Validation of nut processes: An ABC perspective

Tim Birmingham,
Director, Quality Assurance and Industry Services

Presentation Overview

- Pasteurization program research
- The ABC validation process
- Validation of equipment used on almonds

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ABC Pasteurization Program: Baseline Research

Research

- Microbial surveys (Orchard level)
- Risk Assessment
- Pathogen / Surrogate research studies
- Validation research / Protocols / Guidelines
  - Oil Roasting
  - Blanching
  - Dry Roasting
  - Propylene Oxide (PPO)
  - Steam / Moist heat

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Pathogen of Concern – Orchard Surveys

Survey of Almonds from Huller/Shellers (100 g)

Year	Number Positive	% Positive
2001	12 of 2003	0.60
2002	24 of 2012	1.2
2003	15 of 1764	0.80
2004	12 of 1643	0.73
2005	18 of 1852	0.97
2006	30 of 1899	1.6
2007	15 of 1799	0.83
2010	10 of 1000	1.0

Average 0.97% positive samples per year

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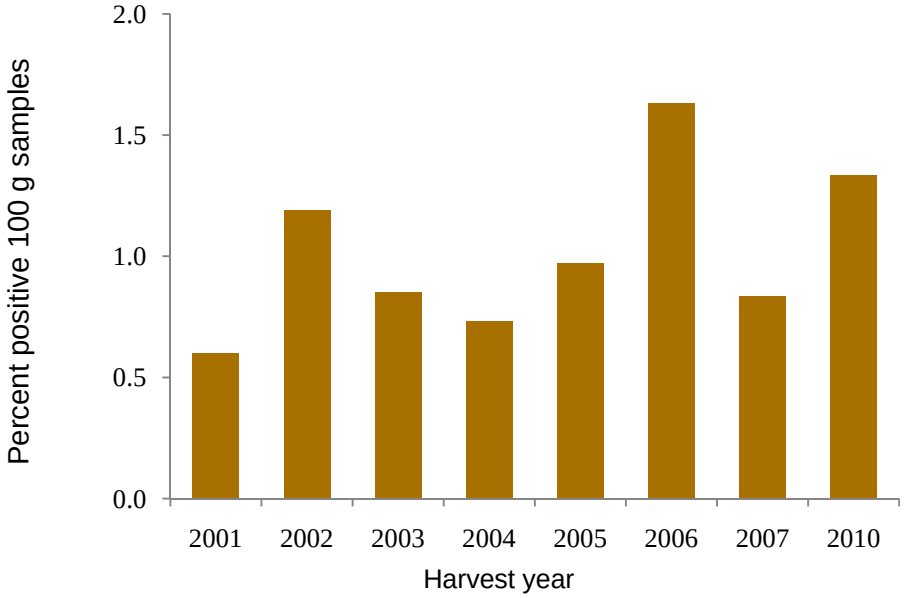
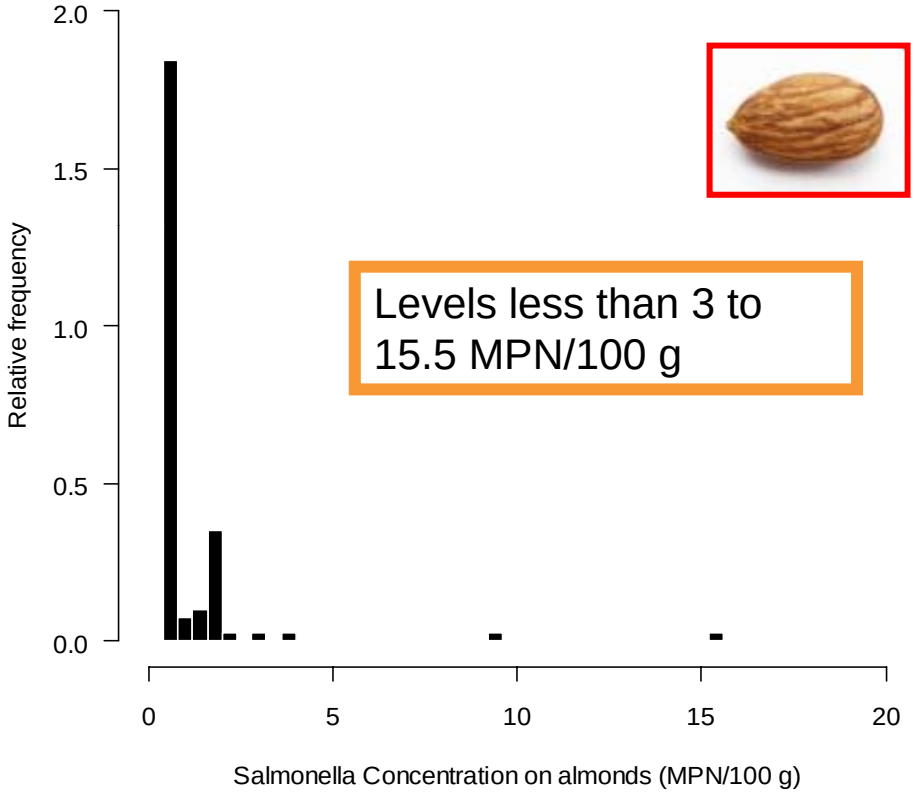
Salmonella prevalence and concentration in almonds





Levels less than 3 to 15.5 MPN/100 g

Lambertini et al. FRI 45 (2012) 1166-1174

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Salmonella serotypes (2001 through 2006 almond samples) characterized by antimicrobial susceptibility profiling and PFGE

Serotype	No. of Times Isolated from Different Almond Lots	No. of Unique Strains Identified
Agona	4	4
Enteritidis	11	7
Montevideo	12	9
Muenchen	10	7
Newport	7	7
Typhimurium	9	6
Total	53	40

Singla and Harris, unpublished

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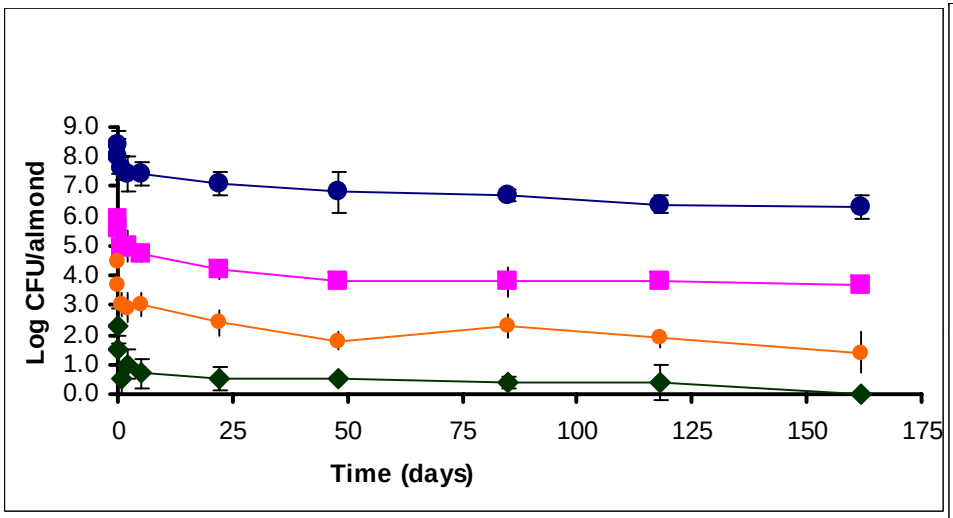
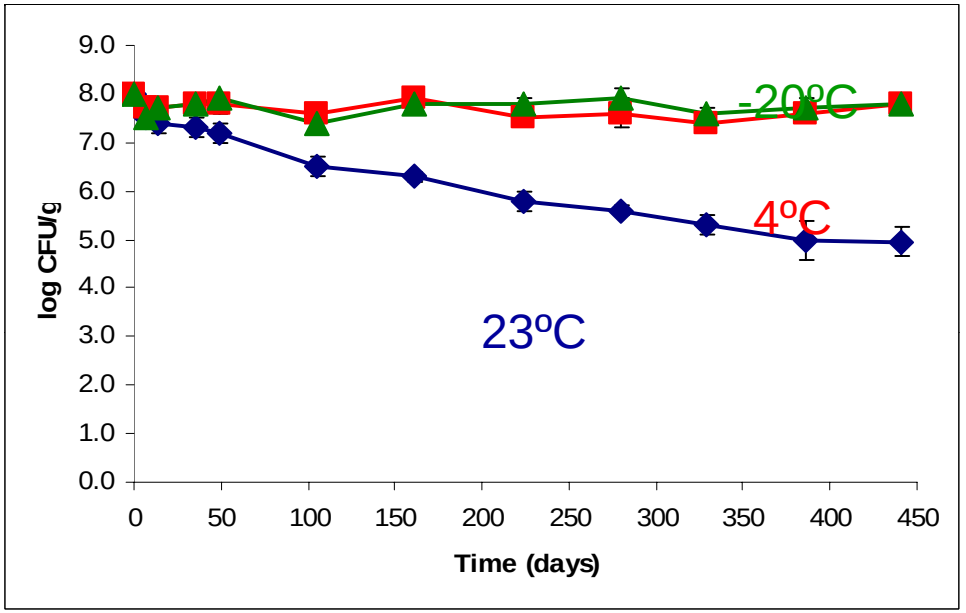
Survival of Salmonella on almonds





The answer is no, Salmonella does not grow in almonds. However, it does survive a really long time!

Infective dose *Salmonella* can be: VERY LOW
10 to 1000 cells per serving

Uesugi, Danyluk and Harris, 2006 JFP 69:1851-1857

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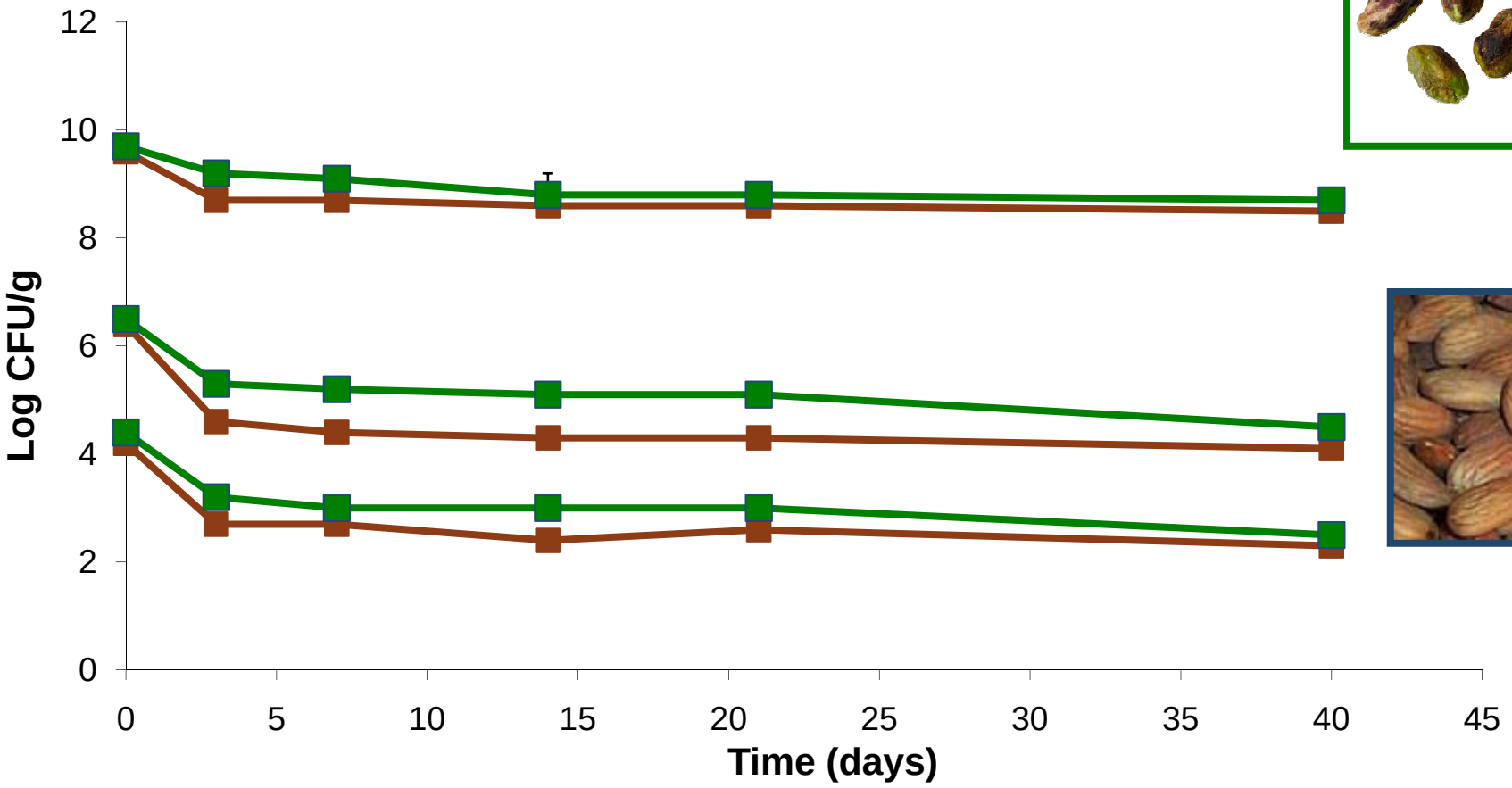
Survival of *Salmonella* spp. (6 serovars) on almonds and pistachios stored at 23°C.



