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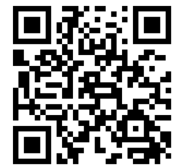

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Early Chemical Markers of Spoilage in Thermally Processed Tomato Paste: A Rapid Approach for Shelf-Stable Foods

Hussein Ali Shaghati *, Rawia Mahmood Yousif

Ministry of Trade/state company for foodstuff trading/Iraq

Abstract

This study aims to reduce the incubation period (commercial sterility test) of tomato paste cans before the bacteriological inspection in quality control laboratories. 62 cans of tomato paste were incubated under temperatures (mesophilic 25,37°C and thermophilic 55°C) to accelerate potential spoilage. Chemical indicators(pH, titratable acidity, and lactic acid) and microbial indicators (Total plate count and yeast/molds) were monitored daily to identify the earliest reliable spoilage signals. Incubation at elevated temperatures accelerated acid buildup. D+L lactic acid levels measured using a rapid enzyme assay increased with titratable acidity, while pH decreased. Meanwhile, total platelet counts remained at very low levels, while yeasts and molds were undetectable. Significant spoilage indicators appeared within 3–5 days at 55°C compared to 7–10 days at 37°C. These results indicate that acidification may be detectable within 5-6 days of incubation at 37°C. However, microbial growth was not consistently identified. We recommend an improved incubation procedure before culture, together with quick lactic acid measurement, to provide an early warning for microbial deterioration in tomato paste.

Keywords: Tomato products, Lactic acid assay, Spoilage, Commercial Sterility, Quality control

1. Introduction

Tomato paste is considered one of the most important foods used worldwide on a daily basis. It is a widely consumed food product known for its shelf-life stability and classified as a food product made from vegetables and rich in antioxidants, some vitamins, and minerals (Ibitomi *et al.*, 2024). Even though thermal processing guarantees that commercial sterility (CS) is maintained, the presence of post-process contamination or the survival of heat-resistant spores can still be of significant concern if not correctly identified during regular quality control checkpoints (USDA, 2005). Some thermophilic spore-forming bacteria under defective sterilization conditions, such as *Bacillus coagulans*, can live in canned tomato products and induce "flat sour" spoilage, which causes a reduction in pH of around 0.3 to 0.5 units owing to lactic acid generation without gas, leaving no visible container deformation. Under stress to canes, these spoilage bacteria can occasionally go into a viable-but-non-culturable (VBNC) state, which keeps them metabolically active (producing acids or enzymes) but prevents them from being detected using standard culture techniques (Squitieri *et al.*, 2023).

Standard CS protocol usually requires the product to be tested by incubation at 25°C for 10 days, in order for the spoilage to become visible, and then culture-based microbial testing needs to be done. To address this issue, the integration of rapid analytical and microbiological techniques has become an area of growing interest. Analytical tools such as lactic acid quantification using a rapid enzymatic assay, titratable acidity, and pH measurement offer chemical indicators of microbial metabolism. A drop in pH is often one of the earliest detectable signs of spoilage in tomato-based products and may correlate with organic acid production by spoilage organisms (Leistner and Gould, 2002). These chemical analyses provide a non-culture-based means of early spoilage detection. At the same time, microbiological testing, like the enumeration of total plate count (TPC) and yeast/mold, gives direct proof of intrinsic

