Spoilage Organisms in the Citrus Industry: Methods, Challenges and Prevention

Emilia Rico, Ph.D.
BCN Research Laboratories, Inc.
Rockford, TN
Emilia.Rico@bcnlabs.com
www.bcnlabs.com

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AGENDA

- Introduction
- Spoilage of heat-processed juices and beverages:
  - TAB/ACB/HRM
  - Human health significance
- Environmental Spoilage of heat-processed juices and beverages:
  - Bacteria/Yeast/Molds
  - Biofilm
  - Human health significance
- Methodology
- Rapid Methods
- Spoilage Prevention

http://www.sflorg.com/sciencenews/scn050307_01.html

Introduction

- Some of the most important spoilage organisms of heat-processed fruit juices and other fruit containing beverages are:
  - *Alicyclobacillus* guaiacol positive (ACB)
  - Heat-resistant molds (HRM)
- Spores (ACB)and ascospores (HRM) are found in soil – contamination of fruits and environment
- These organisms can cause great economic losses to the juice and beverage industry

Spoilage of Heat-processed Juices and Beverages

Hot-filled beverages can be spoiled by heat-resistant molds (HRM) and ACB guaiacol positive

HRM belong to the Ascomycetes and are characterized by the production of heat-resistant spores called ascospores (sexual spores)

Ascospores of HRM and spores of ACB can survive the pasteurization process given to juices and beverages (activation)

HRM can grow at very low oxygen tensions and are capable of producing pectinolytic enzymes.

Heat-Resistant Molds

Fruit juices and beverages that contain fruit juices

- Species most commonly implicated in fruit juices and fruit containing beverages are *Byssochlamys fulva, Byssochlamys nivea, Byssochlamys spectabilis (Paecilomyces variotii), Neosartorya fischeri, Talaromyces macrosporum*, *T. trachyspermus, Eupenicillium spp.*, etc.

Beverages without fruit juices

- In our experience, *Byssochlamys spectabilis* (*Paecilomyces variotii*) is a very common spoilage mold in the U.S.
- In some instances, *B. fulva, B. nivea* and *Talaromyces trachyspermus* have also been isolated
- In other countries, *Neosartorya* sp. can be a problem

Sources of Spoilage

Fruit juices and beverages that contain fruit juices

- Fruit juice concentrates
- Fruit purees
- Liquid sweeteners in sweetened products
- Pectin
- Packaging
- Environment
- Processing water for ACB

Beverages without fruit juices

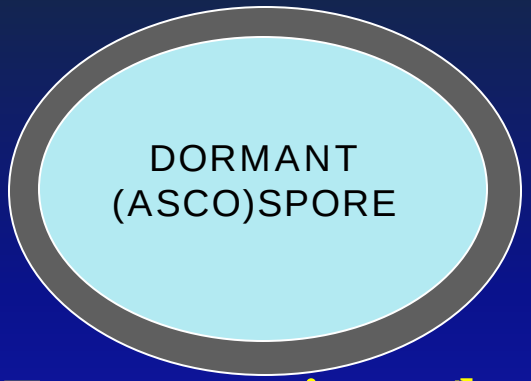
- Liquid sweeteners: *B. spectabilis (Paec. variotii)* ascospores liquid sucrose and HFCS –our study on 2005-06 found highest incidence of ascospores from July to October
- Packaging: PET bottles -- our study on 2006-07 found *Byssochlamys spectabilis* ascospores (after heat shock) in empty PET bottles at a rate of up to 2.5% -- filling temperature (82°C/180°F) was enough to activate ascospores = <u>problem</u>: rinser/filler proximity
- Environment

(Asco)spore Activation

(Asco)spores need to be activated to be able to germinate

(Asco)spores can be activated by the heating during pasteurization or in the lab

After germination, they can grow and spoil the product during storage at room temperature (HRM) or at moderate/high temperature (ACB)



Extreme trigger/heat/high-pressure/?