Slide Courtesy of Dr. Linda Harris, UCD

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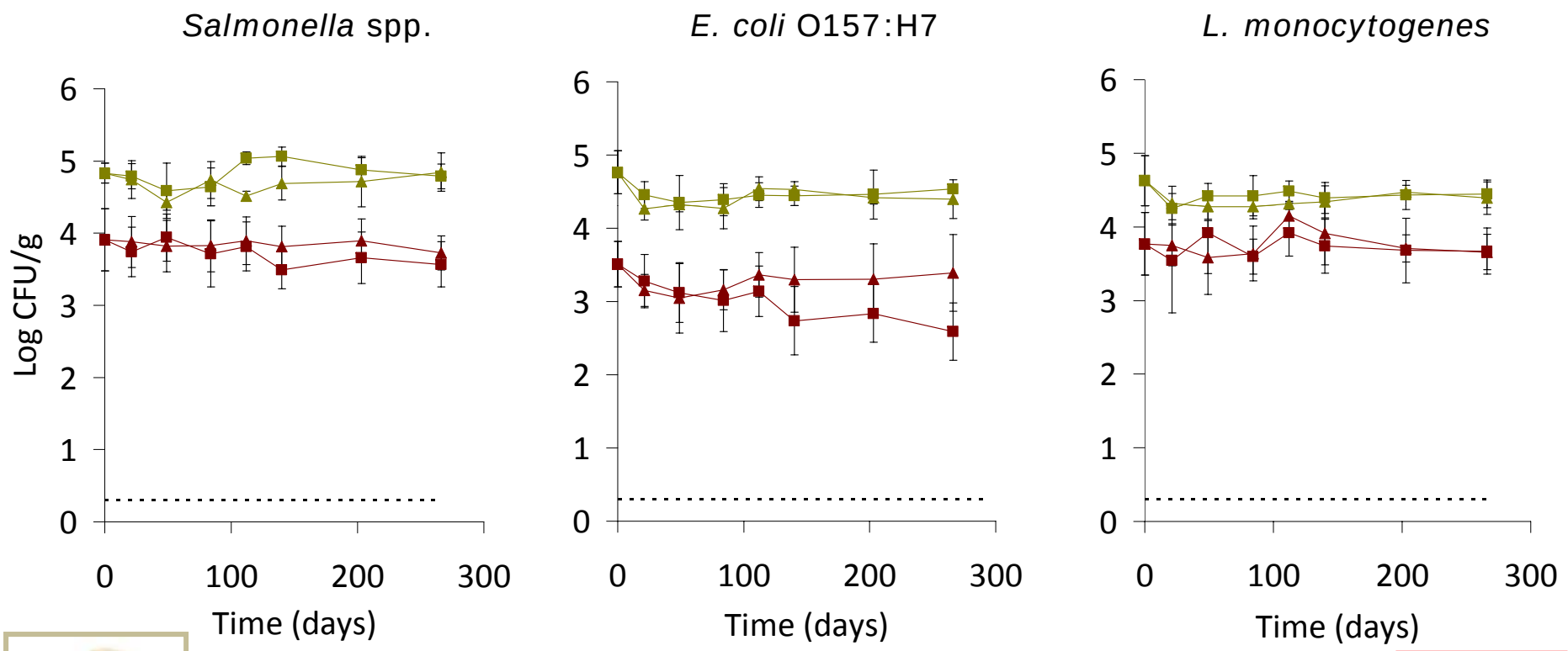
Survival during storage at -20°C (triangle) and 4°C (square) on non-selective agar. No decline over 10 months (exception *E. coli* O157:H7 and almonds (0.09 log CFU/month))



Kimber, Kaur, Danyluk and Harris: IAFP 2011 poster, M.S. Thesis (Kimber) (Slide Courtesy of Dr. Linda Harris, UCD)

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What is an Appropriate Level of Protection?

Survey Work – Backbone of Risk Assessment (RA)

- Showed us that *Salmonella* is present in low levels in 1% of the 100g samples tested
- Doesn't quantify level of risk - helps to define where focus should be

Risk Assessment

- Numerous variables and distributions affecting Salmonella survival modeled to predict risk of illness
  - Levels of contamination per serving, survival characteristics over time, consumption patterns, storage time and conditions, etc.
- RA is a tool to help understand the risk of illness and make informed decisions on how a specific process will reduce that risk
- Helps to define target lethality required to achieve an appropriate level of protection – e.g. 4 log vs. 5 log

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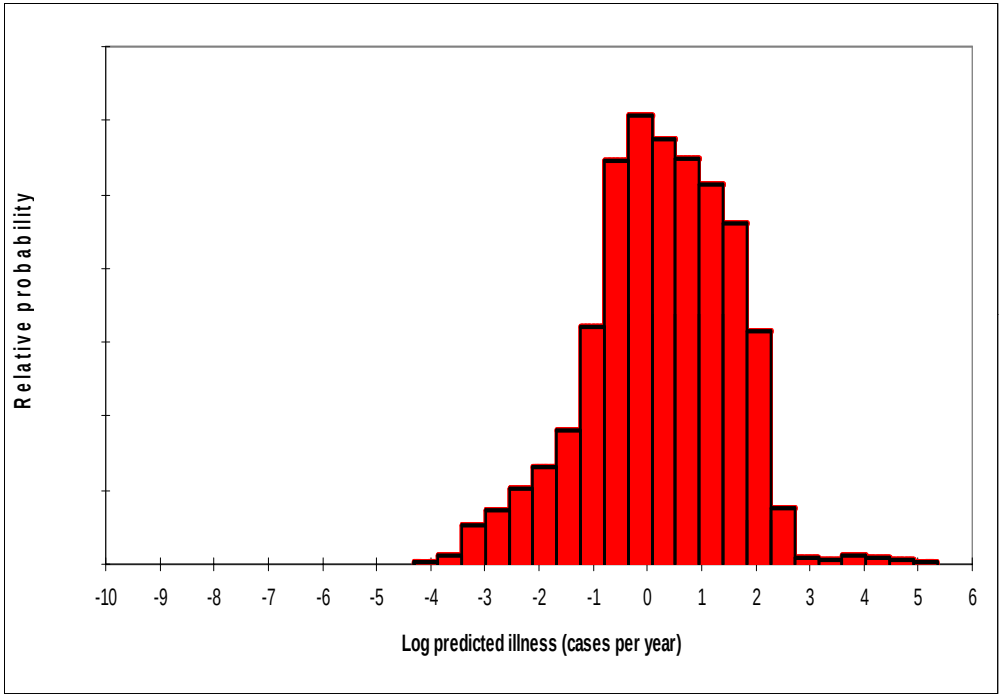
*2005 Risk Assessment Predicted annual illness from consumption of raw almonds.



RA predicted 78% chance of >1 case of salmonellosis per year associated with consumption of raw almonds in the U.S.

Mean predicted cases of 8 per year

*Monte Carlo simulations assessing the risk of salmonellosis from consumption of raw almonds

Michelle D. Danyluk[1], Linda J. Harris[1] and Donald W. Schaffner[2]

[1] Department of Food Science and Technology, University of California, Davis
[2] Food Risk Analysis Initiative, Rutgers University

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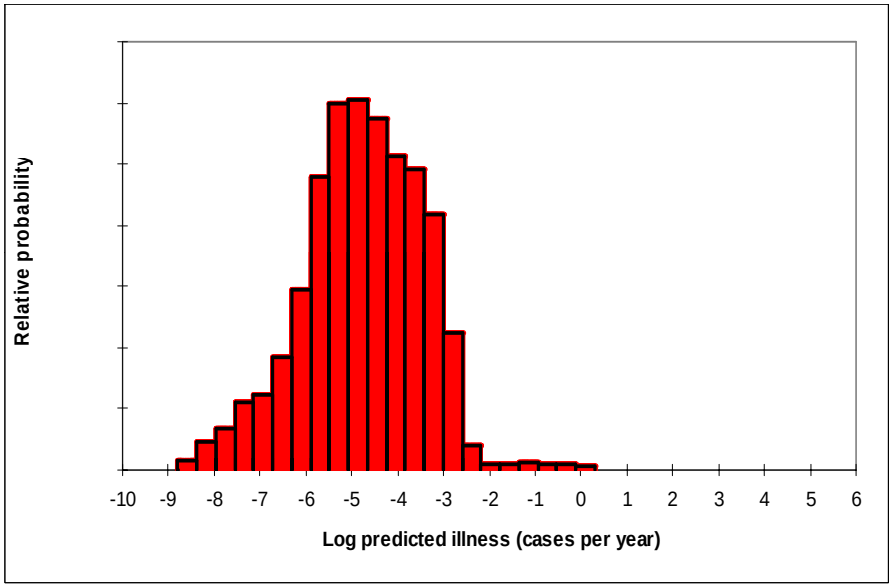
*Predicted annual illness from consumption of almonds treated with a process achieving a 5 ±1-log reduction



Application of a 5 +/- 1 log process reduced that predicted risk to less than 0.69%)

Probability of >1 case/year = <.69% with mean cases per year of 7 x 10-5 per year

*Monte Carlo simulations assessing the risk of salmonellosis from consumption of raw almonds

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Microbial Surrogate Development

Table 1. Potential Surrogates for *Salmonella*	
	Culture Names and Source ID
Salmonella culture	*Salmonella* Enteritidis *Phage Type 30* (ATCC BAA-1045) (SE PT 30)
Surrogate cultures	*Pantoea agglomerans* SPS2F1 (PA)
	Pediococcus spp. NRRL B-2354 (Later reclassified as *Enterococcus faecium*) (PD)
	Enterococcus faecalis (ATCC 49452) (EF)
	Lactobacillus plantarum (ATCC 14917) (LP)
	Lactobacillus fermentum (ATCC 9338) (LF)
	Streptococcus faecalis (ATCC 33186) (SF)

