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Effect of Varying Environmental Conditions on the Growth and Viability of Selected Microorganisms using Conventional Cultures

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ABSTRACT: Four experiments were conducted to examine the effect of heat, temperature, pH, and salt concentration on the growth and viability of *Pseudomonas aeruginosa, Bacillus cereus, Escherichia coli*, and *Staphylococcus aureus* using nutrient agar plate cultures. The heating effect was checked at 50 and 80 ºC for 0, 10, 20, 30, and 60 min, while temperature effects were examined at 4, 25, 30, 37, and 45 ºC. Results showed that *P. aeruginosa* could grow well at 50 ºC for 1 h while only surviving at 80 ºC for 10 min. Growths were recorded from 25 ºC for all the bacteria tested. No growth was recorded at pH 11 for *P. aeruginosa. In contrast*, others exhibited growth at all the pH tested except for S. aureus that could not survive pH 3. All organisms could not survive any of the salt concentrations tested. However, some growth was recorded between 5 – 10 % salt for others, with *S. aureus* showing the highest tolerance to salt. A study of the effect of environmental conditions on the growth and viability of microorganisms is necessary in thr future. These findings provide a more realistic estimation of food safety risks and valuable quantitative data to develop processes for safer food products.

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The growth and survival of microorganisms of economic importance to the food industry can be controlled by the external environment's physical and chemical characteristics (Oliviera *et al.*, 2020, Abebe, 2020). Temperature, oxygen, dissolved gases, salt concentration, pH, and osmotic pressure have been shown to significantly influence microbial activities (Rampelotto, 2010). Several of these factors affect enzymes' activities, which significantly influences microbial growth rates. Among all factors that affect microbial growth, temperature is probably one of the most critical factors directly affecting the growth of microorganisms in foods. Evaluating the effect of temperature on microbial growth is of paramount importance in predictive microbiology and predicting the shelf life of a product (Huang *et al*., 2011). Unlike homeotherms, bacteria lack mechanisms that conserve or dissipate heat generated by metabolism. Consequently, their enzyme systems are directly affected by environmental temperatures (Vandana *et al.*, 2021). Bacteria, as a group, grow in a broad temperature range that extends from approximately 0 °C to above 90 °C. Each species, however, requires a narrower range that is determined by the heat sensitivity of enzyme systems. Specific temperature ranges consist of three cardinal temperature points. The first is the minimum growth temperature, the lowest temperature at which growth will occur. Below this temperature, enzyme activity is inhibited, and the cells are metabolically inactive so that growth is negligible or absent. The second is the maximum growth temperature, the highest temperature at which growth will occur. Above this temperature, most enzymes are denatured, and the organism dies. The last is the optimum growth temperature which refers to the temperature at which the rate of growth is most rapid; however, it is not necessarily optimum for all enzymatic activities of the cell (Su *et al.*, 2020, Moore *et al*., 2021). All bacteria can be classified into one of three major groups: psychrophiles that can grow at temperatures between 0 °C and 15 °C (Rothschild, 2007); mesophiles that grow within a temperature range of 20 °C to 45 °C; and thermophiles that grow at a temperature greater than 45 °C (Madigan, 2006). This experiment will determine the approximate optimum growth temperatures for four microorganisms. A second factor important in regulating microbial activities is the pH of the environment. Most bacteria grow best around neutral pH values of 6.5 - 7.0. However, some can thrive in

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very acid conditions and may even tolerate a pH as low as 1.0, although their internal pH is much closer to neutral values. Some bacteria produce acid as they grow, and this acid becomes excreted, thereby lowering the pH of the surrounding environment. Unless something else in the environment neutralizes the bacterial acid, bacterial growth becomes halted (Rampelotto, 2010). Each microbe species has its characteristic range of pH values in which it grows and reproduces best. Most bacteria are sensitive to the hydrogen ion concentration of their environment. Large proteins, such as enzymes, are affected by pH. They become denatured, which often alters the ionic charges on the molecule, and metabolism is halted (Ahmad *et al.*, 2013).