D-values of some heat-resistant molds and ACB

Byssochlamys fulva	D_{90}= 4-36 min
Byssochlamys nivea	D_{85}= 1.3-4.5 min
	D_{90}= 1.5 min
Byssochlamys spectabilis	D_{85}= 47-75 min*
Talaromyces macrosporus	D_{90}= 2-11.1 min
Neosartorya fischeri	D_{90}= 4.4-6.6 min
Eurotium herbariorum	D_{70}= 1.1-4.6 min
Alicyclobacillus acidoterrestris	D_{95}= 2.3-5.6 min**
	D_{100}= 0.7-1.2 min***

Adapted from Samson *et al.* (2004)

***Houbracken *et al.* (2006); ****Silva and Gibbs (2001)**

*****Bahçeci and Acar (2007)**

Human Health Significance of HRM and ACB

Byssochlamys sp. produce patulin, byssotoxin A and byssochlamic acid; patulin has been reported to be produced mainly by *B. nivea* and viriditoxin by *B. spectabilis* (Houbraken *et a*l., 2006; Puel et al., 2007).

Neosartorya fischeri is known to produce the tremorgenic substances, fumitremorgin A and C, terrain and verruculogen (Horie and Yamakazi, 1981; Nielsen et al., 1988)

There have not been many studies on the production of these metabolites in spoiled beverages – human health significance?

ACB are non-pathogenic

Environmental Contamination of Heat-processed juices and beverages

Heat-treated beverages can also be spoiled by non-heat resistant molds (*Fusarium oxysporum* and others), yeasts, LAB and AAB

Contamination occurs when sterility conditions are broken within the “aseptic” environment (rinser-filler-capper)

Packaging can also play a role (air rinser) -- molds

Environmental Contamination of Heat-processed juices and beverages

When there is a problem with the closures due to overfill of juices with pulp (cooling tunnel) -- **slow down filler!**

When there is a problem with faulty closures (cooling tunnel)

Biofilm accumulation within processing equipment and in the cooling tunnel can be a significant source of contamination -- Bacterial cells in biofilms may be as much as 500 times more resistant to sanitizing chemicals

Human Health Significance of Environmental Contaminants

Molds produce more than 400 toxic secondary metabolites

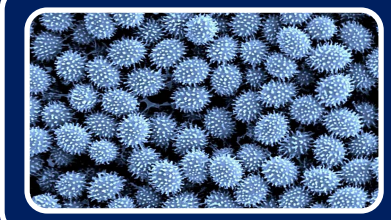
There have not been many studies on the production of these metabolites in spoiled beverages (*Fusarium oxysporum* in juices) – human health significance?

Some molds can be human pathogens or allergenic – human heath significance?

LAB and AAC do not produce toxic metabolites

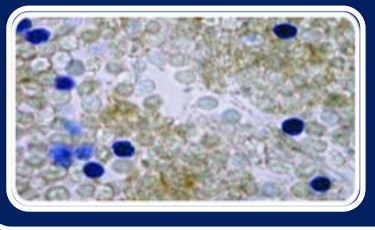
Some yeasts can be human pathogens – human health significance?

HRM/ACB Methodology and Challenges



For HRMs, heat-shock your ingredients at 75º-80ºC for 30 min – incubate plates at 30ºC for 5-21 days – NEED TO USE A LARGE SAMPLE -- challenge

For ACB, heat-shock your ingredients at 80ºC for 10 min – incubate plates at 44-46ºC for up to 7 days – NEED TO USE A LARGE SAMPLE -- challenge

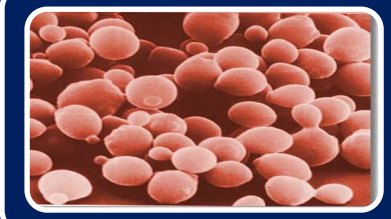


Spoiled product: If a HRM is suspected, transfer growth from bottle to MEA – DO NOT HEAT-SHOCK -- incubate plates at 30ºC for up to 14 days

