

INTRODUCTION:

The gram-positive, thermophilic, acidophilic, spore-forming bacteria *Alicyclobacillus* spp. are known for their ability to cause off-flavors in fructose-sweetened acidic beverages due to the production of guaiacol. Differentiating between guaiacol- and non-guaiacol-producing bacteria is essential for the beverage industry, as guaiacol is a major contributor to product waste and customer dissatisfaction. Due to the ubiquitous occurrence and production of guaiacol, *Alicyclobacillus* contamination can cause the spoilage of beverages during production. A rapid screening test that differentiates between guaiacol- and non-guaiacol-producing *Alicyclobacillus* is of high importance, including for ingredients in lemonade such as fructose.

To avoid spoilage and therefore, product waste, Hygiena® offers the beverage industry the foodproof® *Alicyclobacillus* Detection LyoKit®, a real-time PCR method that detects both guaiacol-producing and non-guaiacol-producing *Alicyclobacillus* strains after a 24-hour enrichment.

PURPOSE:

This study evaluated the foodproof *Alicyclobacillus* Detection LyoKit, a real-time PCR assay for rapid screening for *Alicyclobacillus* spp. in fructose samples and the differentiation of guaiacol-producing and non-guaiacol-producing strains.

REGISTERED TRADEMARKS

foodproof®, StarPrep® and LyoKit® are registered trademarks of Hygiena® Diagnostics GmbH
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DNA Extraction: foodproof StarPrep Two Kit (KIT230177)

PCR Analysis: foodproof *Alicyclobacillus* Detection LyoKit (KIT230151/KIT230152/KIT230153)

Rapid and Reliable Detection and Differentiation of Guaiacol- and Non-Guaiacol- Producing *Alicyclobacillus* in Fructose by foodproof® Real-Time PCR

BAX® System Q7

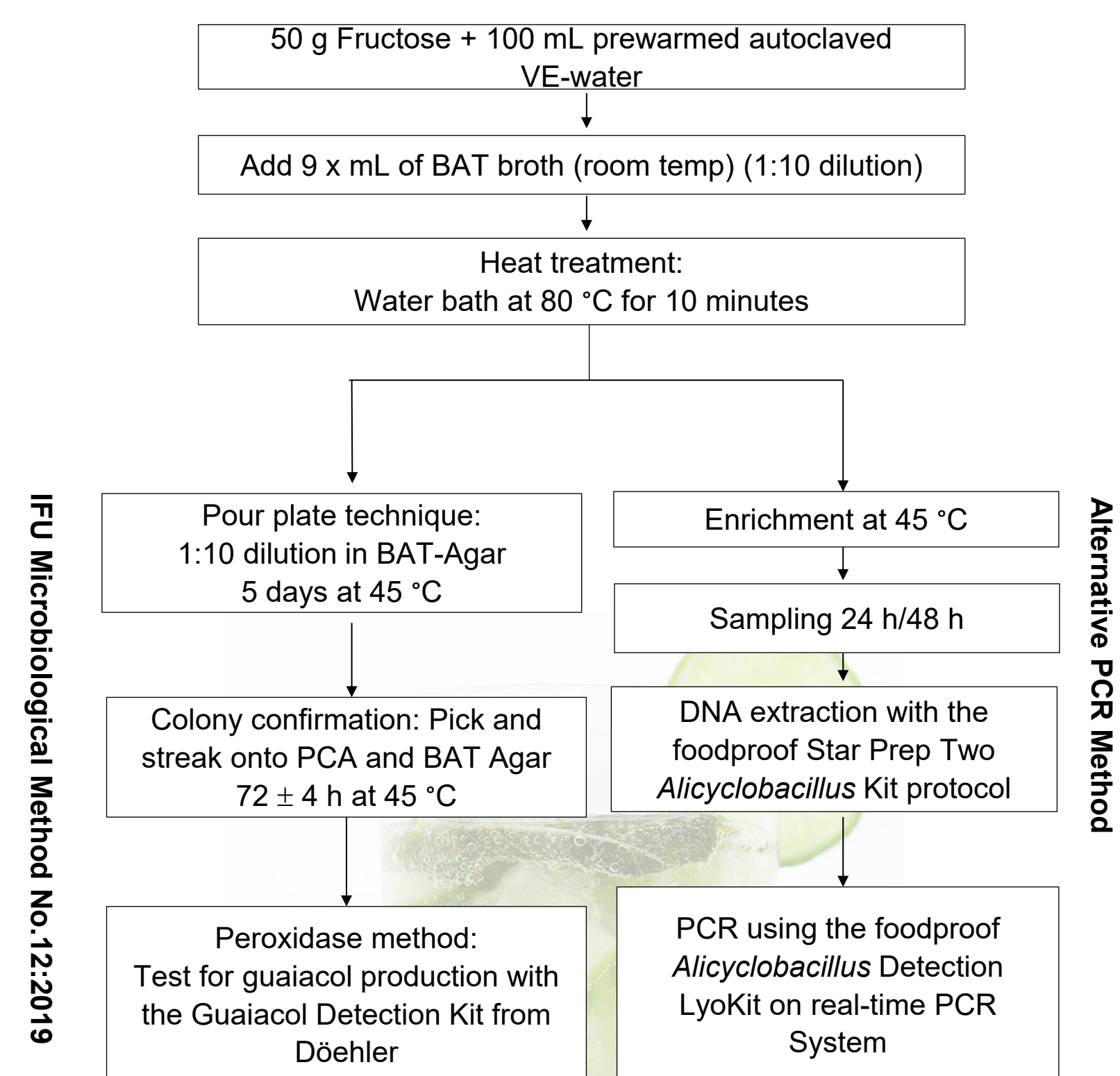
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METHOD:

