

REPORT DOCUMENTATION PAGE

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18200 U.S. Highway 41 North, Evansville, Indiana 47725

Final Project Review

Project Title: Utilization of Novel Processing Technology known as RF to Produce Commercially Sterile Low Acid Rations for Field Trials

Contract Number: SP4701-22-C-0081

Company POC: Shane Shepherd, V.P. R&D and Technical Services, Contracting/Project Manager, 812-867-1444, sshepherd@ameriquial.com

Project Summary

Objective

- The purpose of this project is to validate a novel processing technique, known as radio frequency (RF)
- Two food items will be filed with the Food and Drug Administration and/or United States Department of Agriculture meeting all 9CFR113 Low Acid Canning Regulations
- The two items will be selected, packed and processed in a commercially available 420-gram rectangular tray with similar depth to the existing polymeric tray

Contract Deliverables

- The two items will be produced in a quantity such that they can be field tested alongside conventional retorted items to demonstrate the delta in quality utilizing the novel technology
 - 140 containers of each product for each processing method will be delivered to NSRDEC
- Progress, Status and Management Monthly reports will be provided on the 15th of each month
- Scientific and Technical Final Report will be provided 30 days after completion of the contract

Model Foods

Alfredo Sauce with Pasta and Apple Dessert were identified as the two food matrixes for the project. Both matrixes are significantly impacted by retort processing based on reduced product quality.

Alfredo Sauce with Pasta

INGREDIENT	%
DISODIUM PHOSPHATE	0.26
WATER	6.67
WATER	53.58
BUTTER BULK UNSALTED	1.70
GARLIC POWDER	0.09
CHEESE PARMESAN ALLERGEN	4.25
PEPPER BLACK GROUND	0.09
MW UHT WHIPPING CREAM	21.25
PENNE W/ 7% EW	8.33
THERMFLO	2.13
SALT NON-IODIZED	0.65
STARCH NATIONAL 150	1.00
TOTAL	100.00

Apple Dessert

INGREDIENT	%
WATER	7.46
APPLE JUICE CONCENTRATE	1.09
USDA GRADED SLICED APPLES	80.00
BUTTER BULK UNSALTED	1.17
CINNAMON GROUND	0.05
LEMON JUICE CONCENTRATE	0.06
NUTMEG GROUND	0.05
SALT NON-IODIZED	0.05
STARCH COLFLO	2.67
SUGAR GRANULATED BEET	7.40
TOTAL	100.00

Subcontracting Partners

Microwave Materials Technology, Inc

MMT conducted Heat Penetration testing, Inoculation samples, and Natick Samples with oversight from the AmeriQual team

Eurofins Microbiology Laboratory

Conducted the Experimental design, Microorganism growth and calibration, and incubation of the Inoculation Study

1. Radio Frequency Assisted Thermal Sterilization (RF) System Description and Operation

1.1. General description of the Radio Frequency (RF) system

The RF system consists of two sections, which are utilized for four sequential processing steps of preheating, Radio Frequency (RF) heating, holding, and cooling. Each section is a pressure vessel filled with temperature controlled circulating water from an individually controlled supply. Sealed food product containers are loaded into a plastic 13.5” x 94.25” carrier that travels on a chain conveyor that transports them through the sections during processing. (Figures 1.1, 1.2, 1.3, and 1.4)



Figure 1.1 RF system.

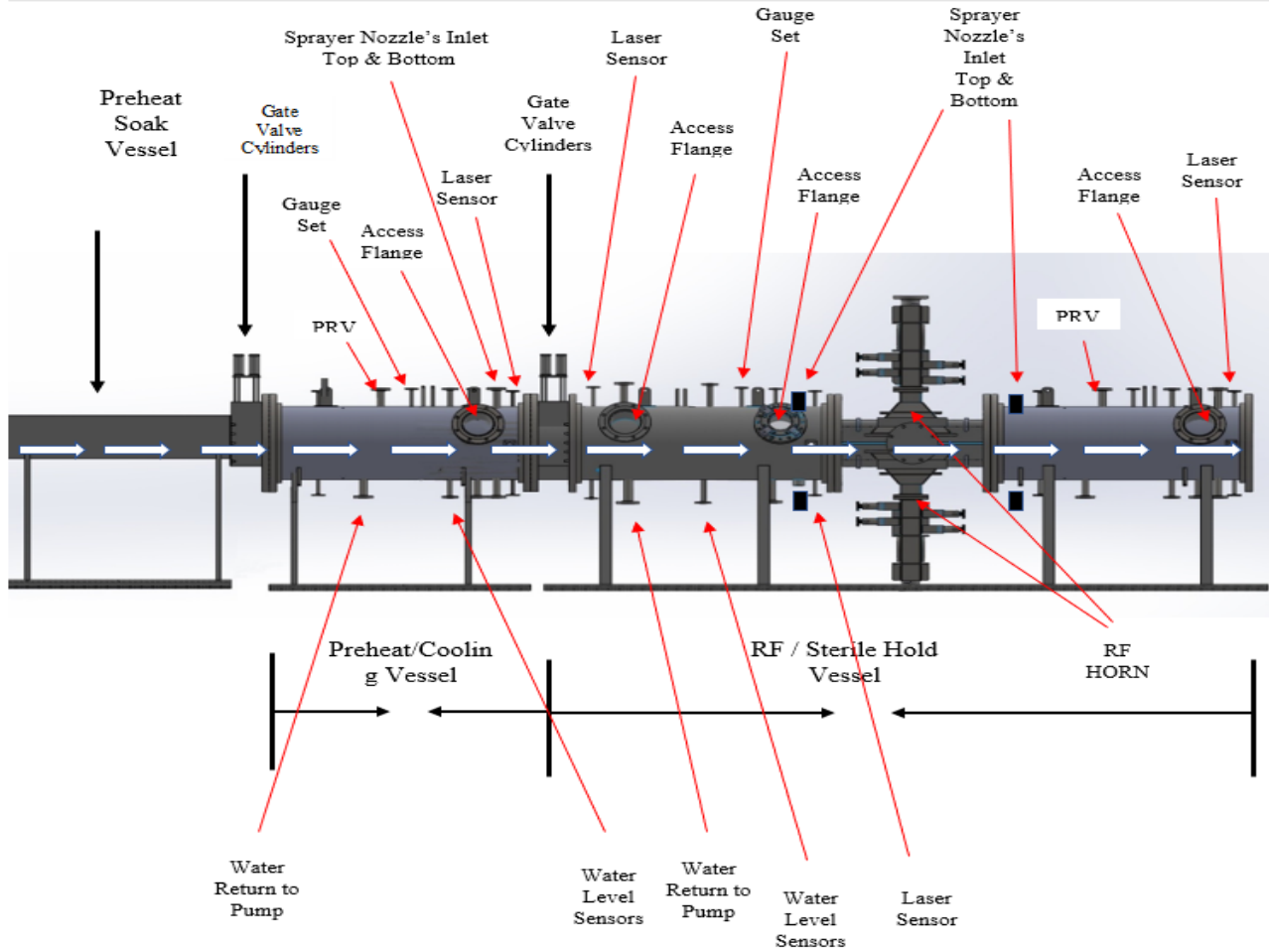


Figure 1.2 RF System graphical representation
 Dashed line represents carrier filled with food trays traveling on chain conveyor.