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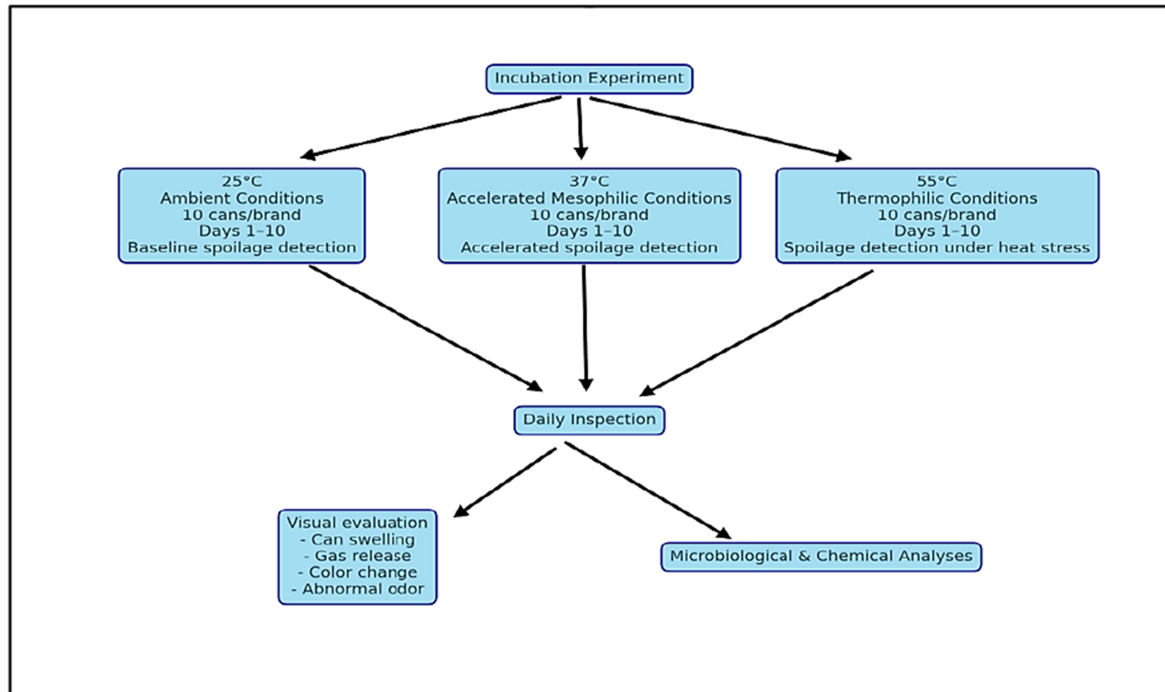

Fig. 1. Experimental design of the study.

contamination. Yeasts and molds, which can become the source of post-processing contamination, are particularly important in the case of acidic, high-sugar matrices like tomato paste (Jay, Loessner, and Golden, 2008).

In recent years, innovative detection technologies like as real-time PCR for microbial DNA and biosensors (e.g., enzymatic tests, electronic noses, and tongues) have been investigated to speed up spoilage detection beyond classic plating methods. These current methods can quickly detect spoiling metabolites or bacteria, even in cases when cells are stressed or VBNC, allowing them to evade regular culture (Kumar *et al.*, 2024).

The use of traditional microbial culture methods allows for the detection of bacterial and fungal contaminants. But these methods require post-plating incubation (e.g., 24 to 72 hours), which further extends the time required for the assessment of quality. Changes to pre-test incubation conditions and temperature can be a major factor in mold growth frequency, which leads to spoilage. A temperature rise in the incubation process has been proven to provoke the action of microorganisms that cause spoilage, and consequently, a faster detection period for the spoilage is needed (Tarlak, 2021). This study aims to employ physicochemical and biological techniques to investigate whether incubation temperature can enhance the effectiveness of quality control procedures by reducing the total time required to identify microbial spoilage in canned tomato paste during commercial sterility testing.

2. Materials and methods

2.1. *Sample material*

Two brands of double-concentrated tomato paste (labeled Brand A and Brand Q) were obtained directly from the production facility. Both brands came in 400 g closed, hermetically sealed cans were used as the test material. A total of 62 cans were utilized. All samples were presumed to be sterile, having undergone industrial thermal processing. For each brand, one can was designated for baseline testing (unincubated) on Day 0, while the remaining cans were incubated under controlled temperature conditions (25°C, 37°C, and 55°C) to investigate the impact of incubation temperature on spoilage detection and test sensitivity. To avoid external contamination, all cans were kept sealed until testing began.

2.2. *Experimental design and incubation protocol*

The experimental design and incubation protocol were carried out as shown in Fig. 1.

2.3. *Chemical analysis*

2.3.1. *Lactic acid measurement (rapid enzymatic assay) REA*

The use of REA follows the standard process stated in EN12631:1999, especially in the use of enzymes and NADH measurement for the determination of D- and L-lactic acids. (CSN EN 12631, 1999) The analyses

were carried out with the CDR FoodLab® system (Italy), which is a photometric analyzer for rapid, simple, and various food tests. The test uses an enzymatic reaction that converts lactic acid to a measurable color change. The analyzer reports total lactic acid in mg/L. The device depends on reagent cuvettes, which are already filled, and an incubation module that allows the processing of more than one sample simultaneously with less preparation. The device detection range (50-3500 mg/L) and the integrated software registered all the measurements automatically, thus providing repeatability and traceability of the data. The test was conducted following the manufacturer's protocol (S. r. I, 2024).