50 grams of naturally- and artificially-contaminated fructose samples were dissolved in 100 mL VE-Water and heat-treated for 10 minutes. Following this, the samples were diluted 1:10 in BAT broth and enriched at 45 °C for 24 and 48 hours. After enrichment, DNA was extracted with the foodproof StarPrep Two Kit, *Alicyclobacillus* protocol and analyzed with the foodproof *Alicyclobacillus* LyoKit PCR assay. In parallel, the microbiological method, IFU Method No. 12:2019, Method on the detection and enumeration of spore-forming thermo-acidophilic spoilage bacteria (*Alicyclobacillus* spp.), Detection Procedure A (Pour plate technique), as established by the International Fruit and Vegetable Juice Association, was performed for comparison to the alternative PCR method. The artificially contaminated samples were inoculated with a low-spiking level of either *A. acidoterrestris* or *A. acidocaldarius*. Detection was tested after 24 and 48 hours of enrichment at 45 °C.

FLOW CHART:



RESULTS:

Results After 24-Hour of Enrichment at 45 °C: Naturally/Artificially Contaminated Fructose Samples						
Sample	FAM Guaiacol positive [Ct]	HEX <i>Alicyclobacillus</i> spp. [Ct]	ROX Internal Control [Ct]	PCR Result	Microbiology IFU 12:2019	Guaiacol Detection Kit (Döehler)
Negative Control PCR	0	0	31.1	Negative	Negative	Negative
Media Control	0	0	30.6	Negative		
Growth Control (<i>A. acidoterrestris</i>)	10,8	9,9	0	<i>Alicyclobacillus</i> spp. positive Guaiacol positive	<i>Alicyclobacillus</i> spp. positive	Positive
Sample 1: Fructose Naturally contaminated	13,0	13,2	37,5	<i>Alicyclobacillus</i> spp. positive Guaiacol positive	<i>Alicyclobacillus</i> spp. positive	Positive
Sample 2: Fructose Naturally contaminated	0	32,6	31,2	<i>Alicyclobacillus</i> spp. positive Guaiacol Negative	Negative	-
Sample 3: Fructose Naturallycontaminated	0	30,3	29,6	<i>Alicyclobacillus</i> spp. positive Guaiacol Negative	<i>Alicyclobacillus</i> spp. positive	Negative
Sample 4: Fructose Naturally contaminated	0	29,7	29,3	<i>Alicyclobacillus</i> spp. positive Guaiacol Negative	<i>Alicyclobacillus</i> spp. positive	Negative
Sample 5: Fructose Artificially contaminated (<i>A. acidoterrestris</i> : 7 CFU/sample)	22,5	23,2	30,6	<i>Alicyclobacillus</i> spp. positive Guaiacol positive	<i>Alicyclobacillus</i> spp. positive	Positive
Sample 5: Fructose Artificially contaminated (<i>A. caldarius</i> : 1.6 cfu/sample)	0	33,6	30,6	<i>Alicyclobacillus</i> spp. positive Guaiacol Negative	<i>Alicyclobacillus</i> spp. positive	Negative

