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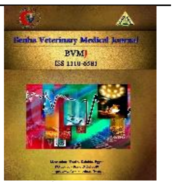

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Original Paper

The effect of a 3D-modeled pyramidal packing shape on the growth of foodborne pathogens inoculated in ribeye-lion at dynamic room temperatures.

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ABSTRACT

Food packaging serves several important tasks, including preserving and controlling foodborne pathogens. One component that contributes significantly to these functions is the design of the food packaging itself. Bacterial retardation of natural meat biota was demonstrated using pyramidal packing patterns designed by the Great Pyramid's dimension ratios at constant chilling temperatures. The current study, based on the Giza-pyramid dimension ratio, used dynamic room temperature to evaluate the consequences of pyramidal packaging on minced ribeye meat experimentally contaminated with foodborne pathogens: *Listeria monocytogenes* and *Salmonella Typhimurium*, as well as naturally occurred *Escherichia coli*. The pyramidal growth curves were compared to cuboidal and cylindrical 3D-printed containers. The APC count increased marginally in all three forms with time, but declined considerably in pyramidal stored beef at 8, 14, 18, and 24 hours and circular stored beef at 32, 48 hours ($P < 0.05$). The result revealed that the packaging shape had no significant effect on the growth curves of both *S. Typhimurium* and *E. coli*. The pyramidal packaging negatively impacted the *L. monocytogens* growth curve, which was more obvious at the end of the storage period than other packaging shapes. The current study was conducted at dynamic room temperature, which may counteract pyramidal effect noticed when storage occurred at constant temperature. Also, the delayed or non-significant impact of the pyramidal package observed here on growth curves of the inoculated pathogen, particularly the Gram-negative one, could be attributable to other factors such as type of packaging material magnetic field stability. Conclusively, the hierarchical shape had optimal and efficient effects on the control of microbial growth introduced in minced meat therefore, it essential to consider incorporate the findings of these studies when developing packaging options that efficiently preserve food.

1. INTRODUCTION

Foodborne pathogens are a significant public health concern due to their association with foodborne diseases, which can lead to severe illness and even death. The most reported foodborne pathogenic bacteria, such as *E. coli*, *L. monocytogenes*, and *Salmonella*, are responsible for most foodborne outbreaks in the USA (Zhang et al., 2021). These pathogens are estimated to cause millions of illnesses, thousands of hospitalizations, and hundreds of deaths annually (Cauteren et al., 2017). *Salmonella* has been identified as a particularly high contributing pathogen to foodborne diseases (Akil, 2021). In addition, foodborne pathogens such as *Salmonella*, *L. monocytogenes*, *Staphylococcus aureus*, and *E. coli* are known to be major causes of foodborne outbreaks (Gyawali and Ibrahim, 2012; Yasmin et al., 2016).

The control of foodborne pathogens is crucial in ensuring food safety. The shape of food packaging plays a crucial role in preserving food and extending its shelf-life. Various studies have highlighted the significance of packaging materials in maintaining food quality, safety, and shelf-life (Peters-Texeira & Badrie, 2005). Furthermore, other research on packaging focused on the challenges encountered in extending the shelf-life of perishable food items (Díaz-Montes & Castro-Muñoz, 2021) and the interaction between food and packaging to determine the packaging's function in fulfilling the daily nutrient requirement demand and its consequences on the food's integrity and quality (Garba, 2023).

Combining preservation and packing shape offers a particularly interesting and maybe more creative approach. The discovery of the pyramid effect dates to 1930, when Antoine Bovis explored the mummification of organic materials (Abdelsamie et al., 2014). The Pyramid shape technique combines preservation and packaging in one process and is poised to be a useful tool for the food industry (Abdelsamie et al., 2012). Pyramid shapes have shown effectiveness in diverse fields, from milk packaging and preservation (Kumar et al., 2005; Gopinath et al., 2008); an Indian research team yielded similar success with an experiment that tested the effect of the pyramid shape on the growth and emergence of fenugreek seeds (Kumar & Nagendra, 2011). The Pyramid-shaped compost bucket can limit microorganisms' growth and reduce the foul smell (Klosko, 2023).

