Meat and Muscle Biology™

Practical Elimination of Raw Meat Microbiological Risk Using Thermal Pasteurization, a Novel Meat-Safety-Driven Technology

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Abstract: Although the safety of raw meat products has improved in recent decades, raw meat is still associated with a considerable incidence of foodborne illnesses and death. Standard raw meat antimicrobial interventions such as chemical sprays can reduce meat quality, and their effectiveness has plateaued. However, a new thermal pasteurization technology implementing direct steam injection into ground meat and subsequent chilling of the meat by expansion under vacuum has the potential to nearly eliminate pathogens in raw ground meat products while preserving the proteins in the raw state. An inoculation (*Escherichia coli* surrogates) study of a full-scale pilot pasteurization system demonstrated the effectiveness of pasteurization to significantly reduce illness-causing pathogens in raw ground beef. High-level (log 6.3 colony-forming units per gram [cfu/g]) inoculations were used to validate the minimum temperature required to achieve a 5 log microorganism reduction, and low-level (log 3.8 cfu/g) inoculations were used to validate the minimum temperature required to achieve a 3 log microorganism reduction. At both levels of inoculation, pasteurization achieved the targeted reduction in inoculated microorganism populations (mean ± SEM log microorganism reductions for high-level = log 5.8 ± 0.04 cfu/g, low-level = log 3.3 ± 0.03 cfu/g). Ground beef protein profile and color were studied to determine functional effects of the thermal pasteurization technology. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and mass spectrometry revealed no significant changes in the protein profile ($P > 0.05$). Colorimetric measurements revealed minor changes that were visually insignificant in the color profile of processed versus unprocessed ground beef. A consumer acceptance study found similar preferences for pasteurized ground beef products compared with retail-available ground beef products.

Key words: pasteurization, meat, intervention, raw preservation
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Industrial Relevance

Historical trends of pathogens in meat

The primary pathogens of concern in the production of raw meat products are *Salmonella* and Shiga toxin-producing *Escherichia coli* (STEC). There has been a steady but slightly increasing incidence of *Salmonella* infections in the United States since a historic low in 1997 (Figure 1) (CDC, N.D.). Of all *Salmonella* infections reported in the US, poultry is estimated to be the source of 10.1% to 29.2% of the *Salmonella* infections (Painter et al., 2013). There has been an increasing incidence of STEC infections in the US since a historic low in 2004 (Figure 1) (CDC, N.D.). Of all STEC infections reported in the US, beef is estimated to be the source of 26.4% to 29.0% of the STEC infections (Painter et al., 2013).

Current antimicrobial intervention technologies used in raw meat production

In addition to the standard processes long employed by the meat industry, such as cooling and freezing, there are many intervention type technologies

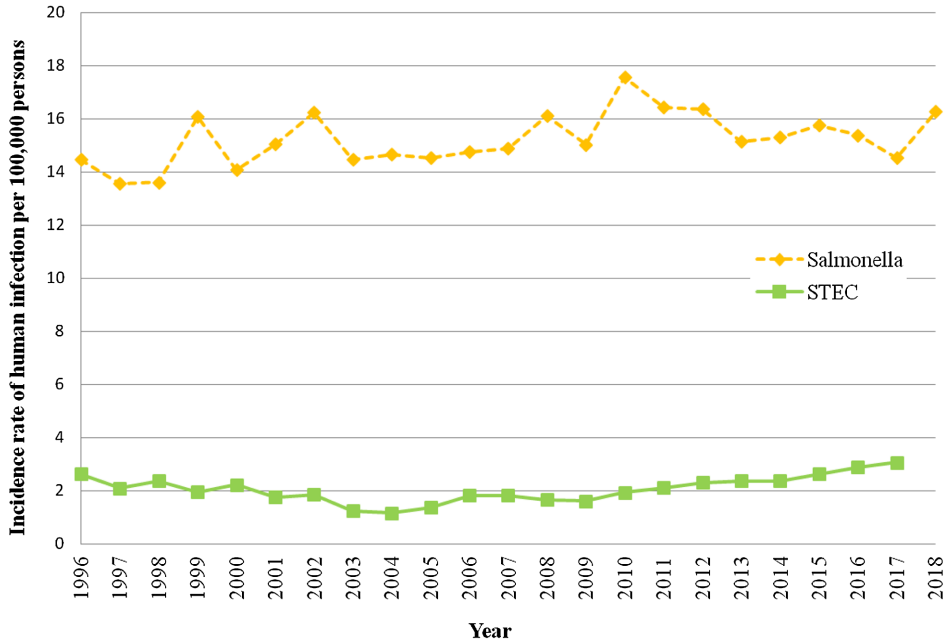

Figure 1. Incidence of foodborne human infections, US. STEC, Shiga toxin-producing *Escherichia coli*.

in use today in meat production, primarily focused on reducing STEC in beef and *Salmonella* in poultry. The most common type of intervention is the use of a chemical antimicrobial. Typically, beef production utilizes formulations of lactic acid, acetic acid, or citric acid at concentrations of 1.5% to 2.5% in the form of carcass sprays to achieve reductions of pathogen populations. At best, these applications result in a 2 log reduction of STEC; more typically, they reduce STEC by 0.5 log to 1 log (Sohaib et al., 2016). Concentrations of 2% and 4% acetic acid or lactic acid have been shown to be effective when applied to beef trimmings, sustaining reductions in the final ground beef by 2.5 log of *E. coli* O157:H7 and 1.5 log of *Salmonella* Typhimurium (Harris et al., 2006). In poultry production, the most common antimicrobial treatment is chlorine water at concentrations of 18 to 25 parts per million (ppm) chlorine applied as a spray or dip (Sohaib et al., 2016). These treatments are effective at reducing surface bacteria but have limited ability to penetrate and treat non-topical surfaces of meat.

Limitations of current intervention technologies used in raw meat production

For an antimicrobial intervention to be effective, it must come into contact with the microorganism. This can be a significant challenge when applying interventions to the whole carcass or to trimmings. A zero-moisture addition requirement for many raw meats, such that there can be no retention of the sprayed or dipped agents on the carcass or trim, typically limits the amount of agent that can be applied, making it difficult to achieve complete surface coverage by the antimicrobial. In addition, acids and chlorine will both reduce the quality of the meat to some degree. At low concentrations of acid (1% to 2%) or chlorine solutions (18 to 25 ppm of chlorine into chill water), the meat quality reduction is negligible. However, at higher concentrations—above 2%-acid solutions and above 25-ppm chlorine in chilled water, where these antimicrobial solutions may be more effective—the resulting treatment can have undesirable effects on the meat quality, including discoloration or bleaching, off-odor, and off-flavor attributes (Sohaib et al., 2016). In many cases, these limitations are insignificant when treating whole muscle cuts; however, they are significant for treating trim or carcass components used in the standard production of ground meat products.

Technological leaps

Although complete elimination of foodborne illness associated with raw meat is not possible, there are examples in other industries in which a technological leap has completely revolutionized food safety. The advent of milk pasteurization completely transformed the dairy industry and has practically eliminated the risk of foodborne illness associated with pasteurized dairy products. Today, unpasteurized milk products cause 840 times more illnesses and 45 times more hospitalizations than pasteurized products (Costard et al.,

2017). Clearly, there is a great opportunity for similar achievement in the meat industry. However, given the current trend of incidence of foodborne illness associated with raw meats and the limitations of existing intervention technologies, an achievement of this type is unlikely to result from iterative developments of existing technologies. A technological leap, similar to the advent of pasteurization in the milk industry, will be required to practically eliminate microbiological risk with raw meats.

For this technology to be effective, it will have to overcome the limitations of the current technologies, namely, the inability to equally treat the entire product and to achieve the concentrations necessary for significant pathogen reductions without negatively impacting meat quality.

Meat Pasteurization Using Steam Injection and Vacuum Expansion Chilling

A direct steam injection and vacuum expansion chilling meat pasteurization system, referred to as refrigerated instantaneous temperature cycling (RITC), has been developed to inactivate microorganisms while preserving meat in the raw state. The process consists of 2 main steps: direct application of steam to meat to instantaneously raise the temperature beyond a minimum of 82.2°C, followed by equally instantaneous chilling of the meat by expansion under vacuum. The entire process, from the point steam contacts meat to the point meat is fully chilled under vacuum, takes only 0.3 s. This sophisticated form of thermal pasteurization can reduce microorganism populations by more than 5 log with only minimal and imperceptible changes to the sensory characteristics of the raw meat. The RITC process is similar to direct-heating ultra-high temperature (UHT) pasteurization of milk in common use today. However, typical direct-heating UHT treatments applied to meat would likely cause denaturing of proteins or other undesirable effects on the meat. There are several key distinctions that make the RITC process unique among UHT pasteurization processes and appropriate for use in raw meat.