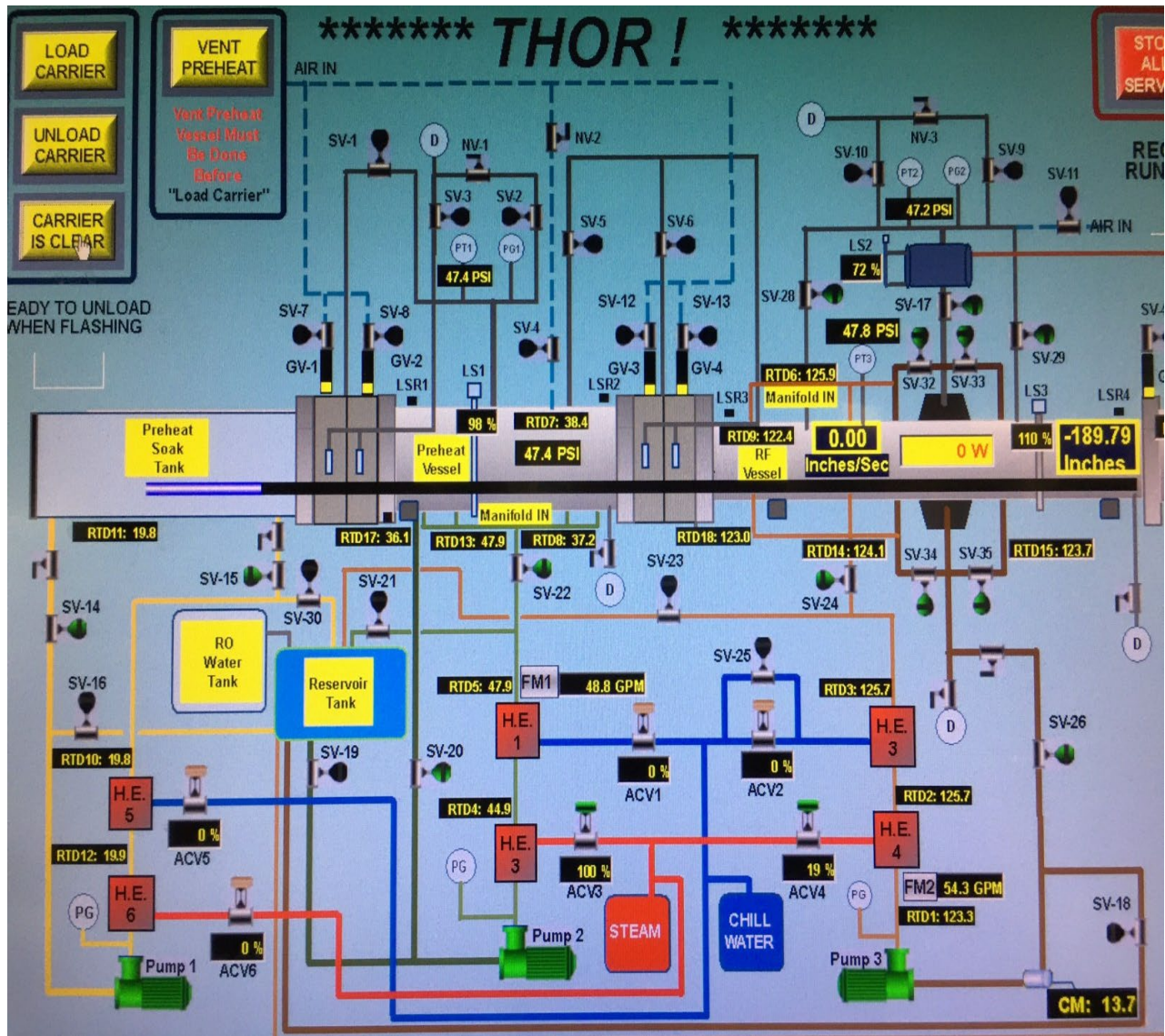


Figure 1.3 RF System HMI graphical representation showing instrumentation

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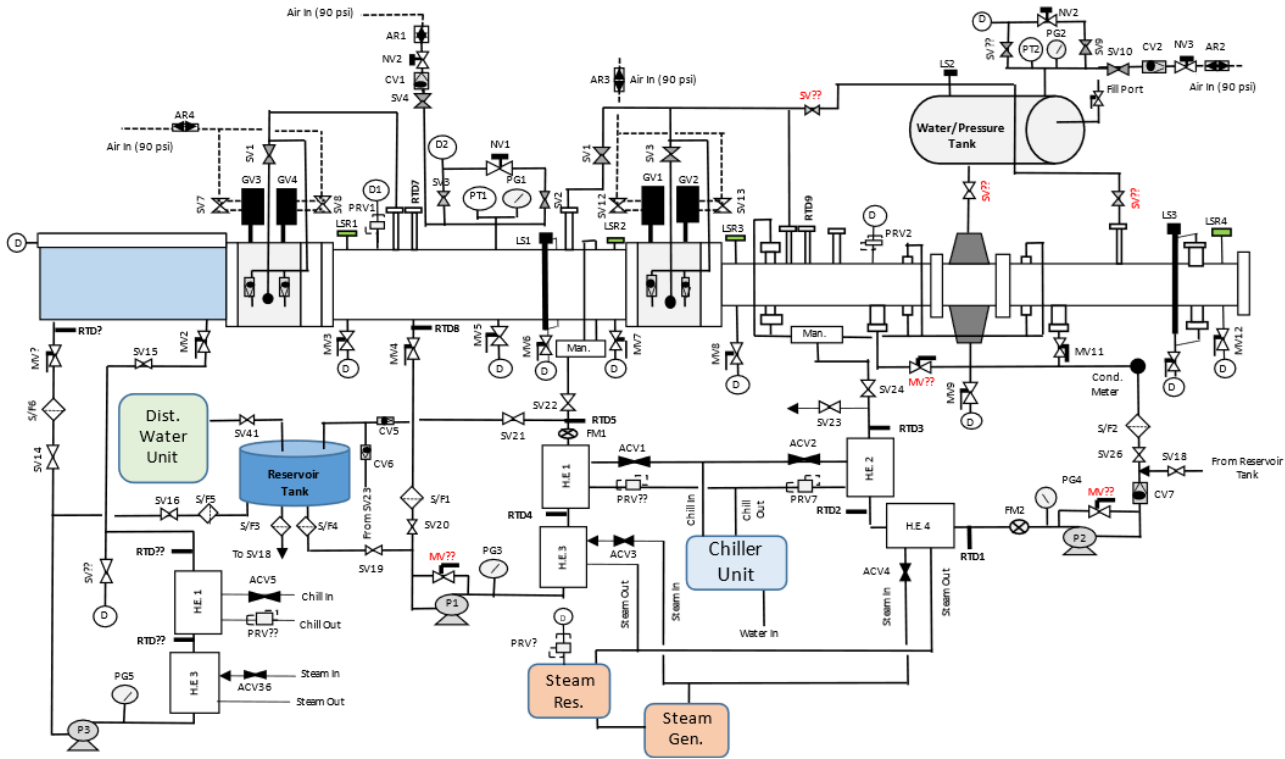


Figure 1.4 RF System P & I D

The preheat vessel (**Figure 1.2**) is used to equilibrate the food to a uniform initial temperature. It consists of a cylindrical vessel connected to RO water supply tank. Before operation, the vessel is filled with the RO water to the top of the vessel. During operation the water is pumped from the bottom end of the vessel (Return Drain) through reverse flow heat exchangers back to a sprayer manifold that feeds sprayers on the top and bottom of the vessel. The water temperature is maintained through a heat exchanger utilizing steam and returned to the vessel. The temperature of the water supplied to the vessel is monitored by an RTD sensor (RTD-7 in **Figure 1.4**) and used to control the valve to the heat exchanger. The vessel outlet temperature is monitored using an RTD sensor (RTD-5 in **Figure 1.3**).

The RF vessel is used to heat the food containers using Radio Frequency energy and circulation water at controlled temperatures. The RF vessel consists of one water filled single mode 99.6 MHz RF applicator. In the applicator, 99.6 MHz radio frequency is applied to the top and bottom of the processing cavity through a pair of waveguide horns (**Figure 1.5**). RF power is supplied to the horn by a RF generator. RF forward power is monitored using calibrated directional couplers. Water at the process temperature is circulated throughout the processing cavity of the applicators to promote uniform RF heat distribution throughout the food container and prevent heat loss. The temperature of the water in the RF vessel is monitored by an RTD sensor (RTD-9 in **Figure 1.3**) and used to control the steam/cooling flow to the heat exchangers. The water circulation loop uses two heat exchangers. One is used for heating the circulation water while the other is for removing excess heat as required. The temperature of the water supplied to the RF vessel is monitored by RTD sensors (RTD-2 for the hot water heat exchanger and RTD-3 for the cool water heat exchanger in **Figure 1.3**). The outlet temperature is monitored using an RTD sensor (RTD-14 in **Figure 1.3**). Similar to the preheat vessel, the RF vessel system water is circulated from the bottom of the vessel through the two heat exchangers back to the sprayer manifolds. In addition, the small amount of headspace of this vessel is used to control the overpressure of the entire system during processing by adding or bleeding compressed air as required.

The RF vessel also serves as the holding section (**Figure 1.2**) and is used to provide additional time for the food containers to accumulate the required lethality at the process temperature. Once the RF process is complete, the plastic carrier containing food trays continues to travel in the same vessel between the upper and lower sprayers.

