reported for *Alicyclobacillus* detection include, (i) capillary electrophoresis on a microchip in Agilent 2100 Bioanalyzer (Funes-Haucca et al., 2004), and (ii) electrochemical conduction in DNA biosensor (Eguiluz et al., 2008).

2.2.2. Non-amplification nucleic acid technique

A random genomic DNA microarray technique was applied for the detection of *Alicyclobacillus* species (Jang et al., 2011). In this method, fluorescent-labelled bacterial genomic DNA obtained from orange juice was hybridized to DNA probe made from randomly fragmented *Alicyclobacillus* genes on a microarray chip, followed by laser-scanning of the microchip to determine the intensity of fluorescence signals emanating from hybridization spots. The microarray system proved to be highly specific for *Alicyclobacillus* species (hybridization rate was > 80%) without cross reactivity with closely-related bacteria such as *B. subtilis* and *B. cereus*. This method also has the potential for shortening analytical time as well as eliminating the need for expensive PCR equipment because genomic DNA was applied directly without the PCR step. In addition, the method allowed rapid and simultaneous analysis of thousands of *Alicyclobacillus* genes on a DNA probe chip that was fabricated without prior knowledge of the probe sequences (Jang et al., 2011).

Table 4
Sensitivity of some sensory and instrumental techniques for detection of off-flavours formed by *Alicyclobacillus* species in fruit juices.

Analytical Technique	Detection medium	Target taint compound	Sensitivity (LOD)/BET[a]	Limit of quantification[a]	Reference
Human taste panel	Apple juice	Guaiacol	0.24 ppb	Not applicable	Eisele and Semon (2005)
	Water	Guaiacol	0.17 ppb	Not applicable	Eisele and Semon (2005)
Human odour/aroma panel	Apple juice	Guaiacol	0.91 ppb	Not applicable	Eisele and Semon (2005)
	Water	Guaiacol	0.48 ppb	Not applicable	Eisele and Semon (2005)
	Orange juice	Guaiacol	2.0 ppb	Not applicable	Pérez-Cacho et al. (2011)
Headspace solid phase micro-extraction GC-MS-selected ion monitoring (HS-SPME-GC MS-SIM)	Apple juice	Guaiacol	0.29 ppb	1.06 ppb	Zierler et al. (2004)
	Apple juice	2, 6-dibromophenol	0.06–0.08 ppb	0.27 ppb	Zierler et al., 2004; Bianchi et al., 2010
	Orange juice	Guaiacol	0.49 ppb	Not reported	Pérez-Cacho et al. (2011)
High performance liquid chromatography (HPLC)	*Bacillus acidoterrestris* Broth	Guaiacol	0.0125 mM	Not reported	Witthuhn et al. (2012)
Electronic Nose (EN)	Apple juice	Guaiacol	0.25 mg/L	Not applicable	Huang et al. (2015b)
Peroxidase Enzyme colorimetric assay (PECA)	*Bacillus acidoterrestris* Broth	Guaiacol	0.0125–0.025 mM	Not reported	Witthuhn et al. (2013)

[a] The lowest value reported in literature, LOD-limit of detection, BET-best estimate threshold, ppb-parts per billion.

2.3. Metabolite based detection

Indirect detection of *Alicyclobacillus* contamination by measuring metabolic activity has been achieved using either of three strategies; (i) assessment of samples for specific phenolic off-flavours (ii) characterization of the total volatile organic compound (VOC) profile of samples, or (iii) detection of non-volatile metabolic by-products.

2.3.1. Assessment of fruit juice for specific phenolic off-flavours

Guaiacol and the halophenols (2, 6-DBP and 2, 6-DCP) which represent the main taint markers for *Alicyclobacillus*-induced spoilage can be detected in contaminated samples using sensory, chromatographic or chemical methods. The limits of detection (LOD) and best estimate threshold for guaiacol and halophenols by some analytical techniques are presented in Table 4.

2.3.1.1. Sensory methods for taint detection. Trained human sensory panels have been able to recognize tainted fruit juices at low concentrations of guaiacol and halophenols (Table 4). Generally, panelists trained to recognize specific taint compound are exposed to suspected contaminated samples and are expected to classify tainted and untainted samples. Most of the studies that reported human sensory detection of guaiacol focused on the odour and aroma of the compound in the analytical samples (Huang, Guo, Yuan, Luo, & Yue, 2015a; Orr, Shewfelt, Huang, Tefera, & Beuchat, 2000; Pérez-Cacho, Danyluk, & Rouseff, 2011). However, Eisele and Semon (2005) reported that the guaiacol's taste best estimate threshold in water and apple juice was lower than that of odour or aroma, indicating that taste-based sensory method may be more sensitive than odour-based technique, and therefore should also be considered when using sensory techniques to evaluate *Alicyclobacillus* contamination. The drawbacks of human sensory panels include the time and financial costs for training, as well as inconsistency in results due to emotion, stress or fatigue of panelists (Cagnasso et al., 2010).

2.3.1.2. Chromatographic methods for taint detection. A widely reported chromatographic technique for detection of specific VOCs in *Alicyclobacillus* contaminated samples is gas chromatography (GC), which separates the volatiles in the analytical samples based on their elution time (retention time) in a GC column, followed by identification of individual VOCs by mass spectrum (GC-MS), olfactometry (GC-O) or flame ionization (GC-FID) detector (Gocmen, Elston, Williams, Parish, & Rouseff, 2005). A critical aspect of chromatographic analysis is the initial extraction of volatiles from the analytical sample. Orr et al. (2000) and Zierler, Siegmund, and Pfannhauser (2004) reported a significant improvement in sensitivity for guaiacol when GC-MS was coupled to headspace solid phase micro-extraction (HS-SPME) for volatiles extraction. During HS-SPME, the analytical sample is mixed with salt and heated to drive-off VOCs into the sample headspace. The VOCs are then adsorbed onto the surface of a coated solid phase micro-extraction (SPME) fiber, after which the fiber is transferred to the GC injection port where the VOCs are thermo-desorbed before GC separation and MS detection (Bianchi et al., 2010; Gocmen et al., 2005). Parameters recommended for optimal HS-SPME are described in Table 5. It was also reported that constant stirring of the analytical sample during SPME increases the dilution of volatiles into the sample headspace (Bianchi et al., 2010; Pérez-Cacho et al., 2011; Zierler et al., 2004). Another important influence on the sensitivity of GC-MS is the operating mode of the mass spectrum (MS) detector. Pérez-Cacho et al. (2011) reported that MS operated at selected ion monitoring mode using only responses from m/z 109 and 124 achieved lower LOD for guaicol in orange juice than MS operated at total ion current mode. Similarly, high performance liquid chromatography (HPLC) has also found wide application for detection of specific taints in *Alicyclobacillus* contaminated samples (Bahçeci & Acar, 2007; Bahçeci, Gokmen, & Acar, 2005; Gocmen et al., 2005; Witthuhn et al., 2011). A major

Table 5
Recommendations for optimal solid-phase microextraction (SPME) of off-flavour compounds in fruit juice.