2.3.2. Titratable acidity (TA)

Titratable acidity was determined using an acid-base titration. Five grams of tomato paste were diluted in 50 mL of distilled water and titrated with 0.1 M NaOH using phenolphthalein as an indicator. The endpoint was the first permanent pink color lasting 15–20 seconds. TA was calculated as grams of lactic acid per 100 g of product, as in Eq. (1) (AOAC, 2005).

$$\text{Lactic Acid } (g/100g) = \text{Volume of NaOH } (mL) \times 0.1 \times 0.09 \backslash \text{Sample Weight } (g) \times 100 \quad (1)$$

Where 0.09 is the equivalent weight of lactic acid

2.3.3. pH measurement

The pH was measured using a calibrated digital pH meter (±0.01 accuracy) at 25°C. Prior to measurement, each tomato paste sample (about 20 g) was homogenized with 80 mL of distilled water to facilitate electrode contact (given the thick consistency of the paste). The pH electrode was rinsed between samples and calibrated with pH 4.00 and pH 7.00 buffers daily. Measurements followed standard protocols for food products. The initial pH (4.3) and daily pH changes were recorded. A drop in pH over time indicates acid production by microbial activity (ISO, 1991).

2.4. Microbiological analysis

Microbiological examination was carried out using the serial dilution approach. For each sample, 25 g of tomato paste was aseptically transferred to 225 mL of sterile buffered peptone water and homogenized. A milliliter of the relevant dilution was plated for each of the following microorganism counts:

2.4.1. Total plate count (TPC)

Aerobic mesophilic counts were performed according to ISO 4833-1:2013. Plate Count Agar (PCA) plates were incubated pour-plated aerobically at 30°C for 24-48 hours, and the colony counts were expressed as CFU/g (ISO, 2013).

2.4.2. Yeast and mold enumeration

Grounding on ISO 21527-1:2008, yeast and mold were cultured on Sabouraud Dextrose Agar (SDA). 0.1 mL was spread on duplicate plates using the identical homogenates as were used for TPC. Plates were incubated in the dark for five days, at 25 ± 1°C. After incubation, colonies were counted and reported as CFU/g of tomato paste (ISO, 2008).

2.5. Physical inspection

At each test point, cans were inspected for external signs of spoilage, including bulging or swelling of the can, any gas release upon opening, off-odors, or discoloration of the product. (USDA, 2001) These observations were performed in order to correlate visual deterioration evidence with chemical and microbiological findings.

2.6. Statistical analysis

The statistical analysis was carried out using IBM SPSS Statistics v26.0 (IBM Corp, 2019) Linear regression analysis (R) was used to analyze changes in lactic acid concentration, TA, and pH throughout a 10-day incubation period at different temperatures (25°C, 37°C, and 55°C) for Brand A and Brand Q. To assess the strength and direction of the relationships, Pearson's correlation coefficients (r) and coefficient of determination (R^2) were calculated. *P*-values were used to evaluate statistical significance, with $p < 0.05$ considered significant. In addition, 95% confidence intervals (CI) were computed to indicate the precision of the regression estimates. All chemical measurements were based on the average of duplicate readings, while microbiological tests were conducted in triplicate for improved reliability.

3. Results and discussion

3.1. Chemical analysis

3.1.1. Lactic acid measurement (REA)

From Table 1, at 25°C, Lactic acid concentration increased steadily from 481 mg/L to a peak of 678 mg/L on Day 5, followed by a decline to 545 mg/L by Day 10. This suggests active but transient acidification under ambient conditions. A similar trend was observed at 37°C. Acid levels rose to 668 mg/L on Day 5, then gradually decreased through Day 10, ending at 558 mg/L. At 55°C, Acidification was more pronounced, with values rising to 687 mg/L on Day 5, followed by a gradual reduction to 607 mg/L on

Table 1. Lactic Acid concentration for Brand A (mg/L) over 10 days of incubation.