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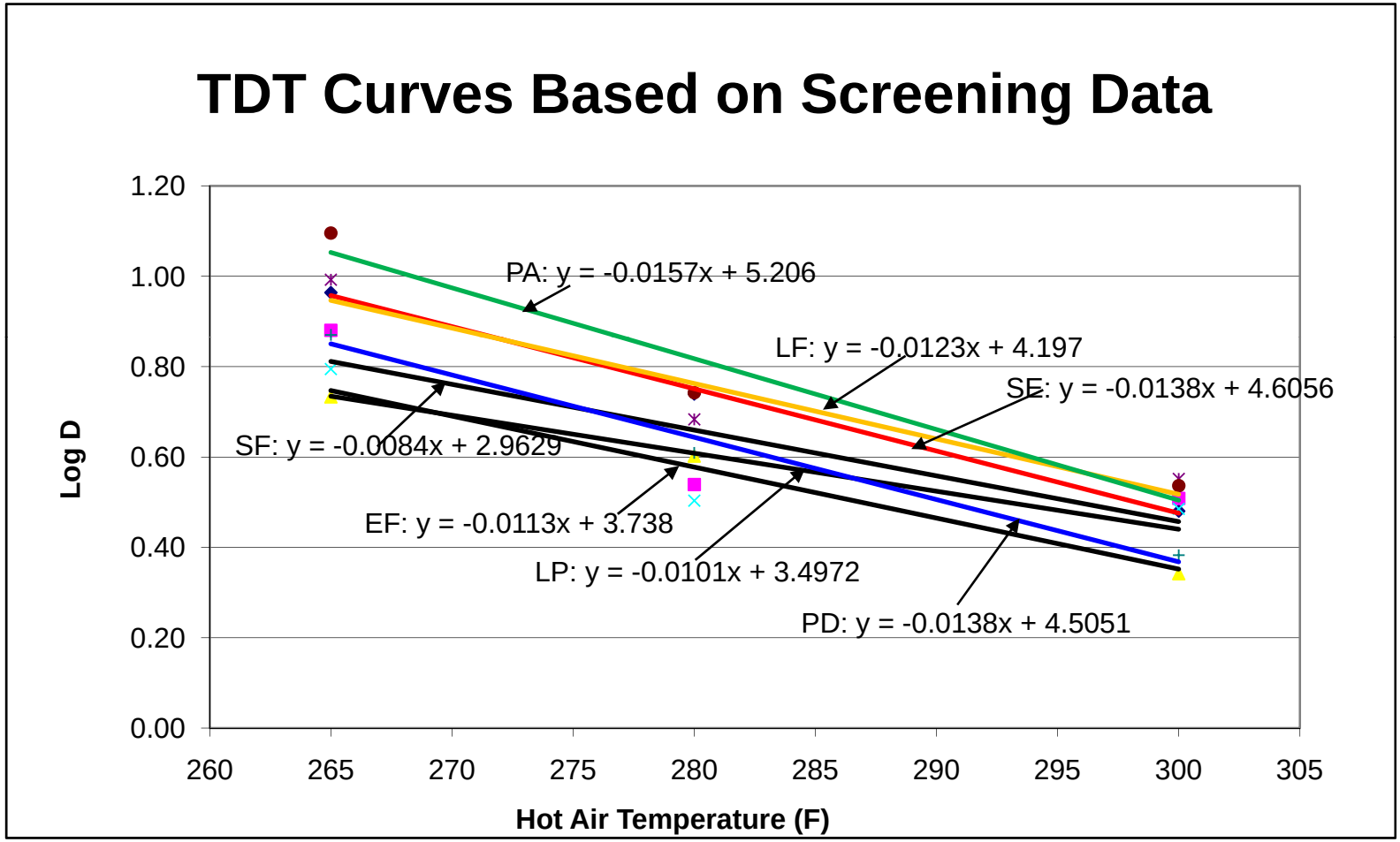
Potential Cultures Screened (Dry Heat)



Pantoea agglomerans (PA) and Enterococcus faecium (PD) were chosen for full TDT study

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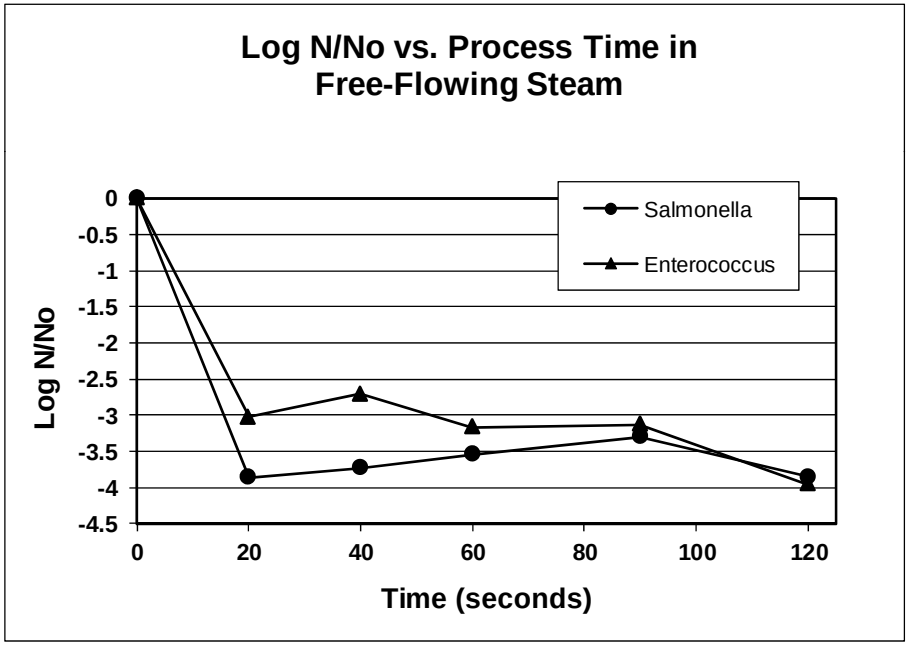
E. Faecium (NRRL B-2354 and ATCC 8459) confirmed for moist and dry heat validation

Moist Heat: Log reduction *S. enteriditis* vs.*E. faecium*



*Dry heat processes for 4-log destruction of SE PT 30 (General Method Calculations)

Time (minutes)	Temperature (°F)
100	250
50	265
23	280
12	295
9	300

*Note: Based on most conservative data from ABC sponsored studies & z-value of 47°F

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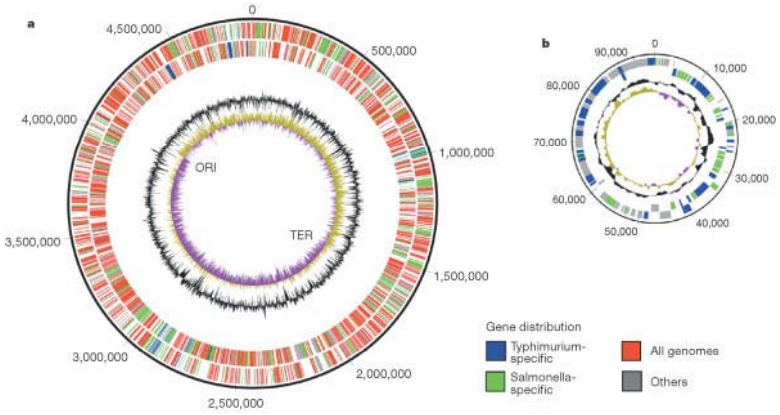
Enterococcus *faecium* NRRL B-2354 & ATCC 8459 : Surrogate Safety Study – Dr. Maria Marco, UCD

Goals

- Clarify taxonomic designations of E. feacium strain NRRL B-2354
- Evaluate potential of these organisms and E. *faecium* ATCC 8459 to cause illness in humans
  - Identify virulence factors
  - Hemolysin Activity (virulence trait)
  - Evaluate antibiotic resistance



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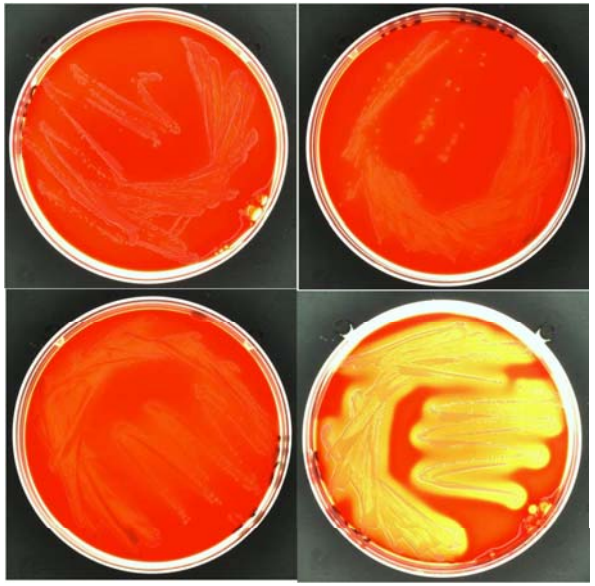
Surrogate Safety

Results

- USDA Strain NRRL B-2354
  - Is a member of the E. *faecium* species
  - Contains few known virulence genes
  - Growth Inhibited by medically relevant antibiotics / lacks genes coding for specific antibiotic resistance
- ATCC 8459 strain
  - Sensitive to majority of antibiotics
  - Exhibited intermediate level of Erythromycin resistance
  - Resistant to cephalothin - B-lactam antibiotics

Hemolytic Activity

- NRRL B-2354 (USDA Strain) and ATCC 9459 showed no hemolytic activity



7/17/12: E. faecium ATCC 8459 is now listed in the ATCC catalog as a BSL1 item – Brian Beck, Manager, Scientist ATCC
http://www.atcc.org/ATCCAdvancedCatalogSearch/ProductDetails/tabid/452/Default.aspx?ATCCNum=8459&Template=bacteria

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7 CFR Part 981.442b Mandatory Pasteurization Program Rule for Almonds– Effective Sept 2007

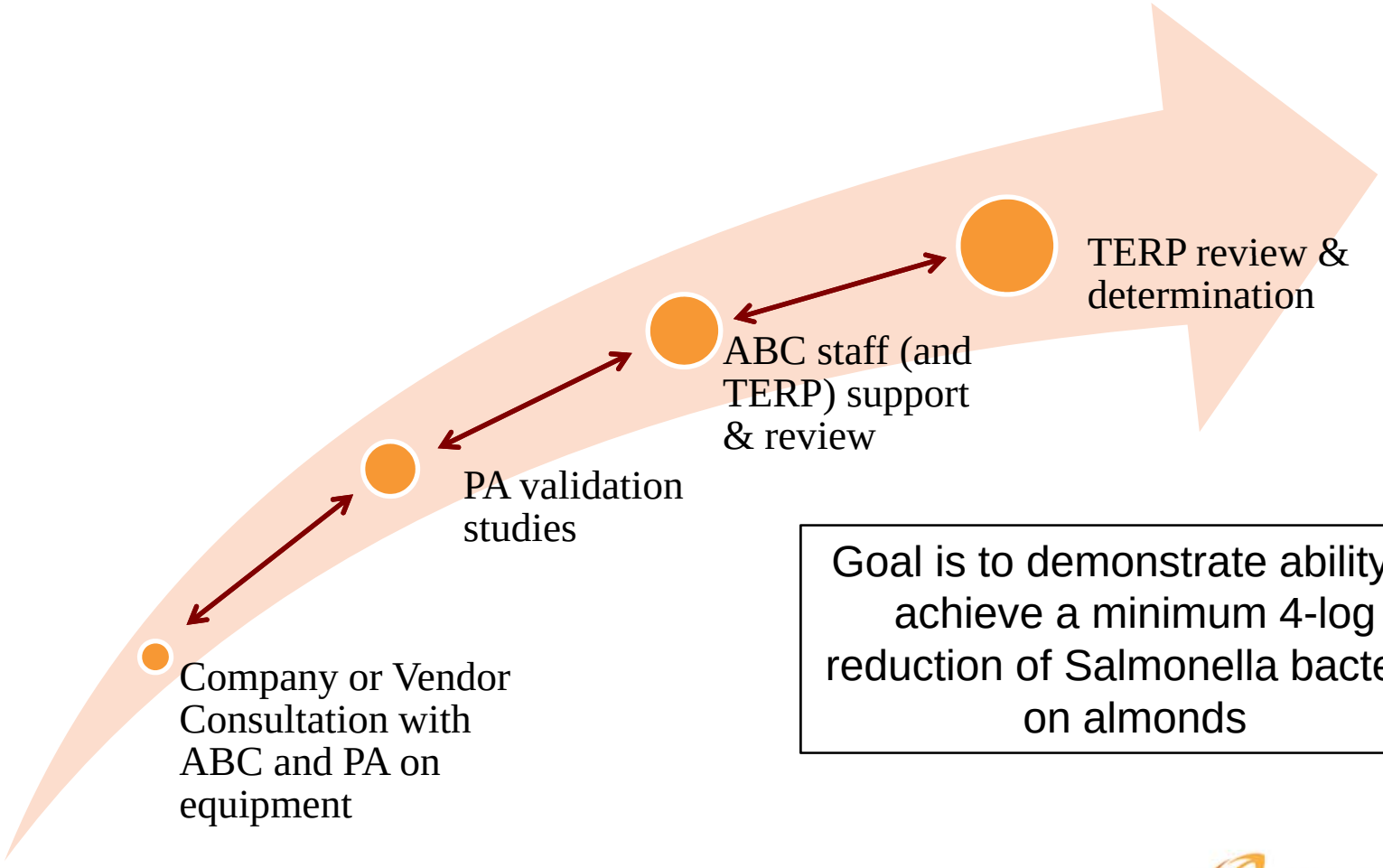
Requires all Handlers to utilize technologies that have been determined to achieve a minimum 4-log reduction of *Salmonella* bacteria in almonds