Parameter	Recommendation	Reference
Length of SPME fiber	2 cm	Zierler et al. (2004)
Coating on SPME fiber	50/30 μm divinylbenzene/carboxenTM/polydimethylsiloxane (DVB/CAR/PDMS) or polyethylene glycol (PEG)	Zierler et al. (2004); Bianchi et al. (2010)
Salt for salting-out VOCs from samples	Sodium sulphate (Na_2SO_4)	Zierler et al. (2004)
Samples equilibration temperature and time	60 °C for 30 min	Zierler et al. (2004)

advantage of chromatographic techniques is the accurate quantification of the target compound. However, these techniques are often complex and expensive to operate, and may be more suitable for laboratory research rather than routine monitoring of products on the production line.

2.3.1.3. Chemical method for detection of specific taint compounds. In contrast to chromatographic techniques, chemical detection of guaiacol by peroxidase enzymatic colourimetric assay (PECA) is cheaper and less cumbersome. PECA is based on the oxidation of guaiacol by hydrogen peroxide in the presence of peroxidase enzyme to form reddish-brown tetraguaiacol, which can be detected visually or spectrophotometrically at wavelength 420 or 470 nm (Bahçeci & Acar, 2007; Witthuhn et al. 2012, 2013). Using a PECA-based miniaturized kit, Niwa (2003) detected *Alicyclobacillus* contamination within 5–24 h, with rapidity varying with contamination levels. Due to stoichiometry between guaiacol concentration and the intensity of colour formed, PECA could be used to determine guaiacol concentrations in sample through a standard curve of turbidity versus guaiacol concentrations (Bahçeci & Acar, 2007; Witthuhn et al. 2012, 2013). In addition, Bahçeci and Acar (2007) reported that there was no significant difference between the guaiacol measurements obtained by HPLC and those obtained by PECA coupled with Minolta spectrophotometry. However, PECA performed poorly when guaiacol concentration was lower than 10 mg/L or higher than 75 mg/L (Bahçeci & Acar, 2007). Also, PECA may be less useful for analyzing juices such as cranberry or red grapes because of colour similarity with tetraguaiacol. Whenever it is desirable to analyze such juice types by PECA, it may be necessary to separate the bacteria from the juice followed by inoculation of cells into clear vanillic acid broth where guaiacol formation can then be measured (Niwa, 2003).

Indirect detection of *Alicyclobacillus* contamination by targeting specific taint compounds is based on the assumption that samples contain *Alicyclobacillus* if guaiacol is present in them. However, there may be exceptions where guaiacol is produced by chemical reactions among juice constituents during storage at elevated temperatures, or by other microbial species such as *B. subtilis*, *B. megaterium*, *Streptomyces* spp., *Sporotrichum thermophile* and lactic acid bacteria (Álvarez-RodrÍgue et al., 2003; Crawford & Olson, 1978; Gocmen et al., 2005; Topakas, Kalogeris, Kekos, Macris, & Christakopoulos, 2003). Where it is necessary to correlate taint formation with *Alicyclobacillus* presence, it is suggested that *Alicyclobacillus* should be isolated from the tainted samples for confirmation. In addition, the relationship between taint concentration and *Alicyclobacillus* population is not yet established, therefore *Alicyclobacillus* contamination levels cannot be reliably predicted from the concentration of specific taint compound, although it is known that phenolic taints are perceived by human senses when *Alicyclobacillus* concentration is $\geq 10^4$ CFU/mL (Bahçeci et l. 2005; Pettipher et al., 1997; Witthuhn et al., 2012).

2.3.2. Assessment of total VOC profile of samples

In contrast to detection of specific taint compounds, assessment of total VOC profile of fruit juices enables the detection of *Alicyclobacillus* contamination before detectable levels of guaiacol or halophenols are formed i.e. before irreparable spoilage of juice product. Such early detection of *Alicyclobacillus* contamination was achieved in one part, by the combination of dynamic headspace extraction with GC-MS, which differentiated contaminated and uncontaminated orange juice by identifying quantitative variations in their total VOC profiles (Bianchi et al., 2010). A cheaper and less cumbersome alternative to this method is the electronic nose (EN), which basically consists of an array of semi selective/conductive metal oxide gas sensors with its readout electronics, a sampling unit and software for data acquisition and processing (Cagnasso et al., 2010; Hartyáni, Dalmadi, & Knorr, 2013). Before analyzing a particular sample, the EN first undergoes a training procedure that exposes each gas sensor to the original VOC profile of the sample, thus enabling the detection of any alterations resulting from product adulteration or microbial contamination (Concina et al., 2010; Gobbi et al., 2010). Huang et al. (2015a) reported that the combination of EN with linear discriminate analysis enabled the detection of *A. acidoterrestris* contamination in apple juice within 4 h of incubation when contamination level was just 200 CFU/mL contrary to human sensory panel, which detected contamination after 12 h when contaminant level was $> 10^4$ CFU/mL and detectable levels of guaiacol were already present. In addition, Huang, Yuan, Wang, Jiang, and Yue (2015b) demonstrated that EN can accurately discriminate contaminated and uncontaminated apple juice when guaiacol concentration is just 0.25–0.5 mg/L. Some drawbacks of EN include its diminutive specificity for individual VOCs compared to chromatographic techniques, the lengthy and laborious training required for the gas sensors, and the instability of the gas sensors for long term application otherwise known as 'the sensor drift phenomenon' (Concina et al., 2010; Gobbi et al., 2010). It was also observed that EN performed poorly with apple juice, indicating that the fruit juice type influences the technique's sensitivity (Cagnasso et al., 2010; Gobbi et al., 2010). In order to mitigate the sensor drift phenomenon, the following were recommended; (i) periodic calibration of the EN sensors, (ii) construction of sensors with better sensing materials, (iii) inclusion of mathematical transactions such as the multiplicative drift correction in data analysis, (iv) maintenance of constant temperature and humidity in sensor chamber during analysis, (v) random placement of samples in carousels to avoid any memory effect on the sensors, and (vi) use of dynamic headspace sampling unit to shorten EN analysis time (Bianchi et al., 2010; Cagnasso et al., 2010). Nonetheless, EN is an artificial sensory system and therefore immune to emotions or fatigue. In addition, the availability of portable EN equipment means that this technique is easily adaptable for routine inspection of products in the production line or on the shelf, particularly when a yes or no response is sufficient (Cagnasso et al., 2010; Concina et al., 2010; Gobbi et al., 2010; Huang et al., 2015a).