Once the process is complete, the carrier travels back into the preheat vessel, which is also utilized for cooling (**Figure 1.2**). The vessel water temperature is maintained by pumping it through a heat exchanger to remove heat. The temperature of the circulation water supplied to (and returned from) the cooling section is monitored by RTD sensors (RTD-5 and 6 in **Figure 1.3**).

Each of the two loops has a flow meter to monitor water flow. Since each vessel's water system operates as a closed loop, transfer of water between vessels is prevented. A dual gate valve is located between the vessels to reduce the mixing of the water to maintain the temperature of each vessel. The pumps for each vessel are stopped before the gate valve opens and return to the pump speed setpoint when the gate valve closes to reduce water mixing during carrier transfer.

The chain drive conveyor is controlled by individual variable speed reversible servo motors located on each vessel. The carrier drive chains in each vessel are timed together and then the servo motors are electronically locked together. The chain position is controlled by a variable speed system using a servo motor with built in optical encoders driving two 90:1 gear boxes. The output of each gear box drives a chain that holds the carrier filled with food carriers.

The control system is a programmable logic control (PLC) unit driven by the ICON^{RMS} software application. A set of process setpoints referred to as the “Process Recipe” are programmed into a series of segments. Set points for the operating limits and critical process parameters (temperature, pressure, power, etc.) are defined in the recipe. Once the recipe has been finalized, the control system ensures the process is conducted in the designed sequence and within the pre-defined parameter limits. Alarms, program hold, and user acknowledgement safeguards are required to remove hold conditions and ensure each critical process segment is completed within the pre-defined limits. In addition, a Eurotherm digital chart recorder is provided to record the critical values of the RF forward and reflected power delivered to the RF applicators, the preheat, heating/holding temperatures, and the chain speed during the entire process. Safety interlocks and stop switches are provided to de-energize the generators and close valves to a failsafe condition as required.

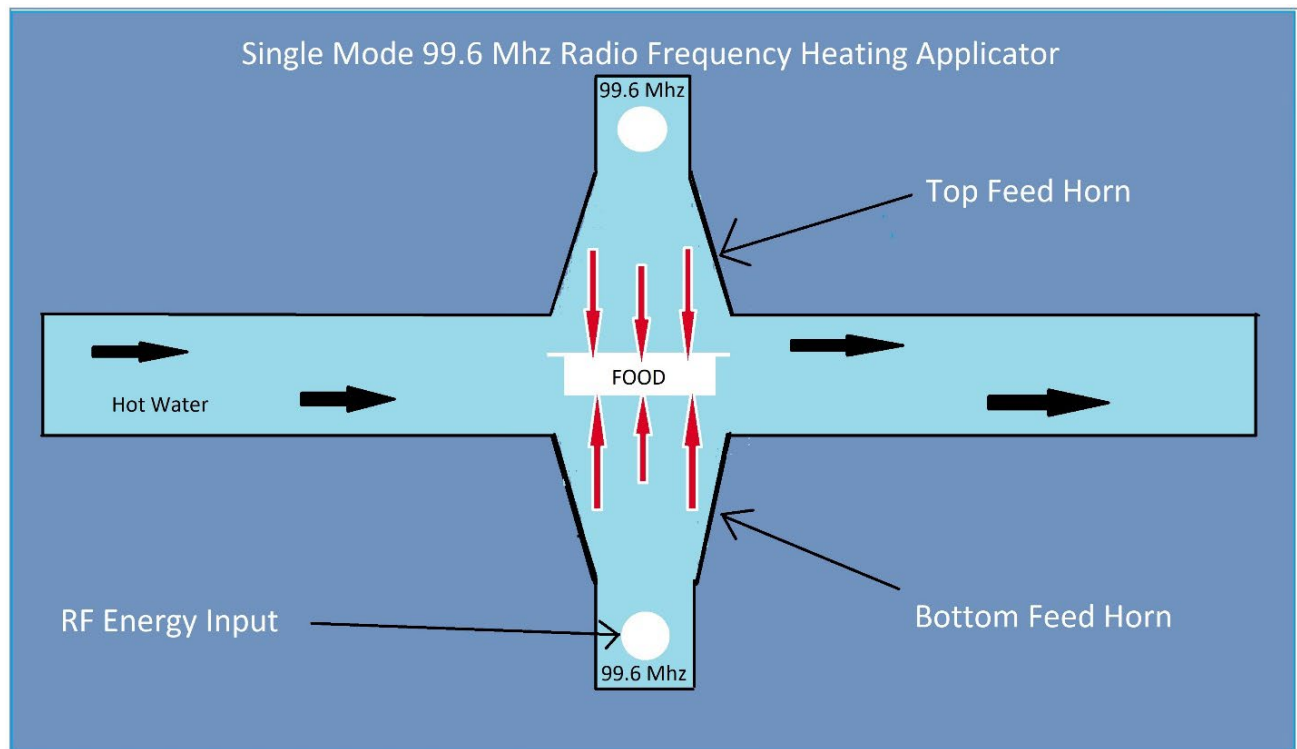


Figure 1.5 RF Applicator

1.2. Operation

System Loading:

Before operation, the preheat soak tank, preheat, and RF/Hold vessels are heated to their operating temperatures by starting a predetermined process recipe. The 13.5" x 94.25" carrier is loaded with food trays and then the carrier is lowered into the preheat soak vessel. The carrier is lowered to a rail that is on the same plane as the preheat vessel. (**Figure 1.1**). The recipe product initial temperatures are recorded. The recipe is started. Once the recipe has been started, the preheat vessel will evacuate all air to atmospheric levels so the gate valve between the preheat soak and the preheat vessel can be opened. When the gate valve opens between the two vessels the conveyor system will start at the load speed. The carrier will move from the preheat soak vessel into the preheat vessel until it reaches the "Load Point" laser sensor. The carrier will stop at this point and the servo system location will be downloaded to the PLC. The gate valve will shut and preheat pressure will be raised to the recipe level.

Process:

After the scheduled process preheat temperature is reached as indicated on the preheat section Digital Reference Thermometer, the program is released to begin the process timing. The process holds for the predetermined time and temperature to equilibrate the product temperature. After it has completed this recipe time, the pressure equalizes between the two vessels and the gate valves open. The carrier filled with food trays will travel from the preheat vessel to the beginning of the RF vessel. Once the servo system records the correct travel distance that is required for the rear of the carrier to clear the gate valve, the servo's driving the chain will stop and the gate valve will close. The carrier full of food trays will stay at the beginning of the RF vessel for a predetermined time (Recipe Slug Time) to allow the water to equilibrate before traveling under the heating horns (note: this carrier position is out of the RF horn heating area). Once the recipe slug timer has ended, the carrier will start moving towards the RF heating horns at the speed used in the recipe. The RF Transmitter's output level (from the recipe) is activated at the required forward power when the front of the carrier enters the RF horn heating section. The carrier makes one pass through the RF applicator to promote rapid uniform heating. Once the RF Pass is complete, the carrier continues to travel through the RF vessel to accumulate the required lethality according to the scheduled process. During the RF heating and Hold phases the preheat/cooling vessel's heat exchanger cools the water temperature to the cooling recipe setpoint for the cooling phase. Once the hot hold time has been met, the pressures in the RF Vessel and Preheat/Cooling vessels equilibrate allowing the gate valves to open. The carrier passes back through into the preheat/cooling vessel to begin the cooling phase. The cooling phase recipe time will not start until the cooling vessel temperature is at or below the recipe setpoint. The carrier filled with food trays stays in the cooling vessel with water circulating coming from the upper and lower sprayer nozzles until the cooling phase timer is complete.

Post Process:

After the food packages have been cooled by the circulating water for the predetermined recipe time in the preheat/cooling vessel, the pressure reduces to atmospheric pressure to open the preheat vessel gate valve giving travel access to the preheat soak vessel that is always at atmospheric pressure. The carrier is transferred from the preheat vessel to the preheat soak vessel. The carrier full of food trays are raised above the water level using a hand crank and the food containers are removed from the carrier.

2. Instrumentation**2.1. Radio Frequency (RF) power measurement devices****Instruments:**

Directional Couplers (DC) measure the RF energy directed into (“**forward**” power) the processing vessel and returning back from (“**reflected**” power) the processing vessel. The difference between the forward power and reflected power is a representation of the net power absorbed by the materials in the processing vessel.

