

Evaluation of detection methods for alicyclobacilli in fruit juice concentrates in Australia

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Introduction

Spoilage of shelf stable fruit juice products by *Alicyclobacillus* bacteria can be very costly to the fruit juice industry. The spores, often present in fruit concentrates, are heat resistant and can survive heat processes used by juice manufacturers. It is therefore important that concentrates and other raw materials are screened for spores of these taint producers to reduce the risk of spoilage of processed products. However confusion exists regarding screening tests, target organisms, and in the interpretation of test results. The IFU (International Federation of Fruit Juice Producers) undertook to develop internationally acceptable methods for the detection of these organisms in concentrates and juices, and has subsequently published a general method for detection of alicyclobacilli. This project was developed to provide Australian input and data to support this method development.

Aims

To evaluate the IFU method and additional media to detect alicyclobacilli in (non filterable) concentrates and juices. To evaluate the reliability of the results for the fruit juice industry, and the suitability of the method for routine use.

Methods

The initial product testing was conducted by industry laboratories from autumn to spring 2003. For concentrates the dilution, heat shock, enrichment, incubation and subculturing procedures prescribed by the IFU method were followed using BAT (*Bacillus acidoterrestris*) broth and agar. In parallel, samples were also tested with SDW (sterile distilled water), K and OSS (orange serum with 0.5% sucrose) agars. Enrichment broths were subcultured at 7 days as prescribed by the IFU method, and also at 2 and 5 days.

Screening and confirmatory tests were performed by Food Science Australia. Presumptive alicyclobacilli were distinguished from other microbes by spore production at pH \leq 4.0 (BAT agar) and no growth at pH $>$ 6. They were presumptively identified to species level using a range of tests, with 5-7 isolates from each group identified by 16S rRNA sequencing. Taint production was also evaluated in apple juice, and confirmed by chemical analysis.

Results

- Only two species of *Alicyclobacillus*, *A. acidoterrestris* and *A. acidocaldarius*, were detected in 156 concentrates tested using 2 enrichment broths and 3 agars. 505 colonies were screened.
- Only strains of *A. acidoterrestris* were shown to produce taints in the test juice. The presence of *A. acidocaldarius* did not impact on juice quality.
- Highest detection rates for *A. acidoterrestris* in orange concentrates were achieved by 5 days with SDW and K agar, detecting 92% of +ve samples (Table 1). Rates did not increase significantly at 7 days in any media.
- In apple concentrates, overall detection rates were lower and isolates more evenly spread than in orange concentrates. Maximum detection rates were achieved by 5 days and did not increase significantly at 7 days. Highest recovery (BAT broth and agar) only detected 83% of the samples that were +ve for *A. acidoterrestris*, indicating the systems trialed were probably not optimal for apple concentrates, and that spore numbers were lower than in orange concentrates.

Table 1: Comparison of incubation times and media for the detection of *A. acidoterrestris* (numbers in blue) and *A. acidocaldarius* (numbers in pink) in concentrates. Results are shown as cumulative totals of +ve samples, and % +ve samples (highest only).

SDW: Sterile distilled water.
OSS: Orange serum agar with 0.5% sucrose.
BAT: *Bacillus acidoterrestris* medium/agar

Sample & broth	2 day broth incubation			5 day broth incubation			7 day broth incubation		
	K	OSS	BAT	K	OSS	BAT	K	OSS	BAT
Orange/SDW	13 0	21 0	20 4	24 (92%) 1	22 4	21 17	24 2	22 4	22 28 (80%)
Orange/BAT	7 0	10 0	11 1	15 1	13 0	18 5	15 2	13 0	18 2
Apple/SDW	5 0	5 0	5 2	7 0	6 0	6 2	7 0	7 0	7 2
Apple/BAT	5 1	6 0	7 3	8 1	9 1	10 (83%) 6	8 2	9 2	10 7

Results (cont)

- Detection of *A. acidocaldarius* was always highest where BAT agar was used. A greater number of colonies will need confirmatory tests if this agar is used routinely.
- Whilst OSS agar performed quite well in detecting *A. acidoterrestris* and in providing limited growth of *A. acidocaldarius*, use of this medium is generally not favoured by most laboratories.
- K agar and SDW provided a good compromise for detecting taint producing *A. acidoterrestris* in apple and orange concentrates and suppressing the growth of the non-taint producing *A. acidocaldarius*. Although some samples tested +ve for *A. acidoterrestris* at 2 days, subculturing at 5 days was necessary for many samples. This is still a distinct advantage over the 7 days prescribed by the current IFU method.

Recommendations

The following amendments to the current IFU method are recommended:

- Change the focus of the method to detect taint producing strains only
- Recommend SDW and K agar for orange concentrates
- Evaluate additional media/samples for apple concentrates, but in the short term recommend the use of K agar in addition to BAT agar
- Prescribe subculturing of enrichment broths after 2 days and again at 5 days if plates from the 2 day broth are -ve for taint producers
- Prescribe confirmatory tests to distinguish potential taint producers.

Additional comments

- The IFU method recommends testing 10 g samples of concentrate. Greater sensitivity and reliability will be achieved if the sample size is increased (e.g. to 100 g concentrate), particularly as spores are likely to be present in low numbers and distributed unevenly.
- A. acidiphilus* has been shown to produce guaiacol in laboratory media (Matsubara *et al.* 2002). This organism also grows on K agar (Jensen, unpublished) so is likely to be detected using these amended methods, if present.

REFERENCES

- IFU (2004). Standard IFU Method 12 - Microbiological detection of *Alicyclobacillus* in fruit juices. International Federation of Fruit Juice Producers, Paris.
- Matsubara *et al.* (2002). *Alicyclobacillus acidiphilus* sp. nov., a novel thermo-acidophilic ω -alicyclic fatty acid-containing bacterium isolated from acidic beverages. Int. J. Syst. Environ. Microbiol. 52, 1681-1685.

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