2.3.3. Measurement of non-volatile metabolic by-products

Fernández, Gabaldón, and Periago (2017) reported the detection of *A. acidoterrestris* in apple juice by indirect electrical impedance (ID-EI) technique. This technique measures changes in electrical conductivity and impedance of culture media caused by microbial metabolite such as carbon dioxide (CO_2). According to the ID-EI method described by Fernández et al. (2017), equal volumes of heat-shocked apple juice, *B. acidoterrestris* broth and potassium hydroxide (KOH) were mixed in sterile vials and incubated at 45 °C in an impedance measurement system. Carbon dioxide produced as by-product of guaiacol formation by contaminating *A. acidoterrestris* reacted with KOH to produce

potassium hydrogen carbonate ($KHCO_3$), which caused a decrease in electrical impedance of the growth medium (Fernández et al., 2017). The limit of detection of ID-EI technique was reported as ≤1 CFU/mL, although rapidity was positively influenced by higher concentrations of the contaminant. In addition, impedance values correlated with *A. acidoterrestris* concentrations; therefore a standard curve of cell concentrations versus impedance values was used to quantify *A. acidoterrestris* in samples (Fernández et al., 2017). Further confirmatory studies are required to establish ID-EI as a useful technique for rapid detection of *Alicyclobacillus*. The inclusion of guaiacol precursors in the broth medium used for impedance measurement may also potentially reduce analysis time by quickening guaiacol formation and CO_2 production, although this is yet to be determined.

3. Innovative methods for *Alicyclobacillus* enrichment

The sensitivity of detection techniques for bacteria in food generally improves with higher contaminant concentration. However, it is natural for bacterial contaminants to occur at very low concentration in food products (Wang et al., 2013a). In addition, interferences from food constituents and non-target microbiota may impair the sensitivity and specificity of certain detection methods. Therefore, innovative separation and enrichment procedures other than centrifugation or filtration, or the lengthy broth enrichment, have been explored for quick enrichment and separation of *Alicyclobacillus* cells/spores from sample constituents and non-target microbiota. One of such enrichment techniques is immunomagnetic separation (IMS), which involves the trapping of target bacteria by immunomagnetic nanoparticles (IMNPs), followed by magnetic decantation in an electric field to separate the IMNP-bacteria complex from the food matrix and non-target microbiota (Malkova, Rauch, Wyatt, & Morgan, 1998; Shields et al., 2012; Špánová et al., 2003). The IMNP-bacteria complex thus isolated may then be analysed by traditional microbiological methods or by other innovative detection techniques (Jeníková, Pazlarová, & Demnerová, 2010; Malkova et al., 1998).

The IMS procedure for *Alicyclobacillus* enrichment involves the development of IMNPs by immobilizing anti-*Alicyclobacillus* IgG antibody onto the surface of magnetic nanoparticles (MNPs), followed by IMNP-trapping of *Alicyclobacillus* cells in samples (Wang et al., 2013a). The conditions for optimal immobilization of anti-*Alicyclobacillus* IgG on MNPs and subsequent trapping of *Alicyclobacillus* in fruit juices or concentrates are presented in Table 6. Wang et al. (2013b) reported that the combination of IMS pre-enrichment with ELISA (IMS-ELISA) resulted in a 100 fold improvement of the sensitivity of ELISA for *A. acidoterrestris* detection in apple juice (LOD reduced from 10^5 to 10^3 CFU/mL) and a 4 h reduction in detection time. In addition, > 95% of *Alicyclobacillus* at ≤ 10^4 CFU/mL concentration was effectively recovered from apple juice within 30 min of IMS enrichment (Wang et al., 2013a). Wang et al. (2014) further observed that the efficiency of IMS enrichment declined with increasing *Alicyclobacillus* concentration in sample. However, this is not a disadvantage because IMS is required when target bacteria population is low in sample, while filtration or centrifugation can generally be applied to concentrate samples when contaminant levels are above 10^4 CFU/mL. Similar improvements in sensitivity and rapidity were observed when IMS was combined with PCR techniques (IMS-PCR) for *Alicyclobacillus* detection in a variety of fruit juices (Table 1) (Cai et al., 2015; Wang et al. 2013c, 2014). In reported IMS-PCR techniques, DNA extraction was achieved simply by boiling IMNP-bacteria suspension followed by centrifugation to obtain supernatants, which were used directly as template for PCR, thereby reducing analytical time and eliminating the cost for expensive commercial DNA extraction kits. However, a further filtration step of the supernatant was recommended to sieve out cell debris, IMNPs and other impurities that may interfere with PCR signals (Cai et al., 2015; Wang et al., 2014).

More recently, enrichment and detection of *Alicyclobacillus* in apple juice was achieved simultaneously by conjugating MNPs with fluorescent quantum dots (QD) (Wang et al., 2018). In this procedure, IMNPs-QDs-bacteria conjugates decanted from juice samples were detected directly by fluorescence spectrophotometry while quantification of *Alicyclobacillus* contaminant levels was determined from a standard curve of fluorescence intensity versus logarithmic number of cells. Due to the direct detection of IMNPs-QDs-bacteria complexes by fluorescence analysis, the detection time for *Alicyclobacillus* in apple juice was significantly reduced to 90 min, although this method gave a detection limit of 10^4 CFU/mL, which was one log-cycle higher than those of other IMS-based *Alicyclobacillus* detection methods (compare IMS-QDs fluorescence with IMS-PCR and IMS-ELISA in Table 1). Wang et al. (2018) suggested that the elution of QDs from the IMNP-QP-bacteria complexes after magnetic decantation may help to reduce background interferences that impaired the sensitivity of IMS-QP-fluorescence at < 10^4 CFU/mL contamination levels.