The importance of salt in food manufacture arises from its preservation roles and its contribution to flavor, texture, and color (Man, 2007; Durack *et al*., 2008). Salt acts as a preservative by lowering water activity, causing microbial cells to experience osmotic shock with a drastic water loss through plasmolysis. However, the amount of salt needed to achieve a reduction in water activity (a_w) varies; a 1.7 % salt solution gives a a_w of 0.99, while an 18.2 % salt solution lowers a_w to 0.86 (Betts *et al*., 2007). Previous researches have shown that salt is another major factor regulating the activities of microbes in foods (Bouttefroy *et al*., 2000; Boziaris *et al*., 2007; Pianetti *et al*., 2008). This study may have significance in manufacturing reduced-salt foods where concerns regarding the impact of salt reduction on microbial quality may preclude a meaningful lowering of added salt levels. While growth models exist for cultures in broths with varying added salt levels, no such data exists for food matrices such as complex ready meals where growth may differ from broth models. Overall, this study of ready meals with altered salt contents suggests that significant salt reduction is achievable without compromising product safety for the microbial strains tested. Data indicate that a large-scale salt reduction program is technically feasible for these products. However, a case-by-case investigation is required to ensure all low salt foods are comparable in microbial safety with commercially available regular salt counterparts. Therefore, these scientific findings have direct practical industrial applications for ready meal manufacturers. According to Lecrec and Moreau (2002), bacteria have a viable but non-cultivable state when in certain environmental conditions. Consequently, a study of the effect of environmental conditions on the growth and viability of microorganisms is necessary. This may lead to a more realistic estimation of food safety risks and provide valuable quantitative data to develop processes that allow safer food products. Therefore, the study aimed to investigate the effect of some environmental conditions of heat, temperature, pH, and salt on the growth and viability of *Pseudomonas aeruginosa* with that for *Bacillus cereus Escherichia coli* and *Staphylococcus aureus*.

MATERIALS AND METHODS

Materials: All experiments were conducted in the Food Microbiology Research Laboratory of Glasgow Caledonian University, Scotland, UK. Bacterial strains and broth cultures of *Pseudomonas aeruginosa, Bacillus cereus, Escherichia coli, Staphylococcus aureus*, Nutrient agar plates, water bath, sterile loop, amongst others, were used.

Experiment A: Effect of Heat on the Viability of Microorganisms: Each broth culture containing each organism tested was labeled 50 ^oC and 80 ^oC, respectively. The underside of two nutrient agar plates was divided into five sectors representing 0, 10, 20, 30, and 60 min. Into the sector marked "0", one loopful each broth culture was transferred to serve as control with the aid of a sterile loop. The labeled broth cultures were then placed in water baths operated at 50 ^oC and 80 ^oC, respectively. At intervals of 10, 20, 30, and 60 min from when the broth cultures were immersed in the water bath, one loopful each was transferred from the tube into the corresponding plate. All plates were then incubated at 30 ^oC for 24 h before being examined for viability.

Experiment B: Effect of Temperature on the Growth of Microorganisms: For each microorganism tested, five nutrient agar plates were labeled 4 ^oC, 25 ^oC, 30 ^oC, 37 ^oC, and 45 ^oC, respectively. Each sample culture was streaked onto each of the plates and then incubated at the corresponding temperatures for 24 h. These were then stored at 4 ^oC examined for growth.

Experiment C: Effect of pH on the Growth of Microorganisms: Each tube of nutrient broths of pH 3, 5, 7, 9, and 11 was inoculated with one loopful of each bacterial culture tested using a sterile loop. All plates were then incubated at 30 ^oC for 24 h before being examined for growth.

Experiment D: Effect of Salt Concentration on the Growth of Microorganisms: Nutrient agar plates containing 5 %, 10 %, and 15 % salt were used. A nutrient agar with no salt added was used as a control. Each plate was inoculated with one loopful of each bacterial culture tested by spreading evenly over the surface of the agar in the plate. All plates were incubated at 30 ^oC for 24 h before being examined for growth.

RESULTS AND DISCUSSION

Effect of Heat on the Viability of Microorganisms: Results from the investigation conducted to determine the effect of heat on the growth and viability of the tested microorganisms are presented in Table 1. From the results in Table 1 above, it can be concluded that among the four microorganisms tested, only *B. cereus* was still viable and capable of surviving heat at temperatures of 50 ºC and 80 ºC for 0, 10, 20, 30, and 60 min.