DAY	A_25	A_37	A_55
DAY0	481	481	481
DAY1	553	550	610
DAY2	588	579	622
DAY3	605	608	624
DAY4	638	629	633
DAY5	678	668	687
DAY6	569	602	656
DAY7	560	610	606
DAY8	568	585	644
DAY9	551	569	631
DAY10	545	558	607
	Slope = 0.85; r = 0.05; $R^2 = 0.003$; $p = 0.874$ (ns)	Slope = 4.15, r = 0.28, $R^2 = 0.081$, $p = 0.396$ (ns)	Slope = 6.97, r = 0.45, $R^2 = 0.202$, $p = 0.165$ (ns)

r = Pearson correlation coefficient; R^2 = Coefficient of determination; $^*p < 0.05$ = statistically significant; ns = not significant. Slope indicates the rate of change per day.

Table 2. Lactic acid concentration for brand Q (mg/L) over 10 days of incubation.

DAY	Q_25	Q_37	Q_55
DAY0	1163	1163	1163
DAY1	1191	1198	1167
DAY2	1197	1203	1213
DAY3	1120	1118	1121
DAY4	1138	1198	1215
DAY5	1164	1224	1248
DAY6	1130	1227	1177
DAY7	1122	1243	1175
DAY8	1139	1220	1174
DAY9	1164	1198	1177
DAY10	1200	1194	1189
	Slope = –0.92; r = –0.10; $R^2 = 0.01$; $p = 0.763$ (ns)	Slope = 4.41; r = 0.43; $R^2 = 0.183$; $p = 0.189$ (ns)	Slope = 1.12; r = 0.11; $R^2 = 0.013$; $p = 0.742$ (ns)

r = Pearson correlation coefficient; R^2 = Coefficient of determination; $^*p < 0.05$ = statistically significant; ns = not significant. Slope indicates the rate of change per day.

Day 10. This higher and more rapid peak indicates accelerated metabolic activity (microbial or enzymatic) under elevated temperature.

Despite apparent increases in lactic acid levels, particularly at higher incubation temperatures, the statistical analysis indicated no significant relationships ($p > 0.05$). Brand A showed a modest association between time and lactic acid buildup at all temperature conditions, notably at 25°C (r = 0.05, $R^2 = 0.003$). The observed peak at 55°C suggests a faster accumulation rate. The subsequent drop after Day 5 might be the result of decreased metabolic activity, which could be brought on by lactic acid transformation through secondary biochemical reactions, nutrient depletion, or inhibition of pH feedback.

From Table 2, Lactic acid levels showed moderate fluctuations at 25°C, increasing from 1163 mg/L to 1200 mg/L by Day 10. A temporary decrease occurred on Day 3 (1120 mg/L), followed by gradual recovery. At 37°C, acid levels increased more sharply, peaking on Day 7 with 1243 mg/L, then slightly declining to 1194 mg/L by Day 10. This temperature showed the most consistent rise during the mid-incubation period (Days 5–7). While at 55°C, the highest values were observed, particularly on Day 5 (1248 mg/L), followed by a slight decrease and then stabilized around 1170–1190 mg/L through Day 10. The maximum value among all readings was recorded at this temperature.

Brand Q showed no statistically significant temporal trends at any temperature ($p > 0.05$), even though the concentrations of lactic acid fluctuated slightly during incubation. With slight peaks at 37°C and 55°C around Days 5–7, the baseline level of 1163 mg/L stayed comparatively constant. These findings imply that Brand Q retained chemical stability during the incubation.

In summary, Brand A showed a distinct increase in lactic acid during incubation (particularly at 55°C), while Brand Q maintained a high beginning lactic acid but showed only slight changes without a major upward trend. These results suggest a variation in metabolic reactions between the two brands, with

Table 3. Titratable acidity (% lactic acid) for brand A.

DAY	A_25	A_37	A_55
DAY0	2.25	2.25	2.25
DAY1	2.25	2.25	2.34
DAY2	2.25	2.25	2.34
DAY3	2.25	2.25	2.34
DAY4	2.25	2.25	2.34
DAY5	2.25	2.25	2.34
DAY6	2.25	2.30	2.34
DAY7	2.25	2.30	2.34
DAY8	2.30	2.30	2.39
DAY9	2.30	2.34	2.39
DAY10	2.30	2.34	2.39
	Slope = 0.01; r = 0.77; R^2 = 0.6; p = 0.005 (*)	Slope = 0.01; r = 0.90; R^2 = 0.81; p = 0.0002 (**)	Slope = 0.01; r = 0.81; R^2 = 0.66; p = 0.002 (**)

r = Pearson correlation coefficient; R^2 = Coefficient of determination; *$p < 0.05$ = significant; ** $p < 0.01$ (highly significant); ns = not significant. Slope indicates the rate of change per day.

