

Impact Of Staged Come-Up Time On Thermal, Nutritional, And Physicochemical Stability Of Retort-Processed Plant-Based Foods

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ABSTRACT

The starting heating phase, known as come-up time (CUT), determines the total thermal exposure at the core of retort-processed products, but its influence on microbial reduction and storage stability in dense plant-protein matrices remains poorly characterized. We investigated how gradual temperature increases during retort processing affects internal temperature profiles, cumulative thermal lethality (F_0), and overall product stability in plant-based sausages. Plant-based sausages were subjected to retort processing at 121.1 °C for 15 minutes under staged CUT conditions, with temperature at the cold spot and cumulative F_0 values supervised throughout. Analyses included a inoculated *Clostridium sporogenes* spores, a surrogate for *Clostridium botulinum* and relevant microbiological counts, physicochemical characterization, and sensory evaluation for storage stability. Staged CUT achieved the target $F_0 \geq 7$, whereas non-staged heating reached only 5.3, illustrating faster and more uniform lethality growing. Incremental heating maintained product structure, contributing to enhanced storage stability preserving nutritional and sensory attributes. Overall, implementing controlled, staged CUT enhances microbial safety and product quality in dense plant-protein systems, offering support the optimization of thermal processing of shelf-stable plant-based meat analogs.

KEYWORDS Come-up time, Thermal lethality (F_0), Retort processing, Microbial safety, Storage stability, Meat analogues

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INTRODUCTION

The rapid expansion of shelf-stable plant-based meat alternatives has intensified the need for thermal processing approaches that can ensure microbial safety while maintaining product quality during storage. Unlike conventional meat systems, plant-based sausages are composed of compact protein networks with restricted water mobility, which can limit heat transfer and create non-uniform temperature distribution during processing. These matrix-dependent constraints are increasingly recognized as critical factors influencing both microbial inactivation and quality outcomes in thermally processed foods^{1,2}.

Retort processing remains a key technology for producing commercially sterile, low-acid foods intended for ambient storage. In such systems, the total lethal effect is governed by the combined thermal exposure accumulated throughout heating, holding, and cooling stages, commonly expressed as the F_0 value. However, the pathway by which this lethality is achieved—particularly during the initial heating phase—can significantly influence process efficiency and product response^{3,4}.

The come-up time (CUT) phase plays a decisive role in establishing internal temperature profiles, especially at the slowest heating location within the product. Modifying this phase through staged or gradual temperature increments has the potential to reduce internal thermal gradients and promote more uniform

heat penetration. Such control over heating trajectories may enhance the distribution of lethal effects while minimizing localized overprocessing, an aspect that becomes particularly relevant in dense, structured plant-based matrices⁵.

Beyond microbial safety, thermal processing conditions strongly influence physicochemical and sensory

attributes that determine product acceptability and shelf life. Changes in protein structure, moisture retention, and matrix integrity during heating can affect texture and overall stability during storage. These quality-related transformations are closely linked to both the intensity and uniformity of thermal exposure, highlighting the need to balance lethality with structural preservation^{6,7}. Shelf stability of retort-processed foods therefore depends on an integrated outcome of sufficient microbial inactivation and the retention of desirable quality attributes over time. In plant-based systems, where composition and structure differ substantially from traditional products, understanding this balance becomes even more important for process optimization. In this context, the present study specifically investigates the influence of staged come-up time on heat penetration characteristics and the development of cumulative thermal lethality in retort-processed plant-based sausages. Particular emphasis is placed on how controlled heating profiles affect the uniformity of lethality delivery and subsequent product stability during storage. Microbial reduction, along with

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physicochemical and sensory attributes, was evaluated to establish the relationship between processing conditions, safety assurance, and quality retention in dense plant-based systems.

MATERIALS AND METHODS

Product Preparation

Plant-based sausages were formulated using tender jackfruit shreds (locally sourced from farms in Kochi, Kerala) and texturized faba bean protein (Beneo India Pvt Ltd, Haryana), combined with a standardized seasoning blend and a binder premix emulsion prepared with pea protein isolate (Synthite Industries Kochi, Kerala), native potato starch (Angel starch, Tamil Nadu), methylcellulose and kappa carrageenan (Merco Hydrocolloids; food grade) which were chosen for their proven ingredient functionality and was prepared by mixing premix, chilled water, and oil (0–4 °C) in a 1:1:3 ratio to form a cold emulsion.