Table 1: Effect of heat on the growth and viability of selected microorganisms

Microorganism	Viability at the corresponding heat and time									
	50 ºC					80 ºC				
	0 min	10 min	20 min	30 min	60 min	0 min	10 min	20 min	30 min	60 min
Pseudomonas aeruginosa	Y	Y	Y	Y	Y	Y	Y	N	N	N
Staphylococcus aureus	Y	Y	Y	Y	N	Y	N	N	N	N
Escherichia coli	Y	Y	Y	Y	Y	Y	N	N	N	N
Bacillus cereus	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y

NB: Y – Yes; N – No

All four organisms exhibited growth at 50 ºC for all heating regimens except *S. aureus,* which showed no growth at 60 min. Both *S. aureus* and *E. coli* did not grow at a temperature of 80 ºC. However, *P. aeruginosa* survived this temperature (80 ºC) for only 10 minutes, after which the organism died. All organisms evaluated in the current study showed growth and viability when samples were kept at 50 ºC for 0 min, 10 min, 20 min, and 30 min. At 60 min under this same temperature condition, no growth was however recorded for *Staphylococcus aureus. Bacillus cereus, Escherichia coli,* and *P. aeruginosa* grew successfully at 50 ºC but with a decreasing trend in colony count regarding heat applied. However, only *Bacillus cereus* and *P. aeruginosa* could survive at 80 ºC for 10 min. This indicates that these organisms can easily be destroyed at cooking temperatures, thereby posing less threat to food safety. At 80 ºC for 20 min, 30 min, and 60 min, no growth was recorded for *Staphylococcus aureus, Escherichia coli,* and *P. aeruginosa*. Among the organisms tested, only *Bacillus cereus* showed growth at all temperature and time combinations evaluated, making it the most viable organism among all the organisms tested. *Bacillus cereus* is a spore-forming organism, and it can form spores capable of withstanding cooking temperatures. *Bacillus cereus* was observed to be highly resistant to heat, which is a possible indication that there is a threat to its elimination, resulting in food poisoning and toxins in foods, especially those not cooked thoroughly under the appropriate temperature and time combination. Both *Staphylococcus aureus* and *Escherichia coli* were easily destroyed when heated to 80 ºC for any given period. This indicates that these two pathogenic bacteria are highly susceptible to cooking temperatures and may not result in food poisoning when present in undercooked food.

Effect of Temperature on the Growth of Microorganisms: Results demonstrating the effect of temperature on the growth of microorganisms are presented in Table 2. The results showed that all the microorganisms tested could only grow at a temperature of 4 ºC. Growth was not recorded at 25 ºC, 30 ºC, 37 ºC, and 45 ºC.

Table 2: Effect of temperature on the growth and viability of selected microorganisms

Microorganism	Growth at Temperature				
	4 ºC	25 ºC	30 ºC	37 ºC	45 ºC
Pseudomonas aeruginosa	N	Y	Y	Y	Y
Staphylococcus aureus	N	Y	Y	Y	Y
Escherichia coli	N	Y	Y	Y	Y
Bacillus cereus	N	Y	Y	Y	Y

NB: Y – Yes; N – No

The current study shows that microorganisms show varied responses to environmental conditions of heat, temperature, pH, and salt concentration. Regarding temperature, none of the organisms tested grew at 4 ºC, indicating that low-temperature storage methods such as refrigeration and freezing are suitable means of preserving foods without bacterial proliferation. These findings confirm research findings by Jahed-Khaniki *et al.* (2014), in which bacteria kept at 4 ºC showed a substantial reduction in their population, an indication of an unfavorable temperature regime. Foods stored under these conditions can be kept for a much more extended period since the activities of microbes are inhibited. This is why temperature and pH have been seen as principal factors regulating the multiplication of microorganisms in foods. Growth was recorded for

all the organisms at 25 ºC, 30 ºC, 37 ºC, and 45 ºC, with the highest recorded for *Staphylococcus aureus* and *Escherichia coli* at 37 ºC and *Bacillus cereus* and *Pseudomonas aeruginosa* at 30 ºC. This suggests that food microbes multiply faster when the temperature of the surrounding medium is between 30 – 37 ºC. This is within the optimum range of temperature supporting microbial growth and survival for mesophilic organisms (Madigan and Martino, 2006) and agrees with the findings of Jahed-Khaniki *et al.* (2014). A substantial increase in bacterial numbers was observed in samples stored at 37 ºC after culture.