RITC Process Distinctions

Time and temperature

Meat proteins, by nature, are more sensitive to high-temperature processing than milk proteins; therefore, it is important to minimize both the time and temperature during thermal pasteurization to maintain meat proteins in a native state and limit loss of color. For instance, heating meat proteins to 82.2°C using common direct-heating UHT milk pasteurization processes (Milk UHT) would likely cause the proteins to be cooked or denatured because of the longer time at high temperature. Typical Milk UHT process hold times range from 2.4 s to 6.7 s (Lewis and Heppell, 2000), depending on holding temperature, which is 8.5 to 24 times longer than the RITC process, for which the hold time is just under 0.3 s. The RITC process also operates at much lower temperatures throughout the process than traditional Milk UHT processes, and the maximum temperature achieved is also lower—85.0°C in RITC compared to 147°C in Milk UHT (Lewis and Heppell, 2000).

Pre-tempering

Pre-tempering, in which the product temperature is raised from a stable storage temperature to a consistent pre-injection temperature by indirect heat exchange, is common in UHT processes. In the RITC process for ground meats, the process of pre-tempering raises the product temperature to approximately 46°C prior to steam injection. A proprietary design heat exchanger using a single tube of small diameter ensures positive flow of all the product to be treated. This is important for precision temperature control and to minimize temperature variability throughout the product as it passes through the heat exchanger, resulting in even tempering without any overheating.

Steam injection

The steam injection step utilizes a patented (U.S. Patent No. 10,674,751) axial flow injector designed to promote rapid mixing without fouling. Fouling is a phenomenon common in pasteurization that refers to the formation of deposits on heat transfer surfaces as a result of reactions that take place as foods are heated (Lewis and Heppell, 2000). Meat proteins are susceptible to rapid fouling and, if not handled properly in an injector, will quickly foul the injector and disrupt the flow and mixing. The specialized injector developed for the RITC process uses a proprietary design that promotes extremely rapid mixing and brings all particles of meat, regardless of dimension (particles must be of an appropriate size, e.g., ground to 1/8", to pass through injector), to the same temperature within hundredths of a second. Additionally, flow-promotion geometries and a proprietary system within

the injector—which controls the heat flux through the materials that make up the injector—prevent protein deposits on heat transfer surfaces and subsequent fouling. These designs achieve the desired mixing without creating undesired flow profiles that lead to surface deposits and fouling.

High-level exposure of vacuum

The expansion chilling step uses a propriety design that creates an immediate, high-level exposure of the proteins to the vacuum environment, allowing instant boiling of the condensed water from the steam injection, which causes the rapid and equal chilling of all particles of the meat. The product entrance design limits the interaction of the product with the surface of the vessel, which prevents interference with the vacuum chilling or localized overheating due to contact with hot surfaces of the vessel.

RITC Development

Previous attempts at pasteurization of meat proteins have taken the form of either carcass steam vacuuming systems or irradiation. Carcass steam vacuum systems work by delivering a stream of water at 7 to 10 pounds per square inch, between 88°C and 94°C, to an area 1.5 × 6.5 cm while simultaneously vacuuming the area around the stream of hot water (Dorsa, 1996). Steam delivered at approximately 45 psi continuously sanitizes the equipment while in use. The method of pasteurization in the RITC process differs from the previous attempts of using steam and vacuum in several regards. By using direct steam injection in RITC, the product temperature can be elevated instantaneously by latent heat transfer, which results in the shortest time possible for the thermal treatment process. By contrast, in carcass steam vacuum systems, the product temperature takes longer to be elevated since the only heat transfer effect is from the specific heat of the sprayed water resulting in a longer overall thermal treatment process. Similarly, the vacuum expansion in RITC causes the instantaneous chilling of the product, while the vacuum in the carcass steam vacuum systems is only intended to remove contamination from the carcass. Finally, since the entire product matrix is thermally treated during the RITC process, the log reductions of pathogen populations are representative of the entire matrix, whereas the log reductions achieved in steam vacuuming only apply to the small areas that are treated, typically areas of visible contamination.

An alternative method of meat pasteurization, irradiation, uses ionizing radiation from an energy source such as gamma rays or X-rays to achieve reduction in pathogen populations. Although irradiation is extremely effective in reducing microorganisms, the treatment produces a characteristic aroma and alters meat flavor, both of which negatively impact consumer acceptance (Ahn et al., 2013). Antioxidant treatments can reduce this effect; however, the labeled disclosure of common antioxidants is required for use in many raw products such as raw ground beef and could deter consumers. The RITC pasteurization process has no associated oxidizing mechanism and does not affect the flavor of meat.

Precedence for wide-scale commercial thermal pasteurization already exists for nondairy animal proteins, e.g., thermal pasteurization of liquid egg, which contains a low-viscosity protein in liquid phase (Froning et al., 2002).

The patent-pending RITC meat pasteurization technology was pioneered by empirical™ Innovations, Inc. (Dakota Dunes, SD) as part of the ground beef production process that it has developed using modern technologies. In this production process, the lean proteins from higher-fat beef cuts are separated into 2 phases, a fibrous component of protein and a component of protein in liquid phase (light protein). The 2 phases of protein are mixed back together to form the finished ground beef. The first application of the RITC technology was to the light protein. The low viscosity of the light protein made it easier to process and less susceptible to immediate fouling.

Throughout the development, there were several challenges that were difficult to predict or to model, therefore an empirical process of design, test, revise and retest was employed to rapidly understand and overcome the different challenges. A summary of some of the more significant challenges and solutions in developing the RITC process is presented here.

Instantaneous mixing

The steam and product must be mixed on the order of hundredths of a second in order to limit the time at high temperature to 0.3 s. Initial design and testing achieved minimal microorganism reductions because the mixing could not take place on the necessary timescale. The challenge to effective mixing is the penetration of the steam into the entire product mix without flow stagnation, which leads to overheating and fouling.

Two years of experimentation resulted in a robust understanding of the flow mechanics, leading to

identification of several critical factors of instantaneous mixing: penetration depth, penetration angle, and flow velocity. Experimenting with the relationship between these factors led to the development of a specialized flow geometry that maximizes surface contact between the steam and the product while preventing flow stagnation.

Localized overheating

Overheating during hold time causes fouling of the injector. The bulk fluid temperature is controlled by the ratio of steam added to the product, but localized overheating can occur where product contacts boundary surfaces, which absorb heat rapidly from the unmixed steam. After experimentation with a variety of techniques, the optimal solution involved controlling the heat flux through boundary surfaces. By using appropriate heat transfer media and channel design around the boundaries, the heat flux through the boundary surfaces can be maintained such that the heat is removed from the boundary surface prior to localized overheating. There is a balance between removing the necessary amount of heat to prevent overheating and removing too much heat, which causes the temperature achieved in the injector to fall below critical limits.

Instantaneous chilling

Direct chilling by expansion under vacuum is considered instantaneous if all of the product being processed is given equal access to the vacuum condition. Conventional processing techniques and vacuum expansion design do not immediately expose sufficient surface area to the vacuum, resulting in non-instantaneous chilling in much of the product. This wide range in chilling times would not be adequate in the RITC process due to the short timescale at high temperatures necessary for retaining meat quality.

An innovative, custom-designed vacuum expansion system closely integrated with steam injection and mixing was developed with the specific intent of maximizing the surface area of product when exposed to the vacuum condition. It was also important to minimize product contact with hot boundary surfaces of the vacuum system to prevent localized overheating. Together, these 2 design elements, properly implemented, facilitate instantaneous chilling of the product when exposed to the vacuum.