The emulsion was then mixed with chopped jackfruit, rehydrated texturized vegetable protein (1:2; TVP:water) and seasonings. The mixture was stuffed into cellulose casings and steam-cooked at 100 °C for 20-25 minutes until the core temperature reached 75 °C,

followed by a dry heat at 80 °C for 2-3 minutes, Products were cooled in ice water, and casings were removed. Each sausage measured approximately 12.5 cm in length and weighed ~40 g. Five sausages (200 g total) were packed into multilayer retort pouches (from Pradeep laminate, Pune) composed of cast polypropylene (70 μm), biaxially oriented nylon (12 μm), aluminum foil (9 μm), and polyethylene (50 μm), arranged from inner to outer layer⁸. All ingredients and packaging materials were sourced from single batches to minimize variability

Retort Processing and Staged Come-Up Time Setting

Thermal processing was performed in a pilot-scale steam-and-water overpressure retort (KM Grand Pack Co., Ltd, Bangkok). Retort cycles targeted 121.1 °C with a 15-minute holding period at 15 psi above atmospheric pressure⁹. Two processing regimes were tested: (i) conventional non-staged heating and (ii) staged CUT, where temperature gradually increased in steps before holding at the target sterilization temperature. The retort cycle parameters are shown in Table 1. Each batch was processed in triplicate with uniform positioning in the retort chamber¹⁰.

Table 1. Retort Cycles processing parameters with staged come-up time (CUT)

Stage	Set Retort Temp(°C)	Time(min)
Come-Up Time (CUT)	1	40
	2	60
	3	80
	4	105
	5	110
	6	118
	7	120
	8	121.1
Cook Step / Holding	121.1	15
Cooling	110	5
	90	2
	60	2
	40	1
	30	2

Note: CUT: staged come-up time; values indicate stepwise temperature increase to 121.1 °C, followed by holding (sterilization) and controlled cooling.

Temperature Monitoring and Thermal Lethality Calculation

K-type thermocouples (KM Grand) were calibrated at 0, 50, and 100 °C against a reference thermometer. Thermocouples were inserted into the geometric center of a representative pouch, with additional probes monitoring the retort chamber temperature (Figure 1). Temperature readings were recorded every second throughout the cycle¹¹.

Cumulative thermal lethality (F_0) at the cold spot was calculated using:

$$F_0 = \int_0^t 10^{\frac{T(t)-T_{ref}}{z}} dt$$

where $T(t)$ is the measured product temperature, $T_{ref} = 121.1 °C$, and $z = 10 °C$, consistent with low-acid food processing standards. 18 F_0 values were monitored directly by the retort system^{12,13}.

Figure 1. Packed plant-based sausages on retort tray with probes



Storage Stability Analysis

Post-retort, sausages were stored under two conditions to simulate ambient and accelerated shelf-life scenarios. Ambient storage was conducted at $30^{\circ}\text{C} \pm 5^{\circ}\text{C}$ and $70\% \pm 5\%$ relative humidity, while accelerated storage was maintained at $45^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and $70\% \pm 5\%$ relative humidity to accelerate stress conditions. Non-retorted and non-spore inoculated samples were stored under frozen conditions ($-18 \pm 2^{\circ}\text{C}$) and evaluated as reference controls to monitor baseline quality attributes in the absence of thermal processing. Samples were analyzed at defined intervals and analysis were performed in triplicate to ensure reproducibility.

Microbiological Evaluation

Preparation of *Clostridium sporogenes* Spores

Clostridium sporogenes (ATCC 7955), a non-toxic surrogate for *Clostridium botulinum*, was revived in reinforced clostridial medium (RCM; HiMedia, India) under anaerobic conditions at 37°C for 24–48 h (Brown, 2000). Cultures were transferred into 50 mL sporulation medium (Duncan–Strong medium) in 250 mL anaerobic bottles and incubated at $30\text{--}37^{\circ}\text{C}$ for 5–7 days to promote spore formation (Duncan & Strong, 1968). Sporulation was confirmed by Schaeffer–Fulton spore staining¹⁴.

Spores were collected by centrifugation at $5000 \times g$ for 10 min at 4°C , washed three times with sterile 0.85% saline, and heat-shocked at 80°C for 10 min to remove remaining vegetative cells¹⁵. The spore suspension ($\sim 10^8$ spores/mL) was quantified by serial dilution and anaerobic plating¹⁶.