Table 4. Titratable acidity (as % lactic acid) for brand Q.

DAY	Q_25	Q_37	Q_55
DAY0	2.30	2.30	2.30
DAY1	2.30	2.30	2.34
DAY2	2.34	2.30	2.34
DAY3	2.34	2.30	2.34
DAY4	2.30	2.30	2.34
DAY5	2.30	2.34	2.39
DAY6	2.34	2.34	2.39
DAY7	2.34	2.34	2.34
DAY8	2.34	2.34	2.39
DAY9	2.30	2.34	2.39
DAY10	2.30	2.34	2.39
	Slope = 0.00; r = 0.06; R^2 = 0.003; p = 0.866 (ns)	Slope = 0.01; r = 0.87; R^2 = 0.75; p = 0.001 (*)	Slope = 0.01; r = 0.81; R^2 = 0.65; p = 0.003 (*)

r = Pearson correlation coefficient; R^2 = Coefficient of determination; *$p < 0.05$ = significant; ns = not significant. Slope indicates the rate of change per day.

Brand A perhaps exhibiting restricted spoiling activity while Brand Q remained chemically more stable under identical incubation conditions.

3.1.2. *Titratable acidity (TA)*

From Table 3, TA remained stable at 2.25% across all temperatures during the initial six days of incubation. This steady pattern suggests minimal to no net acid production during the early incubation phase. At 25°C, TA increased from 2.25% to 2.30% by Day 8 to 10, while at 37°C, TA increased from 2.25% to 2.30% by Day 6 and continued to 2.34% by Day 9–10. At 55°C, TA rose slightly earlier. It reached about 2.34% by Day 1 from 2.25% on Day 0 and later further increased to 2.39% by Day 8, where it stabilized through Day 10. The levels of TA significantly increased at 37°C ($p = 0.0002$) and 55°C ($p = 0.002$), with major positive correlations r = 0.90 and 0.81, respectively. The rise at 25°C was significant ($p = 0.005$); however, there was a considerable relationship (r = 0.77). These patterns show that higher temperatures enhanced acid generation in Brand A, especially after Day 6.

On the other hand, Brand Q (Table 4), TA started slightly higher, 2.30% on Day 0. At 25°C, by Day 5-6, TA reached 2.34% at 37°C and 2.39% at 55°C, indicating slight increases at both temperatures. Brand Q had a rather stable TA with no noticeable trend (readings ranging from 2.30–2.34%). Although the absolute TA increases in Brand Q were small, statistically significant trends were still detected at 37°C ($p = 0.001$, r = 0.87) and 55°C ($p = 0.003$, r = 0.81). No significant trend was found at 25°C ($p = 0.866$), suggesting that meaningful acid development required elevated temperatures.

Both brands saw temperature-dependent increases in titratable acidity. Brand A had earlier and more consistent changes, particularly at 55°C. While Brand Q's TA profile remained more stable overall, statistically significant increasing trends at higher temperatures support the theory that TA may respond to spoiling stress less sensitively than REA. This supports the use

Table 5. pH readings for brand A samples.

DAY	A_25	A_37	A_55
0	4.34	4.34	4.34
1	4.34	4.34	4.34
2	4.34	4.34	4.34
3	4.34	4.34	4.34
4	4.34	4.34	4.33
5	4.34	4.33	4.33
6	4.33	4.33	4.3
7	4.33	4.29	4.28
8	4.29	4.29	4.28
9	4.29	4.28	4.28
10	4.29	4.28	4.28
	Slope = –0.01, r = –0.84, R^2 = 0.71, p = 0.001 (*)	*Slope = –0.01, r = –0.90, R^2 = 0.81, p = 0.000 (**)*	*Slope = –0.01, r = –0.93, R^2 = 0.86, p = 0.000 (**)*

r = Pearson correlation coefficient; R^2 = Coefficient of determination; **p* < 0.05 = significant; ** p < 0.01 (highly significant); ns = not significant. Slope indicates the rate of change per day.

Table 6. pH readings for brand Q samples.