Radio Waves are conveyed through “coaxial waveguides” designed to efficiently deliver RF energy to the processing vessel. There are two independent DC instruments installed on the waveguide prior to the guide splitting into top and bottom “horns” that provide power pathways to the processing vessel. The forward and reflected power is measured in millivolts (mV), and output is displayed in kilowatts (kW). All DC circuits are individually calibrated using NIST traceable instruments.

Measured Parameters:

The Directional Coupler (DC) instrument installed on the RF system measures forward and reflected energy throughout the RF process segment when the RF transmitter is delivering radio waves to the processing vessel. The measured values from the Directional Coupler (DC) instrument is displayed in real time on the Human Machine Interface (HMI) panel. The values are also saved in electronic data files and can be displayed as discreet segment values (beginning and end of each segment) on sterilization reports, and graphically as continuous values on trend reports.

Monitoring the Radio Wave (RF) Power Critical Factor:

RF power is a critical factor of the process, and unique to Radio Frequency (RF) processing systems. It is important to know the amount of RF energy delivered to the processing vessel as it directly affects the rate of product heating, and subsequently process lethality. Critical limits must be established that provide for a conservative amount of RF energy necessary to provide a minimum sterilizing value to the processed product. Operationally, monitoring procedures must be identified that allow for accurate (and preferably simple) measurement of process parameters that ensure critical limits are met.

Radio Wave (RF) Energy Heating Under Load:

A product in a specific heating medium will heat at a faster rate as the amount of RF energy absorbed by the product increases. Therefore, the critical limit for RF power must be established at a minimum value, under which the required lethality may not be achieved.

The following should be confirmed and considered for establishing the RF power critical limits:

- Forward RF power, measured by the dual coupler, directly affects heating of the product.
- The reflected RF power, also measured by the dual coupler, is a portion of the forward power that does not contribute to product heating.

2.2. Temperature measurement devices***Temperature measurement devices for RF system***

The temperature measurement devices used in the Radio Frequency (RF) sterilization system include RTD sensors and Digital Reference Thermometers. One RTD sensor is used for monitoring the supply water temperature in each of the preheating and RF heating vessels. Another RTD sensor is used for monitoring the temperature of the circulation water returned from each vessel. One RTD sensor is installed at the outlet end for monitoring the outlet water temperature in each of the preheating and RF heating vessels. Readings from RTD sensors are recorded in an electronic data file and readings from DART sensors are also recorded on a Eurotherm digital chart recorder during a process cycle.

Temperature measurement devices for temperature-distribution (TD) tests and process development

TMI wireless temperature sensors were used for measuring temperature distribution in circulation water within preheat and RF vessels. TMI wireless temperature sensors were placed in the food packages for recording product temperature profiles during RF processes.

Calibration for temperature measurement devices

All temperature sensors, RTD's, and Digital Reference Thermometers are calibrated against NIST standards.

Equipment / Lab Calibration Program

All inspection, measuring and testing equipment is identified, calibrated / verified, and adjusted at prescribed intervals or prior to use against equipment/standards traceable to nationally recognized standards. If no standards exist, the basis for calibration / verification is documented.

Inspection, measuring and test equipment have the necessary accuracy and precision based on standard test methods (such as ASTM), manufacturer's recommendations, and / or internal procedures. Vessel MIG's / TID's are calibrated annually. Retort Pressure gauges are verified annually. Scales are calibrated annually.

Equipment is identified with suitable indicators or records to show status. Calibration / verification records are maintained on site in the Calibration database. Certificates of calibration are maintained from external source calibrations and actual calibration / verification readings are recorded in-house. All records are maintained per the record retention guidelines. Environmental conditions are suitable for the test being performed. Handling, preservation, and storage practices ensure that accuracy is maintained.

If equipment is found to be out of calibration, validity of a sample prior to calibration / verification is assessed.

3. Water Temperature Distribution (TD) inside the RF System

3.1. Placement of thermocouples

15 wireless TMI probes were placed with probe tips sticking slightly outside the edge of the trays allowing water to fill trays and move freely around the probe tips (See **Figure 3.1**). Probes were also attached to the front and rear of the carrier to act as free leads.



Figure 3.1 Sensor setup during RF System Temperature Distribution Tests

3.2. Test conditions

Table 3.1-3.4 outline the RF System Setpoints used during the Alfredo Sauce with Penne Pasta & Apple Dessert Temperature Distribution Tests. RF Power was turned off during Temperature Distribution Testing to represent a worst-case scenario.

Pressurized Preheat Vessel	
Time (min)	35
Temperature (°C)	70
Pressure (psi)	45
Pump speed (Hz)	45
RF Sterilization Vessel	
Temperature Stabilization (Slug) Time	6
Temperature (°C)	122
Pressure (psi)	45
Pump speed (Hz)	45
RF Power (Watts)	0
Carrier Speed (in/sec)	0.28
Sterile Hold Time	15
Pressure Cool	
Time (min)	30
Temperature (°C)	30
Pressure (psi)	45
Pump speed (Hz)	45

Table 3.1 RF System Setpoints for Alfredo Sauce with Penne Pasta Temperature Distribution

Pressurized Preheat Vessel	
Time (min)	35
Temperature (°C)	70
Pressure (psi)	45
Pump speed (Hz)	37
RF Sterilization Vessel	
Temperature Stabilization (Slug) Time	6
Temperature (°C)	122
Pressure (psi)	45
Pump speed (Hz)	36
RF Power (Watts)	0
Carrier Speed (in/sec)	0.28
Sterile Hold Time	15
Pressure Cool	
Time (min)	30
Temperature (°C)	30
Pressure (psi)	45
Pump speed (Hz)	37

Table 3.2 RF System Setpoints for Alfredo Sauce with Penne Pasta Low Flow Temperature Distribution

Pressurized Preheat Vessel	
Time (min)	35
Temperature (°C)	60
Pressure (psi)	45
Pump speed (Hz)	45
RF Sterilization Vessel	
Temperature Stabilization (Slug) Time	6
Temperature (°C)	95
Pressure (psi)	45
Pump speed (Hz)	45
RF Power (Watts)	0
Carrier Speed (in/sec)	0.28
Sterile Hold Time	20
Pressure Cool	
Time (min)	30
Temperature (°C)	30
Pressure (psi)	45
Pump speed (Hz)	45

Table 3.3 RF System Setpoints for Apple Dessert Temperature Distribution

Pressurized Preheat Vessel	
Time (min)	35
Temperature (°C)	60
Pressure (psi)	45
Pump speed (Hz)	37
RF Sterilization Vessel	
Temperature Stabilization (Slug) Time	6
Temperature (°C)	95
Pressure (psi)	45
Pump speed (Hz)	36
RF Power (Watts)	0
Carrier Speed (in/sec)	0.28
Sterile Hold Time	20
Pressure Cool	
Time (min)	30
Temperature (°C)	30
Pressure (psi)	45
Pump speed (Hz)	37

Table 3.4 RF System Setpoints for Apple Dessert Low Flow Temperature Distribution

3.3. Procedures

15 TMI wireless probes were placed in the trays with the tips of the probes slightly outside the edge of the trays through small cut-outs that allow the trays to fill with vessel water when in the machine. Probes were also attached to the front and rear of the carrier to act as free leads. The trays were sealed and placed in the tray carrier. The lid of the carrier was secured to the base of the carrier and lowered into the full atmospheric tank. Once full the carrier was moved into the preheat tank and the gate valve was closed. The recipe was then started. Temperature distributions were carried out in normal flow conditions in the RF/ hot hold vessel as well as lower flow conditions. Trays were tested in both sides of the carrier to gather all necessary data around the trays for each product.

3.4 Results and observations

Figures 3.3 - 3.5 show samples of TD test results that indicate a uniform distribution in both the preheat and the RF vessels. All the temperature distribution data shows temperature uniformity was achieved by the start of the hold segment and was at or above sterility set points for the duration of the hold segment on all the tempature distribution tests.