Inoculation

Spores were then aseptically mixed into the sausage formulation to achieve $10^5\text{--}10^6$ spores/g. Inoculated mixtures were held at 4°C for 30 min for equilibration before stuffing into casings. Non-inoculated samples were prepared in parallel for physicochemical and sensory analyses¹⁷.

Microbial analysis

Microbiological analyze were performed following standard AOAC methods, for aerobic plate count

(AOAC 990.12), yeast and mold (AOAC 997.02), coliforms (AOAC 991.14), detection of *Salmonella* spp. (AOAC 967.26), and Enterobacteriaceae (AOAC 2003.01) (AOAC International, 2019a). Samples were aseptically homogenized, serially diluted, and analyzed in duplicate. Outcomes were expressed as $\log \text{CFU g}^{-1}$. The survival and inactivation of the inoculated organism, *Clostridium sporogenes* (ATCC 7955), were assessed following ISO 15213-1:2017 (ISO, 2017). Samples were heat-shocked at 80°C for 10 min to eliminate residual vegetative cells, and serial dilutions were plated on Tryptose Sulfite Cycloserine (TSC) agar. Plates were incubated anaerobically at 37°C for 48 h, and surviving spores were enumerated. Log reductions were calculated relative to the initial inoculum to determine thermal lethality¹⁸.

Physicochemical Analysis

To correlate microbial inactivation with product characteristics, physicochemical analysis were measured at each sampling intervals. pH and salt determination, 5 g of sample was homogenized in 50 mL of deionized water. pH was measured using a calibrated portable pH meter following AOAC 981.12, and salt content was resolute using a digital salt meter in accordance with AOAC 971.27. Moisture content was determined using a gravimetric hot air oven method according to AOAC 950.46^{19,20}.

Proximate Composition

Proximate composition of plant-based sausages subjected to freezing and retort processing was determined according to standard AOAC methods. Moisture content was estimated by hot air oven drying (AOAC 950.46), crude protein by the Kjeldahl method using a conversion factor of 6.25 (AOAC 981.10), and crude fat by Soxhlet extraction (AOAC 960.39). Carbohydrate content was calculated by difference. Energy value (kcal/100 g) was computed using Atwater factors (4 kcal/g for protein and carbohydrates and 9 kcal/g for fat). Total dietary fiber was determined by the enzymatic–gravimetric method (AOAC 991.43) (Boye et al., 2012; AOAC, 2019; van Boekel et al., 2021). All determinations were carried out in triplicate, and results

were expressed on both fresh weight and dry weight basis²¹.

Sensory Evaluation

For sensory analysis non-inoculated samples were used. Evaluation was carried out by a trained panel (n = 30) and an untrained panel (n = 30) employing a 15-point intensity scale (1 = extremely low, 15 = extremely high) (ISO, 2012). Panelists evaluated samples in randomized order under controlled lighting and temperature conditions to minimize bias. Informed consent was obtained from all participants prior to evaluation²².

Statistical Analysis

All experimentation was conducted in triplicate. Data was analyzed using one-way analysis of variance (ANOVA), with significance established at p < 0.05. Results are demonstrated as mean ± standard deviation^{23,24}.

RESULTS AND DISCUSSION

Heat Penetration and Thermal Lethality

Under non-staged come-up retort conditions, heat perception at the product cold spot was significantly slower than the retort air temperature (Datta & Teixeira, 1988). The cold-spot temperature reached only 115.7 °C after 35 min, corresponding to an F₀ value of 5.4 min. In contrast, the staged CUT cycle facilitated progressive heat transfer, with the cold-spot temperature reaching 121.1 °C and achieving a cumulative F₀ value of 7.2 min under identical holding conditions.

As presented in Table 2 and illustrated in Figures. 2 staged CUT improved cumulative lethality despite equivalent target holding temperatures. The contribution of the come-up phase to overall lethality was evident in the staged cycle, where controlled temperature increments raised gradual heat distribution within the dense plant-protein matrix. This has likely reduced internal thermal gradients and enhanced conductive heat transfer, thereby supporting greater lethality accumulation prior to the holding phase indicating that come-up time contributes significantly to cumulative lethality and heat distribution efficiency in retort-processed foods, particularly in dense products.