DAY	Q_25	Q_37	Q_55
0	4.3	4.3	4.3
1	4.3	4.34	4.3
2	4.3	4.3	4.3
3	4.3	4.3	4.29
4	4.3	4.3	4.27
5	4.3	4.28	4.27
6	4.3	4.28	4.27
7	4.3	4.28	4.26
8	4.3	4.27	4.25
9	4.27	4.27	4.26
10	4.27	4.27	4.26
	Slope = –0.00; r = –0.67; R^2 = 0.45; *p* = 0.024 (*)	Slope = –0.01; r = –0.83; R^2 = 0.7; *p* = 0.001 (*)	Slope = –0.01; r = –0.92; R^2 = 0.9; *p* = 0.000 (**)

r = Pearson correlation coefficient; R^2 = Coefficient of determination; *p < 0.05 = significant; ** p < 0.01 (highly significant); ns = not significant. Slope indicates the rate of change per day.

of direct lactic acid measurement as a more responsive signal in early-spoiling detection techniques.

3.1.3. pH measurement

From Table 5, throughout the first three days, the pH stayed constant at 4.34 at all temperatures. A statistically significant decrease was noted at all incubation temperatures starting on Day 4. By Day 10, the pH had slightly decreased to 4.29 at 25 °C (r = –0.84, R^2 = 0.71, *p* = 0.001). The decline was pronounced at 37°C (r = –0.90, *p* = 0.000), and the highest negative correlation was observed at 55°C (r = –0.93, R^2 = 0.86), suggesting that the acidification process was highly time-dependent. These results indicate that, even in the absence of microbial growth, high temperatures promote pH reduction, most likely as a result of thermal hydrolysis or residual enzymatic activity.

Table 6 displays the corresponding values for Brand Q. At 25°C, the pH remained stable at 4.30 - 4.27 but was statistically significant (*p* = 0.024). At 37°C, the pH decreased slightly to 4.27, with a significant difference (r = –0.83, R^2 = 0.70, *p* = 0.001). At 55°C, the strongest correlation was observed with a sharp pH decline to 4.25 (r = –0.92, R^2 = 0.90, *p* = 0.000). In comparison to Brand A, Brand Q demonstrated slower acidification and greater thermal stability under stress conditions. Although all pH readings remained above the spoiling threshold (pH < 4.0), the statistically significant decreasing trends, notably at 55°C, show the sensitivity of pH as a spoilage indicator during thermal stress. The high R^2 values indicate a major time-temperature relationship. These results match with lactic acid and titratable acidity profiles, highlighting the importance of integrated chemical indicators in early spoilage identification.

3.2. Microbiological analysis

3.2.1. Total plate count (TPC)

Table 7 shows the aerobic mesophilic total plate counts for both Brands A and Q incubated at three

Table 7. Aerobic total plate count (TPC) of brands A and Q (CFU/g).

Days	A 25	A37	A55	Q25	Q37	Q55
Day 0	2×10^1	2×10^1	2×10^1	0	0	0
Day 1	2×10^1	2×10^1	6×10^1	0	0	0
Day 2	1×10^1	0	3×10^1	5×10^1	0	5×10^1
Day 3	0	0	0	0	0	0
Day 4	0	0	0	0	0	0
Day 5	0	0	0	0	0	0
Day 6	0	0	7×10^1	0	0	0
Day 7	0	0	0	0	0	0
Day 8	0	0	0	0	0	0
Day 9	0	0	0	0	0	0
Day 10	5×10^1	0	0	0	0	0

CFU values represent colony-forming units per gram; "0" indicates no colonies detected on plates (detection limit $\sim 10^1$ CFU/g).

temperatures over the 10 days (before any further culture enrichment).

Brand A (TPC): Since all Brand A samples had low initial counts on Day 0 (about 1-2 $\times 10^1$ CFU/g), the microbial load was very low. Some counts in Brand A varied at low levels on Days 1–2. For example, at 25°C and 37°C, they stayed at 1–2 $\times 10^1$, while at 55°C, they slightly increased to 6×10^1 CFU/g on Day 1 and 3×10^1 on Day 2. All conditions for Brand A exhibited no visible development from Day 3 to Day 5 (plates had no colonies). On Day 6, the highest count for Brand A throughout the experiment was 7×10^1 CFU/g, which was only identified at the 55°C setting.