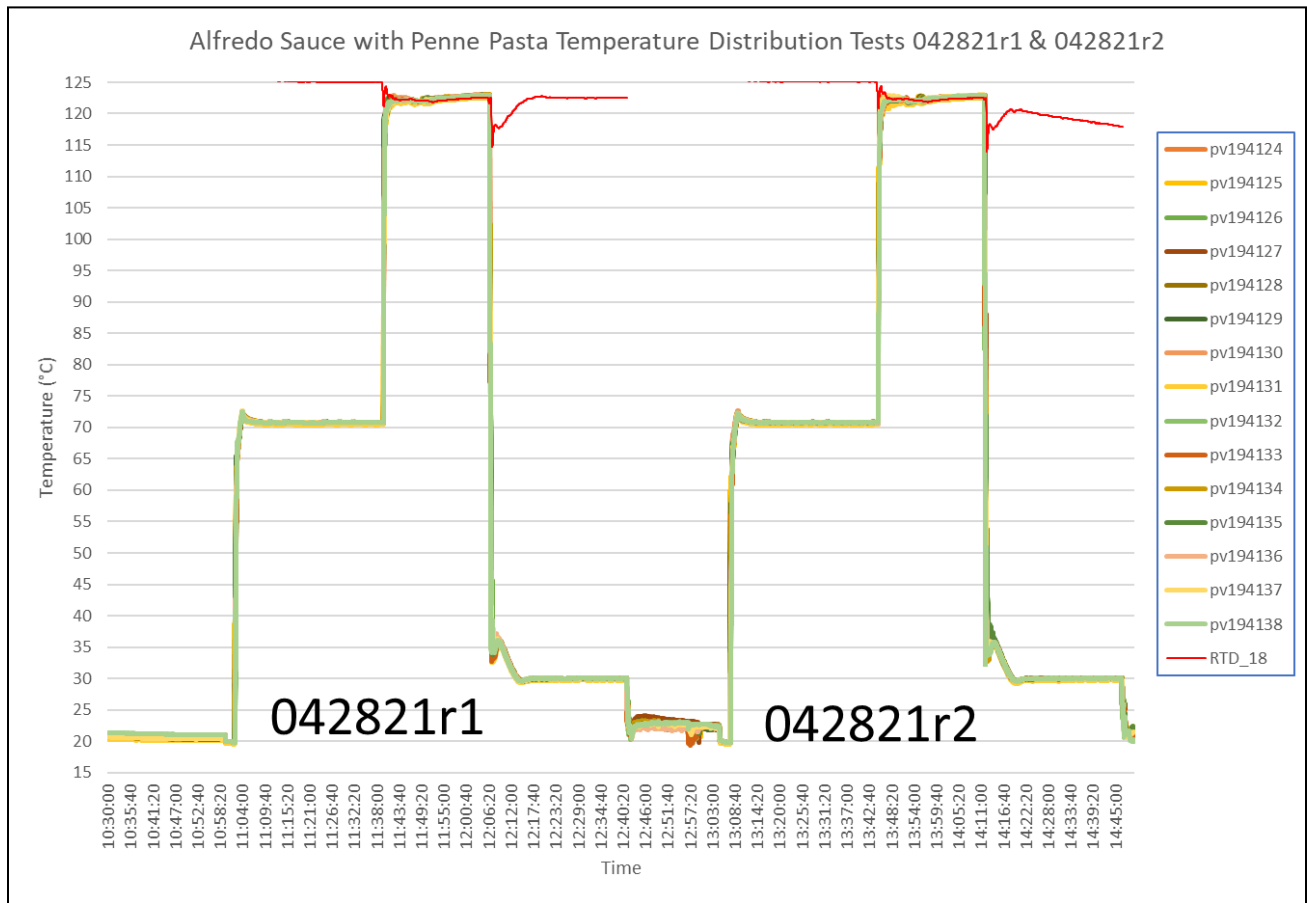


Figure 3.3 Data Chart from 4-28-2021 Alfredo TD runs with water circulation only

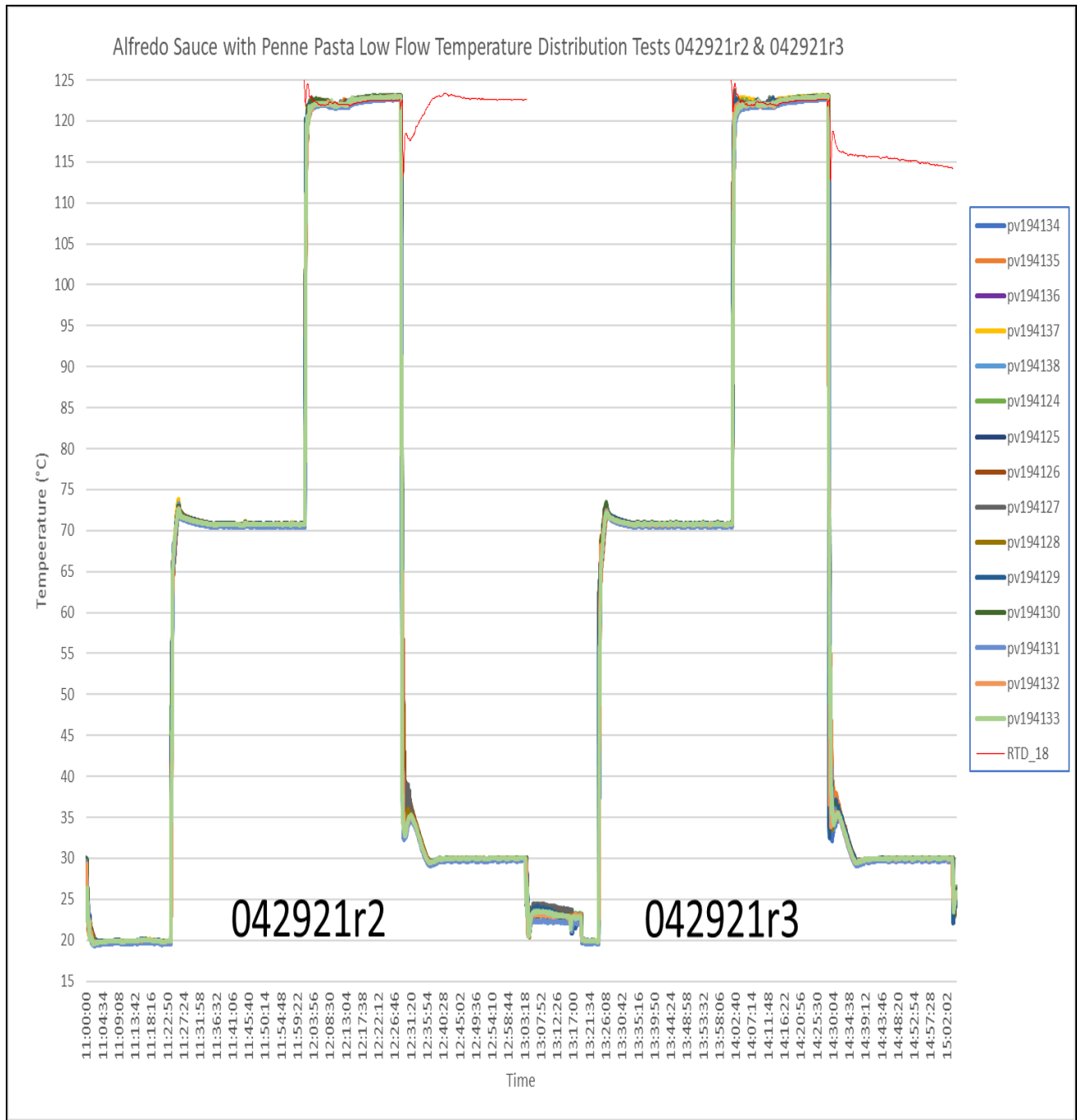


Figure 3.4 Data Chart from 4-29-2021 Alfredo TD runs with water circulation only and low flow (43 GPM) to represent a worst-case scenario.