Table 2. Staged CUT thermal processing profile and F₀ values during retort sterilization as monitored at retort and product cold spot

Stage	Set Temp °C	Retort Time in minutes	Probe Retort °C	1 temp	Probe Cold Spot temp °C		2	Probe Cold Spot temp °C		3
					Product Core Temp	F0 Value		Product Core Temp	F0 Value	
Come-Up Time (CUT)	1	40.0	2	41.00 ± 0.86 ^a	32.73 ± 1.27 ^a	0.00 ± 0.00 ^{a, b}	±	33.73 ± 1.33 ^a	0.00 ± 0.00 ^{a, b}	±
	2	60.0	6	63.24 ± 1.17 ^a	53.00 ± 4.58 ^a	0.00 ± 0.00 ^{a, b}	±	54.63 ± 5.59 ^a	0.00 ± 0.00 ^{a, b}	±
	3	80.0	4	82.04 ± 1.47 ^a	75.00 ± 2.65 ^a	0.57 ± 0.15 ^a	±	75.87 ± 3.35 ^a	0.60 ± 0.20 ^a	±
	4	105.0	8	105.23 ± 1.27 ^{a, b}	98.23 ± 0.74 ^a	1.10 ± 0.10 ^{a, b}	±	98.50 ± 0.20 ^a	1.30 ± 0.00 ^{a, b}	±
	5	110.0	6	110.00 ± 0.33 ^a	107.33 ± 0.76 ^a	2.13 ± 0.15 ^a	±	107.10 ± 0.6 ^a	2.37 ± 0.15 ^a	±
	6	118.0	4	118.57 ± 0.52 ^{a, b}	114.53 ± 0.25 ^a	2.83 ± 0.15 ^a	±	114.20 ± 0.52 ^a	2.73 ± 0.23 ^a	±
	7	120.0	4	120.23 ± 0.18 ^a	119.70 ± 0.20 ^a	5.27 ± 0.12 ^a	±	119.20 ± 0.70 ^a	5.60 ± 0.26 ^a	±
	8	121.1	1	120.87 ± 2.14 ^{a, b}	120.47 ± 0.3 ^a	6.23 ± 0.15 ^{a, b}	±	119.97 ± 0.47 ^a	6.77 ± 0.15 ^{a, b}	±
Cook Step Holding	121.1	15	121.20 ± 0.22 ^a	121.20 ± 0.22 ^a	7.2 ± 0.10 ^{a, b}	±	121.20 ± 0.22 ^a	7.3 ± 0.15 ^{a, b}	±	
	110.0	5	110.53 ± 0.77 ^{a, b}	111.24 ± 0.75 ^{a, b}	-	±	110.43 ± 0.67 ^{a, b}	-	±	
	90.0	2	90.45 ± 2.25 ^{a, a}	88.44 ± 2.25 ^{a, a}	-	±	86.52 ± 2.18 ^{a, a}	-	±	
	60.0	2	60.25 ± 2.55 ^a	63.00 ± 1.23 ^a	-	±	62.25 ± 1.55 ^a	-	±	
	40.0	1	40.23 ± 1.10 ^a	42.22 ± 2.1 ^a	-	±	40.73 ± 2.32 ^a	-	±	
Cooling	30.0	2	32.24 ± 2.28 ^{a, b}	30.24 ± 2.25 ^{a, b}	-	±	32.00 ± 2.25 ^{a, b}	-	±	

Note: *Values are expressed as mean ± SD (n = 3).

Means within the same row followed by different superscript letters differ significantly (p < 0.05) based on one-way ANOVA.

Temperature data were monitored online at the cold spot using calibrated retort probes. F₀ values were obtained directly from the retort system, where they were automatically calculated using pre-installed programming based on the recorded time–temperature history, at a reference temperature of 121.1 °C.