Due to the drawbacks of antibody-based techniques mentioned earlier, DNA aptamers (aptamers) have been proposed as alternatives to animal-derived antibodies for enrichment and magnetic separation of *Alicyclobacillus* spores in fruit juices (Hünniger et al., 2015a). Aptamers

Table 6
Recommendations for (A) immobilization of anti-*Alicyclobacillus* IgG on magnetic nanoparticles (MNPs) and MNPs-quantum dots (MNPs-QDs) for the formation of immuonmagnetic nanoparticles (IMNPs) and IMNPs-QDs, and (B) IMS enrichment of *Alicyclobacillus* in sample (fruit juice or concentrate).

A		B*	
Parameter	Recommendation	Parameter	Recommendation
Immobilization method	'Oriented' immobilization[a]/esterification reactions between COOH-group of MNPs-QD and amine group of Carbodiimide hydrochloride/N-Hydroxysuccinimide (EDC/NHS)[f1]	Final IMP concentration	2.5 mg/mL[abcde]
Magnetic nanoparticle	silica-coated magnetic Fe_3O_4 nanoparticles (SMNPs)[a]	Sample volume	2 mL [abcde]
Adsorbent coating on magnetic nanoparticle	(3-Aminopropyl) triethoxysilane (APTES)[a]/EDC/NHS [f1]	Reaction temperature	37 °C[abcde]
Concentration of magnetic nanoparticle	2.0 mg/mL[a]/5.0 mg[f1]	Immuno-capture time	30[bde] - 60 minutes[ac]
Antibody concentration	2.0 mg/mL[af]	Re-suspension medium after magnetic decantation	PBS[abf] or TE- buffer[cde]
Blocking agent to prevent non-specific binding	PBS plus 5% BSA and 0.05% Tween-20[af]		
Reaction temperature	37 °C[af]		
Reaction time	30–60 minutes[af]		

*-Conditions applicable when *Alicyclobacillus* contamination level is ≤ 10^4 CFU/mL.
[a]-Wang et al., 2013a; [b]-Wang et al., 2013b; [c]-Wang et al., 2013c; [d]-Wang et al., 2014; [e]-Cai et al., 2015; [f]-Wang et al., 2018.
[1]-Recommendations applicable to MNPs-QDs particles.

are single stranded DNA molecules, which form distinct three-dimensional structures that interact specifically with various biological targets, including bacterial spore coat. Although, their binding affinity is comparable to that of antibodies, aptamers have higher stability, wider target range and lower production cost. Application of aptamers for bacterial-trapping generally involves the selection of target-specific DNA aptamers using the process of systematic evolution of ligands by exponential enrichment (SELEX), followed by the immobilization of the selected aptamers on magnetic nanoparticles for target-trapping and separation by magnetic decantation. Using a spore-SELEX strategy, Hünniger et al. (2015a) selected four DNA aptamers with specific binding affinity for *Alicyclobacillus* spore coat (AliApt1, AliApt3, AliApt5 and AliApt9, Table 3). Subsequently, Hünniger et al. (2015b) combined aptamer-based enrichment with SYBR Green I real-time PCR for direct detection of *Alicyclobacillus* spores in orange juice. This detection approach presented a detection limit of < 10 CFU/mL and was highly specific for *Alicyclobacillus* without any cross reactivity with common food-borne bacteria such as *Bacillus, Clostridium* or *Paenibacillus* species (Hünniger et al., 2015b).

4. Concluding remarks

Early detection of *Alicyclobacillus* in raw materials and finished products is crucial for effective quality control management in fruit juice industries. In this review, various innovative techniques for rapid detection of *Alicyclobacillus* in fruit juices and concentrates were discussed. The majority of these techniques gave excellent agreement with standard traditional microbiological methods. In addition, the low limits of detection reported for most of the innovative techniques indicate that they can achieve quick detection of *Alicyclobacillus* when contamination levels are low and without prolonged enrichment procedure. In cases where enrichment is necessary, it was widely recommended that steps should be taken to provide conditions that encourage rapid growth of *Alicyclobacillus* cells in the analytical samples or enrichment broth. Otherwise, rapid enrichment methods such as IMS or aptamer-binding can be combined with detection techniques to improve sensitivity.

It was also observed that *Alicyclobacillus* detection by the innovative techniques described in this article was mostly validated with apple juice. However, other juice types such as tomato, grapefruit, pineapple and juice blends have also been reported to readily support *Alicyclobacillus* growth. Therefore, *Alicyclobacillus* detection using these techniques should also be validated in these fruit juice types in order to ascertain assay responses in varying analytical samples. In addition, the techniques should be validated with other non-juice materials such as soils, water, and surface swabs that represent potential sources of *Alicyclobacillus* contamination.

Lastly, it is important to emphasise the need for improvement of the reported innovative techniques as well as the development of new ones with improved sensitivity and rapidity for *Alicyclobacillus* detection and furthermore depict genetic diversity as to support food quality. Also, use of taste-based methods for early detection of *Alicyclobacillus*-induced spoilage is also needed. The use of taste-based sensory technique that is highly sensitive for guaiacol detection in apple juice and water is recommended and artificial taste-based sensory technique such as electronic tongue can be used to expand the scope of techniques available for early detection of *Alicyclobacillus* contamination and spoilage. The adoption of appropriate detection technique for *Alicyclobacillus* is crucial for enhancing fruit juice quality and shelf life, as well as safeguarding the reputation of commercial juice brands.

Authors' contributions

Conception of study: FAO and RCW; Planning: FAO, BAO and GAA; Drafting and outlining of review: FAO, BAO and GAA; Sourcing for papers: BAO and GAA; Data compilation and preparation of tables: BAO, GAA and OOK; Writing, review, and fine-tuning of draft: FAO, RCW, BAO, GAA and OOK.

Conflicts of interest

The authors declare no conflicts of interest regarding this work.

References

Al-Qadiri, H. M., Lin, M., Cavinato, A. G., & Rasco, B. A. (2006). Fourier transform infrared spectroscopy, detection and identification of *Escherichia coli* O157:H7 and *Alicyclobacillus* strains in apple juice. *International Journal of Food Microbiology, 111*, 73–80.

Álvarez-Rodrígue, M. L., Belloch, C., Villa, M., Uruburu, F., Larriba, G., & Coque, J. (2003). Degradation of vanillic acid and production of guaiacol by microorganisms isolated from cork samples. *FEMS Microbiology Letters, 220*, 49–55.

Bahçeci, K. S., & Acar, J. (2007). Determination of guaiacol produced *by Alicyclobacillus acidoterrestris* in apple juice by using HPLC and spectrophotometric methods, and mathematical modeling of guaiacol production. *European Food Research and Technology, 225*, 873–878.