Effect of pH on the Growth of Microorganisms: Results showing the response of microorganisms based on growth to pH are as presented in Table 3. It can be concluded from the results presented in Table 3 above that both *E. coli* and *B. cereus* are capable of growing at pH of 3, 5, 7, 9, and 11. While *P. aeruginosa* showed growth at all pH except 11, *S. aureus* survived all pH except 3. Apart from temperature, the hydrogen ion concentration of the environment of microbial organisms is also a major factor regulating their growth and survival. Most bacteria can grow at their fastest rates at pH between 6 and 8 (Pazlarová, 2015). Among the four organisms tested, *E. coli* and *B. cereus* could tolerate acidic, neutral, and alkaline conditions by showing growth at pH 3, 5, 7, 9, and 11. This reflects the importance of these two organisms to food safety due to their ability to survive broad pH values on the scale. Their control in foods becomes challenging as the pH status cannot be solely used to target their reduction and possible elimination. *Pseudomonas aeruginosa* did not survive at pH 11, indicating that growth is more favorable under acidic and neutral conditions. S. aureus showed no growth at pH 3. This shows that the organism is not capable of tolerating high acid conditions.

Table 3: Effect of pH on the growth and viability of selected microorganisms

Microorganism	**Growth at pH**				
	3	5	7	9	11
Pseudomonas aeruginosa	Y	Y	Y	Y	N
Staphylococcus aureus	N	Y	Y	Y	Y
Escherichia coli	Y	Y	Y	Y	Y
Bacillus cereus	Y	Y	Y	Y	Y

NB: Y – Yes; N – No

Effect of Salt Concentration on the Growth of Microorganisms: Results demonstrating the effect of salt concentration on the growth of microorganisms are presented in Table 4. From the results presented in Table 4, it can be concluded that *P. aeruginosa* cannot grow under salt concentrations of 5 %, 10 %, and 15 %. However, *S. aureus* could exhibit growth at 5 % and 10 % salt concentrations, while *E. coli* and *B. cereus* only showed growth at a 5 % salt concentration.

Table 4: Effect of salt concentration on the growth and viability of selected microorganisms

Microorganism	**Growth at Salt concentration**			
	Control (0 %)	5 %	10 %	15 %
Pseudomonas aeruginosa	Y	N	N	N
Staphylococcus aureus	Y	Y	Y	N
Escherichia coli	Y	Y	N	N
Bacillus cereus	Y	Y	N	N

NB: Y – Yes; N – No

Reducing salt levels on the growth of food spoilage bacteria was investigated, and salt addition levels over the concentration range of 5 – 15 % were shown to significantly affect the growth of the bacterial organisms tested. This is indicated by reducing the number of colonies observed in each plate for various bacterial species in the culture plate systems that tolerate salt. This correlates with Alqahtani's (2016) findings, in which it was observed that after 24 hours, increased salinity in the media produced a moderate decline of culture growth for S*tenotrophomonas maltophilia*. P. aeruginosa could not survive salt levels of 5 % in the nutrient agar and thus could not grow in any of the salt concentrations used. This is an indication that the organism cannot thrive under saline conditions. At 5 % salt concentrations, final populations of S. aureus, E. coli, and B. cereus, which in contrast were able to tolerate this level of salt, were well-reduced. However, S. aureus produced some colonies at a salt concentration of 10 %, indicating that it has the highest ability to tolerate salt among all organisms tested.

Conclusion: The findings of this study infer that microorganisms differ in their growth response to heat, temperature, salt concentration, and pH. Understanding the optimum range, minimum and maximum values of these environmental factors that can support the growth and viability of bacterial species considered agents of food poisoning, foodborne infection, or food spoilage would assist in preventing food contamination. It was considered highly useful for protecting ready-to-eat foods, usually classified as high-risk foods, from contamination.

REFERENCES

Abebe, GM (2020). The role of bacterial biofilm in antibiotic resistance and food contamination. *Int. J. Microbiol. 2020*.

Ahmad, E; Rabbani, G; Zaidi, N; Khan, MA; Qadeer, A; Ishtikhar, M; et al. (2013). Revisiting ligand-induced conformational changes in proteins: essence, advancements, implications and future

challenges. *J. Biomol. Struct.Dynam*. 31(6): 630-648.