Spoiled product: If an ACB is suspected, plate using K or YSG plate WITH AND WITHOUT HEAT-SHOCK -- incubate plates at 44-46ºC for up to 7 days

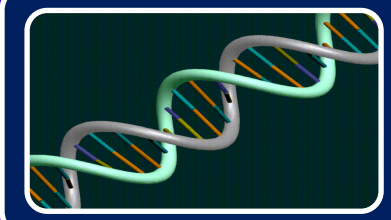
HRM/ACB Methodology and Challenges

Identify correctly the fungi or bacteria spoiling your product – challenge



Molecular methods for the identification of bacteria and yeasts work very well

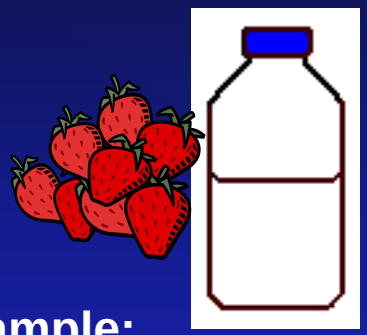
Identification of food-borne filamentous fungi relies on very specialized knowledge, requiring years of training and experience – FoodMold CD



Traditional fungi identification methods should be used in conjunction of molecular identification methods

Detection method for heat-resistant molds

(courtesy of Rob Samson, CBS, Utrecht, The Netherlands)



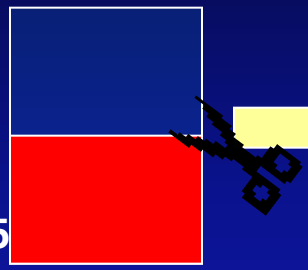
Sample:

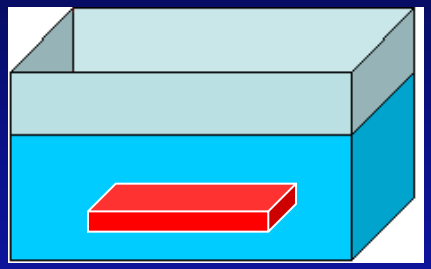
1. Pectin: 12.5 g + 230 ml H_2O
2. Fruit (concentrate): 100 g + 150 ml H_2O
3. Solid samples (eg powdery ingredients, soil): 25 g + 225 ml H_2O

Homogenize

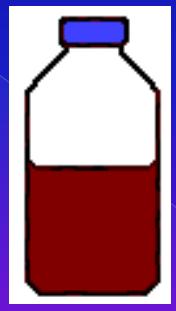
and seal

Adjust pH >3.5



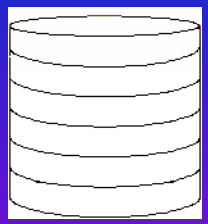


Heat treatment, 30 min 75-80 C



1. Cool the sample rapidly
2. Mix the sample with 250 ml handwarm double strength MEA agar + chloramphenico

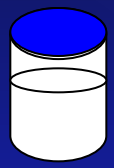
Mix thoroughly and disperse agar/product mass into approx. 7-8 Petri-dishes (diam. 14.5 cm)



1. Incubate for 14 -21 d. at 28-30 C
2. Check every 7 days

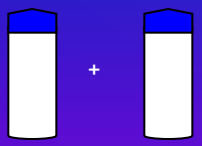
HRM/ACB Method for Sugars

BCN Labs (2002):