Bahçeci, K. S., Gokmen, V., & Acar, J. (2005). formation of guaiacol from vanillin by *Alicyclobacillus acidoterrestris* in apple juice: A model study. *European Food Research and Technology, 220*, 196–199.

Baumgart, J., Husemann, M., & Schmidt, C. (1997). *Alicyclobacillus acidoterrestris*: Occurrence, importance and detection in beverages and raw materials for beverages. *Flussiges Obst, 64*, 178–180.

Bevilacqua, A., Corbo, M. R., & Sinigaglia, M. (2010). *In vitro* evaluation of the antimicrobial activity of eugenol, limonene and citrus extract against bacteria and yeasts, representative of the spoiling microbiota of fruit juices. *Journal of Food Protection, 73*, 888–894.

Bevilacqua, A., Sinigaglia, M., & Corbo, M. R. (2008). *Alicyclobacillus acidoterrestris*: New methods for inhibiting spore germination. *International Journal of Food Microbiology, 125*, 103–110.

Bianchi, F., Careri, M., Mangia, A., Mattarozzi, M., Musci, M., Concina, I., et al. (2010). Characterization of the volatiles of orange juice contaminated with *Alicyclobacillus acidoterrestris*. *Food Chemistry, 123*, 653–658.

Borlinghaus, A., & Engel, R. (1997). *Alicyclobacillus* incidences in commercial apple juice concentrate (AJC) supplies – method development and validation. *Fruit Processing, 7*, 262–271.

Cagnasso, S., Falasconi, M., Previdi, M. P., Franceschini, B., Cavalieri, C., Sberveglieri, V., et al. (2010). Rapid screening of *Alicyclobacillus acidoterrestris* spoilage of fruit juices by electronic nose: A confirmation study. *Journal of Sensors*. https://doi.org/10.1155/2010/143173.

Cai, R., Wang, Z., Yuan, Y., Liu, B., Wang, L., & Yue, T. (2015). Detection of *Alicyclobacillus* spp. in fruit juice by combination of immunomagnetic separation and a SYBR green I real-time PCR assay. *PLoS One*. https://doi.org/10.1371/journal.pone.0141049.

Cerny, G., Hennlich, W., & Porolla, K. (1984). Spoilage of fruit juices by bacilli: Isolation and characterization of the spoiling microorganisms. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung A, 179*, 224–227.

Chang, S., & Kang, D. (2004). *Alicyclobacillus* spp. in fruit juice industry: History, characteristics and current isolation/detection procedures. *Critical Reviews in Microbiology, 30*, 55–74.

Chen, J., Ma, X., Yuan, Y., & Zhang, W. (2011). Sensitive and rapid detection of *Alicyclobacillus acidoterrestris* using loop-mediated isothermal amplification. *Journal of the Science of Food and Agriculture, 91*, 1070–1074.

Concina, I., Bornšek, M., Baccelliere, S., Falasconi, M., Gobbi, E., & Sberveglieri, G. (2010). *Alicyclobacillus* spp.: Detection in soft drinks by electronic nose. *Food Research International, 43*, 2108–2114.

Connor, C. J., Luo, H., McSpadden Gardener, B. B., & Wang, H. H. (2005). Development of a real-time PCR-based system targeting the 16S rRNA gene sequence for rapid detection of *Alicyclobacillus* spp. in juice products. *International Journal of Food Microbiology, 99*, 229–235.

Crawford, R. L., & Olson, P. P. (1978). Microbial catabolism of vanillate: Decarboxylation to guaiacol. *Applied and Environmental Microbiology, 36*(4), 539–543.

Danyluk, M. D., Friedrich, L. M., Jouquand, C., Goodrich-Schneider, R., Parish, M. E., & Rouseff, R. (2011). Prevalence, concentration, spoilage, and mitigation of *Alicyclobacillus* spp. in tropical and subtropical fruit juice concentrate. *Food Microbiology, 28*, 472–477.

Daron, H. H. (1970). Fatty acid composition of lipid extracts of a thermophilic *Bacillus* species. *Journal of Bacteriology, 101*, 145–151.

Deinhard, G., Blanz, P., Poralla, K., & Altan, E. (1987). *Bacillus acidoterrestris* sp. nov., a new thermotolerant acidophile isolated from different soils. *Systematic & Applied Microbiology, 10*, 47–53.

Durak, M. Z., Churey, J. J., Danyluk, M. D., & Worobo, R. W. (2010). Identification and haplotype distribution of *Alicyclobacillus* spp. from different juices and beverages. *International Journal of Food Microbiology, 142*, 286–291.

Eguiluz, K. I. B., Salazar-Banda, G. R., Funes-Huacca, M. E., Alberice, J. V., Carrilho, E., Machado, S. A. S., et al. (2008). Sequence-specific electrochemical detection of *Alicyclobacillus acidoterrestris* DNA using electroconductive polymer-modified fluorine tin oxide electrodes. *Analyst, 134*, 314–319.

Eisele, T., & Semon, M. (2005). Best estimate aroma and taste detection threshold for guaiacol in water and apple juice. *Journal of Food Science, 70*(4), 267–269.