Many factors could be responsible for the pyramidal-structure microbial retardation effect, with the dimensions and structure ratio being the most important. However, the surrounding environment, including temperature, moisture, and many other factors, could also contribute to or hinder this effect. Based on these observations, the current study investigates the impact of commercially designed

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hierarchical packaging, based on a cosmic proportion of the Giza Great Pyramids, on the microbial growth curves of food-borne pathogens, including *Salmonellae* and *L. monocytogenes*, in minced meat maintained at dynamic room temperature. This study aims to improve our knowledge of the prospective preservation characteristics of pyramidal packaging on meat. It may aid in the creation of novel preservation strategies or the refinement of already effective ones.

2. MATERIAL AND METHODS

Ethical approval

The protocols used in this study were approved by the Research Ethics Committee at the Faculty of Veterinary Medicine, Benha University with ethical approval *number (BUFVM15-05-2023),*

2.1. Shapes Design and Manufacturing:

First, three groups of different geometrical package containers were designed (square pyramids, rectangular cuboids, and cylindrical containers) using CAD software (FUSION 360, Autodesk, USA) with specific dimensions listed in Table 1. The three shapes were designed to be almost equal in volume to accommodate the same size as the meat samples. Secondly, fused deposition modeling (FDM) 3D printing technology was used to manufacture four packages made of white-colored polylactic material (PLA) from each geometry using Ender -3 V2 3D printer (Creality 3D Technology, Shenzhen, China).

Table 1 3D printed container dimensions and configuration

Shape	Number	Base (cm)	Slant height (cm)	Vertical height (cm)	Volume (cm^3)
Square pyramids	4	15.7 Length (cm)	14.9 Width (cm)	10 Height (cm)	828.97
Rectangular cuboids	4	20 Radius (cm)	20	10 Height (cm)	4,000
Cylinders	4	10		10	3141.59

2.2. Bacterial strains and inoculum preparation

Salmonella Typhimurium (ATCC 14028) and *L. monocytogenes* (ATTC 35152), all pathogenic bacteria, were procured from the Animal Health Research Institute (AHRI) in Dokki, Egypt. The growth of *Salmonella Typhimurium* was observed on Hektoen enteric agar (Hi Media, India), which was incubated at 37°C for 24 hours. For *L. monocytogenes*, Palcam Listeria selective agar base (Oxoid, UK) was supplemented with Palcam Listeria selective supplement (Oxoid, UK), which was incubated at 35°C for 48 hours. Separately, three to five single colonies of each pathogen were transferred to 1 ml of tryptic soy broth (TSB) (BioLife, USA) in a test tube. The tubes were then incubated at 37°C for 18 hours. To obtain 3 $\log_{10}$ CFU/gm of 1×10^3 targeted pathogens, *L. monocytogenes*, and *Salmonella Typhimurium* in minced meat, tenfold serial dilution was conducted to obtain 10^5 or 5 $\log_{10}$ CFU/mL dilution.

2.3. Meat Samples Inoculation and Preparation

In this experiment, minced beef rib eye samples (2kg) were procured at a nearby meat market in Qalyubia governorates, Egypt, 24 hours postmortem. Subsequently, the samples were promptly transferred cold to the Food Hygiene and Control Laboratory, Food Hygiene and Control Department, Faculty of Veterinary Medicine, Benha University. The minced meats were inoculated with a bacterial strain cocktail (mixed bacterial strains) with a rate of 2ml /100gm minced meat and left for 20 minutes to facilitate bacterial attachment. The samples were then separated into three equal batches, sealed in sterile polyethylene bags, ten grams each, and stored individually in three distinct packaging form models at dynamic temperatures. The room temperature of the entire experiment period was recorded using a thermo data logger (Testo, Germany). The average temperatures obtained from the record were then examined at 0, 2, 4, 8, 10, 14, 18, 24, 32, and 48 hrs.

2.4. Microbial Analysis

Ten grams of meat sample was homogenized with peptone water diluted, and aerobic plate count (APC) was performed according to ISO 4833-1 (2013). *Salmonella Typhimurium* (ISO, 2017a) and *L. monocytogens* (ISO, 2017b) were isolated. *E. coli* was detected naturally and estimated in sample packages (ISO, 2018).

2.5. Bacterial growth prediction modeling

Growth at a dynamic temperature was predicted using a new logistic predictive model primary equation, according to Sabike et al. (2014). Briefly, the average bacterial counts for the three trials of the dynamic temperature studies (Fig. 1) were determined and analyzed. The counts of the samples during storage were then examined using the logistic model, and numerical data of bacterial counts were analyzed using a computer program to fit the growth model. The model's estimated microbial populations (CFU/g) were then logarithmically transformed to create a growth curve.