- Equipment must be validated by a Board approved Process Authority
- Data must be reviewed and accepted pursuant to a letter of determination by the FDA or ABC Technical Expert Review Panel (TERP)

Exemptions currently exist for shipments to DV Users and for shipments outside of North America

- DV Users are manufacturers with pasteurization equipment that has been subjected to the same validation and review process through the ABC TERP
- Typical DV users are treating product with dry roasting, oil roasting, or blanching processes to deliver a minimum 4-log reduction of *Salmonella*
- Any product shipped under exemption must be labeled as “unpasteurized”

The ABC Validation Process



Goal is to demonstrate ability to achieve a minimum 4-log reduction of Salmonella bacteria on almonds

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Technical Expert Review Panel Established – Oct. 2004

ABC Technical Expert Review Panel (TERP)

- Experts in microbiology, process engineering, pasteurization, process validation, and regulatory food safety requirements
- Conduct technical reviews of equipment and validation reports to determine ability to deliver a minimum 4-log reduction of *Salmonella* bacteria on almonds
  - Provide written reports outlining the Panel's determination that a process has or has not met the established criteria

Panel Members

- Dr. Ken Stevenson (Chairman)
- Dr. Larry Beuchat
- Dr. Brad Marks
- Mr. Keith Ito
- Dr. Dave Ashton
- Mr. Rick Stier

- Dr. John Larkin – FDA Ex-Officio

- Tim Birmingham – ABC Staff Liaison

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Pasteurization - Equipment Validated (As of 02/26/2013)

Equipment Type	# of Distinct units		
	Active	Inactive	Total
Blanching	28	0	28
Oil Roasting	82	6	88
PPO	23	0	23
Dry Roasting	17	1	18
Pasteurizer (Steam or Moist Heat)	8	0	8
Steam Plasticizer	3	0	3
Others	12	0	12
Total of 168 validations at 69 Locations			

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Manufacturer/Technology in use or in the Pipeline (Treatments that maintain raw characteristics)

Technologies Currently In Use for Almonds

- H20 Express – Vacuum steam
- JSP-1 (Formerly FMC) – Moist heat
- Ventilex- Atmospheric steam
- MRL – Moist Heat
- PPO
- Revetech – Steam/hot air
- RF Biocidics – Radio Frequency/heat

Technologies Under TERP Review or testing

- Buhler/Barth – Moist heat
- Napasol

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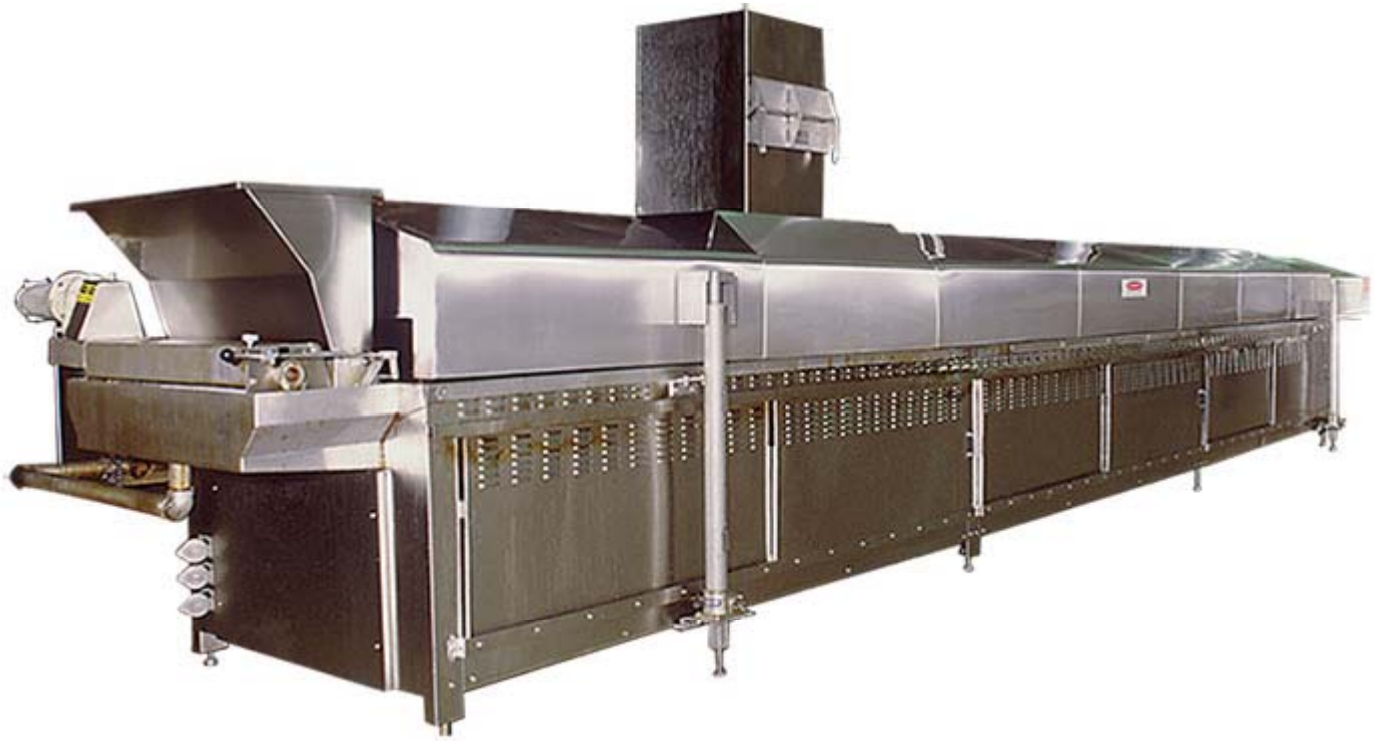
Oil Roasting Validation



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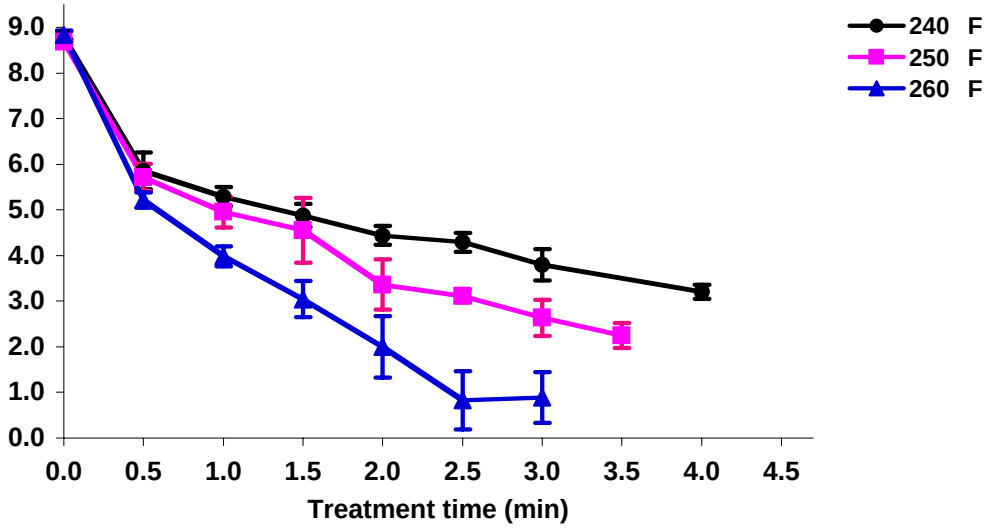
Validation Studies / Protocols – Oil Roasting

Minimum Critical Factors

- Time: Minimum 2 minutes
  - Critical factor will be speed setting
- Temperature: 260°F
  - Temperature must remain at or above 260°F throughout roaster

Survival of *Salmonella* Enteritidis PT 30 on inoculated almonds after exposure to oil heated to 240, 250 and 260 F (116, 121, and 127 C). TSA, n=6.



Oil Roasting Validation

Validation Objective

- To verify almonds are exposed to oil temperatures exceeding 260°F for a minimum time of 2 minutes

Validation Methodology

- Temperature mapping and/or temp profile during production
- Residence time confirmation
- Product loading (Throughput)
- Verification of Controls

Typical Critical Factors

- Belt/Chain Speed
- Belt Depth
- Temperature

Common Issues

- Cold Spots or lack of cold spot identification
- Process recordation
- Poor cooling air filtration
- Fixed bed depth

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Oil Roasting: 1. Demonstrate residence time > 2.0 minutes

Table 2 Meeting the Requirement

Time	Row	Location	South	Center	North
9:46	A	Time in oil (m:ss)	0:11	0:11	0:11
	B	Dwell time	3:10	3:10	3:10
	C	Time out of oil (m:ss)	3:21	3:21	3:21
	D	Time @ 260°F (:ss)	0:41	0:26	0:24
	E	Total time @ 260°F (m:ss)	2:40	2:55	2:57
10:07	A	Time in oil (m:ss)	0:11	0:11	0:11
	B	Dwell time	3:10	3:10	3:10
	C	Time out of oil (m:ss)	3:21	3:21	3:21
	D	Time @ 260°F (m:ss)	0:41	0:38	0:46
	E	Total time @ 260°F (m:ss)	2:40	2:43	2:35
10:29	A	Time in oil (m:ss)	0:11	0:11	0:11
	B	Dwell time	3:10	3:10	3:10
	C	Time out of oil (m:ss)	3:21	3:21	3:21
	D	Time @ 260°F (m:ss)	0:32	0:28	0:41
	E	Total time @ 260°F (m:ss)	2:49	2:53	2:40