Fernández, P., Gabaldón, J., & Periago, M. J. (2017). Detection and quantification of *Alicyclobacillus acidoterrestris* by electrical impedance in apple juice. *Food Microbiology, 68*, 34–40.
Funes-Huacca, M., De Almeida Regitano, L. C., Mueller, O., & Carrilho, E. (2004). Semiquantitative determination of *Alicyclobacillus acidoterrestris* in orange juice by reverse-transcriptase polymerase chain reaction and capillary electrophoresis-laser induced fluorescence using microchip technology. *Electrophoresis, 25*, 3860–3864.
Gill, P., & Ghaemi, A. (2008). Nucleic acid isothermal amplification technologies - a review. *Nucleosides, Nucleotides & Nucleic Acids, 27*(3), 224–243.
Gobbi, E., Falasconi, M., Concina, I., Mantero, G., Bianchi, F., Mattarozzi, M., et al. (2010). Electronic nose and *Alicyclobacillus* spp. spoilage of fruit juices: An emerging diagnostic tool. *Food Control, 21*, 1374–1382.
Gocmen, D., Elston, A., Williams, T., Parish, M., & Rouseff, R. L. (2005). Identification of medicinal off-flavours generated by *Alicyclobacillus* species in orange juice using GC-olfactory and GC-MS. *Letters in Applied Microbiology, 40*, 172–177.
Goto, K., Mochida, K., Asahara, M., Suzuki, M., & Yokota, A. (2002b). Application of the hypervariable region of the 16S rDNA sequence as an index for the rapid identification of species in the genus *Alicyclobacillus*. *Journal of General and Applied Microbiology, 48*, 243–250.
Goto, K., Tanaka, T., Yamamoto, R., & Tokuda, H. (2007). Characteristics of *Alicyclobacillus*. In A. Yokota, T. Fujii, & K. Goto (Eds.). *Alicyclobacillus. Thermophilic Acidophilic Bacilli* (pp. 9–48). Tokyo: Springer.
Goto, K., Tanimoto, Y., Tamura, T., Mochida, K., Arai, D., Asahara, M., et al. (2002a). Identification of thermoacidophilic bacteria and a new *Alicyclobacillus* genomic species isolated from acidic environments in Japan. *Extremophiles, 6*, 333–340.
Grande, M. J., Lucas, R., Abriouel, H., Ben Omar, N., Maqueda, M., Martínez-Bueno, M., et al. (2005). Control of *Alicyclobacillus acidoterrestris* in fruit juices by enterocin AS-48. *International Journal of Food Microbiology, 104*, 289–297.
Grasso, E. M., Yousef, A. E., Castellvi, S. L., & Rodriguez-Saona, L. E. (2009). Rapid detection and differentiation of *Alicyclobacillus* species in fruit juice using hydrophobic grid membranes and attenuated total reflectance infrared microspectroscopy. *Journal of Agricultural and Food Chemistry, 57*, 10670–10674.
Groenewald, W. H., Gouws, P. A., & Witthuhn, R. C. (2009). Isolation, identification and typification of *Alicyclobacillus acidoterrestris* and *Alicyclobacillus acidocaldarius* strains from orchard soil and the fruit processing environment in South Africa. *Food Microbiology, 26*, 71–76.
Hartyáni, P., Dalmadi, I., & Knorr, D. (2013). Electronic nose investigation of *Alicyclobacillus acidoterrestris* inoculated apple and orange juice treated by high hydrostatic pressure. *Food Control, 32*, 262–269.
Hiraishi, A., Inagaki, K., Tanimoto, Y., Iwasaki, M., Kishimoto, N., & Tanaka, H. (1997). Phylogenetic characterization of a new thermo-acidophilic bacterium isolated from hot springs in Japan. *Journal of Applied Microbiology, 43*, 295–304.
Huang, X., Guo, C., Yuan, Y., Luo, X., & Yue, T. (2015a). Detection of medicinal off-flavour in apple juice with artificial sensing system and comparison with test panel evaluation and GC-MS. *Food Control, 51*, 270–277.
Huang, X., Yuana, Y., Guoa, C., Gekasb, V., & Yuea, T. (2014). *Alicyclobacillus* in the fruit juice industry: Spoilage, detection, and prevention/control. *Food Reviews International, 31*, 91–124.
Huang, X., Yuan, Y., Wang, X., Jiang, F., & Yue, T. (2015b). Application of electronic nose in tandem with chemometric analysis for detection of *Alicyclobacillus acidoterrestris*-spawned spoilage in apple juice beverage. *Food and Bioprocess Technology, 8*, 1295–1304.
Huch, M., Müller, A., Strohhäcker, J., Saskia, V., Hanak, A., Gräf, V., et al. (2010). Use of UV-C Treatment for the inactivation of microorganisms. *Fruit processing, 22*, 196–201.
Hünniger, T., Felbinger, C., Wessels, H., Mast, S., Hoffmann, A., Schefer, A., et al. (2015b). Food targeting: A real-time PCR assay targeting 16S rDNA for direct quantification of *Alicyclobacillus* spp. spores after aptamer-based enrichment. *Journal of Agricultural and Food Chemistry, 63*(17), 4291–4296.
Hünniger, T., Fischer, C., Wessels, H., Hoffmann, A., Paschke-Kratzin, A., Haase, I., et al. (2015a). Food sensing: Selection and characterization of DNA aptamers to *Alicyclobacillus* spores for trapping and detection from orange juice. *Journal of Agricultural and Food Chemistry, 63*(8), 2189–2197.
Jang, J. H., Kim, S., Yoon, B. H., Ryu, J., Gu, M. B., & Chang, H. (2011). Detection of *Alicyclobacillus* species in fruit juice using a random genomic DNA microarray chip. *Journal of Food Protection, 74*, 933–938.
Jeníková, G., Pazlarová, J., & Demnerová, K. (2010). Detection of Salmonella in food samples by the combination of immunomagnetic separation and PCR assay. *International Microbiology, 3*, 225–229.
Jensen, N., & Whitfield, F. B. (2003). Role of *Alicyclobacillus acidoterrestris* in the development of a disinfectant taint in shelf-stable fruit juice. *Letters in Applied Microbiology, 36*, 9–14.
Kannenberg, E., Blume, A., & Poralla, K. (1984). Properties of ω-cyclohexane fatty acids in membranes. *FEBS Letters, 172*, 331.
Kannenberg, E. L., & Poralla, K. (1999). Hopanoid biosynthesis and function in bacteria. *Naturwissenschaften, 86*, 168–176.
Karavaiko, G. I., Bogdanova, T. I., Tourova, P. T., Kondrat'eva, T. F., Tsaplina, I., Egorova, M. A., et al. (2005). Reclassification of '*Sulfobacillus thermosulfidooxidans* subsp. *thermotolerans*' strain K1 as *Alicyclobacillus tolerans* sp. nov. and *Sulfobacillus disulfidooxidans* Dufresne *et al.* 1996 as *Alicyclobacillus disulfidooxidans* comb. nov., and emended description of the genus *Alicyclobacillus*. *International Journal of Systematic and Evolutionary Microbiology, 55*, 941–947.
Komitopoulou, E., Boziaris, I. S., Davies, E. A., Delves-Broughton, J., & Adams, M. R. (1999). *Alicyclobacillus acidoterrestris* in fruit juices and its control by nisin. *International Journal of Food Science and Technology, 34*, 81–85.