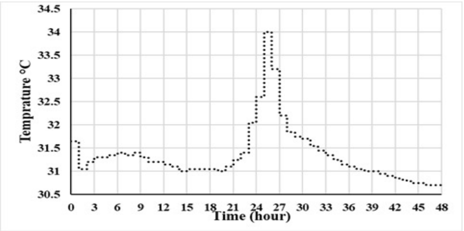

Figure (1): Dynamic temperature patterns recorded through 48 hr.

2.6. Statistical Analysis

The statistical analysis program (SAS version 9.1, SAS Institute, Inc., 2003) was used to analyze the data using ANOVA variance analysis using the general linear Model's approach. At $p < 0.05$, the least significant differences were utilized to split means. As fixed variables, the Model incorporated storage time, treatment, and their interaction. Duncan test ($P < 0.05$) was used to examine differences between effects (Mahgoub et al., 2017).

3. RESULTS

The effect of packaging shape on microbial proliferation in the form of Aerobic Plate count (APC) and the growth of *Listeria monocytogenes*, *E. coli*, and *Salmonella Typhimurium* in minced meat were investigated in this study. Table 2 illustrates the effect of various packaging model shapes (circular, pyramidal, and square) on the number of aerobic plates (APC) in stored beef at dynamic room temperature. There was a substantial effect of time, type of packaging, and their interactions on the APC of room-temperature-stored beef. The APC count exhibited a marginal rise in the three shapes time-dependent manner, but it decreased significantly in pyramidal stored beef at 8, 14, 18, and 24 hours and circular stored beef at 32,48 hours ($P < 0.05$).

The effect of the three model shapes on the count of *Salmonella Typhimurium (S.Typhimurium)* inoculated on beef was depicted in Table 3. Only storage time significantly affected the *S. Typhimurium* count among the three forms. While all three shapes show no discernible increase in *S. Typhimurium* count during storage, the square shape shows the most dramatic drop at 48 hours ($P < 0.05$).

In addition, three different packing styles affected the count of *Listeria monocytogenes (L.monocytogenes)* artificially inoculated at room-temperature beef ($P < 0.05$), as shown in Table 4. Packing shape, duration, and their interactions substantially impact the *L.monocytogenes* count. In circular-shaped meat, the number of *L. monocytogenes* dropped noticeably after 14 hrs and rose again after 48 hours ($P < 0.05$). Nevertheless, after 32 hours of incubation, the count of *L. monocytogenes* begins to diminish in a pyramidal pattern ($P < 0.05$).In square-shaped models, the count of *L. monocytogenes* only decreased at 18 hrs of incubation ($P < 0.05$).

The impact of three distinct model shapes on the naturally present *E. coli* count in beef meat held at dynamic room temperature is illustrated in Table 5. There was a strong correlation between the amount of *E. coli* in preserved beef and the duration of preservation. Following 14 and 18 hrs of dynamic room temperature incubation, the *E. coli* count in pyramidal-stored meat began to decline substantially ($P < 0.05$). While circular-stored meat dropped significantly at 14 and 48 hr of dynamic room temperature incubation, and square-stored beef showed a considerable decline at18, 24, and 48 ($P < 0.05$).

The measured growth curves for all bacterial indices were then compared to the predicted growth curve using a new logistic predictive model. The mean standard error (MSE) average between the estimated and the predicted growth curve of APC was 0.120, 0.091, and 0.058 for the Pyramidal, cylindrical, and cuboidal packages, respectively (Fig. 2A-C). However, the MSE average between the estimated and the predicted growth curve of Salmonella Typhimurium 0.198, 0.149, and 0.202 was for the Pyramidal, cylindrical, and cuboidal packages, respectively (Fig. 2D-F). Listeria monocytogens' MSE average between the estimated and the predicted growth curve was 0.263, 0.296, and 0.282 for the Pyramidal, cylindrical, and cuboidal packages, respectively (Fig. 2G-I). E. coli MSE average between the estimated and the predicted growth curve was 0.139, 0.163, and 0.260 for the Pyramidal, cylindrical, and cuboidal packages, respectively (Fig. 2J-L). Here, the greater deviation in measured curves from the predicted one indicated that packaging shape influenced the estimated growth curve; the larger the main standard deviation, the greater the difference between the estimated growth curve from the predicted one.

Table 2 Aerobic Plate count (APC, Log10/g.) in beef minced meat influenced by different shape packages.