RITC Process Description

Figure 2 represents a common RITC process utilized for the pasteurization of light protein from an empirical™ ground beef process. Proteins are held in a chilled storage tank (1), typically at 7°C, prior to treatment. Prior to steam injection, proteins are tempered in a single-tube, one-pass heat exchanger (3), which tempers the proteins to approximately 46°C. Direct application of steam in the injector (5) raises the temperature of the proteins to over 82.2°C. The steam condenses and is rapidly mixed into the protein and held at this temperature for an extremely short time: 0.3 s. The holding time at high temperature takes place in a specialized mixing tube (7) designed to promote rapid mixing and prevent fouling. After mixing and holding, the protein and condensed steam mixture flow into an expansion cooling vessel (9), which causes instantaneous chilling due to the low-temperature boiling of water from the condensed steam. The expansion chilling step is intentionally designed to remove all of the steam (condensed and non-condensed) through boiling and

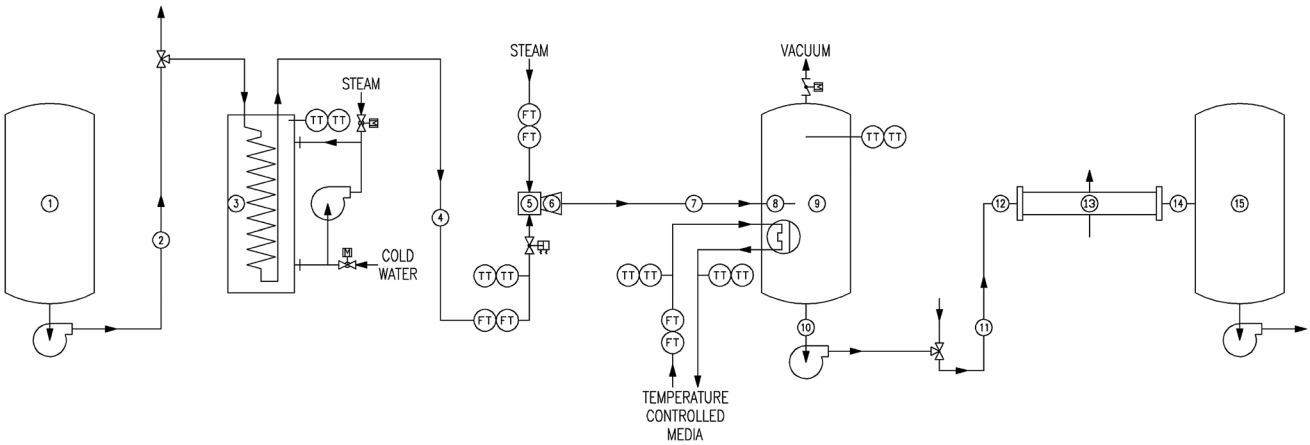

Figure 2. RITC thermal pasteurization of raw meat process. RITC, refrigerated instantaneous temperature cycling.

vapor removal. The treated proteins are pumped out of the expansion vessel and chilled to low temperature in a single-tube, one-pass, refrigerated heat exchanger (13) to bring the proteins to a stable storage temperature. The profile of the time versus temperature of the process is illustrated in Figure 3.

System control

The 2 key control attributes of the system are temperature control and mass differential control, both of which must be maintained within limits to ensure the effectiveness of the heat treatment and quality of the products.

Temperature control

A control volume and energy balance analysis determines the appropriate equations for controlling the temperature achieved in the steam injection step.

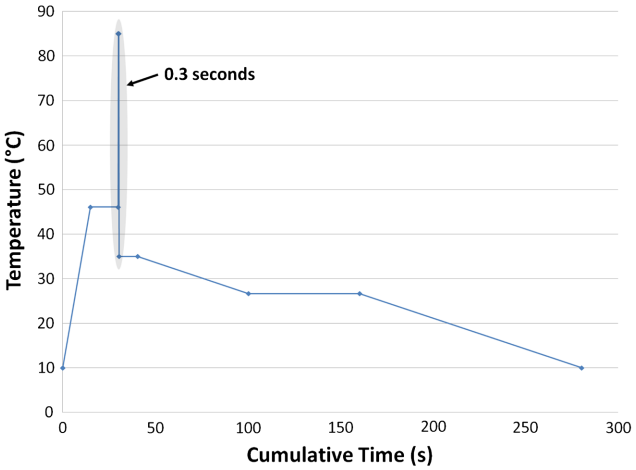

Figure 3. Time and temperature profile for RITC thermal pasteurization of raw meat process. RITC, refrigerated instantaneous temperature cycling.

The dashed line around the steam injector (5) in Figure 4 illustrates the control volume ("Control Volume 1" in Figure 4) for this energy balance. The terms and constants of the system are listed in Table 1. In a direct steam injection system, an inline temperature resistive device typically senses the temperature achieved in the injection step. However, this becomes impractical when processing meat due to the propensity to fouling on the device and unstable system operation. Instead, an energy balance around the injector (Figure 4) can be used to calculate the temperature of the product achieved in the injection step. The temperature of the product out of the injector is then given by

$$T_{op} = \frac{m_s h_{latent}}{m_{ip} C_{pp}} + T_{ip}.$$

Sensors are used to monitor the mass flow of steam, mass flow of product, and inlet temperature of the product. Each input is adjustable to maintain the outlet temperature of the injector within certain limits.

Moisture control

A control volume and energy balance analysis determines the appropriate equations for controlling the mass differential in the process. The dashed line around the steam injector (5) and expansion cooling vessel (9) in Figure 4 illustrates the control volume ("Control Volume 2" in Figure 4) for this energy balance. The terms and constants of the direct steam heating and expansion cooling system are listed in Table 1.

Moisture addition to the product during raw meat production processes is typically unacceptable. It is therefore important to have a simple and reliable method of tracking the mass change in order to ensure

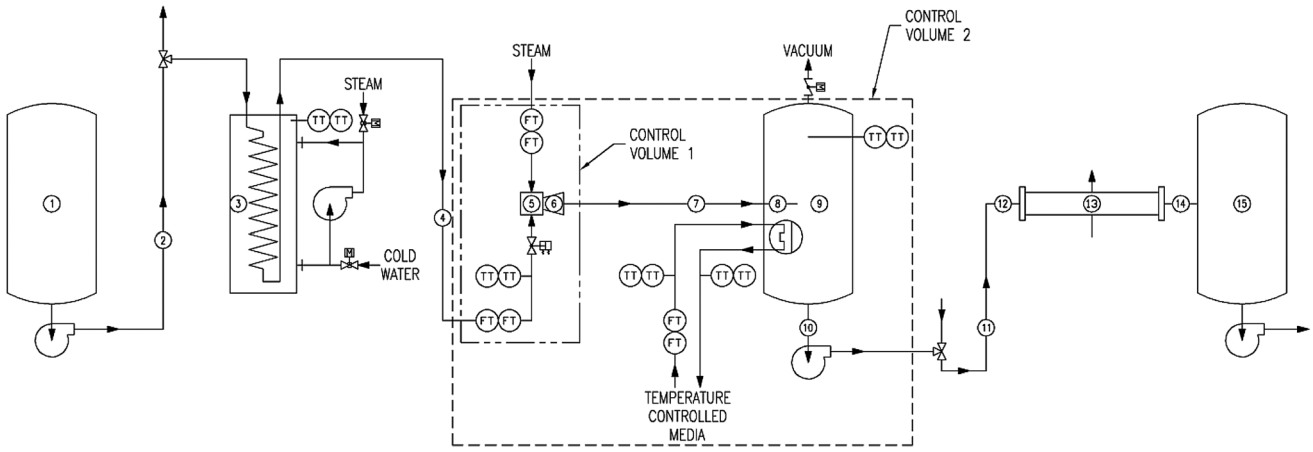

Figure 4. Control volumes for analysis of RITC thermal pasteurization of raw meat process. RITC, refrigerated instantaneous temperature cycling.

Table 1. Terms and constants of the RITC thermal pasteurization of raw meat process

Term	Description	Source
Mass flow terms		
m_{ip}	Mass flow of product into the control volume	Data
m_s	Mass flow of steam into the control volume	Data
m_m	Mass flow of temperature-controlled media	Data
Δm	Difference of mass flow of product entering and leaving the control volume	Variable
Temperatures		
T_{ip}	Temperature of product into the control volume	Data
T_{im}	Temperature of temperature-controlled media into the control volume	Data
T_{op}	Temperature of product out of the control volume[1]	Data
T_{om}	Temperature of temperature-controlled media out of the control volume	Data
Constants		
C_{pp}	Specific heat of product	Table
C_{pm}	Specific heat of temperature-controlled media	Table
h_f	Enthalpy of flashed water	Table
h_s	Enthalpy of steam input	Table
h_{latent}	Enthalpy of vaporization of water	Table

RITC, refrigerated instantaneous temperature cycling.