Lee, S. Y., Gray, P. M., Dougherty, R. H., & Kang, D. H. (2004). The use of chlorine dioxide to control Alicyclobacillus acidoterrestris spores in aqueous suspension and on apples. *International Journal of Food Microbiology, 92*, 121–127.
Li, J., Huang, R., Xia, K., & Liu Liu, L. (2014). Double antibodies sandwich enzyme-linked immunosorbent assay for the detection of *Alicyclobacillus acidoterrestris* in apple juice concentrate. *Food Control, 40*, 172–176.
Lin, M., Al-Holy, M., Chang, S.-S., Huang, Y., Cavinato, A. G., Kang, D.-H., et al. (2005). Rapid discrimination of *Alicyclobacillus* strains in apple juice by Fourier transform infrared spectroscopy. *International Journal of Food Microbiology, 105*, 369–376.
Liu, Y., Li, J., Zhang, L., Wu, X., Wang, F., & Song, B. (2017). Development of colloidal gold-based immunochromatographic assay for rapid detection of *Alicyclobacillus acidoterrestris* in apple juice. *CyTA - Journal of Food, 15*(3), 352–356.
Li, J. K., Xia, K., & Yu, C. Z. (2013). Detection of *Alicyclobacillus acidoterrestris* in apple juice concentrate by enzyme-linked immunosorbent assay. *Food Control, 30*, 251–254.
Luo, H., Yousef, A. E., & Wang, H. H. (2004). A real-time polymerase chain reaction based method for rapid and specific detection of spoilage *Alicyclobacillus* spp. in apple juice. *Letters in Applied Microbiology, 39*, 376–382.
Maldonado, C. M., Aban, M. P., & Navarro, R. A. (2013). Chemicals and lemon essential oil effect on *Alicyclobacillus acidoterrestris* viability. *Brazilian Journal of Microbiology, 44*(4), 1133–1137.
Malkova, K., Rauch, P., Wyatt, G., & Morgan, M. (1998). Combined immunomagnetic separation and detection of *Salmonella enteritidis* in food samples. *Food and Agricultural Immunology, 10*, 271–280.
Mandal, P. K., Biswas, A. k., Choi, K., & Pal, U. K. (2011). Methods for detection of foodborne pathogens. *American Journal of Food Technology, 6*(2), 87–102.
Muyzer, G., De Waal, E. C., & Uitterlinden, A. G. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology, 59*, 695–700.
Nakauma, M., Saito, K., Katayama, T., Tada, M., & Todoriki, S. (2004). Radiationheat synergism for inactivation of *Alicyclobacillus acidoterrestris* spores in citrus juice. *Journal of Food Protection, 67*, 2538–2543.
Niwa, M. (2003). *Control of hazardous bacteria in acidic beverages by using a guaiacol detection kit (peroxidase method)*. [WWW document]. URL 79 http://www.cosmobio.co.jp/export_e/products/kits/products_KYO_20050412/Guaiakol_detection_kit_reference.pdf. 29 January 2007.
Orr, R. V., & Beuchat, L. R. (2000). Efficiency of disinfectants in killing spores of *Alicyclobacillus acidoterrestris* and performance of media for supporting colony development by survivors. *Journal of Food Protection, 63*, 1117–1122.
Orr, R. V., Shewfelt, R. L., Huang, C. J., Tefera, S., & Beuchat, L. R. (2000). Detection of guaiacol produced by *Alicyclobacillus acidoterrestris* in apple juice by sensory and chromatographic analyses and comparison with spore and vegetative cell populations. *Journal of Food Protection, 63*, 1517–1522.
Osopale, B. A., Witthuhn, R. C., Albertyn, J., & Oguntoyinbo, F. A. (2016). Culture dependent and independent genomic identification of *Alicyclobacillus* species in contaminated commercial fruit juices. *Food Microbiology, 56*, 21–28.
Osopale, B. A., Witthuhn, R. C., Albertyn, J., & Oguntoyinbo, F. A. (2017). Inhibitory spectrum of diverse guaiacol-producing *Alicyclobacillus acidoterrestris* by poly dimethyl ammonium chloride disinfectant. *LWT- Food Science and Technology, 84*, 241–247.
Palop, A., Alvarez, I., Razo, J., & Condon, S. (2000). Heat resistance of *Alicyclobacillus acidocaldarius* in water, various buffers and orange juice. *Journal of Food Protection, 61*, 1377–1380.
Pei, J., Yue, T., & Yuan, Y. (2014). Control of *Alicyclobacillus acidoterrestris* in fruit juices by a newly discovered bacteriocin. *World Journal of Microbiology and Biotechnology, 30*, 855–863.
Pérez-Cacho, P. R., Danyluk, M. D., & Rouseff, R. (2011). GC-MS quantification of and sensory thresholds of guaiacol in orange juice and its correlation with *Alicyclobacillus* spp. *Food Chemistry, 129*, 45–50.
Pettipher, G. L., Osmundson, M. E., & Murphy, J. M. (1997). Methods for the detection and enumeration of *Alicyclobacillus acidoterrestris* and investigation of growth and production of taint in fruit juice and fruit juice-containing drinks. *Letters in Applied Microbiology, 24*, 185–189.
Rhoads, A., & Au, K. F. (2015). PacBio sequencing and its applications. *Genomics, Proteomics and Bioinfomatics, 13*, 278–289.
Shields, M. J., Hahn, K. R., Janzen, T. W., Goji, N., Thomas, M. C., Kingombe, C. B. I., et al. (2012). Immunomagnetic capture of *Bacillus anthracis* spores from food. *Journal of Food Protection, 75*, 1243–1248.
Simpson, R. F., Amon, J. M., & Daw, A. J. (1986). Off-flavour in wine caused by guaiacol. *Food Technology in Australia, 38*, 31–33.
Špaňová, A., Rittich, B., Horák, D., Lenfeld, J., Prodělalová, J., Sučiková, J., et al. (2003). Immunomagnetic separation and detection of *Salmonella* cells using newly designed carriers. *Journal of Chromatography A, 1009*, 215–221.
Spinelli, N. F. A., Sant'Ana, A. S., Rodrigues-Junior, S., & Massaguer, P. R. (2009). Influence of different fillig, cooling, and storage conditions on the growth of *Alicyclobacillus acidoterrestris* CRA7152 in orange juice. *Applied and Environmental Microbiology, 75*(23), 7409–7416.
Splittstoesser, D. F., Churey, J. J., & Lee, C. Y. (1994). Growth characteristics of aciduric sporeforming bacilli isolated from fruit juices. *Journal of Food Protection, 57*, 1080–1083.
Steyn, Y., Cameron, M., Venter, P., & Witthuhn, R. C. (2011). *Alicyclobacillus* spoilage and isolation–A review. *Food Microbiology, 28*, 331–349.
Topakas, E., Kalogeris, E., Kekos, D., Macris, B. J., & Christakopoulos, P. (2003). Bioconversion of ferulic acid into vanillic acid by the thermophilic fungus *Sporotrichum thermophile*. *Lebensmittel-Wissenschaft und -Technologie, 36*, 561–565.
Torlak, E. (2014). Efficacy of ozone against *Alicyclobacillus acidoterrestris* spores in apple juice. *International Journal of Food Microbiology, 172*, 1–4.