Time (hours)	Packaging shapes			SEM	Packaging type (P)	Time (T)	P*T
	Pyramidal	Circular	Square				
Zero	4.14^{E}	4.14^{E}	4.14^{H}	0.018	0.00	000	000
2	4.47^{E}	4.24^{E}	4.25^{G}				
4	4.25^{E}	4.85^{D}	4.68^{F}				
8	6.50^{Db}	6.60^{aC}	6.56^{abE}				
10	6.52^{D}	6.46^{C}	6.52^{E}				
14	7.51^{bC}	7.56^{bB}	7.64^{aD}				
18	7.46^{cC}	7.55^{bB}	7.68^{aD}				
24	8.29^{bB}	8.30^{bA}	8.42^{aC}				
32	8.63^{aB}	7.82^{bB}	9.00^{aB}				
48	9.41^{aA}	8.55^{bA}	9.37^{aA}				

Table 3 Growth of *Salmonella Typhimurium* (Log10/g.) in beef minced meat influenced by different shape packages.

Time (hours)	Packaging shapes			SEM	Packaging type (P)	Time (T)	P*T
	Pyramidal	Circular	Square				
Zero	2.70^{D}	2.70^{D}	2.70^{E}	0.055	0.96	000	0.94
2	2.70^{D}	2.70^{D}	2.70^{E}				
4	2.94^{D}	2.94^{D}	2.85^{E}				
8	4.43^{C}	4.10^{C}	4.57^{D}				
10	4.95^{C}	5.09^{B}	5.07^{C}				
14	5.82^{ABC}	5.61^{B}	5.35^{C}				
18	5.07^{BC}	5.32^{B}	5.50^{C}				
24	5.53^{BC}	5.55^{B}	5.38^{C}				
32	6.44^{AB}	7.03^{A}	7.26^{A}				
48	7.16^{aA}	7.01^{aA}	6.73^{bB}				

Table 4 Growth of *Listeria monocytogenes*(Log10/g.) in beef minced meat influenced by different shape packages.

Time (hours)	Packaging shapes			SEM	Packaging type (P)	Time (T)	P*T
	Pyramidal	Circular	Square				
Zero	2.44^{F}	2.44^{E}	2.44^{E}	0.036	000	000	000
2	3.45^{EF}	3.15^{D}	2.94^{E}				
4	3.57^{DEF}	3.22^{D}	2.50^{E}				
8	3.42^{abF}	3.29^{bCD}	3.85^{aD}				
10	3.96^{DE}	3.90^{C}	4.42^{CD}				
14	4.67^{aC}	3.72^{bCD}	4.99^{aC}				
18	4.04^{aD}	3.62^{bCD}	3.74^{bD}				
24	5.57^{A}	4.78^{B}	5.02^{C}				
32	4.85^{bBC}	4.85^{bB}	5.83^{aB}				
48	5.20^{bAB}	7.07^{aA}	7.49^{aA}				

Table 5 Growth of *E. coli* (Log10/g.) in beef minced meat influenced by different shape packages.

Time (hours)	Packaging shapes			SEM	Packaging type (P)	Time (T)	P*T
	Pyramidal	Circular	Square				
Zero	2.63^{G}	2.63^{G}	2.63^{F}	0.029	0.07	000	0.05
2	$3.00F^{G}$	2.85^{G}	2.85^{EF}				
4	3.44^{F}	3.40^{F}	3.39^{E}				
8	4.13^{E}	4.50^{E}	4.83^{D}				
10	4.94^{D}	4.94^{D}	5.63^{C}				
14	6.29^{bC}	6.02^{cC}	6.67^{aB}				
18	6.36^{cBC}	6.89^{aB}	6.63^{bB}				
24	6.67^{aBC}	6.71^{aB}	6.34^{bB}				
32	6.82^{B}	6.86^{B}	7.03^{B}				
48	8.40^{aA}	8.35^{bA}	8.33^{bA}				