LIQUID OR GRANULATED SUGAR

50 g 50 g

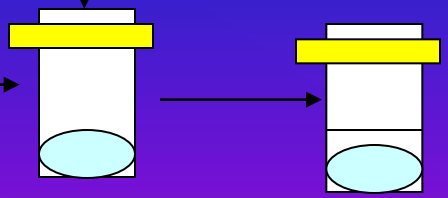


0.1% PEPTONE BUFFER

WASHED TWICE

ADD 10 ML 0.1% PEPTONE BUFFER

ADD 10 ML ALI BUFFER



HEAT AT 75°C FOR 30 MIN, IMMERSED

HEAT AT 80°C FOR 10 MIN, IMMERSED

COOL DOWN

COOL DOWN TO 45°C

MEA + Ab

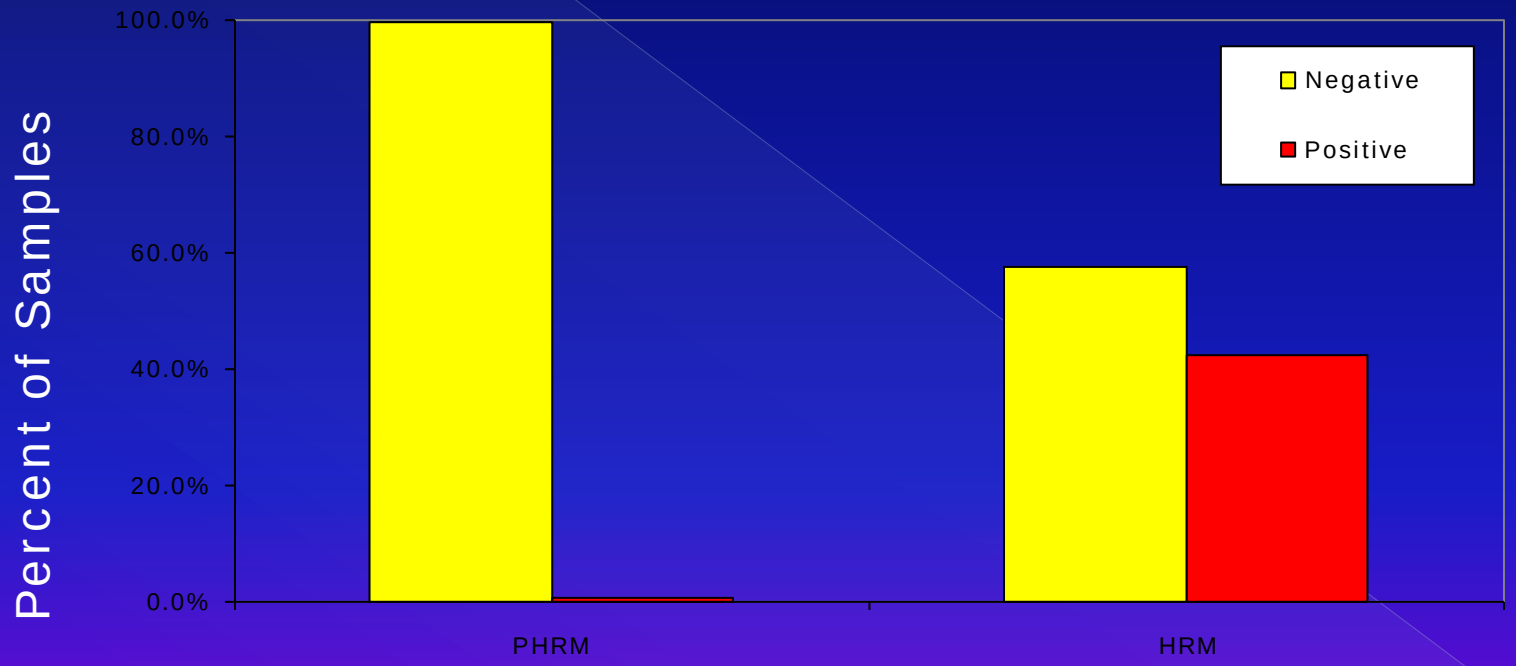
K AGAR OR YSG AGAR

INCUBATE IN BAG AT 30°C, 5-21 DAYS

AT 44-46°C, 7 DAYS

Methodology comparison for liquid sweeteners

June-October 2005



Plating using OPDA **Heat-shocking**

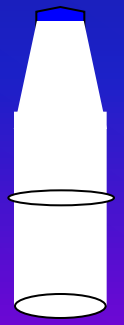
HRM/ACB Method for water

BCN Labs (2002):