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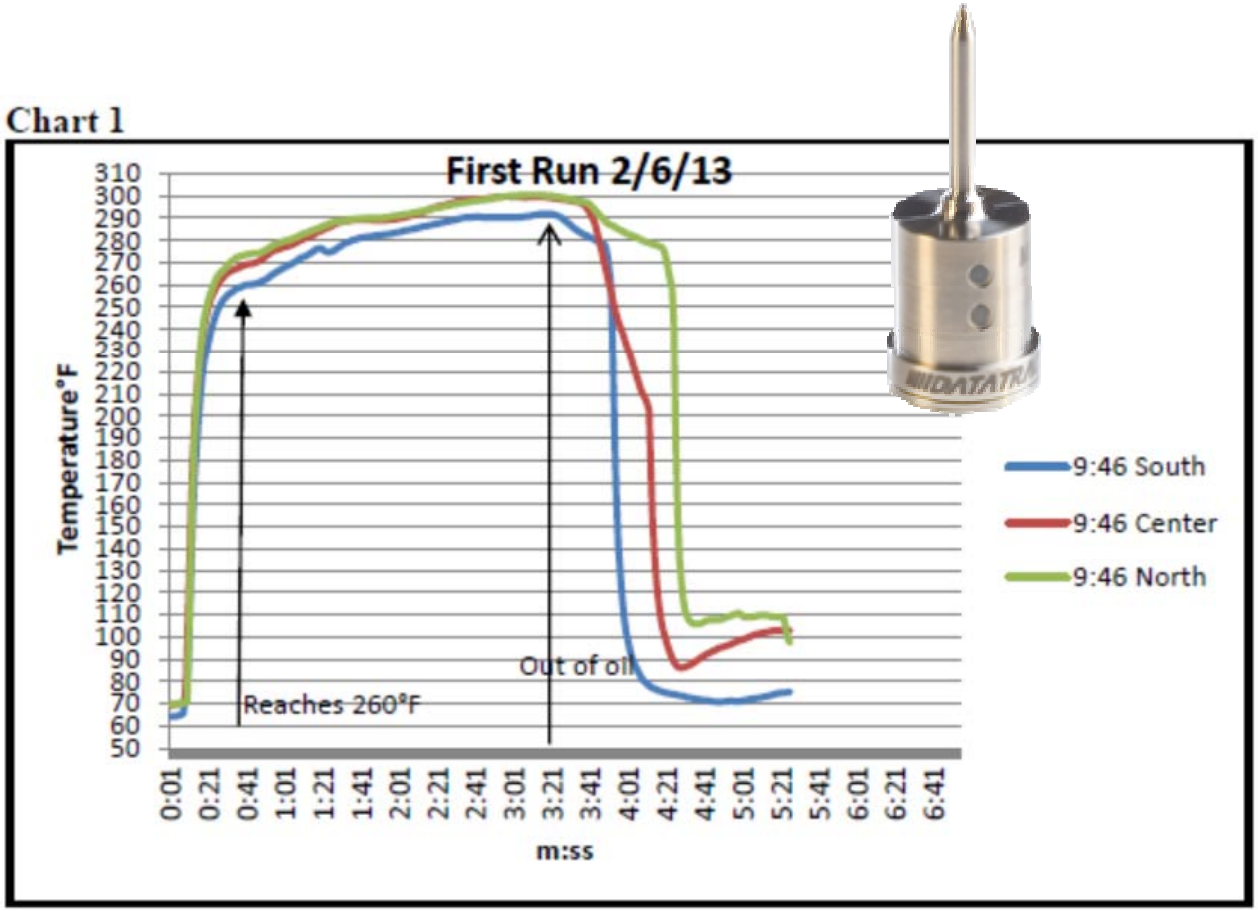
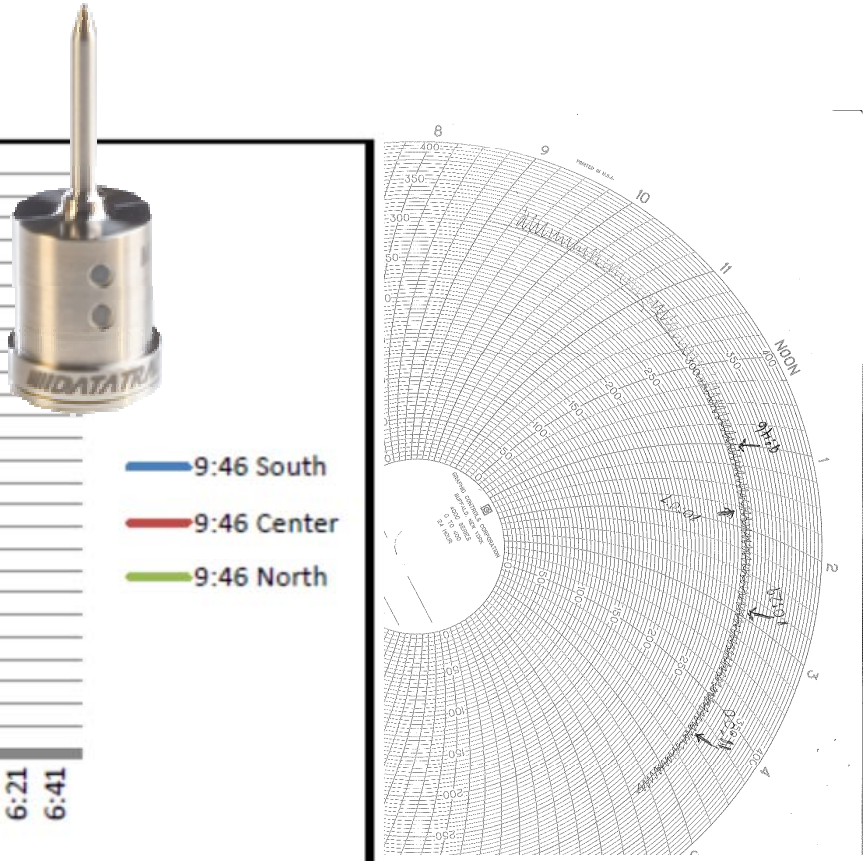
Oil Roasting: 2. Temperature Profile





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Oil Roasting: 3. Establish Critical Factors

Manufacturer:	

Equipment Information

Description	Equipment Manufacturer	Model #	Serial #	Validation #	ID#
Oil Roaster					

Scheduled Process

Process	Process Lethality	Minimum Oil Roast Temperature(°F)	Minimum Time (m:ss))
Oil Roasting	5-Log	Zone One=307°F Zone Two =300°F	3.10

Operating Critical Parameters
(To ensure the scheduled process is reached)

Process		Critical Parameters	
Zone One Oil Temperature °F Zone Two Oil Temperature °F Oil Roaster Dwell Setting, Dial Gauge Bed Depth, Inches		307 (Minimum) 300 (Minimum) 55 (Maximum) 2 (Maximum)	
Monitor Parameter		Frequency	Method
Oil Temperature ,dwell time, bed depth		Beginning and end of shift	Circular Chart Recording

Product Identification: Roasted Whole Almonds

Product Segration: Raw almonds are stored in a separate cooler location in 50 pound cartons; roasted product is stored in stainless bins covered with sanitary breathable liners.

Product Packing: 25, 50 lbs carton, bulk bins or sacks and various retail sizes.

Deviation Instructions: Management to review all process deviations with the PA before making a disposition.

Process Authority: Name: Signature: Date:

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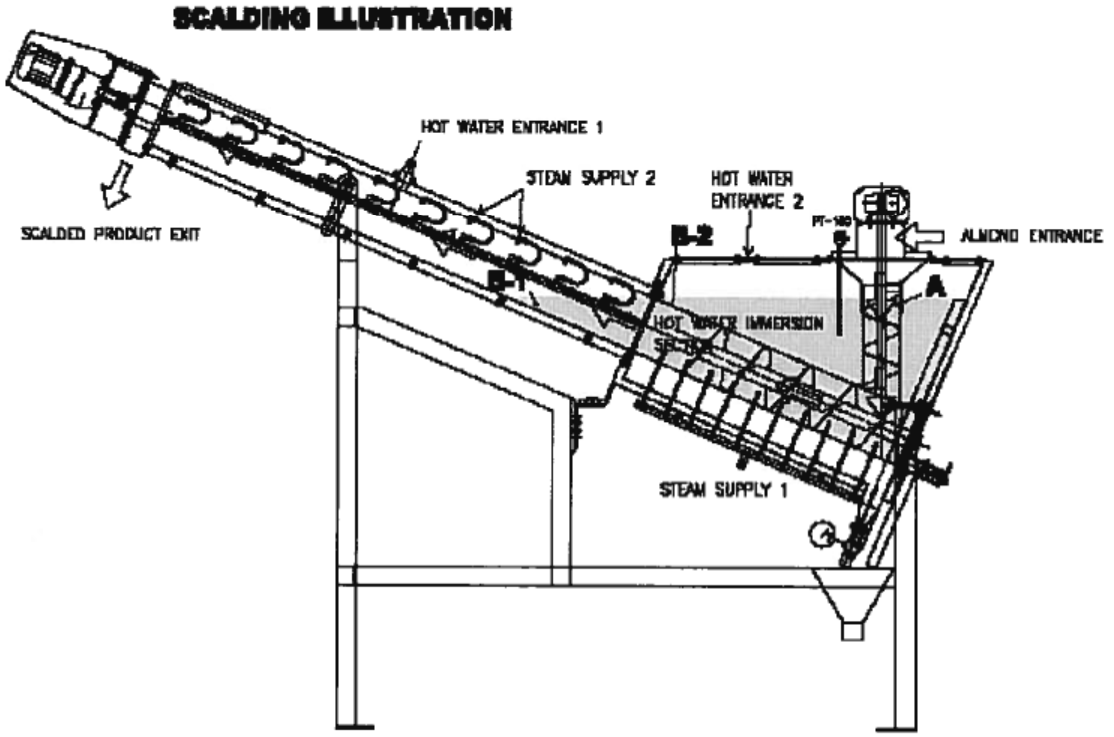
Blancher Validation



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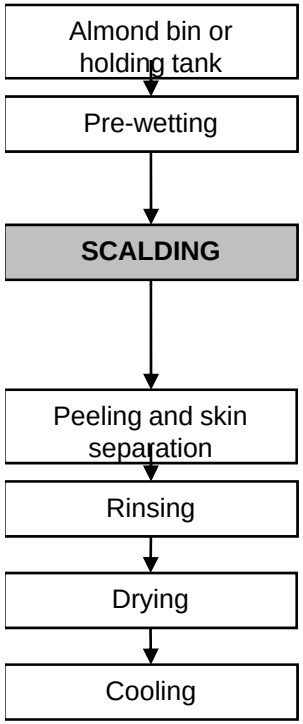
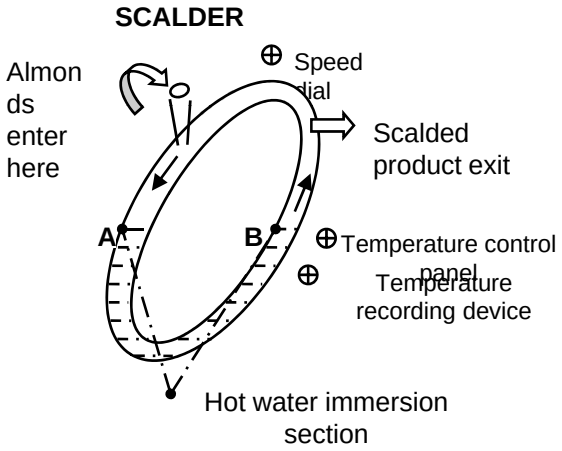
Validation Studies / Protocols - Blanching

Table 1. Minimum Time/Temperature Requirement for Blanching Processes		
Hot Water Temperature (°F)	Time to Meet Required Lethality (min)**	
	4-Log	5-Log
180	2.47	3.09
181	2.37	2.96
182	2.27	2.83
183	2.17	2.71
184	2.08	2.60
185	1.99	2.49
186	1.90	2.38
187	1.82	2.28
188	1.75	2.18
189	1.67	2.09
190	1.60	2.00

** z = 53 F°





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Blacnching Validation

Validation Objective

- To verify almonds are immersed it hot water at temperatures exceeding 190°F for a minimum time of 2 minutes

Validation Methodology

- Temperature mapping and/or temp profile during production
- Residence time confirmation (2.0 minutes minimum in hot H2O)
- Product loading (Throughput)
- Verification of Controls