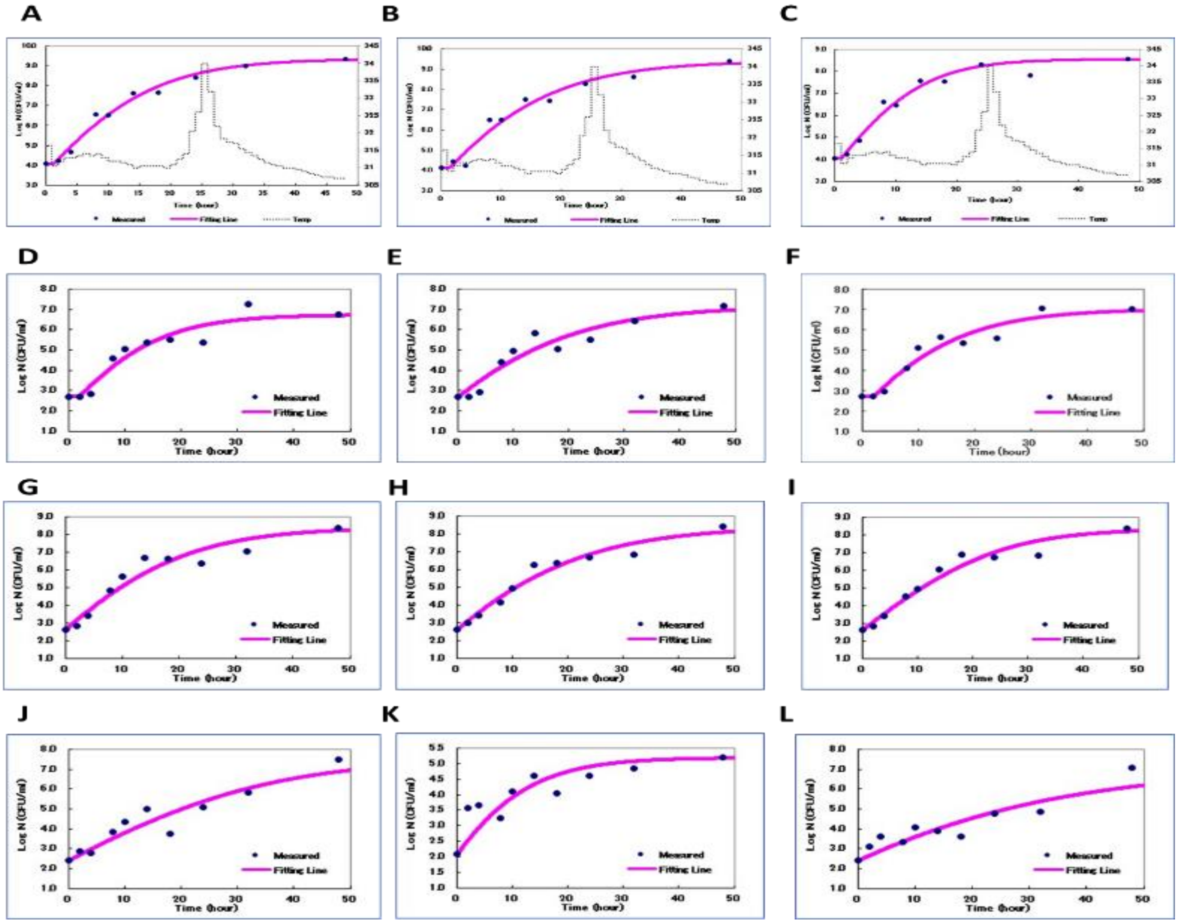

Figure (2): Prediction of growth of (A): APC in cuboidal package (B): APC in Pyramidal package (C) APC in cylindrical package (D): *SalmonellaTyphimurium* in cuboidal package (E): *SalmonellaTyphimurium* in Pyramidal package (F): *Salmonella Typhimurium* in cylindrical package (G): *E.coli* in cuboidal package (H): *E.coli* in Pyramidal package (I): *E.coli* in cylindrical package (J*): Listeria monocytogens* in cuboidal package (K): *Listeria monocytogens* in Pyramidal package (L): *Listeria monocytogens* in cylindrical package in ribeye loin at dynamic patterns of temperature using new logistic model compared to experimental results.