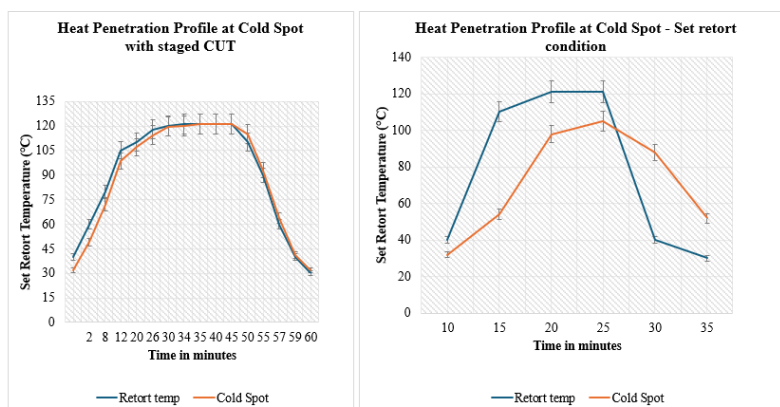
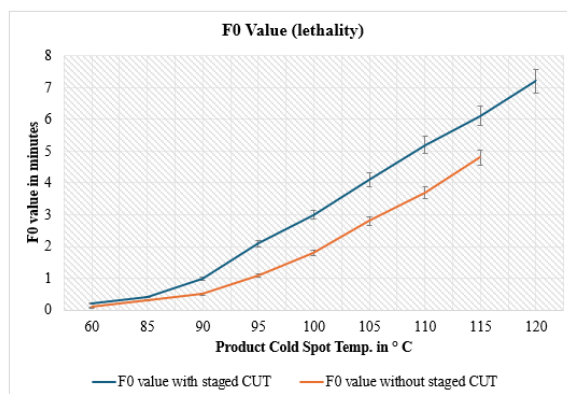


Figure 2. Heat Penetration profile and Thermal lethality (F₀ value)



Proximate Composition

Proximate composition of plant-based sausages prepared and thermally processed is presented in Table 3. Frozen samples had 59.7% moisture, which decreased slightly after retort processing, with staged CUT retaining marginally more water (59.6%) than non-staged CUT (59.4%). Protein, fat, carbohydrate, and dietary fiber on a fresh weight basis were slightly higher in non-staged CUT due to modest water redistribution, whereas dry weight values were comparable across treatments, indicating minimal nutrient loss of macro nutrients during thermal processing.

Table 3. Proximate composition of plant-based sausages prepared and thermally processed.

Parameter	As fresh weight basis			As dry weight basis		
	Frozen (-18 °C)	Retort, Staged CUT	Retort, Non-Staged CUT	Frozen (-18 °C)	Retort, Staged CUT	Retort, Non-Staged CUT
Moisture (%)	59.72 ± 0.1	59.60 ± 0.1	59.40 ± 0.1	-	-	-
Protein (%)	12.8 ± 0.3	12.9 ± 0.3	13.0 ± 0.3	32.0 ± 0.8	32.1 ± 0.8	32.2 ± 0.9
Fat (%)	8.5 ± 0.2	8.7 ± 0.2	8.8 ± 0.2	21.3 ± 0.6	21.6 ± 0.6	21.8 ± 0.7
Carbohydrate (%)	14.2 ± 0.5	14.4 ± 0.5	14.5 ± 0.5	35.5 ± 1.0	35.7 ± 1.0	35.8 ± 1.1

Total dietary fiber (%)	2.0 ± 0.1	2.1 ± 0.1	2.2 ± 0.1	5.0 ± 0.2	5.2 ± 0.2	5.3 ± 0.2
Energy (kcal/100 g)	168 ± 3	170 ± 2	171 ± 3	-	-	-

Note* Values are mean ± SD of three replicates. statistically significant differences ($p < 0.05$)

Storage Stability – Physico-chemical, Microbiological and Sensory attributes.

Storage stability data as in Table 4 were evaluated under ambient, accelerated, and frozen control conditions at 0th day, 1, 2, 3, and 4 months. Physicochemical parameters did not show significant differences ($p > 0.05$) during storage, showing that staged thermal exposure did not adversely affect product composition or chemical stability.

Differences in cumulative F₀ values were reflected in microbiological behavior during storage. Products

processed with staged CUT, which achieved higher lethality values, exhibited no detectable microbial growth under both ambient and accelerated conditions. In contrast, products subjected to lower lethality showed a gradual increase in total plate counts, although levels stayed within acceptable limits. While the study utilized indicator organisms rather than inoculated challenge organisms, the observed trend suggests enhanced microbiological stability associated with staged CUT processing.