Typical Critical Factors

- Feed Rate Setting
- Temperature

Common Issues

- Process recordation
- Poor cooling air filtration

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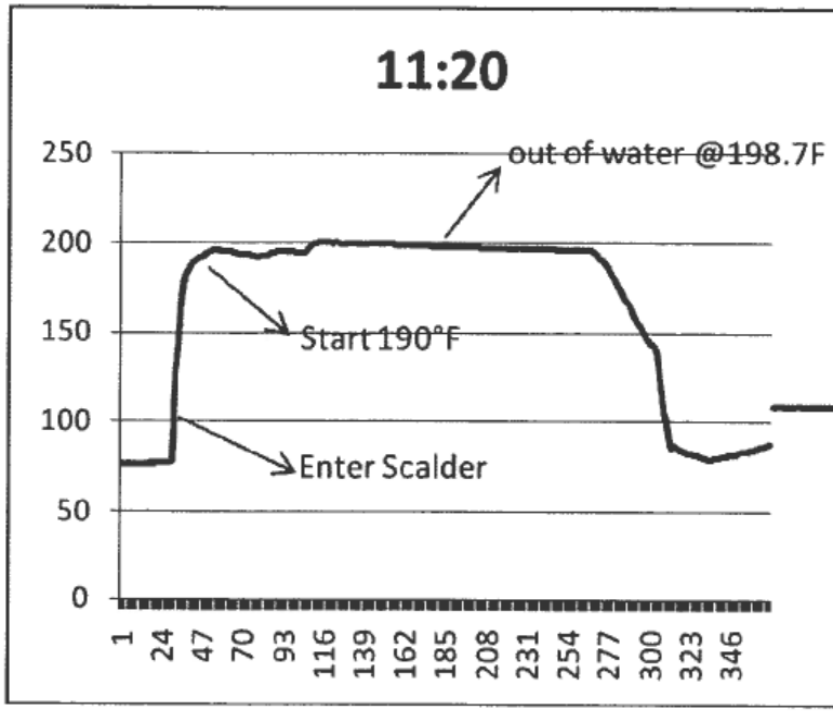
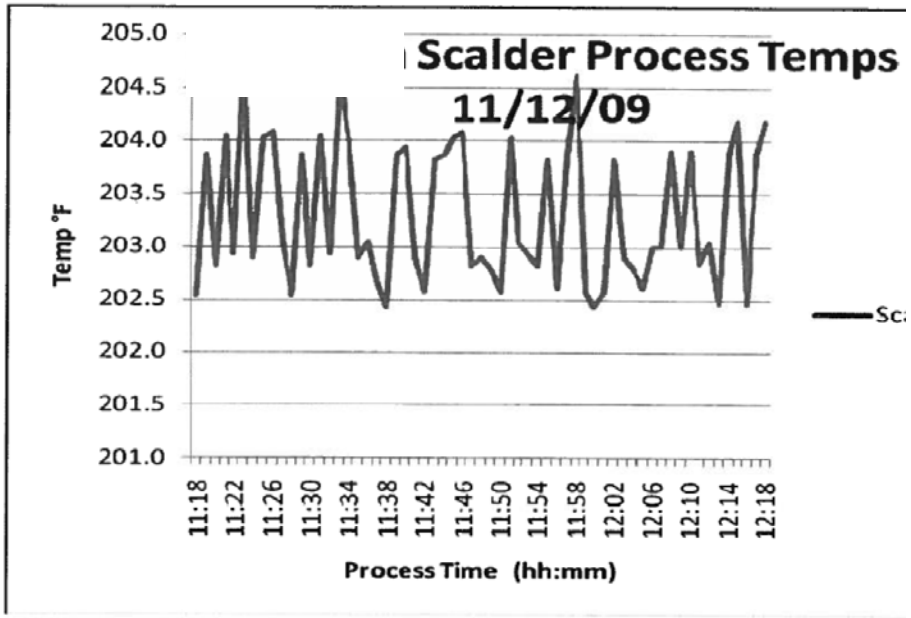
Blancher Validation

Blancher Heating Profile Trial





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PPO Validation

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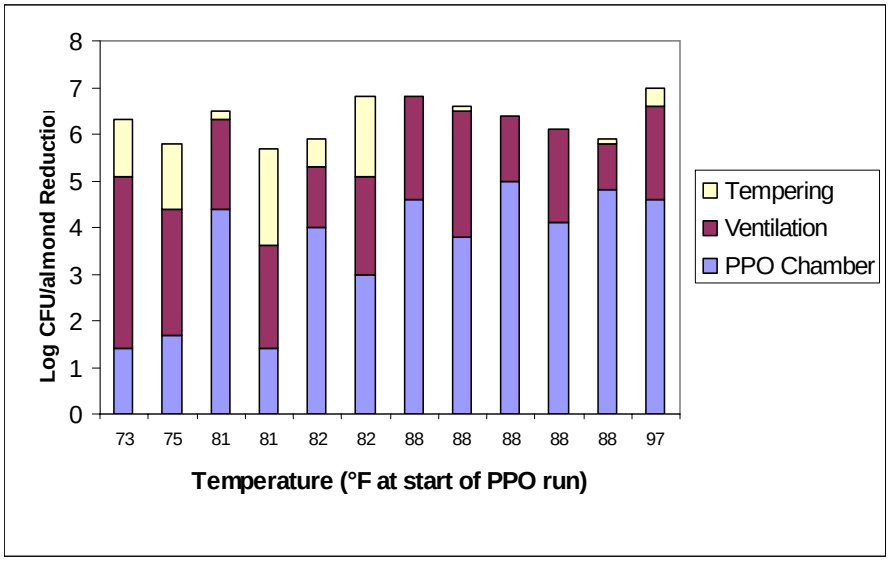
Validation Studies / Protocols - Propylene Oxide (PPO)

TABLE 2. *Reductions in concentration of* Salmonella *Enteritidis PT 30 on inoculated Mission almonds before and after PPO treatment, ventilation, and tempering*

		Sampling times[b]:											
		A		B				C, C1			D, D1		
Trial no.	Sample[a]	TSA	BSA	TSA	BSA	BΔ (TSA)[c]	Treatment[d]	TSA	BSA	C, C1Δ (TSA)[c]	TSA	BSA	D, D1Δ (TSA)[c]
7	U	<0.1	<0.1	1.9	<0.1	NA[e]		<0.1	<0.1	NA	<0.1	<0.1	NA
	I	8.2	8.0	8.1	7.9	NA		8.1	7.7	NA	7.9	7.7	NA
	T (n = 2)	8.0 ± 0.2	7.7 ± 0.3	4.4	4.3	3.6	V (n = 2)	0.6	0.8	7.4	2.1	1.1	5.9
							T (n = 2)	2.8	2.7	5.2	2.5	2.3	5.5
8	U	<0.1	<0.1	1.8	<0.1	NA		1.5	<0.1	NA	<0.1	<0.1	NA
	I	8.7	8.3	8.7	8.3	NA		8.4	8.0	NA	8.4	8.0	NA
	T (n = 2)	8.6 ± 0.1	8.3 ± 0.1	3.7	3.7	4.9	V (n = 2)	1.0	0.3	7.6	<0.1[f]	<0.1[f]	>8.6
							T (n = 2)	1.3	0.4	7.3	<0.1[f]	<0.1[f]	>8.5



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PPO Parameters necessary to achieve > 5-log reduction - The (SOP)

PPO Pasteurization Operating Parameters

Parameters	Operational Level
Initial product temperature	Not less than 86°F (30°C)
Temperature inside chamber at start and during sterilization	117-125°F(47-51°C)
Chamber vacuum before PPO injection	At least 27"Hg vacuum
PPO vaporizer temperature at point of PPO injection	140 -160°F (60-71°C)
Initial PPO concentration in Chamber	Not less than 0.5oz PPO/ft^3
Chamber vacuum at completion of inert gas injection	5-6 inch Hg vacuum
Duration of pasteurization	4 hours
Aeration cycles	Not <4 and not >14
Post ventilation	100-110°F (38-43°C) for 2 days or above 59°F (15°C) for 5 days.

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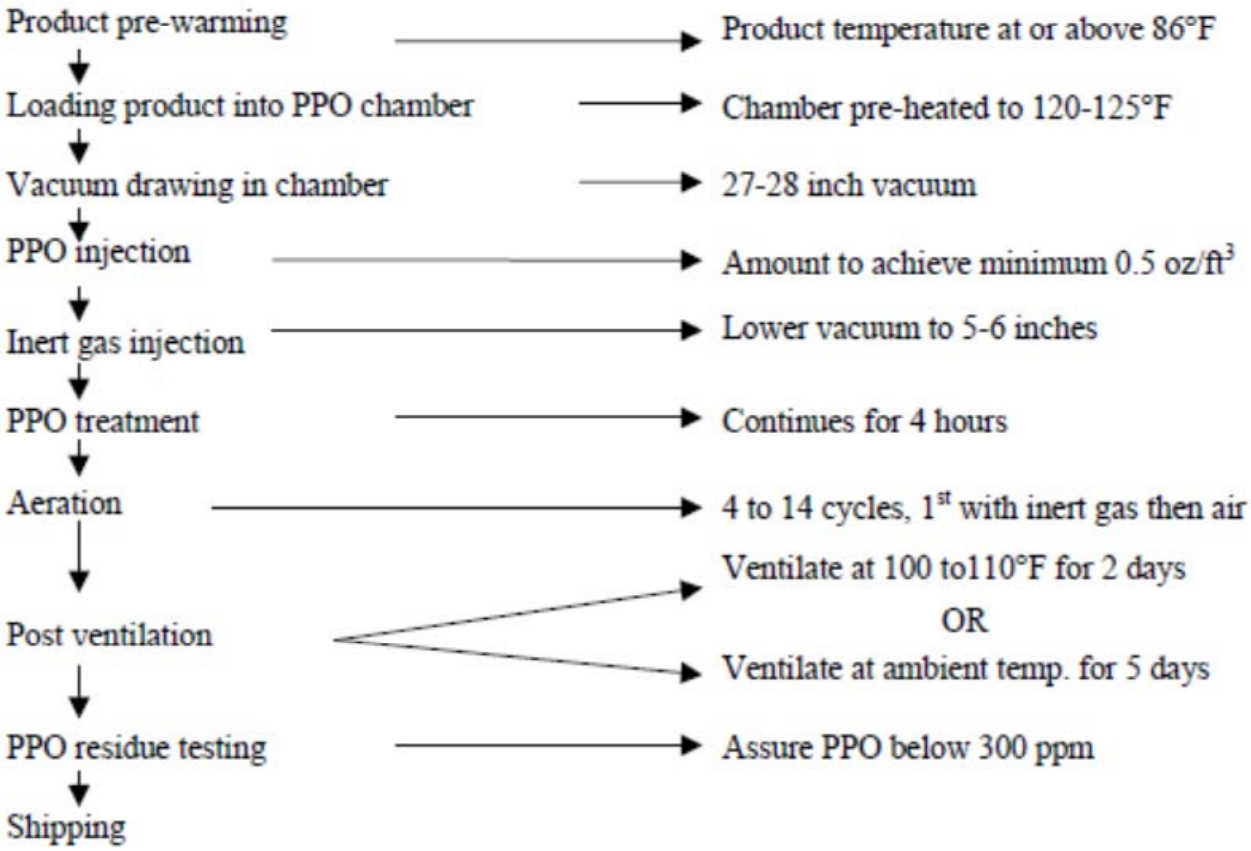
The PPO Process

Chart 1. PPO Pasteurization Process Flow Chart and Respective Parameter



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PPO Validation

Validation Objective

- Verify the PPO treatment facility equipment and operations meet parameters specified in SOP

Validation Methodology (Confirming conditions during PPO cycle)

- Pre warming control / monitoring (>86°F)
- PPO injection vaporization temp verify controls / monitoring (140-160°F)
- PPO dosage (0.5 oz per cubic foot)
- Chamber temperature distribution – 117-125°F (with inert gas only)
- Chamber vacuum (pre PPO injection and post inert gas injection)
- Pasteurization time (minimum 4 hours)
- Post ventilation process temperature and time

Critical Factors

- Initial Product Temperature
- Chamber temperature
- Chamber vacuum
- PPO vaporization temperature
- Initial PPO concentration
- Pasteurization time
- Aeration cycles
- Post ventilation

Common Issues

- Inadequate verification of controls
- Occasional temperature distribution in chamber