4. DISCUSSION

The current investigation was conducted to elucidate aspects that may contribute to the probable preservation effect of pyramidal designs based on the dimension ratio of Giza pyramid constructions on stored food intentionally contaminated with a foodborne pathogen. The current study found that package form statistically significantly affected the growth curves of artificially infected *listeria monocytogens* and naturally occurring APC. In the first four hours, APC was similar across shapes. However, pyramidal stored beef had a lower APC count than other shapes until 24 hours, emphasizing the influence of pyramidal containers on microbial growth in milk (Gopinath et al., 2008). Cylindrical, kept beef showed a similar APC curve to pyramidal, but remained lower than other package shapes until the conclusion of room temperature storage. The growth curve of *L. monocytogens* also began to change after four hours of storage. The growth curve for *L. monocytogens* in cylindrical meat was, therefore, plainly larger than that of pyramidal meat at the last 48-hour storage point; conversely, the growth curve for cuboidal meat was the highest, while the growth curve for beef stored in pyramids was the lowest. For *L. monocytogens*, significant differences were observed in time and interaction, indicating the pyramid's unique energy accumulation capable of influencing bacterial growth (Abdelsamie et al. 2014). However, the shape did not affect the growth curves of either gram-negative infused *SalmonellaTyphimurium*; prolonged preservation revealed a decrease in bacterial growth during the last hours, suggesting an energy-related impact on *Salmonella* acceleration (Stock and Stolle 2001). Regarding *E. coli*, no immediate impact of pyramidal containers was observed, attributed to the ideal growth conditions provided by ground meat (Díaz et al. 2001; Jang et al. 2017).

At dynamic temperatures, the findings could not give a consistent influence associateed with a certain package design. Circular meat, on the other hand, demonstrated slower growth curves for several introduced and naturally occurring pathogens during preservation. Pyramidal-contained meat comes next, with lower growth curves. Our early experiment revealed that at a constant temperature, there was a considerable reverse effect on the growth curve of spoiling flora at 5°C. Many additional factors may have contributed to the current unanticipated pyramidal outcomes due to the dynamic temp-. Furthermore, current findings suggest that the pyramidal effect is tied to a steady temperature rather than a dynamic one.

It is important to note that time was a factor in this case because, in comparison to other types, bacterial growth was reduced in the final hours (24–32) of preservation. It's possible that the temperature accelerated the growth of the bacteria to the point where the experiment's first findings between the storage packaging did not show a significant difference; however, over time, the energy of the pyramid shape affected the acceleration of *Salmonella* bacterial growth in comparison to other shapes. The current study predicted that summer would see a rise in *Salmonella* because of the high temperatures promoting microorganisms' growth. However, according to current research findings, there isn't a plausible answer. Specified for isolation rooms containing *Salmonellae* in the wintertime (Stock and Stolle 2001).

Pyramid shapes can induce a magnetic field, as evidenced by studies on nanocrystal evolution under magnetic induction (Kuo et al., 2010; Peng and Hwang, 2013). Based on the previous literature, pyramid shapes can induce a magnetic field. This phenomenon has been explored in food safety and quality domains, particularly in controlling foodborne pathogens. Magnetic fields have shown potential in controlling these pathogens, as demonstrated in the biocontrol of *Listeria innocua* (Novickij et al., 2021). Recent advances in magnetic-based methods for dealing with food pollutants, including pathogens, highlight the potential of magnetic nanoparticles for food safety analysis (Yu et al., 2021).

Exploring the relationship between three-dimensional forms and magnetic fields, the study highlighted the potential of pyramid shapes in reducing bacterial growth, particularly in APC (Abdelsamie et al., 2012; Abdelsamie et al., 2013). The research delved into material aspects, emphasizing the role of composite PLA polymers in microbial control (Oyelaja et al., 2020). The intricate interplay between structure, material, and external factors underscores the need for a multidimensional approach in microbial control design.

In summary, the complex interaction between design characteristics, material composition, and external influences such as magnetic fields significantly impact microbial proliferation. This comprehensive understanding emphasizes the importance of using a multidimensional approach when constructing microbial control structures, considering physical and environmental elements to enhance performance. The findings highlight the need for holistic considerations in designing structures for effective microbial control.

5. CONCLUSIONS

In this study, we assessed hierarchical shape and its potential uses in meat foodborne pathogen and microbial proliferation control. Utilizing a pyramidal model container with specific dimensions directly related to those of the Great Pyramid yielded intriguing results, revealing an unknown energy that possesses the remarkable ability to preserve organic materials without the need for external additives. This discovery raises numerous questions regarding the existence and nature of this energy and its capacity to induce positive variations in various materials. The results of our study have provided valuable realizations into the potential of utilizing set universal ratios in meat packages. The hierarchical shape had optimal and efficient effects on the control of microbial growth introduced in minced meat. The pyramidal packaging had a detrimental impact on *the L. monocytogens* growth curve, which was more noticeable at the end of the storage period than other packaging types. It directly, additive-free, influenced the growth of Listeria bacteria added to minced beef. It is critical to incorporate the outcomes of these studies when developing packaging options that efficiently preserve food.

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