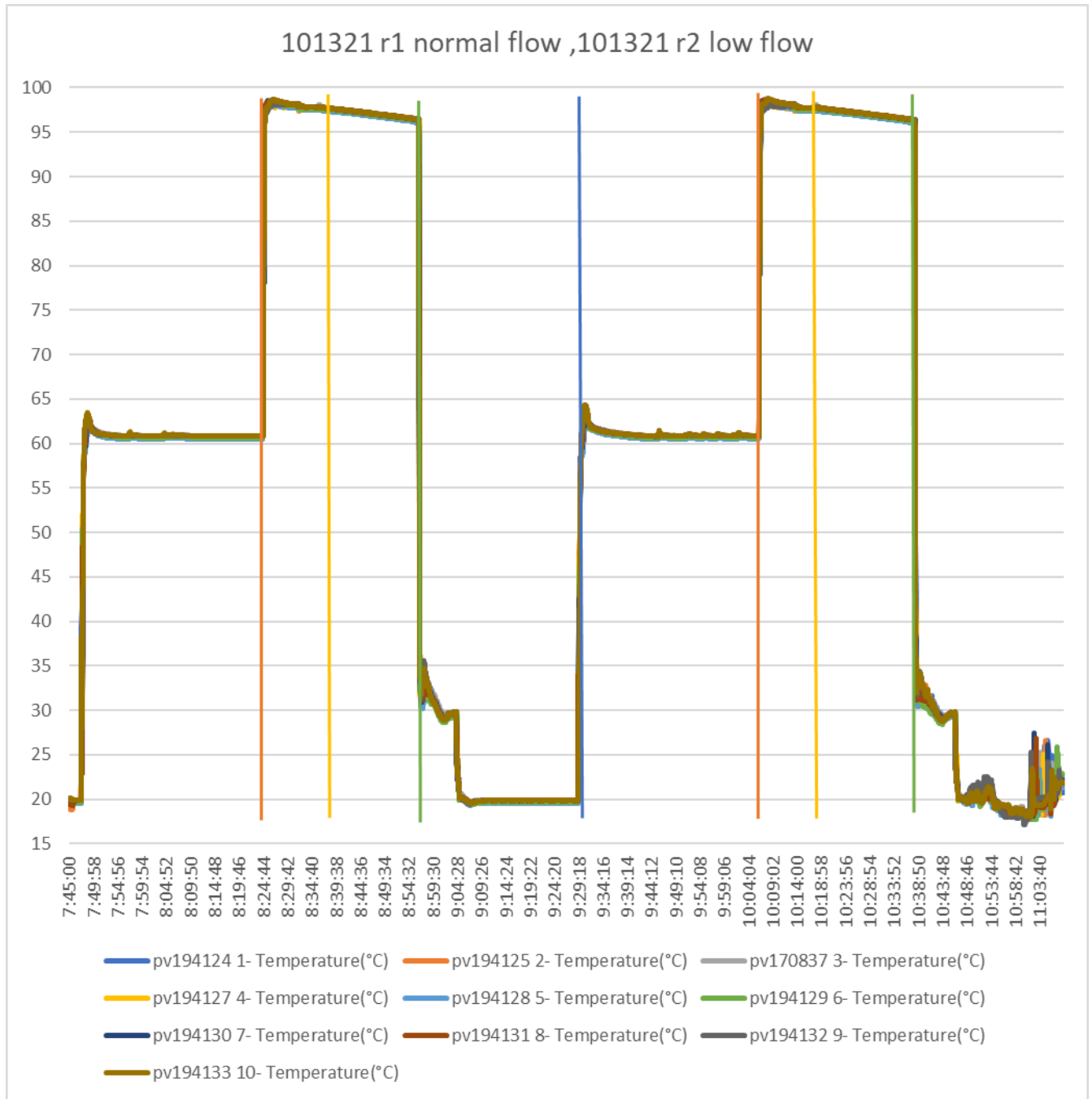


Figure 3.5 Data Chart from 10-13-21 Apple Dessert TD runs with water circulation only and low flow (43 GPM) to represent a worst-case scenario.

4. Determination of Heating Pattern

4.1. Heating pattern and locations of cold spots in Alfredo Sauce with Penne Pasta & Apple Dessert

To determine the slowest heating trays and the slowest heating areas within each tray, temperature probes were mounted in each tray in several locations and then processed under their respective RF System setpoints (see **Tables 4.1-A & 4.1-B** for RF Setpoints for Heat Pattern Mapping & Cold Spot Testing). Initial heat mapping tests included 45 runs with probes in 15 different grid locations throughout each tray. These 15 locations are shown as red dots in **Figure 4.1**.

Pressurized Preheat Vessel	
Time (min)	35
Temperature (°C)	75
Pressure (psi)	45
Pump speed (Hz)	45
RF Sterilization Vessel	
Temperature Stabilization (Slug) Time	6
Temperature (°C)	122
Pressure (psi)	45
Pump speed (Hz)	45
Radio Frequency (MHz)	99.6
RF Power (Watts)	11000
Carrier Speed (in/sec)	0.28
Sterile Hold Time	15
Pressure Cool	
Time (min)	30
Temperature (°C)	30
Pressure (psi)	45
Pump speed (Hz)	45

Table 4.1-A RF System Setpoints during Alfredo Heat Pattern Mapping & Cold Spot Testing

Pressurized Preheat Vessel	
Time (min)	35
Temperature (°C)	60
Pressure (psi)	45
Pump speed (Hz)	45
RF Sterilization Vessel	
Temperature Stabilization (Slug) Time	6
Temperature (°C)	95
Pressure (psi)	45
Pump speed (Hz)	45
Radio Frequency (MHz)	94
RF Power (Watts)	8000
Carrier Speed (in/sec)	0.28
Sterile Hold Time	8
Pressure Cool	
Time (min)	30
Temperature (°C)	30
Pressure (psi)	45
Pump speed (Hz)	45

Table 4.1-B RF System Setpoints during Apple Dessert Heat Pattern Mapping & Cold Spot Testing

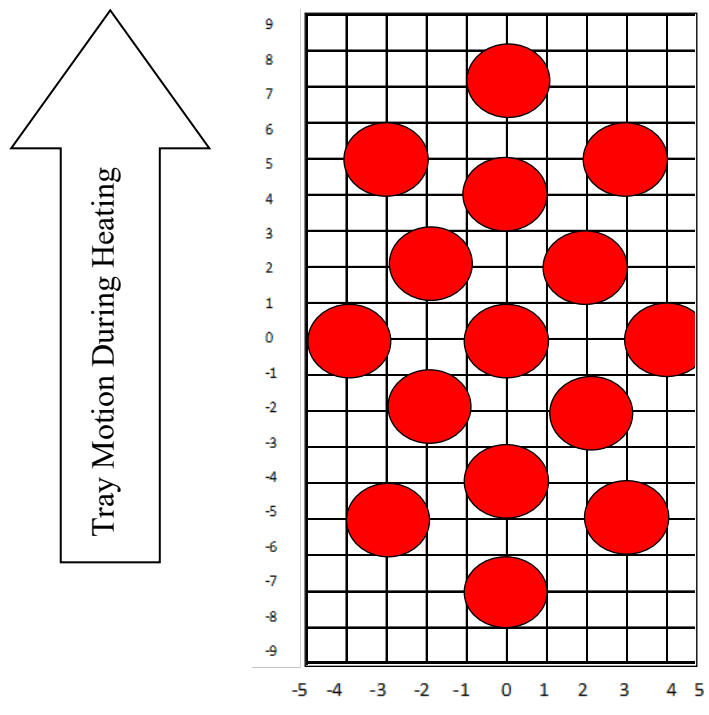


Figure 4.1 Red dots represent probe placement within the gridded 13oz trays during the initial heat mapping tests.

4.2. Cold Spot Validation Testing

The areas surrounding the probe locations with the lowest Fo values from the 45 initial heat map runs were then tested in triplicate under the same machine conditions (see **Table 4.1** for machine setpoints during heat pattern mapping and cold spot testing). This testing method continued until tests of surrounding grid locations resulted in higher Fo values than all previously tested locations. The lowest lethality locations within each tray were then determined to be the cold spot of that tray (See **Figure 4.2**).

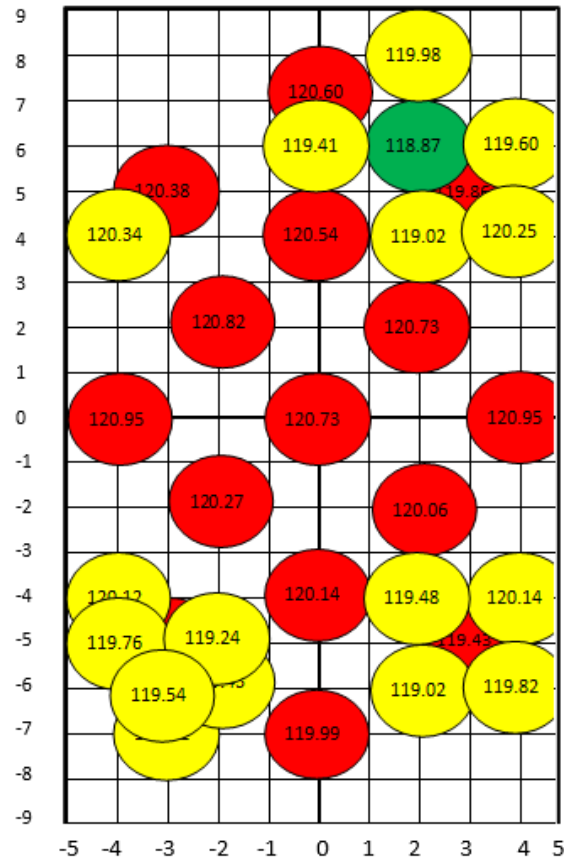


Figure 4.2 This figure depicts the minimum temperatures (°C) collected at each location tested within tray B5 (the coldest Alfredo tray). The tray location (2,6) received lower thermal treatment than all other tray locations and was therefore determined to be the cold spot.