Table 4. Storage Stability with physio-chemical and microbiological parameters

Parameter	Condition	0th Day	1st Month	2nd Month	3rd month	4th month
Physio-chemical analysis						
Moisture, % by wt.	Frozen (-18 C)		59.6 ± 0.1 ^a	59.6 ± 0.1 ^a	59.6 ± 0.1 ^a	59.6 ± 0.1 ^a
	Non - Amb.		59.6 ± 0.1 ^b	59.6 ± 0.1 ^b	59.6 ± 0.1 ^b	59.6 ± 0.1 ^b
	Staged CUT	59.73 ± 0.12 ^a	59.4 ± 0.1 ^b	59.4 ± 0.1 ^b	59.4 ± 0.1 ^b	59.4 ± 0.1 ^b
	Staged CUT	Amb.	59.5 ± 0.1 ^b	59.5 ± 0.1 ^b	59.5 ± 0.1 ^b	59.5 ± 0.1 ^b
		Acc.	59.3 ± 0.1 ^b	59.3 ± 0.1 ^b	59.3 ± 0.1 ^b	59.3 ± 0.1 ^b
	pH by 10% solution	Frozen (-18 C)		5.51 ± 0.02	5.50 ± 0.02	5.49 ± 0.03
Non - Amb.			5.50 ± 0.02	5.48 ± 0.03	5.46 ± 0.03	5.43 ± 0.04
Staged CUT		5.52 ± 0.02	5.48 ± 0.03	5.44 ± 0.04	5.39 ± 0.05	5.33 ± 0.06
Staged CUT		Amb.	5.51 ± 0.02	5.50 ± 0.02	5.49 ± 0.02	5.48 ± 0.03
		Acc.	5.50 ± 0.02	5.48 ± 0.03	5.46 ± 0.03	5.45 ± 0.03
Salt % (as NaCl)		Frozen (-18 C)		1.85 ± 0.02	1.84 ± 0.02	1.84 ± 0.02
	Non - Amb.		1.84 ± 0.02	1.83 ± 0.02	1.82 ± 0.03	1.80 ± 0.03
	Staged CUT	1.85 ± 0.02	1.83 ± 0.02	1.81 ± 0.03	1.79 ± 0.03	1.76 ± 0.04
	Staged CUT	Amb.	1.84 ± 0.02	1.84 ± 0.02	1.83 ± 0.02	1.82 ± 0.02
		Acc.	1.84 ± 0.02	1.83 ± 0.02	1.82 ± 0.03	1.81 ± 0.03
	Microbiological Analysis					
Total Count (cfu /g)	Frozen (-18 C)	3.38 ± 0.05	3.41 ± 0.03	3.46 ± 0.04	3.51 ± 0.05	3.65 ± 0.02
	Non - Am		<1.0	<1.48	<1.70	<1.90
	Staged CUT	<1.0	<1.70	<1.90	<2.30	<2.70
	Staged CUT	Am	<1.0			
		b.				
		Acc.				
Yeast & Mould (cfu/g)	Frozen (-18 C)	2.26 ± 0.04	2.32 ± 0.05	2.40 ± 0.03	2.46 ± 0.04	2.51 ± 0.04
	Non - Am	<1.0	<1.0	<1.0	<1.30	<1.70
	Staged CUT	<1.0	<1.0	<1.70	<2.00	<2.30
		b.				
		Acc.				

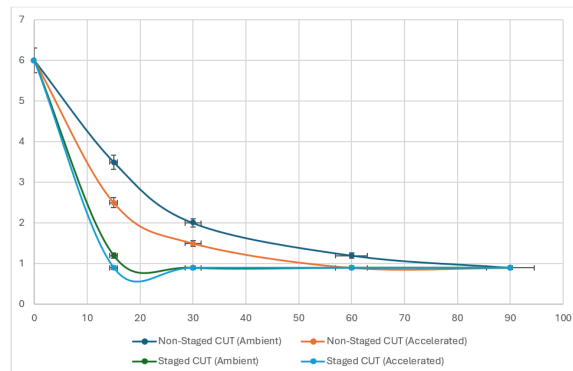
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	Staged CUT	Am b.	<1.0
		Acc.	
	Frozen (-18 C)		
			<10
Coliforms (cfu/g)	Non Staged CUT	Am b.	
		Acc.	
	Staged CUT	Am b.	
		Acc.	
	Frozen (-18 C)		
			<10
Enterobacteriaceae (cfu/g)	Non Staged CUT	Am b.	
		Acc.	
	Staged CUT	Am b.	
		Acc.	
	Frozen (-18 C)		
			Absent
Salmonella (25g)	Non Staged CUT	Am b.	
		Acc.	
	Staged CUT	Am b.	
		Acc.	
Note: *Values represent mean SD (n = 3). Counts below detection limit are expressed as <1.0 log cfu/g (TPC and Y&M) and <10 cfu/g for coliforms and Enterobacteriaceae. Ambient storage temperature = 30°C ± 5°C and 70% ± 5% RH; Accelerated storage temperature = 45°C ± 2°C and 70% ± 5% RH.			