HRM Method for PET Bottles

EMPTY PET BOTTLE

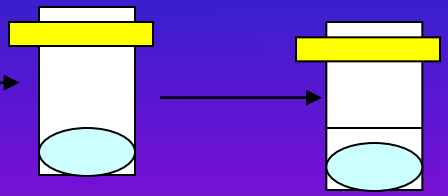
ADD 50 ML 0.1% PEPTONE BUFFER



WASHED TWICE

FILTER

ADD 10 ML 0.1% PEPTONE BUFFER



HEAT AT 75°C FOR 30 MIN, IMMERSED

COOL DOWN

MEA + Ab

INCUBATE IN BAG AT 30°C, 5-21 DAYS

Methodology for ACB for samples that cannot be filtrated

- Use centrifugation – challenges
- Use enrichment – presence or absence
  - 5 day enrichment (original):
    - 50 mL into 450 mL BAT with heat shock
    - 5 day enrichment (BAT) 5 day plate (K agar)
    - Guaiacol confirmation in 4 hours post 5 day plating
  - 3 day enrichment (Rule *et al*., 2010):
    - 50 mL into 450 mL YSG broth with heat shock
    - YSG Broth (3 day) and YSG plates (3 day)
    - Guaiacol confirmation in 4 hours post 3 day plating

Rapid Methods

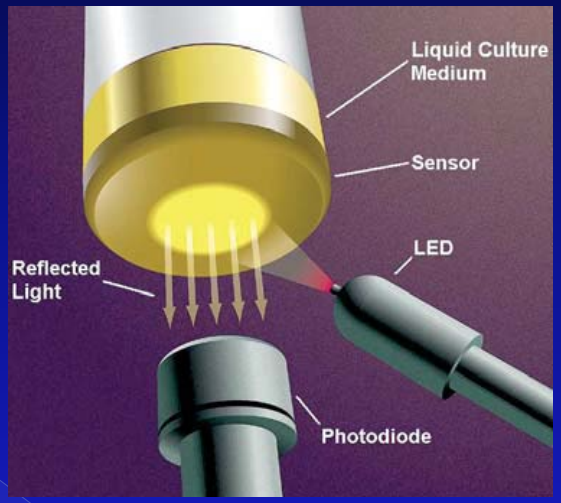
- BacT/ALERT 3D® (bioMérieux) -- Colorimetric growth detection method (CO_2 production)
- TEMPO® (bioMérieux) – fully automated enumeration system based on the MPN method
- DiversiLab (bioMérieux) – strain typing
- Molecular methods for identification of bacteria, yeast and molds

BacT/ALERT (bioMérieux)

- Colorimetric growth detection method -- color change being produced in a growth medium as a result of CO_2 production of organism (metabolic growth)
- Continuous monitoring of growth in 5 - 20 mL of product
- Direct or after a pre-incubation/hold time – results in 4 days after 3 d enrichment in YSG broth

BacT/ALERT (bioMérieux)

- As additional CO_2 is generated within the culture bottle, the built-in sensor changes from a grayish color to a lighter yellow color.
- The BacT/ALERT system utilizes disposable culture bottles - in both aerobic and anaerobic media formulations - for testing a variety of low and high acid food and beverage products.