STEP 1 - Red circles represent initial heat map runs.

STEP 2 - Yellow circles represent cold spot testing around the coldest red circles

STEP 3 - The green circle is the coldest location in the tray, with all surrounding locations reaching higher temps. The standard deviation of temperatures collected at the coldest location [B5 (2,6)] was 0.0287

5. Heat Penetration (HP) Tests

Heat penetration tests were conducted to achieve sterility. The location of cold spots for each product was determined by the heat pattern mapping and cold spot testing described in Section 4. RF sterile hold time was increased to achieve sterility in the cold spots of all trays.

Table 5.2 RF System setpoints during Alfredo Pasta Heat Penetration Testing

Pressurized Preheat Vessel	
Time (min)	35
Temperature (°C)	75
Pressure (psi)	45
Pump speed (Hz)	45
RF Sterilization Vessel	
Temperature Stabilization (Slug) Time	6
Temperature (°C)	122
Pressure (psi)	45
Pump speed (Hz)	45
Radio Frequency (MHz)	99.6
RF Power (Watts)	11000
Carrier Speed (in/sec)	0.28
Sterile Hold Time	17.5
Pressure Cool	
Time (min)	30
Temperature (°C)	30
Pressure (psi)	45
Pump speed (Hz)	45

Table 5.3 RF System setpoints during Apple Dessert Heat Penetration Testing

Pressurized Preheat Vessel	
Time (min)	35
Temperature (°C)	60
Pressure (psi)	45
Pump speed (Hz)	45
RF Sterilization Vessel	
Temperature Stabilization (Slug) Time	6
Temperature (°C)	95
Pressure (psi)	45
Pump speed (Hz)	45
Radio Frequency (MHz)	94
RF Power (Watts)	8000
Carrier Speed (in/sec)	0.28
Sterile Hold Time	8
Pressure Cool	
Time (min)	30
Temperature (°C)	30
Pressure (psi)	45
Pump speed (Hz)	45

5.2. Procedures

- The appropriate recipe was loaded into the HMI. See **Tables 5.2** and **5.3** for the process recipe setpoints used for each product.
- The TMI probes were programmed, placed on ½” stands, then secured to the cold spots. The ½” stands were used to ensure the probe tips were located at a central depth. Probe tips were impaled with product then filled to a net weight of 420g, then vacuum sealed (See **Figure 5.1**).

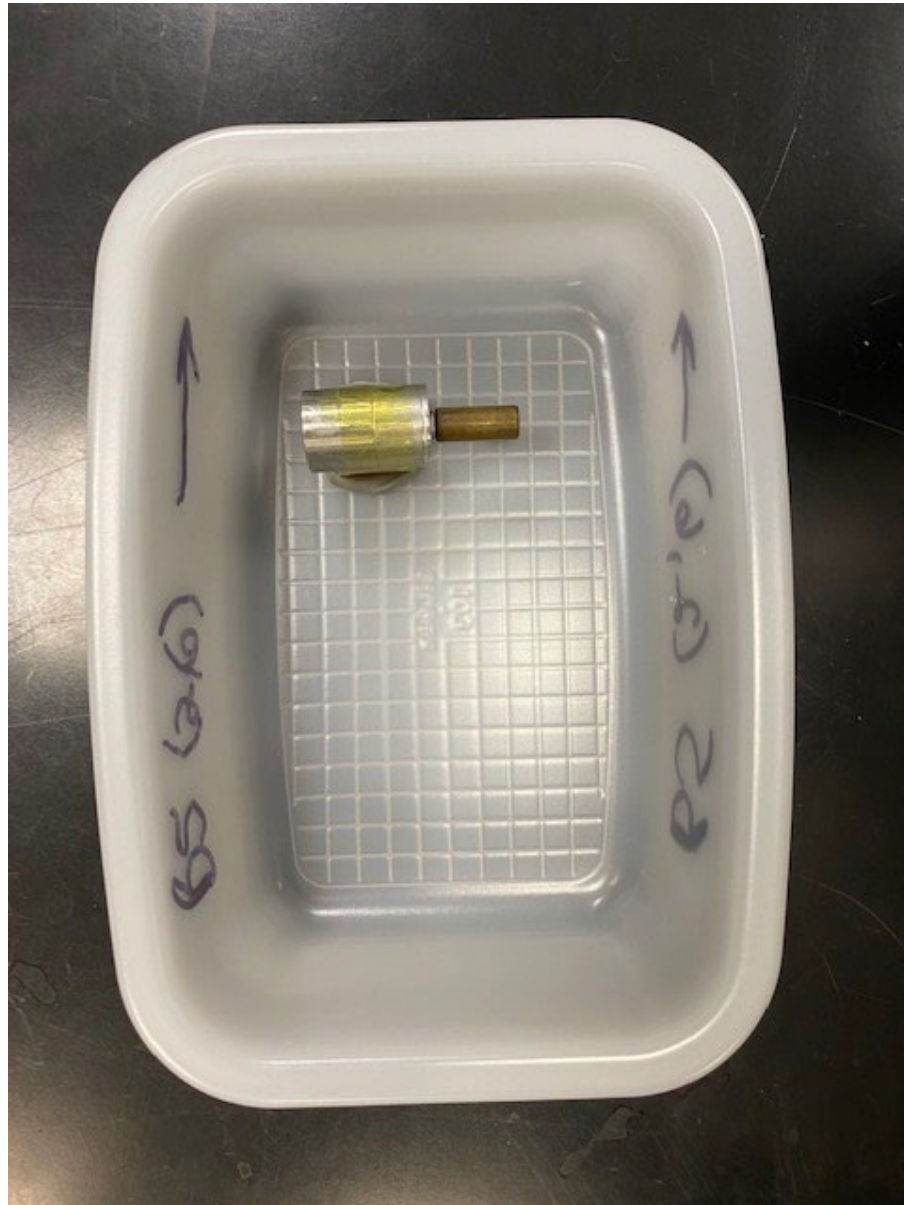


Figure 5.1. TMI temperature probe placed on a half inch stand & taped into the cold spot (2,6) of tray B5.

- 10 trays containing wireless TMI probes and 420g of product were then placed into the RF tray carrier (carrier layout depicted in **Figure 5.2**).
- Ballast trays contained 420g of product that were not probed, as they were not intended to receive full RF treatment. Instead, ballast trays are used as a “dummy load” to allow the RF generator to ramp up to full and stable power.

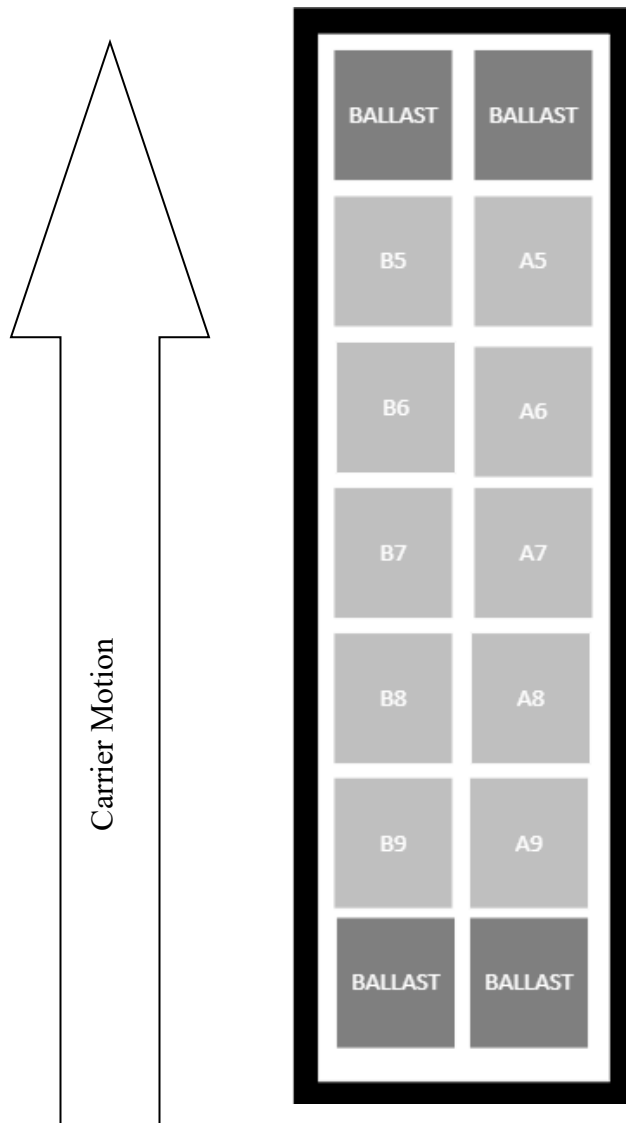


Figure 5.2 Tray layout within the carrier. The arrow indicates the direction the carrier travels through the RF System during heating. The light grey rectangles labeled A5-B9 represent heat penetration tray locations within the carrier. The dark grey rectangles on either side of the heat penetration trays represent ballast tray/dummy load locations.