Tremarin, A., Brand, T. R. S., & Silva, C. L. M. (2017). Application of ultraviolet radiation and ultrasound treatments for *Alicyclobacillus acidoterrestris* spores inactivation in apple juice. *LWT-Food Science and Technology, 78*, 138–142.

Van Dijk, E. L., Auger, H., Jaszczyszyn, Y., & Thermes, C. (2014). Ten years of next-generation sequencing technology. *Trends in Genetics, 30*(9), 418–426.

Walker, M., & Phillips, C. A. (2005). The effect of intermittent shaking, headspace and temperature on the growth of *Alicyclobacillus acidoterrestris* in stored apple juice. *International Journal of Food Science and Technology, 40*, 557–562.

Walker, M., & Phillips, C. A. (2008). *Alicyclobacillus acidoterrestris*: An increasing threat to the fruit juice industry? *International Journal of Food Science and Technology, 43*, 250–260.

Walls, I., & Chuyate, R. (1998). *Alicyclobacillus* – historical perspective and preliminary characterization study. *Dairy, Food, and Environmental Sanitation, 18*, 499–503.

Walls, I., & Chuyate, R. (2000). Spoilage of fruit juices by *Alicyclobacillus acidoterrestris*. *Food Australia, 52*, 286–288.

Wang, Z., Cai, R., Yuan, Y., Niu, C., Hu, Z., & Yue, T. (2014). An immunomagnetic separation-real-time PCR system for the detection of *Alicyclobacillus acidoterrestris* in fruit products. *International Journal of Food Microbiology, 175*, 30–35.

Wang, Z., Li, X., Zhao, Y., Yuan, Y., Cai, R., & Yue, T. (2018). Synthesis of multifunctional fluorescent magnetic nanoparticles for the detection of *Alicyclobacillus* spp. in apple juice. *Food Research International, 114*, 104–113.

Wang, Z., Wang, J., Yue, T., Yuan, Y., Cai, R., & Niu, C. (2013c). Immunomagnetic separation combined with polymerase chain reaction for the detection of *Alicyclobacillus acidoterrestris* in apple juice. *PLoS One, 8*(12), e82376. https://doi.org/10.1371/journal.pone.0082376.

Wang, Z., Yue, T., Yuan, Y., Cai, R., Guo, C., Wang, X., et al. (2012). Development of polyclonal antibody-based indirect enzyme-linked immunosorbent assay for the detection of *Alicyclobacillus* strains in apple juice. *Journal of Food Science, 77*, 643–649.

Wang, Z., Yue, T., Yuan, Y., Cai, R., Niu, C., & Guo, C. (2013a). Preparation of immunomagnetic nanoparticles for the separation and enrichment of *Alicyclobacillus* spp. in apple juice. *Food Research International, 54*, 302–310.

Wang, Z., Yue, T., Yuan, Y., Cai, R., Niu, C., & Guo, C. (2013b). Development and evaluation of an immunomagnetic separation–ELISA for the detection of *Alicyclobacillus* spp. in apple juice. *International Journal of Food Microbiology, 166*, 28–33.

Wang, J., Yue, L., Yuan, Y., Lu, X., Shin, J., & Rasco, B. (2011). Discrimination of *Alicyclobacillus* strains using nitrocellulose membrane filter and attenuated total reflectance fourier transform infrared spectroscopy. *Journal of Food Science, 76*, 137–142.

Whitfield, F. B. (1998). Microbiology of food taints. *International Journal of Food Science and Technology, 33*, 31–51.

Wisotzkey, J. D., Jurtshuk, P., Fox, G. E., Deinhart, G., & Poralla, K. (1992). Comparative sequence analyses on the 16S rRNA (rDNA) of *Bacillus acidocaldarius, Bacillus acidoterrestris*, and *Bacillus cycloheptanicus* and proposal for creation of a new genus, *Alicyclobacillus* gen., nov. *International Journal of Systematic Bacteriology, 42*, 263–269.

Witthuhn, R. C., Smit, Y., Cameron, M., & Venter, P. (2013). Guaiacol production by *Alicyclobacillus* and comparison of two guaiacol detection methods. *Food Control, 30*, 700–704.

Witthuhn, R. C., Smit, Y., Caneron, M., & Venter, P. (2011). Isolation of *Alicyclobacillus* and the influence of different growth parameters. *International Journal of Food Microbiology, 146*, 63–68.

Witthuhn, R. C., Van der Merwe, E., Venter, P., & Cameron, M. (2012). Guaiacol production from ferulic acid, vanillin and vanillic acid by *Alicyclobacillus acidoterrestris*. *International Journal of Food Microbiology, 157*, 113–117.

Yamazaki, k, Murakami, M., Kawai, Y., Inoue, N., & Matsuda, T. (2000). Use of nisin for inhibition of *Alicyclobacillus acidoterrestris* in acidic drinks. *Food Microbiology, 17*, 315–320.

Yamazaki, K., Teduka, H., Inoue, N., & Shinano, H. (1996b). Specific primers for detection of *Alicyclobacillus acidoterrestris* by RT-PCR. *Letters in Applied Microbiology, 23*, 350–354.

Yamazaki, K., Teduka, H., & Shinano, H. (1996a). Isolation and identification of *Alicyclobacillus acidoterrestris* from acidic beverages. *Bioscience Biotechnology and Biochemistry, 60*, 543–545.

Yang, Y., Yang, Q., Ma, X., Zhang, Y., Zhang, X., & Zhang, W. (2017). A novel developed method based on single primer isothermal amplification for rapid detection of *Alicyclobacillus acidoterrestris* in apple juice. *Food Control, 75*, 187–195.

Zhang, J., Yue, T., & Yuan, Y. (2013). *Alicyclobacillus* contamination in the production line of kiwi products in China. *PLoS One, 8*(7), e67704. https://doi.org/10.1371/journal.pone.0067704.

Zierler, B., Siegmund, B., & Pfannhauser, W. (2004). Determination of off-flavour compounds in apple juice using headspace solid phase microextraction-gas chromatography-mass spectrometry. *Analytica Chimica Acta, 520*, 3–11.