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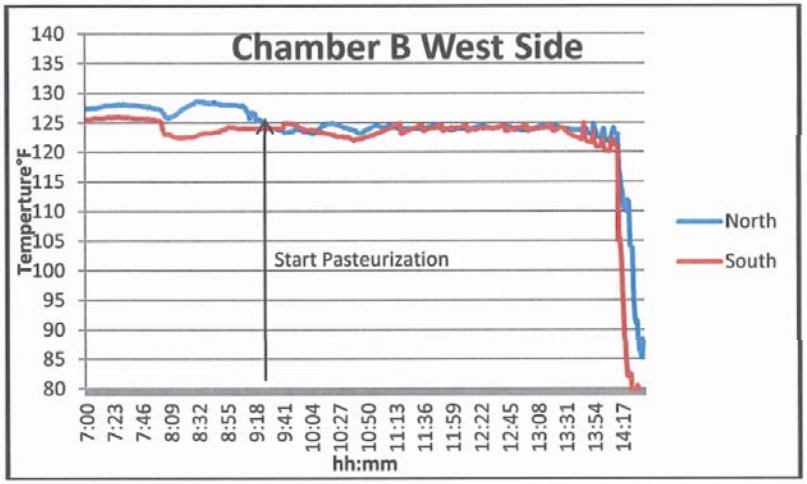
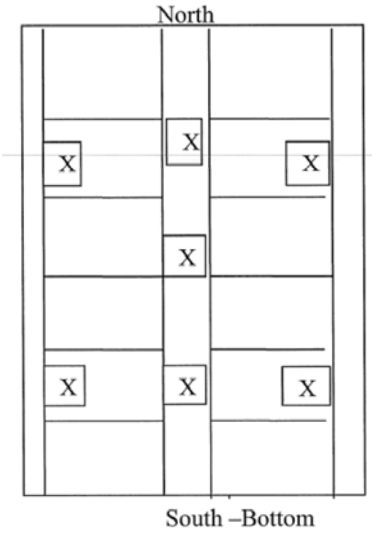
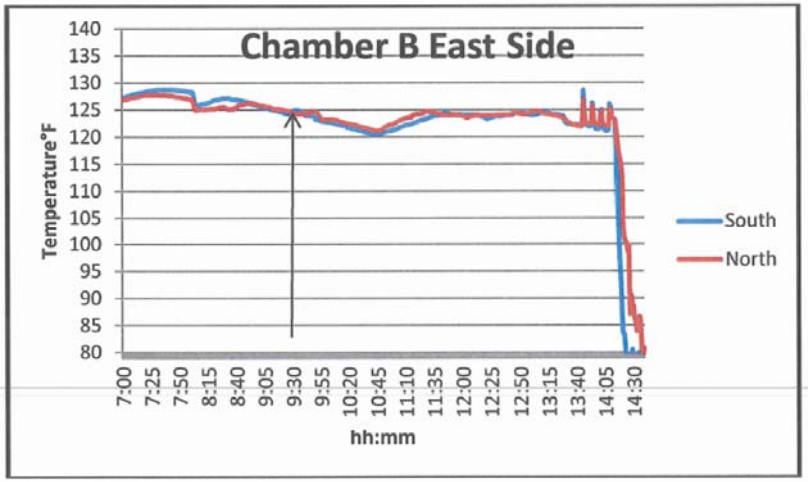
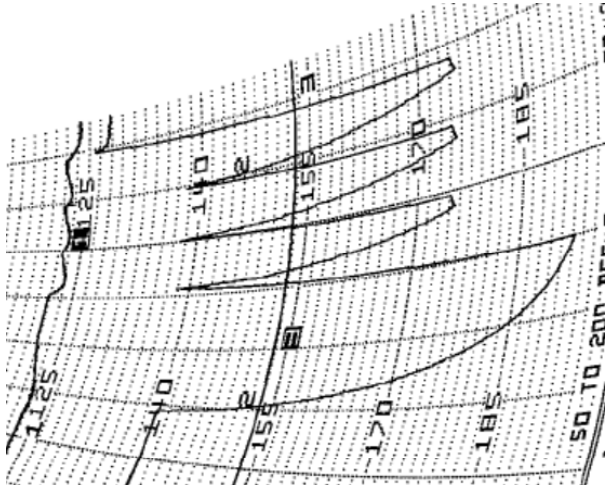
PPO Validation









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PPO – Lots of Steps and Critical factors

Scheduled Process			
Process	Process Lethality	Minimum Temperature(°F)	Minimum Time (hh:mm))
PPO	5-Log	117°F	04 :00

Operating Critical Parameters (To ensure the scheduled process is reached)	
Process	Critical Parameters
1. Almond Input Temp °F	86 (minimum)
2.Chamber Temp at start and during pasteurization, °F	117-125
3.Chamber vacuum before PPO injection ,inches of HG	27 (minimum)
4.PPO vaporizer temp, °F	140-160
5.Initial PPO concentrations, ounces/ft^3	.50 (minimum)
6.Chamber vacuum at completion of inert gas injection, inches of HG	5-6
7.Duration of pasteurization, hours	4.0 (minimum)
8.Aeration cycles	Not <4 and not >14
9.Post ventilation , °F/days	100-110, 2 days minimum
10. Post ventilation **INSHELL**, °F/day	59-65, 5 days minimum
Monitor Frequency	Method
Numbers 1, 9 &10 are done for each chamber load and documented. Number 5 is done automatically once it is manually started for each load. Numbers 2, 3, 4, 6, 7, & 8 are automatically recorded for each chamber load.	Manual Open valve for PPO Circular chart A & B
Product Identification: PPO Treated Whole Almonds/In-shell/Manufactured almonds	
Product Segregation: Raw almonds are stored in a pre-warm room; treated product in off-gas room.	
Product Packing: Fiber bulk bins, wooden bins, plastic bins, super sacks, pallets of 25, 30 and 50# cartons. **In-shell** (hard & soft shell) - 50# bags on pallets	
Deviation Instructions: Management to review all process deviations with the PA before making a disposition.	

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Dry Roaster Validation

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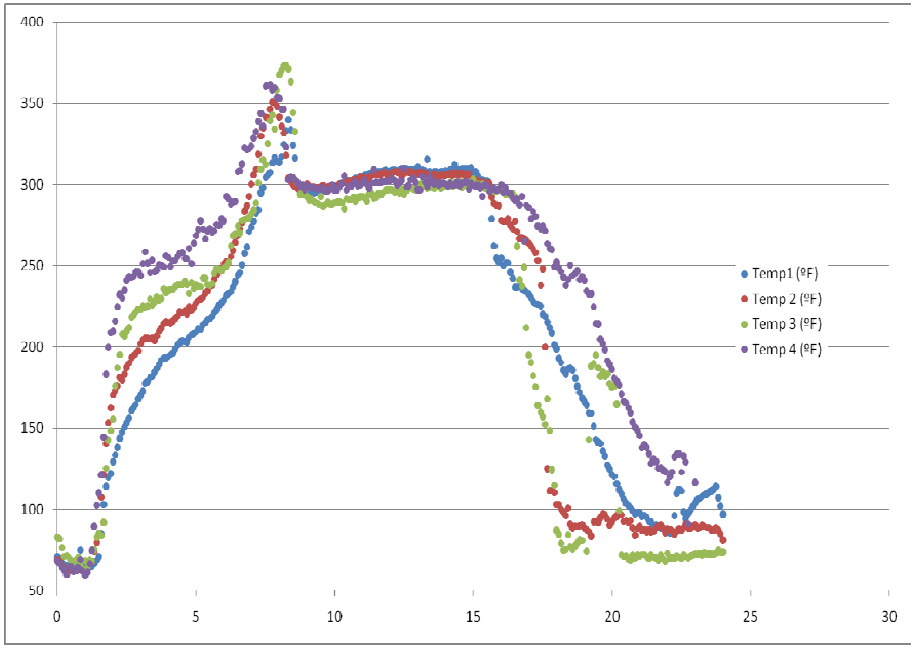
Validation Studies / Protocols

Hot Air Roasters

Hot air roasting may achieve a ≥4-log destruction of SE PT30 under certain conditions

- Aluminum almond temperatures that are equal to general method calculations
  - 100 min at 250°F
  - 50 min at 265°F
  - 23 min at 295°F
  - 9 min at 300°F

Most Validations conducted using microbial challenge testing



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Dry Roaster Validation (Microbial challenge Testing)

Validation Objective

- Identify controllable operating conditions which will consistently deliver a minimum 4-log destruction of SE PT 30

Validation Methodology

- Temperature mapping and/or temp profile to determine heat distribution
- ID worst case scenario product (ie. Low moisture)
- Conduct micro challenge testing (minimum 3 runs with multiple samples/run)
  - Ensure set points are adjusted to test under conditions which can be exceeded during normal production (Temp, Cooling air, belt speed, etc)
- Residence time confirmation
- Product loading (Throughput)
- Verification of Controls

Typical Critical Factors

- Belt Speed (Time)
- Air temperature
- Air flow / Direction
- Product loading
- Initial product temperature
- Burner Temperatures
- Cooling flow and temperature
- Nut Moisture

Common Issues

- Large temperature differentials across bed
- Data anomalies (outliers)
- Lack of abundant data at cold spots
- Lack of adequate controls / Process recordation
- Inadequate ID of critical factors (want to operate at validation conditions)
- Poor cooling air filtration

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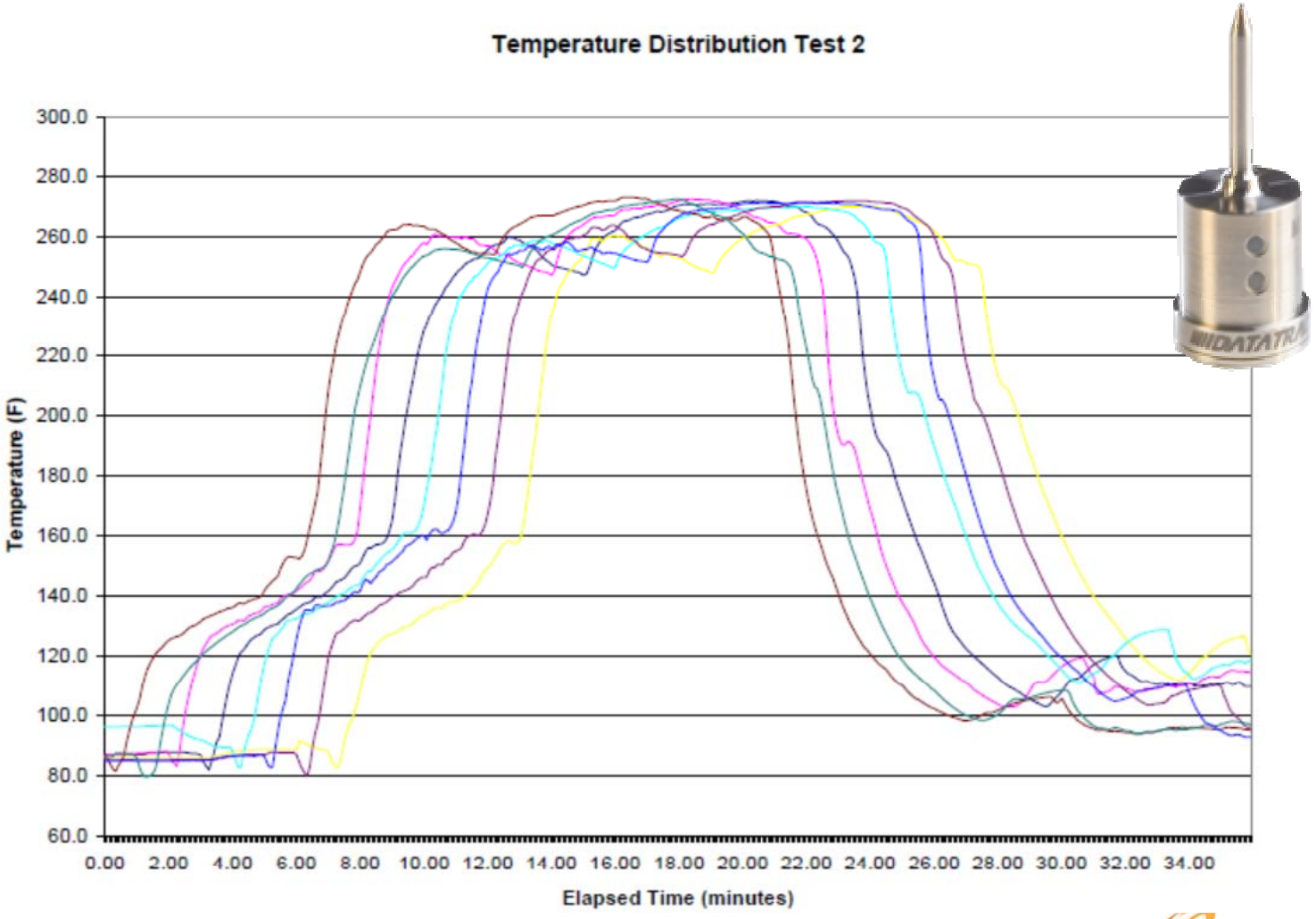
Dry Roaster Validation – Cold Spots an Issue