- The carrier containing probed, filled, and sealed trays was loaded into the vessel.
- The process recipe was started and completed the program set points as is detailed above.

5.4. Summary

Under the heat penetration test conditions and using the procedures described in **Section 5.2**, the RF system delivered a thermal process to achieve sterility.

5.5 Submission for FDA Filing (Form FDA 2541d)

An electronic submission for the RF process based on the HP test data was submitted to FDA for filing on March 23, 2022. In the submission, a recommended RF process on the Alfredo Sauce with Penne Pasta packaged in 13-oz trays was proposed. As the process is based on a general method calculation, the critical factors recommended for the process filing are based on conservative values relative to the measured HP test values. Separately, HP studies were conducted to ensure critical limits for residual air and flow rate are within a range that ensures the filed process delivers the target lethality (F_0) of at least 6.0 minutes.

Regarding RF power, the variations of DC measurements in the test results were considered when setting the lower limit for forward RF power and the upper limit for reflected RF power. The minimum forward RF power and the maximum reflected RF power values from all heat penetration runs that resulted in sterility will be used as critical limits for RF power.

The Apple Dessert process was not filed with the FDA due to the product meeting the requirements of an acid food with an equilibrated pH of less than 4.6. However, the product followed the testing protocol of Temperature distribution, Cold Spot testing, and Heat Penetration testing to verify the product met all requirements to be considered commercially sterile.

6. Microbial Validation of RF Process:

The Inoculation Study was completed on the Alfredo Sauce with Pasta product only. Apple Dessert was not included in the study due to deficient microorganisms to demonstrate growth and sufficient processing associated with the acidic product matrix.

The information below was prepared by Shirin Adb from Euofins Microbiology Laboratory.

Table 3. Study design for the Alfredo Sauce with Noodles product inoculated pack study.

Test Day	Trial	Inoculated Samples ¹		Uninoculated Samples ¹		Thermocouple	
		Number of Packages	Carrier Location	Number of Packages	Carrier Location	Number of Thermocouple	Carrier Location
1	1	9	B5, B6, B7, B8, B9 A5, A6, A7, A9	x	x	1	A8
	2	9	B5, B6, B7, B8, B9 A5, A6, A7, A9	x	x	1	A8
	3	x	x	9	B6, B7, B8, B9 A5, A6, A7, A8, A9	1	A8
2	1	9	B5, B6, B7, B8, B9 A5, A6, A7, A9	x	x	1	A8
	2	9	B5, B6, B7, B8, B9 A5, A6, A7, A9	x	x	1	A8
	3	x	x	9	B6, B7, B8, B9 A5, A6, A7, A8, A9	1	A8
3	1	9	B5, B6, B7, B8, B9 A5, A6, A7, A9	x	x	1	A8
	2	9	B5, B6, B7, B8, B9 A5, A6, A7, A9	x	x	1	A8
	3	x	x	9	B6, B7, B8, B9 A5, A6, A7, A8, A9	1	A8

¹X: No sample was tested.

Monitoring the Product Temperature. Thermocouples were placed into one uninoculated sample per run (location A8) to monitor the product temperature during the inoculated pack study. The location and number of thermocouples was selected by AmeriQual's Process Authority team.

Shipment of Samples after the Inoculated Pack Study. All of the inoculated and uninoculated samples (18 inoculated and 9 uninoculated per day) collected for Day 1, Day 2, and Day 3 were shipped overnight on September 21st, 22nd, and 23rd, 2021, respectively in coolers with ice packs to the Eurofins facility

Incubation of Samples. Upon arrival at the Eurofins facility, all of the 54 inoculated treated samples) and 9 out of the 27 uninoculated treated samples (3 samples per test day) were incubated at 30°C for a minimum of 90 days (Table 4). The samples were checked every 2 weeks for signs of container swelling. If swelling was observed, swollen packages were opened under a biosafety hood and the product was examined under a microscope for the presence of *Clostridium* cells.

Evaluation of Uninoculated Treated Samples. Immediately upon arrival at the Eurofins facility, 3 uninoculated treated samples per test day were aseptically opened under a biosafety hood and tested for pH and water activity level.

Positive Control Samples. At the Eurofins facility, one uninoculated treated sample per test day was inoculated with 1-ml of *C. sporogenes* PA 3679 spore crop to serve as positive control samples. Prior inoculation, the *C. sporogenes* PA 3679 spore crop was heat-shocked at 181°F/83°C for 13 minutes. Two septa were placed at two different locations on the top of the tray and 2 x 0.5 ml aliquots of the spore inoculum were injected into the product through the septa. The septa were immediately sealed using sealant after product inoculation. These samples were considered positive control samples and were incubated at 86°F/30°C along with other samples. It was expected that *C. sporogenes* PA 3679 would germinate and grow in these samples within two weeks and cause the trays to swell. Once signs of

swelling were observed, the swollen trays were opened under a biosafety hood and the product was examined under a microscope for the presence of *Clostridium* cells. The purpose of this control was to demonstrate that *C. sporogenes* PA 3679 spores could germinate and grow in the product inside its final packaging.

Inoculated and Uninoculated Samples after 90 Days of Incubation. All of the samples were negative throughout the incubation period and no visible signs of *C. sporogenes* PA3679 growth (container swelling) were observed in any of the samples.

Conclusion

The results of the study indicated that the RF process applied to the Alfredo Sauce with Noodles product (at the process parameters selected by the AmeriQual process authority team) was adequate to produce a commercially sterile product by providing a minimum 6-log reduction of *C. sporogenes* spores during the process.

7. Critical Factor Considerations

As a novel processing technology, identifying the appropriate critical factors for the RF sterilization system required a thorough evaluation of the engineering design, mechanical configuration, operating characteristics, and attributes of the product and package selected for the first model application.

Processing system critical factors:

- Water level and temperature in preheating and RF/holding vessel
- Time in preheating, RF heating, and holding sections
- Conveyor speed during RF
- RF energy and frequency applied in the RF heating section
- Conductivity of the RF vessel water
- Over pressure in the system
- Heating medium flow rate

Product/package critical factors:

- Formulation
- Initial product temperature
- Package orientation
- Fill weight
- Residual air

8. Critical Limit Determination

Once process critical factors were identified, heat penetration tests were conducted using process conditions and product formulations that were relative to target production limits.

Heat penetration tests to establish an adequate process schedule were conducted using TMI sensors, which required validation for use under RF energy to ensure they would be accurate and repeatable.

The TMI sensors were placed in the predetermined cold spots and temperature data was collected. Time/Temperature data were collected at 1 second intervals, and general method lethality was calculated incrementally and cumulatively using that interval. Time and temperature cold spot data collected in the preheating, RF heating, and holding sections were used for the calculation. Lethality attributable to the cooling section was not included in the sterility value calculation to provide a safety margin.

9. Natick Product Samples

RF and Retort Process samples were produced of Alfredo Sauce and Pasta and Apple Dessert. Natick received 140 samples of each product from each processing method. All samples have been placed in incubation to be evaluated at 6 months, 1 year, 2 years, and 3 years by the Natick team.



10. Commercialization Strategy

Next Steps to be reviewed following shelf-life evaluation and sensory testing of the comparative processing methods by the Natick

11. Company's POC's

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