Example of CO_2 Detection by BacT/ALERT

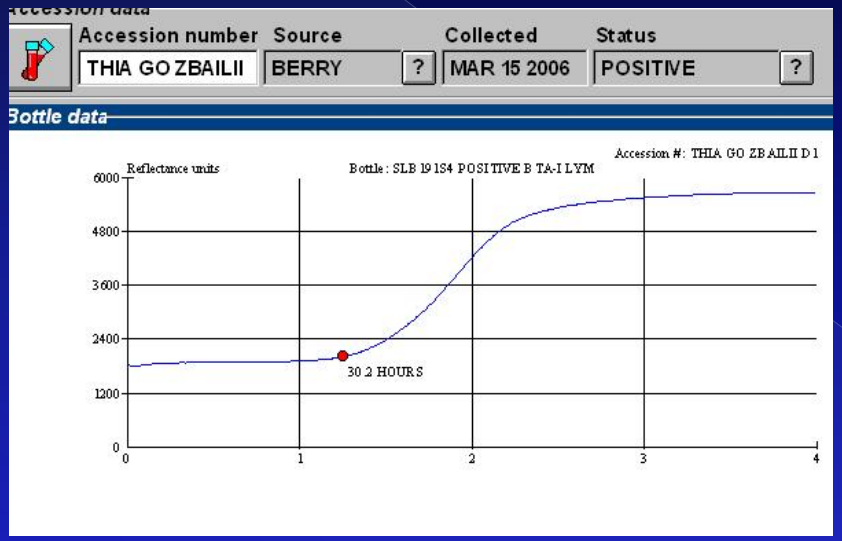


Figure 1. Graph Example of CO2 detection of *a yeast (Zygosaccharomyces bailii)*

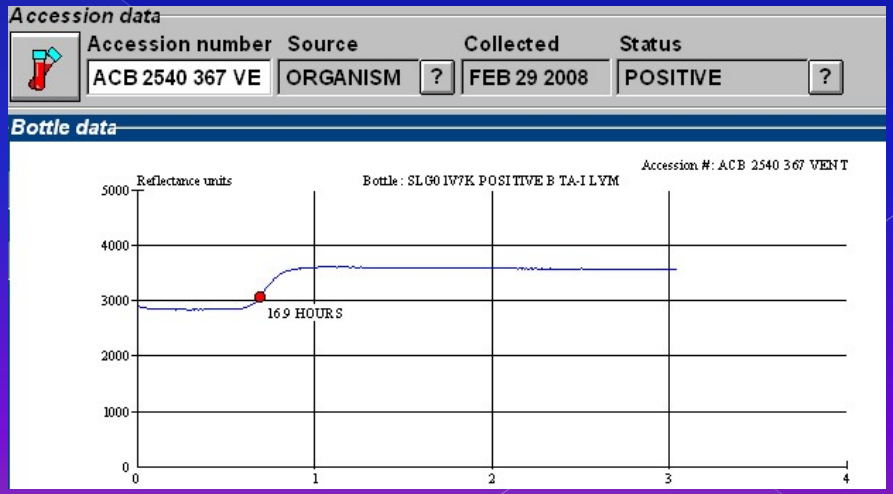


Figure 2. Graph Example of CO2 detection of *Alicyclobacillus acidoterrestris in apple juice post 3 day pre-incubation off line in YSG*

Prevention of Spoilage

The best method to prevent or reduce spoilage is to prevent or control contamination -- better said than done!

Monitor raw ingredients, packaging materials, equipment, cooling tunnel water, and environment – establish critical limits and corrective actions

Use adequate thermal processing – you may not be able to produce product that is 100% spoilage free

Prevention of Spoilage

Cool down product as fast as possible and store it in a cool place -- better said than done!

Prevent biofilm formation and remove it when necessary – cleaning frequently and using the proper cleaner and sanitizer

Implement a strong sanitation program (equipment, overheads, ceilings, airveyors, coolers, drains, floors) – use the right sanitizer (iodine, stabilized chlorine dioxide)

Additional Information

- International Commission on Food Mycology (ICFM) for methodology in food and beverage mycology methodology and member contact information:

  www.foodmycology.org

- ICFM workshops are held every 3 years and are open to the industry: June 2013 in Freising, Germany