Results After 48-hour Enrichment at 45 °C: Naturally/Artificially Contaminated Fructose Samples						
Sample	FAM Guaiacol positive [Ct]	HEX <i>Alicyclobacillus</i> spp. [Ct]	ROX Internal Control [Ct]	PCR Result	Microbiology IFU 12:2019	Guaiacol Detection Kit (Döehler)
Negative Control PCR	0	0	31.5	Negative	Negative	Negative
Media Control	0	0	30.8	Negative		
Growth Control (<i>A. acidoterrestris</i>)	10,2	9,1	0	<i>Alicyclobacillus</i> spp. positive Guaiacol Positive	<i>Alicyclobacillus</i> spp. positive	Positive
Sample 1: Fructose Naturally contaminated	12,9	12,3	0	<i>Alicyclobacillus</i> spp. positive Guaiacol Positive	<i>Alicyclobacillus</i> spp. positive	Positive
Sample 2: Fructose Naturally contaminated	0	19,4	30,5	<i>Alicyclobacillus</i> spp. positive Guaiacol Negative	Negative	-
Sample 3: Fructose Naturally contaminated	0	15,3	29,7	<i>Alicyclobacillus</i> spp. positive Guaiacol Negative	<i>Alicyclobacillus</i> spp. positive	Negative
Sample 4: Fructose Naturally contaminated	0	13,0	29,7	<i>Alicyclobacillus</i> spp. positive Guaiacol Negative	<i>Alicyclobacillus</i> spp. positive	Negative
Sample 5: Fructose Artificially contaminated (<i>A. acidoterrestris</i> : 7 CFU/sample)	9,5	9,8	30,6	<i>Alicyclobacillus</i> spp. positive Guaiacol Positive	<i>Alicyclobacillus</i> spp. positive	Positive
Sample 5: Fructose Artificially contaminated (<i>A. caldarius</i> : 1.6 CFU/sample)	0	30,7	29,6	<i>Alicyclobacillus</i> spp. positive Guaiacol Negative	<i>Alicyclobacillus</i> spp. positive	Negative

In Fructose Sample 1 (naturally contaminated) and Sample 5 (artificially contaminated with 7 CFU/sample), guaiacol-positive *Alicyclobacillus* was detected by the alternative PCR method: the FAM channel, for the detection of guaiacol-producing *Alicyclobacillus*, and HEX channel, for the detection of *Alicyclobacillus* spp., were positive with Ct values < 23 after 24 hours of enrichment. This is in accordance with the reference method, with an enrichment of 8 days and additional colony enrichment of 3 days. The Doehler test showed a colour change from beige to brown, indicating the presence of guaiacol in the colony. Samples 2, 3, 4 and 6 had been analyzed as guaiacol-negative *Alicyclobacillus* using the alternative real-time PCR method: positive in HEX, but negative in FAM channel. The IFU-reference method confirmed these results except for sample 2. For sample 2, it is assumed that the contamination limit is below the reference method's detection limit of 1 CFU/g but above the alternative method's detection limit of 1 CFU/sample, corresponding to 1 CFU/10 mL after a 24-hour enrichment. Low contamination levels are likely because the high Ct value of 32 in the HEX channel after 24 hours of enrichment indicates a low organism concentration in the original sample. The Ct value shift of Sample 2 from 24 hours to 48 hours shows an active growth of the guaiacol-negative *Alicyclobacillus* that was present. The artificially contaminated samples confirm the ability to detect low-level contamination in fructose samples by PCR. After just 24 hours of enrichment, with a low spike level of 7 CFU/sample, a Ct value of 23 was reached in both channels for *A. acidoterrestris*; for *A. caldarius*, the Ct value was 33.6 with a low spike level of 1.6 CFU/sample. These results confirm that the foodproof PCR method exhibits a higher sensitivity after 24 hours than the alternative method provides after 8 days.

SUMMARY:

In summary, the detection and differentiation between guaiacol-positive, and guaiacol-negative *Alicyclobacillus*, can be achieved after 24 hours of enrichment at 45 °C with the foodproof *Alicyclobacillus* LyoKit (KIT230151) in combination with the DNA isolation kit foodproof StarPrep Two Kit, *Alicyclobacillus* Protocol (KIT230177). This alternative method saves 7 days of time-to-results, requires less hands-on time than the reference method, and shows more sensitive detection of contaminants.

SIGNIFICANCE:

The foodproof *Alicyclobacillus* Detection LyoKit® offers the beverage industry a rapid, reliable and easy-to-use PCR-based technology for the detection of *Alicyclobacillus* spp. in fructose samples with differentiation option between guaiacol-producing and non-guaiacol-producing strains preventing false-negative results with the internal PCR control and preventing carry-over contamination due to integrated Uracil-N-Glycosylase in the assay.

No. 10 Envelope