[1]For the condition where the temperature of product out of the control volume is the temperature of the product out of the expansion vessel, then it is equal to the temperature of the vapor in the expansion vessel.

that all of the moisture added in the steam application stage is removed in the vacuuming chilling stage. The change in mass in the RITC process is given by

$$\Delta m = \frac{m_{ip}C_{pp}(T_{op} - T_{ip}) + m_s(h_f - h_s) + m_m C_{pm}(T_{om} - T_{im}) + K}{(h_f - C_{pp}T_{op})}.$$

Sensor input is monitored by the control system, and adjustments are made to maintain the correct change in mass. In this case, the outlet temperature is set by closed-loop control to maintain the change in mass to be less than or rounded to zero.

Uncertainty

Temperature and moisture control of the system both rely on several sensor inputs that all inherently have a degree of uncertainty associated with the measurement. This impact of the uncertainty on the control formulas is taken into account by known methods, but care must be taken to identify all sources of uncertainty and set appropriate control limits. The temperature limit of the injector is maintained above the low limit by a 0.4°C margin of error. The change in mass of the product is maintained below limit by a margin of error of 0.1% of the product flow.

Full-scale control tests

Full-scale production tests were conducted on the RITC system to verify the control scheme capability within the 6-Sigma framework. The mass differential during the RITC process was evaluated against upper (+0.5%Δm) and lower (−0.5%Δm) control limits bounding the desired variability on this process parameter. Process capability index (Cpk) values were calculated to describe the average and spread of the data with respect to the specification limits (Ishikawa, 1989). The accepted minimum Cpk value for a process that is under control is 1.33 (2.0 for a 6-sigma process). The process was determined to be in control with Cpk values of 4.52, 3.33, 3.23, and 3.64 for the 4 periods.

Microorganism Reduction

The product temperature out of the injector must be maintained within certain limits to achieve the desired microorganism reductions. Inoculated challenge studies were performed to determine the appropriate temperature limits to achieve a suitable reduction for high and low levels of inoculated non-pathogenic *E. coli*.

Microorganism Reduction Analysis Methods

Thermal inactivation of the 5 non-pathogenic *E. coli* surrogates has been evaluated (Keeling et al., 2008). SGS Vanguard Sciences (North Sioux City, SD) provided laboratory support and analyzed all samples. Testing included multiple replications with different source materials and occurred on multiple processing dates. The studies were conducted utilizing a full-scale pilot system located in a segregated area of the site.

Preparation of inoculum

The 5 non-pathogenic *E. coli* surrogates allowed for use in processing establishment (BAA-1427, BAA-1428, BAA-1429, BAA-1430, and BAA-1431) (USDA FSIS, 2020) were grown individually in 9 mL trypticase soy broth (TSB) at 37°C for 18 h. The cultures were subsequently transferred individually to 9 mL TSB and incubated at 37°C for 18 h. The individual cultures were then combined into a single inoculum and vortexed for 15 s. The combined culture was used as an inoculum for 3.5 L of TSB. The 3.5 L culture was incubated at 37°C for 22–24 h and then cooled to <5°C for a minimum of 72 h. The cultures were grown independently, such that each 3.5 L container represented

a unique group of 9 mL TSB cultures and a unique combined culture.

Inoculation and sample collection

For the high population inoculation, 317.5 kg of light protein was inoculated with 3.5 L of the combined culture of the non-pathogenic *E. coli* surrogates. This resulted in an average initial population of log 6.3 colony-forming units per gram (cfu/g) (Table 2). For the low population inoculation, a similar procedure to the high population inoculation was followed; however, the average initial population was approximately log 3.8 cfu/g (Table 3).

The light protein and inoculum mixture was recirculated for 30 min, and then 5 replicate control samples were taken. The RITC treatment was applied using a set point to reach a temperature of 85°C out of the injector for the high inoculation level and 82.2°C for the low inoculation level, and 5 replicate treated samples were collected. All of the samples, control and treated, were collected in sterile Whirl-Pak® (Uline, Pleasant Prairie, WI) bags and immersed in an ice-water bath within 5 s of collection. The ice water bath consisted of a standard meat tote with 4.5 kg of ice and approximately 4.5 kg of water. The samples were thoroughly chilled and transported to the laboratory, where they were analyzed within 24 h of collection.

Laboratory methods

The samples were homogenized and serially diluted in Butterfield's Phosphate buffer (6.4% KH_2PO_4 suspended in deionized water, titrated to pH 7.2 and autoclaved) and enumerated using the method of Kang and Fung (2000) to recover thermally injured cells. The detection limit of the enumeration assay was log 0.5 cfu/g.

Table 2. Mean populations (log cfu/g) of non-pathogenic *E. coli* surrogates in inoculated light protein before and after RITC thermal pasteurization processing (high inoculation)

	Mean ± SEM log cfu/g		Processed Samples Below Detection Limit[1]
Replication	Control	Processed	
1	6.3 ± 0.19	0.5 ± 0.00	3/5
2	6.2 ± 0.13	0.5± 0.00	4/5
3	6.2 ± 0.17	0.5 ± 0.06	5/5
4	6.5 ± 0.11	0.5 ± 0.06	1/5
5	6.2 ± 0.17	0.5 ± 0.00	5/5
6	5.9 ± 0.21	0.5 ± 0.00	5/5
7	6.5 ± 0.05	0.5 ± 0.00	5/5
Average	6.3 ± 0.06[a]	0.5 ± 0.01[b]	28/35

RITC, refrigerated instantaneous temperature cycling.

[1]Number of processed samples below the detectable limit of the enumeration assay divided by total number of samples.

[a,b]Lowercase letters indicate significant difference at $P < 0.05$.

cfu, colony-forming units.

Table 3. Mean populations (log cfu/g) of non-pathogenic *E. coli* surrogates in inoculated light protein before and after RITC thermal pasteurization processing (low inoculation)

	Mean ± SEM log cfu/g		Processed Samples Below Detection Limit[1]
Replication	Control	Processed	
1	4.1 ± 0.19	0.5 ± 0.00	5/5
2	3.8 ± 0.13	0.5 ± 0.00	5/5
3	3.7 ± 0.17	0.5 ± 0.06	4/5
4	3.7 ± 0.11	0.5 ± 0.00	5/5
5	3.8 ± 0.17	0.5 ± 0.00	5/5
Average	3.8 ± 0.05[a]	0.5 ± 0.01[b]	24/25

RITC: refrigerated instantaneous temperature cycling.

[1]Number of processed samples below the detectable limit of the enumeration assay divided by total number of samples.

[a,b]Lowercase letters indicate significant difference at $P < 0.05$.

cfu, colony-forming units.

Statistical analysis

The high inoculation experiment was independently replicated 7 times, with 5 replicate control samples and 5 replicate treated samples for each independent replication. The low inoculation experiment was independently replicated 5 times, with 5 replicate control samples and 5 replicate treated samples analyzed for each independent replication. The microbial populations were converted to log colony forming units per gram, and descriptive statistics were computed using WINKS SDA software version 7.07 (TexaSoft; www.texasoft.com/). Additionally, independent samples *t* tests were used to compare the grand mean of control and treated samples. Unless otherwise stated, $P < 0.05$ indicates statistical significance. Samples with populations below the detectable limit of the enumeration assay (<log 0.5 cfu/g) were analyzed as log 0.5 cfu/g.

Microorganism Reduction Analysis Results

The average populations for each replication of the control and processed samples are presented in Table 2 (high inoculation) and Table 3 (low inoculation). Samples below the detection limit of the assay (<log 0.5 cfu/g) were entered as log 0.5 cfu/g for statistical

analysis. The log reductions for each replication are presented in Table 4 (high inoculation) and Table 5 (low inoculation). To analyze the variability of the results, the minimum, average, and maximum reductions were calculated as follows:

Table 4. Minimum, average, and maximum reduction (log cfu/g) in the populations of non-pathogenic *E. coli* surrogates in inoculated light protein after RITC thermal pasteurization processing (high inoculation) ($N = 35$ samples)

Replication	Minimum[1]	Average[2]	Maximum[3]
1	5.3	5.8	6.3
2	5.4	5.7	6.1
3	5.1	5.6	6.2
4	5.3	6.0	6.2
5	5.4	5.8	6.2
6	5.0	5.4	6.1
7	5.8	6.0	6.1
Average[4]	5.3 ± 0.09	5.8 ± 0.08	6.2 ± 0.03

RITC, refrigerated instantaneous temperature cycling.