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Cold Spots Can & Do Impact Lethality

- Majority of samples need to be placed in cold spots identified
- Roaster temperature probes may need to be relocated for better control and/or monitoring at cold spots
  - Air flow is crucial and may need to be altered
- Maximum roaster recorded values become the new minimum operating critical factors

11.11.08	RESIDENCE TIME				
	>270° F		>270° F		>270° F
L1	4:25	M1	6:15	R1	1:45
L2	5:45	M2	7:50	R2	8:25
L3	6:10	M3	8:05	R3	5:15

11/24/08	Micro-Challenge Study with Wire Mesh Baskets			LOG CFU/g Dryer Flow ↑			
Trial Number	Start	End	Data Trace run w/baskets	Left Side	Left Middle	Right Middle	Right Side
1	*7:40	9:43	L1 - 7:36 M1 - 7:38	>7.00	5.15	5.85	6.22
2	*9:30	10:33	L3 - 9:31 M3 - 9:32	>7.00	6.52	5.80	6.52
3	*11:05	11:44	R3 - 11:06	6.70	5.77	5.96	4.96

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Steam & Other Processes Validation

Validation Objective

- Identify controllable operating conditions which will consistently deliver a minimum 4-log destruction of SE PT 30

Validation Methodology

- Temperature mapping and/or temp profile to determine heat distribution
- ID worst case scenario product (ie. Low moisture)
- Conduct micro challenge testing (with multiple runs with multiple samples/run)
  - Ensure set points are adjusted to test under conditions which can be exceeded during normal production (Temp, Cooling air, belt speed, etc)
- Residence time confirmation
- Product loading (Throughput)
- Verification of Controls

Possible Critical Factors

- Belt Speed (Time)
- Air temperature
- Air flow / Direction
- Steam flow / volume
- Product loading
- Initial product temperature
- Burner Temperatures
- Cooling flow and temperature
- Nut Moisture
- Product size
- Vacuum

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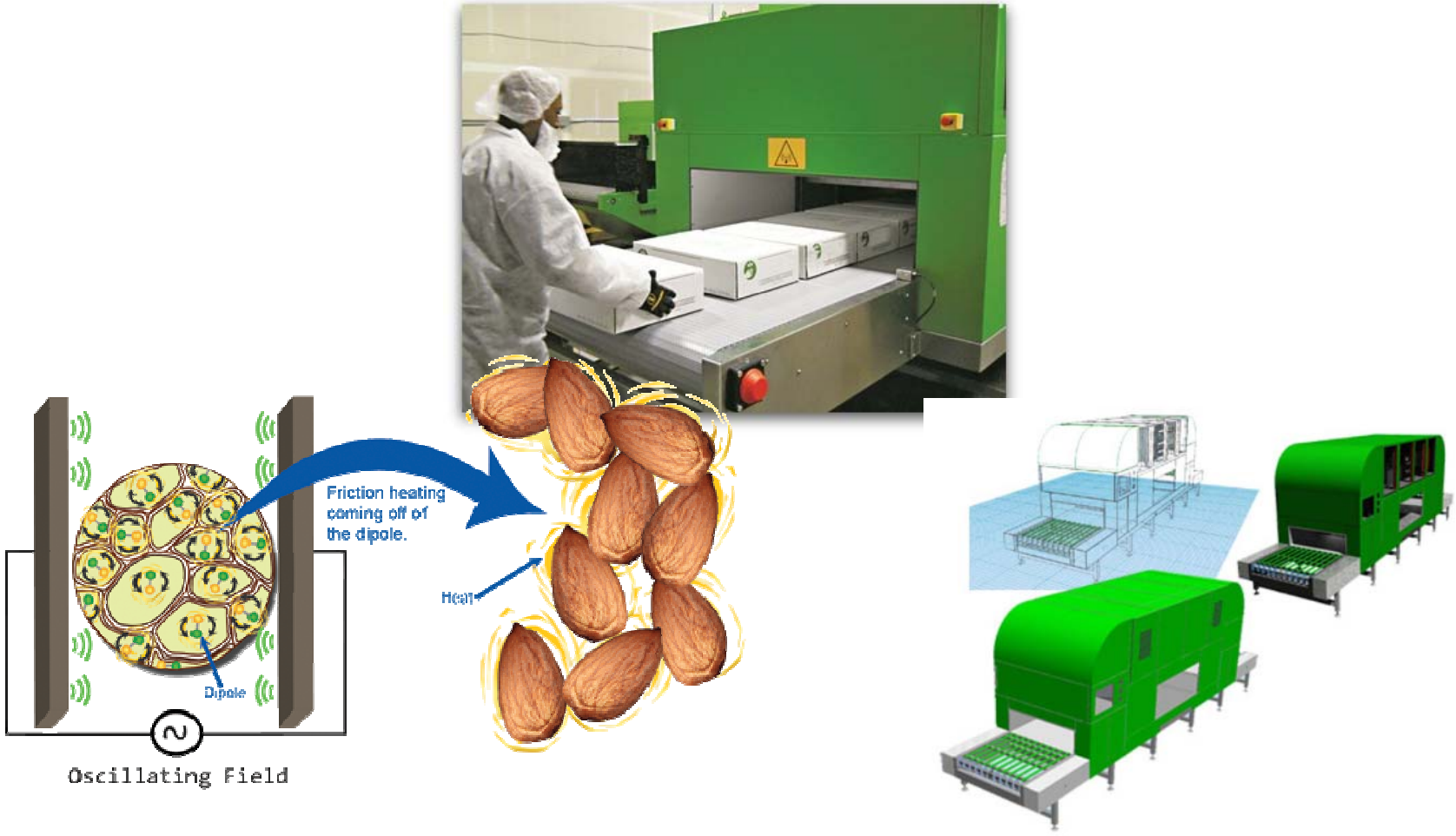
RF Biocidics – Radio Frequency (Heating)



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Validation – Steam and Moist Heat & Other processes

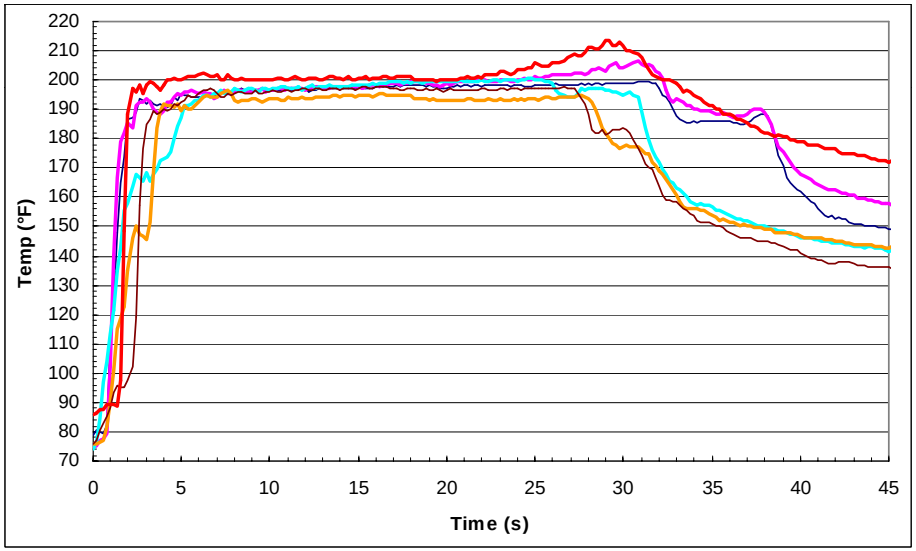
A number of steam processes have been studied and validated

- Ambient Steam continuous product flow
- Moist heat
- Vacuum Steam
- Combination Steam / Drying

Critical factors are specific to the equipment

- Time
- Steam temperature
- Air temperature
- Drying time/temp exposure
- Vacuum
- Product loading
- Type of container

Typical Ambient Steam Pasteurization Process- Average Almond Surface Temperatures



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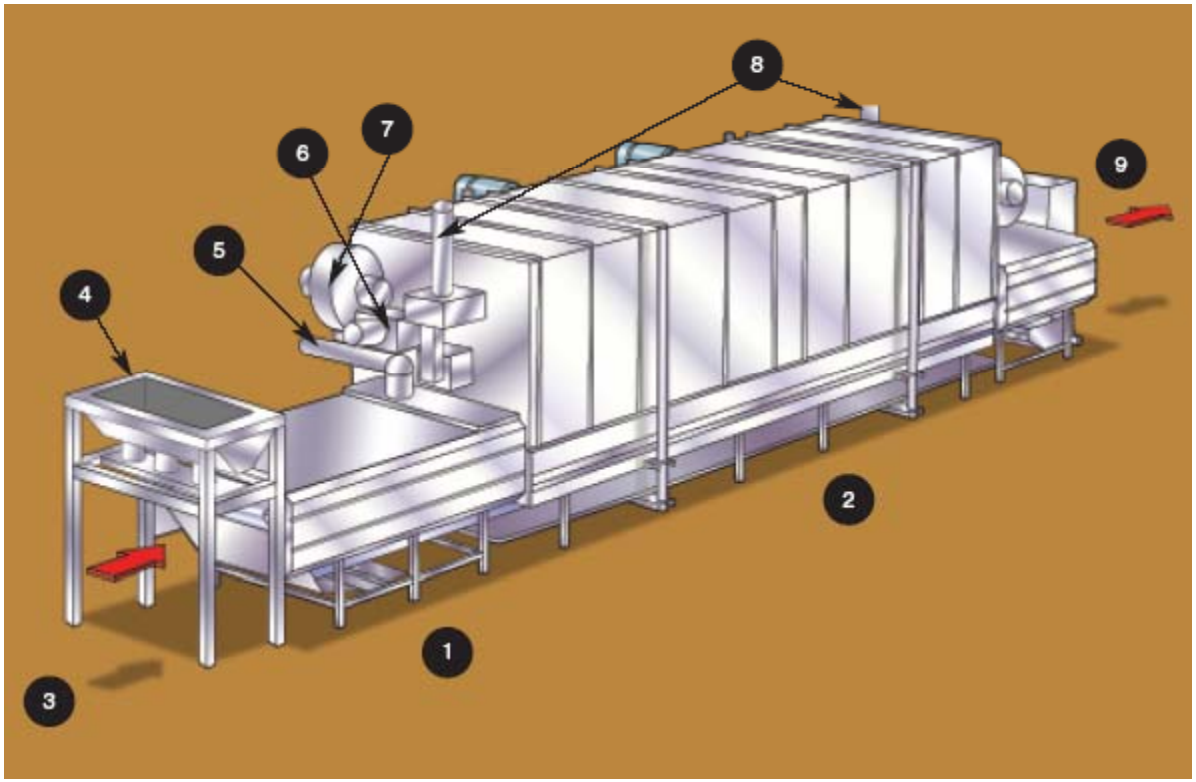
JSP-1 (JBT Food Tech formerly FMC) – Steam/Drying



1) Steam Zone	6) Burner
2) Impingement Zone	7) Combustion Blower
3) Infeed	8) Flue Vents
4) Vibratory Feeder	9) Discharge
5) Exhaust	

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Other Steam / Moist Heat

H2O express
Dry Steam Pasteurization

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Thank-You!