Alqahtani, N (2016). The Effect of Varying Environmental Conditions on the Growth of Stenotrophomonas Maltophilia. *Theses & Dissertations*. *University of the Incarnate Word*. Available at: http://athenaeum.uiw.edu/uiw_etds/2

Betts, G; Everis, L; Betts, R (2007). *Microbial Issues in Reducing Salt in Food Products: Practical Strategies*. D. Kilcast and F. Angus, Eds. 174–200.

Bouttefroy, A; Mansour, M; Linder, M; Milliere, JB (2000). Inhibitory Combinations of Nisin, Sodium Chloride, and pH on *Listeria Monocytogenes* Atcc 15313 In: *Broth by an Experimental Design Approach. Int. J. Food Microbiol.* 54: 109–115

Boziaris, IS; Skandamis, PN; Anastasiadi, M; Nychas, GJE (2007). Effect of NaCl and KCl on Fate and Growth/No Growth Interfaces of *Listeria Monocytogenes* Scott A at Different pH And Nisin Concentrations. *J. Appl. Microbiol.* 102: 796–805.

Durack, E; Alonso, MM; Wilkinson, MG (2008). Salt: A Review of its Role in Food Science and Public Health. *Curr. Nutr. Food Sci.* 4: 290–297.

Huang, L; Hwang, A; Phillips, J (2011). Effect of Temperature on Microbial Growth Rate-Mathematical Analysis: The Arrhenius and Eyring – Polanyi Connections. *J. Food Sci.* 76(8): 553–560.

Jahed-Khaniki, GR; Aghaee, EM; Alimohammadi, M; Dehghani, MH (2014) Effects Of Environmental Conditions On Growth And Permanence Of *Pseudomonas Aeruginosa* In Bottled Water. *J. Appl. Biol. Sci.* 8(2): 91-95.

Lecrec, H; Moreau, A (2002). Microbiological Safety of Natural Mineral Water. *FEMS Microbiol. Rev*. 26: 207–222.

Madigan, MT; Martino, JM (2006). *Brock Biology of Microorganisms*, 11th ed.; Pearson Education: Upper Saddle River, NJ, USA

Man, CM (2007). Technological Functions of Salt in Food Products. In: *Reducing Salt in Foods: Practical Strategies* (D. Kilcast And F. Angus, Eds.) Pp. 157–173, Woodhead Publishing Ltd, Boca Raton, Fl; Boston, Ma; New York, Ny; Washinton, DC

Moore, CE; Meacham-Hensold, K; Lemonnier, P; Slattery, RA; Benjamin, C; Bernacchi, CJ; et al. (2021). The effect of increasing temperature on crop photosynthesis: from enzymes to ecosystems. *J. Exp. Bot.* 72(8): 2822-2844.

Oliveira, M; Tiwari, BK; Duffy, G (2020). Emerging Technologies for Aerial Decontamination of Food Storage Environments to Eliminate Microbial Cross-Contamination. *Foods*, 9(12): 1779.

Pazlarová, J (2015) Factors affecting microbial growth in food [Online] Accessed 22 August, 2015.

Pianetti, A; Manti, A; Boi, P; Citterio, B; Sabatini, L; Papa, S; et al. (2008). Determination of Viability of Aeromonas Hydrophila in Increasing Concentrations of Sodium Chloride at Different Temperatures by Flow Cytometry and Plate Count Technique. *Int. J. Food Microbiol.* 127: 252–260.

Rampelotto, PH (2010). Resistance of microorganisms to extreme environmental conditions and its contribution to astrobiology. *Sustain.* 2:1602–1623.

Rothschild, L (2007). Extremophiles: Defining the envelope for the search for life in the universe. In *Planetary Systems and the Origins of Life*; Pudritz, R., Higgs, P., Stone, J., Eds.; Cambridge University Press: Cambridge, UK.

Su, Z; Luo, J; Li, X; Pinelo, M (2020). Enzyme membrane reactors for production of oligosaccharides: A review on the interdependence between enzyme reaction and membrane separation. *Sep. Purif. Technol.* 243: 116840.

Vandana, GD; Sejian, V; Lees, AM; Pragna, P; Silpa, MV; Maloney, SK (2021). Heat stress and poultry production: impact and amelioration. *Inter. J. Biometeorol*. 65 (2): 163-179.