[1]Minimum reduction = [lowest population observed in control samples] – [highest population observed in processed samples]; log colony-forming units per gram (cfu/g).

[2]Average reduction = [average of population observed in control samples] – [average of population observed in processed samples], log cfu/g.

[3]Maximum reduction = [highest population observed in control samples] – [lowest population observed in processed samples]; log cfu/g.

[4]Mean ± SEM; log cfu/g.

Table 5. Minimum, average, and maximum reduction (log cfu/g) in the populations of non-pathogenic *E. coli* surrogates in inoculated light protein after RITC thermal pasteurization processing (low inoculation) ($N = 25$ samples)

Replication	Minimum[1]	Average[2]	Maximum[3]
1	3.3	3.6	3.8
2	2.8	3.3	3.8
3	2.5	3.2	3.5
4	3.0	3.2	3.4
5	3.0	3.3	3.6
Average[4]	2.9 ± 0.13	3.3 ± 0.07	3.6 ± 0.07

RITC, refrigerated instantaneous temperature cycling.

[1]Minimum reduction = [lowest population observed in control samples] – [highest population observed in processed samples]; log colony-forming units per gram (cfu/g).

[2]Average reduction = [average of population observed in control samples] – [average of population observed in processed samples], log cfu/g.

[3]Maximum reduction = [highest population observed in control samples] – [lowest population observed in processed samples; log cfu/g.

[4]Mean ± SEM; log cfu/g.

- Minimum reduction = [lowest population observed in control samples] – [highest population observed in processed samples]; log cfu/g.
- Average reduction = [average of population observed in control samples] – [average of population observed in processed samples], log cfu/g.
- Maximum reduction = [highest population observed in control samples] – [lowest population observed in processed samples]; log cfu/g.

The average across the 7 replicates of the high inoculation experiments were 5.3, 5.8, and 6.2 for the minimum, average, and maximum log reductions. The minimum log reduction recorded was for replication 6, for which the minimum log reduction was 5.0. The average across the 5 replicates of the low inoculation experiments were 2.9, 3.3, and 3.6 for the minimum, average, and maximum log reductions. The minimum log reduction recorded was for replication 3, for which the minimum log reduction was 2.5. It is important to note, while interpreting the results of the low inoculation experiments, that 24 of the 25 treated samples that were analyzed had populations below the detectable limit of the assay (log 0.5 cfu/g) and that the minimum detection limit was entered for all of those 24 samples. The one treated sample that did have a population above the detection limit had a population of log 0.8 cfu/g. Because of this, the determining factor in calculating the log reduction was the initial population in the control samples.

Based on the average log reductions achieved at the different temperature set points of the high inoculation and low inoculation, maintaining the temperature achieved in the injection step above 85°C is sufficient to achieve a 5 log microorganism reduction and above 82.2°C is sufficient to achieve a 3 log microorganism reduction.

Protein Functionality Analysis Methods

Protein profile

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) analysis demonstrated the protein profile of soluble proteins in samples with and without the incorporation of the RITC process. Independent samples (~250 mL) were collected to represent the protein profile with ($n = 12$) and without ($n = 12$) the incorporation of the RITC process. A representative sample (400 µL) was solubilized with

10 mM sodium phosphate (pH 7.0) 2% SDS solubilization buffer (10 mL). Samples were clarified (1,500 × *g* for 15 min at 25°C). Protein concentration was determined using a modified Lowry (Lowry et al., 1951) method using premixed reagents (detergent compatible protein assay, Bio-Rad, Hercules, CA). Samples were diluted to 1.6 mg/mL using the solubilization buffer and then diluted to a final concentration of 1.0 mg/mL with 0.5 vol Wangs tracking dye (3 mM ethylenediamine tetraacetic acid, 3% SDS, 30% glycerol, 0.001% pyronin Y, 30 mM Tris-HCl [pH 8.0]) and 0.1 vol of 2-mercaptoethanol (Carlson et al., 2017). Samples were incubated at 50°C for 15 min. One-dimensional SDS-PAGE (12% polyacrylamide gels; Bio-Rad catalog 456-1043, Bio-Rad, Hercules, CA) on Bio-Rad Mini-Protean II gel units (Bio-Rad, Hercules, CA) was performed to examine the profile of proteins in all samples. Gels were stained using Coomassie Blue staining solution (0.1% Coomassie Blue [w/v], 10% acetic acid, 40% methanol) and de-stained in de-staining solution (10% acetic acid, 40% methanol). Image analysis was conducted to determine the abundance of distinct bands in each lane for each sample using ImageQuant TL 1D Version 8.1 (GE Healthcare Life Sciences, Piscataway, NJ). Bands were selected based on their predominance and ability to be isolated. There were 11 bands (9 independent and 2 combined) identified for comparison (Figure 5). The reported results are the means of 12 independent samples. Comparisons were made between bands grouped by lane percentages with and without RITC treatment. Statistics were performed using WINKS SDA software version 7.0.9 (Texasoft, Cedar Hill, TX). Unless otherwise stated, $P < 0.05$ indicates statistical significance.

Protein identification

SDS-PAGE was conducted as described, and the distinct gel slices (Figure 5) were removed from one sample of each treatment. Protein was digested with trypsin. After digestion, the solution was dried and reconstituted in 25 µL water containing 0.1% formic acid. The peptides were separated by liquid chromatography (Thermo Scientific EASY nLC-1200 coupled to a Thermo Scientific Nanospray FlexIon source [Thermo Fisher Scientific, Waltham, MA]) using a pulled glass emitter 75 µm × 20 cm (Agilent capillary, part #160-2644-5 [Agilent, Santa Clara, CA]), with the tip packed with Agilent SB-C18 Zorbax 5 µm packing material (part #820966-922) (Agilent) and the remaining portion of the emitter packed with nanoLCMS Solutions UChrom C18 3 µm packing material (part #80002) (nanoLCMS Solutions, Gold River, CA); peptides were analyzed by tandem mass spectrometry on a Thermo Scientific Q Exactive Hybrid Quadrupole-Obritrap Mass Spectrometer (Thermo Fisher Scientific). Raw data were analyzed using Thermo Scientific's Proteome Discoverer Software (version 2.4). The data were searched using Mascot and Sequest HT using SprotBos Taurus.

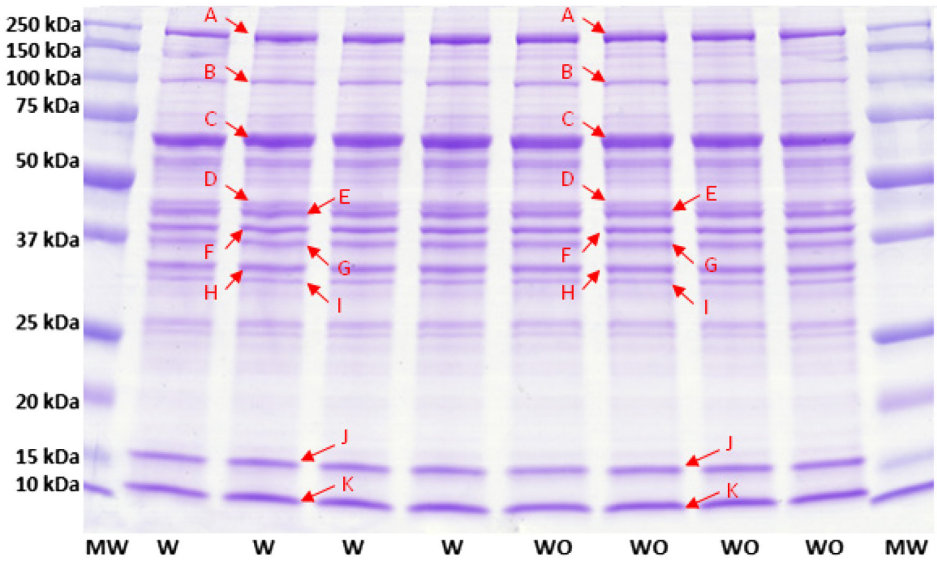

Figure 5. Representative SDS-PAGE gel of light protein. MW molecular weight marker; SDS-PAGE, sodium dodecyl sulfate -polyacrylamide gel electrophoresis; W with refrigerated instantaneous temperature cycling thermal pasteurization process; WO without refrigerated instantaneous temperature cycling thermal pasteurization process.