The inoculated *Clostridium sporogenes* spores in staged CUT products, spore counts declined rapidly to near or below detection limits (<1 log CFU/g) by day 15 and remained undetectable throughout storage, reflecting the higher cumulative F₀ achieved (Figure 3). In contrast non-staged CUT products exhibited slower spore inactivation, with residual spores detected up to 1.5 log CFU/g at day 60 under ambient storage, and slightly

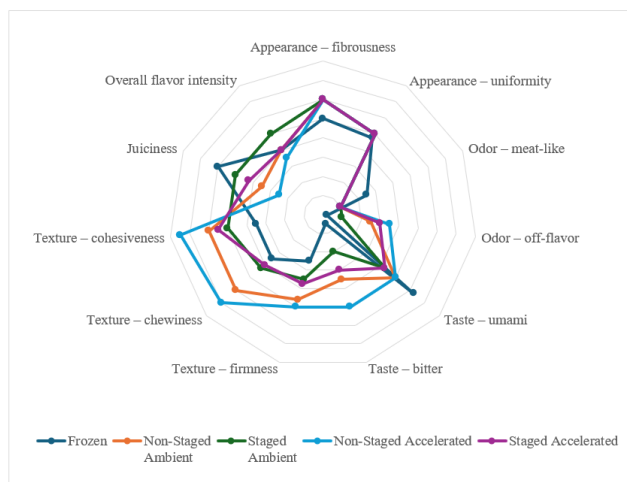
lower counts under accelerated storage due to enhanced thermal stress. These observations indicate that staged CUT processing not only ensures rapid inactivation of highly heat-resistant spores but also enhances microbiological stability during extended storage, supporting the safety and shelf-life of retort-processed plant-based meat analogs.

Figure 3. Inoculated Spore of *Clostridium sporogenes* survival over time



Sensory evaluation as in figure . 4 revealed noticeable textural differences in textural attributes between process conditions. The non-staged cycle, characterized by quickly temperature escalation, resulted in a firmer and slightly rubbery texture, likely due to abrupt protein network tightening under sudden thermal stress.

Figure 4. Sensory Score of analyzed products



In contrast, the staged CUT profile provided gradual thermal disclosure, minimizing structural shock and maintaining a more desirable bite and mouth feel (Damodaran & Parkin, 2017; Tornberg, 2005). Overall sensory scores of the retorted products maintained acceptable quality compared to the frozen reference, particularly in terms of texture and mouth feel²⁵.

The influence of staged come-up time on lethality development should be interpreted in the context of the constructional characteristics of plant-protein based formed products. Gradual temperature staging during CUT may reduce thermal gradients within the product, thereby promoting more uniform heat distribution and cumulative lethality.

This study utilized a single plant-protein sausage formulation and packaging configuration, and heat transfer behavior may vary with changes in composition, geometry, or fill weight. Furthermore, although accelerated and ambient storage investigation provided preliminary insight into stability, extended real-time storage evaluation would strengthen shelf-life prediction. Despite these limitations, the findings offer controlled comparative evidence demonstrating the impact of staged CUT on thermal lethality development and microbial stability in dense plant-protein systems, highlighting its potential to optimize safety and quality in retort-processed meat analogs.

CONCLUSION

Within the conditions examined, implementing a staged come-up time (CUT) significantly enhanced cumulative thermal lethality by effectively inactivating the inoculated spores, including highly heat-resistant *Clostridium sporogenes*. The staged profile resulted in higher F_0 values and enhanced microbiological stability during storage without adversely affecting physicochemical or sensory attributes. These

conclusions underscores the significance of optimizing come-up time, in addition to holding temperature and duration, in the design of retort processing strategies for dense plant-based products intended for ambient storage.

Overall, staged CUT represents a practical and effective approach to enhance safety and shelf stability while maintaining product quality in retort-processed plant-based meat analogs

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