Protein Functionality Analysis Results

Protein profile—based on the primary bands in the SDS-PAGE analysis—was not altered by the RITC process ($P > 0.05$, Table 6). The predominant proteins in each band were the same both with (Table 7) and without the RITC process (Table 8). Together, these results suggest that the RITC process did not result in significant fragmentation, shearing, or coagulation of the meat proteins.

Color Preservation Analysis

To evaluate the impact of the process on the meat color, samples of finished ground beef were taken from the empirical™ production process both with and without incorporation of the RITC process on the light protein. There is natural variability of the concentration of the protein between fibrous and liquid phases during the production process which can affect finished ground beef color. To eliminate this variability from the analysis, the concentration of the protein in liquid phase was maintained at 40% of the finished ground beef during sample collection. A total of 6 sample batches each with and without the incorporation of the RITC process were collected on 3 different production days. Five samples were taken from each batch, and the beef was ground and prepared into a retail tray. Pictures of the sample tray preparation for batches of ground beef made with and without the incorporation of the RITC process are depicted in Figure 6. Colorimetric measurements were taken of all the ground beef samples using a Nix Pro Color Sensor (Nix Sensor, Ltd., Hamilton, Ontario, Canada). Three scans of each ground beef sample were obtained with illuminant A and 10° observer angle to collect L^* (lightness), a^* (redness), and b^* (yellowness) color measurements (Table 9). Statistics were performed using WINKS SDA software version 7.0.9. (Texasoft, Cedar Hill, TX.). Unless otherwise stated, $P < 0.05$ indicates statistical significance.

Table 6. Effect of treatment on abundance (percentage of protein bands in each sample) of primary protein bands separated by SDS-PAGE

	Mean ± SEM Abundance of Band[2]		
Band[1]	With RITC Thermal Pasteurization Process	Without RITC Thermal Pasteurization Process	$P^{3,4}$
A	6.10 ± 0.21	5.75 ± 0.27	0.32
B	3.06 ± 0.06	3.14 ± 0.11	0.54
C	8.95 ± 0.14	9.10 ± 0.12	0.41
D and E	6.92 ± 0.13	7.03 ± 0.09	0.50
F	4.00 ± 0.06	4.02 ± 0.06	0.78
G	4.54 ± 0.07	4.50 ± 0.07	0.68
H	4.43 ± 0.08	4.41 ± 0.07	0.83
I	2.25 ± 0.04	2.38 ± 0.07	0.12
J	5.26 ± 0.11	5.40 ± 0.07	0.31
K	6.23 ± 0.12	6.31 ± 0.08	0.56

[1]Band label corresponds to bands labeled in Figure 5.

[2]Abundance of selected bands based on percentage of total bands in lane ($N = 12$ samples)

[3]Independent t test, statistics were performed using WINKS SDA software (Texasoft, Cedar Hill, TX).

[4]$P < 0.05$ are significantly different.

RITC, refrigerated instantaneous temperature cycling.

SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Color Preservation Analysis Results

The L^* (lightness) and b^* (yellowness) values were unaffected by the RITC process (L^*: $P = 0.37$; b^*: $P = 0.06$; Table 9). There was some difference, however, in the a^* (redness) value ($P < 0.001$, Table 9). The a^* (redness) value was higher in the sample without the incorporation of the RITC process (22.45) than the sample with the incorporation of the RITC process (19.26). The colorimetric difference in the redness between the samples has not been well studied at this point as it is not visually apparent in the current finished products (Figure 6). Since there is no statistical difference in the protein profile—specifically in the band that included myoglobin (Band J, $P = 0.31$, Figure 5, Tables 6–8)—when incorporating the RITC process, it is likely that any difference in redness is primarily related to protein oxidation during prior processing and the lower oxygen pressure due to the vacuum condition during RITC. Meat color can be dramatically affected by the oxygen partial pressure and can be particularly problematic when packaging a bloomed product in vacuum or ultra-low-oxygen atmospheres (Mancini and Hunt, 2005). A part of the RITC process operates in a vacuum, which can produce very low-oxygen partial pressure in the product. Additionally, during the vacuum processing step, the liquid phase of the meat has typically been oxygenated or bloomed during prior processing. Preventing the blooming of the protein by isolating the proteins from oxygen during processing would likely mitigate any undesired effects of operating at low-oxygen partial pressure.

Table 7. Proteins identified in distinct bands (labeled A–K in Figure 5) from sample with RITC thermal pasteurization ($N = 12$ samples)

Band	Total PSMs	Accession	Description	Coverage, %	No. Peptides	No. PSMs	Percentage of Total PSMs	No. Unique Peptides	No. AAs	MW, kDa	Calc. pI	Score Mascot: Mascot
A	24,147	Q9BE40	Myosin-1	63	146	2,924	12.11	13	1,938	222.9	5.72	38,244
		F1MRC2	Myosin-2	63	149	2,912	12.06	0	194	223.2	5.8	36,089
		Q9BE41	Myosin-2	63	147	2,875	11.91	0	1,940	223.2	5.8	35,638
		F1MM07	Myosin-7	61	142	2,369	9.81	54	1,935	223.1	5.74	29,412
		F1N2G0	Myosin heavy chain 6	42	98	1,828	7.57	3	1,938	223.5	5.67	22,905
		E1BP87	Myosin heavy chain 4	45	104	1,924	7.97	1	1,935	222.4	5.73	20,476
		F1N775	Myosin heavy chain 8	42	95	1,842	7.63	1	1,937	222.6	5.77	22,423
B	8,232	B0JYK6	Alpha-1,4 glucan phosphorylase	67	59	1,565	19.01	48	842	97.2	7.14	11,996
		Q3ZC55	Alpha-actinin-2	68	52	603	7.33	33	894	103.7	5.45	8,041
		Q0III9	Alpha-actinin-3	68	51	453	5.50	37	901	103.1	5.45	6,213
		Q0VCY0	Sarcoplasmic/endoplasmic reticulum calcium ATPase 1	44	43	525	6.38	31	993	109.2	5.29	6,325
C	1,449	P02769	Serum albumin	83	64	5,330	36.89	7	607	69.2	6.18	43,076
		B0JYQ0	ALB protein	82	62	4,608	31.89	5	607	69.2	6.33	32,206
D	7,706	A0A452DI31	Beta-enolase	66	32	2,271	29.47	24	444	48.3	7.72	21,075
		P68138	Actin, alpha skeletal muscle	67	19	400	5.19	10	377	42	5.39	3,522
		F1MB08	Alpha-enolase	35	15	759	9.85	9	500	54.1	9.1	7,880
		P60712	Actin, cytoplasmic	41	14	312	4.05	4	375	41.7	5.48	2,562
E	10,131	P68138	Actin, alpha skeletal muscle	81	22	1,705	16.83	3	377	42	5.39	12,254
		Q3T0P6	Phosphoglycerate kinase 1	75	27	587	5.79	19	417	44.5	8.27	6,716
		Q3ZC07	Actin, alpha cardiac muscle 1	75	20	1,624	16.03	1	377	42	5.39	10,037
		A0A452DI31	Beta-enolase	54	23	539	5.32	20	444	48.3	7.72	7,082
		P60712	Actin, cytoplasmic 1	63	18	1,053	10.39	7	375	41.7	5.48	8,712
F	7,786	Q9XSC6	Creatine kinase M-type	69	29	3,230	41.48	28	381	43	7.12	32,122
		A6QLL8	Fructose-bisphosphate aldolase	78	24	372	4.78	21	364	39.4	8.19	4,106
		Q3T0P6	Phosphoglycerate kinase 1	65	22	195	2.50	22	417	44.5	8.27	2,165
G	7,828	A6QLL8	Fructose-bisphosphate aldolase	93	41	3,315	42.35	37	364	39.4	8.19	27,194
		Q9XSC6	Creatine kinase M-type	74	22	488	6.23	22	381	43	7.12	4,746
		A0A3S5ZPB0	Fructose-bisphosphate aldolase	20	8	424	5.42	4	510	55.6	8.47	2,684
H	7,417	P10096	Glyceraldehyde-3-phosphate dehydrogenase	92	32	2,857	38.52	29	333	35.8	8.35	19,209
		A0A3Q1M5R4	L-lactate dehydrogenase	51	22	580	7.82	17	341	37.4	6.25	4,272
		P19858	L-lactate dehydrogenase A chain	71	21	280	3.78	16	332	36.6	8	1,985
I	7,686	P19858	L-lactate dehydrogenase A chain	88	35	1,653	21.51	29	332	36.6	8	10,492
		P10096	Glyceraldehyde-3-phosphate dehydrogenase	67	18	557	7.25	16	333	35.8	8.35	4,313
		P04272	Annexin A2	66	26	650	8.46	26	339	38.6	7.31	8,342
		A0A3Q1M5R4	L-lactate dehydrogenase	50	21	589	7.66	16	341	37.4	6.25	4,090
J	3,441	A0A1K0FUF3	Myoglobin	99	28	1,925	55.94	25	154	17.1	7.46	14,383
K	8,709	D4QBB4	Globin A1	97	28	2,675	30.72	14	145	15.9	7.59	23,519
		P01966	Hemoglobin subunit alpha	100	19	2,022	23.22	18	142	15.2	8.44	14,011
		D4QBB3	Hemoglobin beta	97	17	1,756	20.16	3	145	16	6.89	16,721

AA, amino acid; ALB, albumin; ATPase, adenosine triphosphatase; Calc. pI, calculated isoelectric point; MW, molecular weight; PSM, peptide spectrum match; RITC, refrigerated instantaneous temperature cycling.

Table 8. Proteins identified in distinct bands (labeled A–K) from sample without RITC thermal pasteurization ($N = 12$ samples)

Band	Total PSMs	Accession	Description	Coverage, %	No. Peptides	No. PSMs	Percentage of Total PSMs	No. Unique Peptides	No. AAs	MW, kDa	Calc. pI	Score Mascot: Mascot
A	26,215	F1MRC2	Myosin-2	64	154	3,400	12.97	0	1,940	223.2	5.8	40,897
		Q9BE40	Myosin-1	64	149	3,282	12.52	15	1,938	222.9	5.72	42,806
		Q9BE41	Myosin-2	64	152	3,365	12.84	0	1,940	223.2	5.8	40,585
		F1MM07	Myosin-7	62	145	2,950	11.25	57	1,935	223.1	5.74	37,515
		E1BP87	Myosin heavy chain 4	47	108	2,378	9.07	3	1,935	222.4	5.73	25,836
		F1N775	Myosin heavy chain 8	43	101	2,111	8.05	2	1,937	222.6	5.77	25,420
		F1N2G0	Myosin heavy chain 6	43	100	2,081	7.94	3	1,938	223.5	5.67	27,846
B	10,197	B0JYK6	Alpha-1,4 glucan phosphorylase	67	59	2,044	20.05	49	842	97.2	7.14	14,736
		Q3ZC55	Alpha-actinin-2	65	51	738	7.24	33	894	103.7	5.45	9,215
		A0A3Q1M2X5	Alpha-actinin-3	62	49	548	5.37	36	888	101.7	5.44	6,711
		A5D7D1	Alpha-actinin-4	59	47	354	3.47	27	911	104.9	5.44	3,484
		Q0VCY0	Sarcoplasmic/endoplasmic reticulum calcium ATPase 1	38	40	556	5.45	28	993	109.2	5.29	6,479
C	16,683	P02769	Serum albumin	85	66	5,923	35.50	7	607	69.2	6.18	49,541
		B0JYQ0	ALB protein	83	64	5,139	30.80	5	607	69.2	6.33	38,408
D	8,856	A0A452DI31	Beta-enolase	70	33	2,027	22.89	23	444	48.3	7.72	18,387
		P68138	Actin, alpha skeletal muscle	67	19	628	7.09	2	377	42	5.39	4,769
		Q3ZC07	Actin, alpha cardiac muscle	66	18	606	6.84	1	377	42	5.39	4,337
		F1MB08	Alpha-enolase	39	15	586	6.62	8	500	54.1	9.1	6,439
		P60712	Actin, cytoplasmic 1	49	15	457	5.16	6	375	41.7	5.48	3,674
E	8,919	P68138	Actin, alpha skeletal muscle	70	21	1,476	16.55	2	377	42	5.39	13,377
		Q3T0P6	Phosphoglycerate kinase 1	79	29	694	7.78	21	417	44.5	8.27	8,593
		P62739	Actin, aortic smooth muscle	70	20	1,326	14.87	2	377	42	5.39	10,943
		P60712	Actin, cytoplasmic 1	66	19	946	10.61	9	375	41.7	5.48	9,697
		A0A452DI31	Beta-enolase	62	27	383	4.29	19	444	48.3	7.72	5,477
F	7,824	Q9XSC6	Creatine kinase M-type	72	33	2,412	30.83	32	381	43	7.12	22,683
		A6QLL8	Fructose-bisphosphate aldolase	80	25	351	4.49	23	364	39.4	8.19	4,240
		Q3T0P6	Phosphoglycerate kinase 1	70	25	274	3.50	25	417	44.5	8.27	3,357
G	7,566	A6QLL8	Fructose-bisphosphate aldolase	97	42	2,877	38.03	36	364	39.4	8.19	20,640
		Q9XSC6	Creatine kinase M-type	66	23	397	5.25	22	381	43	7.12	4,186
		A0A3S5ZPB0	Fructose-bisphosphate aldolase	21	10	421	5.56	4	510	55.6	8.47	2,078
H	8,425	P10096	Glyceraldehyde-3-phosphate dehydrogenase	90	31	2,759	32.75	28	333	35.8	8.35	19,955
		A0A3Q1M5R4	L-lactate dehydrogenase	62	25	780	9.26	19	341	37.4	6.25	6,433
		P19858	L-lactate dehydrogenase A chain	74	23	385	4.57	18	332	36.6	8	2,497
I	7,338	P19858	L-lactate dehydrogenase A chain	88	36	1,559	21.25	31	332	36.6	8	9,079
		P10096	Glyceraldehyde-3-phosphate dehydrogenase	62	18	542	7.39	15	333	35.8	8.35	4,565
		P04272	Annexin A2	66	26	531	7.24	26	339	38.6	7.31	6,413
		A0A3Q1M5R4	L-lactate dehydrogenase	48	20	563	7.67	16	341	37.4	6.25	3,411
J	3,618	A0A1K0FUF3	Myoglobin	92	22	2,205	60.95	22	154	17.1	7.46	17,595
K	10,084	D4QBB4	Globin A1	97	24	2,480	24.59	8	145	15.9	7.59	26,483
		P01966	Hemoglobin subunit alpha	100	18	2,077	20.60	17	142	15.2	8.44	14,123
		D4QBB3	Hemoglobin beta	97	16	1,692	16.78	3	145	16	6.89	20,671

AA, amino acid; ALB, albumin; ATPase, adenosine triphosphatase; Calc. pI, calculated isoelectric point; MW, molecular weight; PSM, peptide spectrum match; RITC, refrigerated instantaneous temperature cycling.

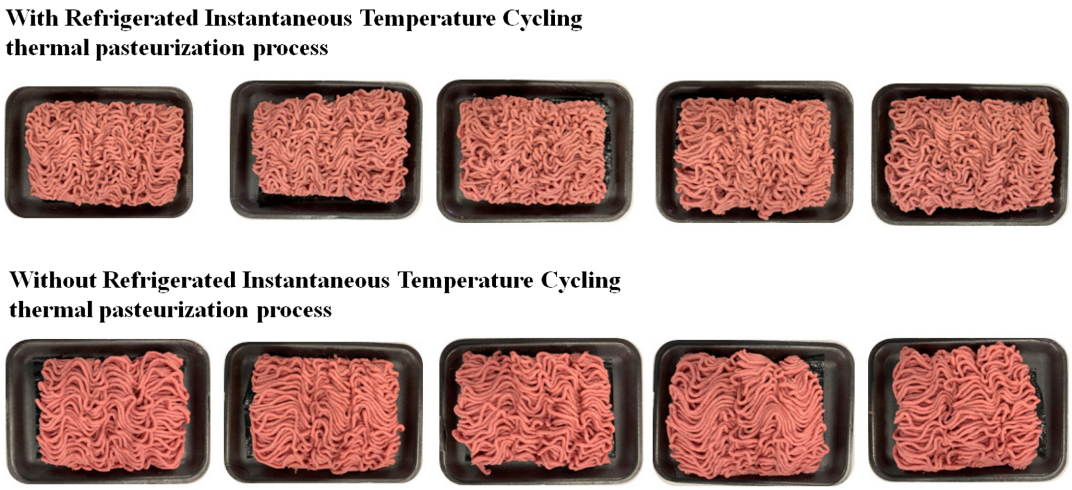

Figure 6. Photograph of tray preparation of batches of ground beef with and without the incorporation of the refrigerated instantaneous temperature cycling thermal pasteurization process.

Table 9. Color measurements of ground beef samples

	Mean ± SEM		
Color Trait	With RITC[1] Thermal Pasteurization Process	Without RITC[1] Thermal pasteurization Process	$P^{2,3}$
Lightness (*L)**	51.33 ± 0.78	50.25 ± 0.90	0.37
Redness (*a)**	19.26 ± 0.27	22.45 ± 0.34	<0.001
Yellowness (*b)**	14.92 ± 0.22	15.66 ± 0.31	0.06

[1]RITC, refrigerated instantaneous temperature cycling.

[2]Independent *t* test, statistics were performed using WINKS SDA software (Texasoft, Cedar Hill, TX).

[3]$P < 0.05$ are significantly different. $N = 30$.

Consumer Study Methods

Research design

An independent, third-party sensory research provider developed a protocol to measure the acceptance of ground beef patties produced exclusively using an empirical™ system (including the RITC pasteurization of the light protein) compared with retail-available ground beef patties known not to contain any empirical™ ground beef as a component. The 3 products tested were Great Value™ 100% Pure Beef Burgers produced at Establishment 18076 (Jensen Meat Company, Inc., San Diego, CA), Great American™–All Natural Beef Burgers produced at USDA Establishment 1899 (American Foods Group, Green Bay, WI), and 100% empirical™ ground beef patties produced at USDA Establishment 19872 (empirical Foods, Inc., South Sioux City, NE). All 3 products tested were one-quarter-pound and 80% lean, 20% fat blend patties cooked to 74°C on a flat grill. The consumer sample consisted of 105 adults who are primary grocery shoppers and primary food preparers, have eaten hamburgers at least once per month that were cooked at home with ground beef purchased from a grocery store (frozen or refrigerated), and are willing to try 100% ground beef patties cooked well done. Participants were served 3 test products in a fully rotated and balanced monadic-sequential presentation and were asked their like or dislike and rating of specific attributes (Table 11) of the products as well as to rank each product in order of preference. Liking data were analyzed with a repeated measures analysis of variance. Mean rank preferences were compared with Friedman and Wilcoxon signed-rank tests.

Consumer Study Results

Ground beef patties produced exclusively using an empirical™ system (including the RITC pasteurization of the light protein) was the second most preferred patty tested (Table 10) and ranked first or second in all liking attributes (Table 11).

Future Development

Other species

Due to vast differences in protein composition and viscosity of meat products, adapting the RITC technology to other species (including poultry and pork) will require customizations at every step of the process.

Table 10. Ground beef patty consumer preference ranking

Preference	Great Value™	empirical™	Great American™
***N* = 105**			
Most Preferred (%)	61	34	5
2nd Preferred (%)	31	50	19
3rd Preferred (%)	8	16	76
Mean Rank[1]	1.5[a]	1.8[b]	2.7[c]

[1]Mean rank refers to weighted mean preference ranking of the 3 patties.

[a–c]Lowercase letter superscripts indicate significant differences between mean ranks at $P < 0.001$.

Product information: Great Value™ (Jensen Meat Company, Inc., San Diego, CA); empirical™ (empirical Foods, Inc., South Sioux City, NE); Great American™ (American Foods Group, Green Bay, WI).

Table 11. Ground beef patty liking attributes

Mean Liking Attributes[1]	Great Value™	empirical™	Great American™
***N* = 105**			
Overall Liking	6.9[a]	6.2[b]	4.2[c]
Overall Appearance	6.8[a]	6.5[a]	5.2[b]
Color	6.9[a]	6.9[a]	5.8[b]
Aroma	6.7[a]	6.8[a]	6.3[b]
Overall Flavor	6.9[a]	6.2[b]	4.5[c]
Grilled Flavor	6.5[a]	6.1[a]	4.7[b]
Overall Texture	6.7[a]	5.7[b]	3.6[c]
Juiciness	6.9[a]	6.0[b]	3.9[c]

[1]Liking attributes were scored on a scale of 9 different selections ranging from 1 (Dislike Extremely) to 9 (Like Extremely).

[a–c]Lowercase letter superscripts indicate significant differences between mean ranks at $P < 0.001$.

Product information: Great Value™ (Jensen Meat Company, Inc., San Diego, CA); empirical™ (empirical Foods, Inc., South Sioux City, NE); Great American™ (American Foods Group, Green Bay, WI).

The process is complex, and inferring the design requirements for other types of meat products is impractical. Developing the appropriate customizations to the process for other meat products will require robust, trial-and-error experimentation and redesign. The experimentation and redesign processes will focus on several aspects:

- Optimizing steam injection penetration and mixing
- Identifying friction-reducing and flow-promoting geometries/surfaces to accommodate high-viscosity proteins
- Defining relevant microorganisms and suitable target reductions for different products
- Controlling heat flux at boundary layer surfaces
- Ensuring the effectiveness of vacuum expansion cooling and moisture removal

Pasteurization claims on consumer-ready packages

The RITC process is consistent with current regulatory pasteurization descriptions, namely, retaining a raw appearance after receiving a process that achieves a 5 log reduction of pathogens (USDA FSIS, 2017). However, this regulatory description has been limited to ready-to-eat meats, and claiming pasteurization on labels of consumer-ready raw meat products has not been well defined. It will be necessary to develop a consensus description of pasteurization for labels on consumer-ready raw meat products in order to ensure a benefit to public health. One common definition of pasteurization is "any process, treatment, or combination thereof, that is applied to food to reduce the most resistant microorganism(s) of public health significance to a level that is not likely to present a public health risk under normal conditions of distribution and storage" (USDA FSIS, 2004, p. 7). In the dairy industry, the definition of pasteurization includes heating every particle of milk to the appropriate time and temperature (FDA, 2017). Regulatory pasteurization descriptions also prohibit post-pasteurization pathogen exposure prior to final packaging (USDA FSIS, 2017). Considering these definitions, thermal pasteurization of meat as it relates to labels on consumer-ready packages could be defined as thermal exposure applied to every particle of meat at a temperature and a period of time to cause a 5-log or greater reduction of *Salmonella* and enteropathogenic *E. coli* in the final finished package while maintaining the raw appearance of the meat.

Shelf-stable

Non-refrigerated distribution and sale of milk and milk products (shelf-stable milk) were historically accomplished by in-container sterilization processes that produced significant chemical changes in the milk and milk products. Milk UHT was developed as an alternative method of shelf-stable milk that has substantially fewer chemical changes in the milk than traditional in-container sterilization (Burton, 1988).

Since RITC technology is based on similar principles as Milk UHT, it is reasonable to expect that the technology could be used to extend the refrigerated shelf life of raw meat products and even further developed to achieve non-refrigerated distribution and sale of raw meat products. The important first step in production of shelf-stable raw meats is the prevention of the presence of pathogenic organisms in meat during the time prior to consumption.

Daily tests for the presence of generic *E. coli* were performed on the raw meat proteins treated with the RITC process over a duration of 41 d of refrigerated storage. *E. coli* organisms were not detected in any of the tests, warranting further work toward shelf-stable raw meats.

Conclusions

Meat pasteurization using direct steam injection and expansion chilling under vacuum overcomes the limitations of current intervention technologies in use in the industry; namely, it significantly reduces the microbiological populations of the entire product with only minimal, imperceptible changes to the sensory characteristics of the raw meat. Development of the technology has largely been oriented toward a specific production process of ground beef in use by empirical™; however, there is great potential to use the technology to significantly reduce microbiological risk associated with any raw ground meat products as well as other beneficial impacts such as increased shelf life.

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