Overall, Brand A's plate count data show that there was no significant microbial proliferation and that the main cause of spoiling was biochemical (acid generation) rather than significant CFU increases. This implies that either the plate count method did not capture all metabolically active cells (e.g., viable but non-culturable bacteria) or a small number of metabolically active bacteria (possibly sub-lethally damaged or in spore form initially) that produced acids without large population growth were responsible for the spoilage found in Brand A.

Brand Q (TPC): Brand Q originally showed no microbiological development. There were no colonies found at Day 0 (0 CFU/g). On Days 2 and 3, two instances each showed a small peak: 5×10^1 CFU/g at 25°C and 55°C (25°C on Day 2 and 55°C on Day 3). These isolated low numbers were not seen in the days that followed and did not last. For the rest of the 10-day test, all plate counts for Brand Q at all temperatures were nil starting on Day 4.

As a result, Brand Q had basically no recoverable bacteria and remained microbiologically stable during the incubation period. The brief one-day increases in Q (just 50 CFU/g) might have been caused by a small amount of contamination, a handling error, or even the activation of some background spores that stopped growing. Crucially, they were unrelated to any chemical alterations, and the next day, the numbers were zero once more.

The TPC data show that neither of the brands had significant microbial growth under the conditions being evaluated. Brand A did have a few colonies intermittently, especially at higher temperatures, but the numbers were extremely low and temporary. Brand Q maintained a virtually zero viable count throughout. These findings support the hypothesis that the items were initially commercially sterile and indicate that even under abusive storage settings (up to 55°C), bacteria did not bloom to high levels after 10 days.

3.2.2. Yeast and mold counts

Yeast and mold enumeration revealed zero colonies for both Brand A and Brand Q at all temperatures and on all days (Table 8). There were no detected fungi in any of the samples during the study. This implies that the paste products were free of fungal contamination to begin with, and that the incubation conditions (including 25°C, which would be ideal for mold development if spores were present) did not promote fungal growth. The lack of yeast/mold development is consistent with the product's acidity, the efficacy of heat processing in killing fungal spores, and the use of sterile handling.

The absence of yeast and mold activity demonstrates that the spoiling events seen (in Brand A) were not caused by fungi. If yeasts caused swelling or fermentation, CO_2 generation and colony expansion on SDA would be expected, but this was not observed. The stability of Brand Q in relation to fungus demonstrates that it has stayed entirely unspoiled.

4. Discussion

Significant chemical changes were noted, especially in Brand A at 55°C, even though no visible microbial growth was found in any of the brands or

conditions. The most sensitive indicator of quality changes among the analytical parameters was the concentration of lactic acid, which was determined by both TA and REA. After Day 3 at 55°C, lactic acid levels in Brand A significantly rose, while Brand Q stayed chemically stable in all conditions. This implies non-microbial acidification, which is supported by earlier research and is probably caused by enzymatic or oxidative degradation pathways under heat stress (Sionek *et al.*, 2024; Dash *et al.*, 2022).

The correlation analysis between lactic acid and pH in Brand A revealed a strong inverse relationship, despite the fact that TA remained mostly unchanged and pH only showed slight declines. This supports the idea that acidification was taking place in the absence of significant microbial growth. Products that are shelf-stable and still have enzymatic activity have been shown to exhibit this behavior. (Sorathiya *et al.*, 2025; Mafe *et al.*, 2024). During the study period, all samples had TPC, yeasts, and molds that were consistently below detection limits (<10 CFU/g) in accordance with ISO 4833-1 and ISO 21527-1. These results rule out microbial spoiling as a reason for the observed acidification and validate the products' commercial sterility. This is consistent with research showing that *Bacillus coagulans* and related thermophilic bacteria can occasionally exhibit low levels of metabolic activity without developing visible colonies. (Zawawi *et al.*, 2022). An Incubation period at 37°C for 5-6 days was found to be effective in identifying chemical changes, including lactic acid production, without the risk of overestimating spoiling due to excessive thermal influence. In contrast, at 55°C, acidity increased significantly. Such increased levels of acid might exceed the standard thresholds, thus hiding the true spoilage risk.

This supports the guidelines of Codex Alimentarius and other investigations (Li, Zhang, and Wang, 2022). Codex Alimentarius advises for the CS test the incubation at 35-37°C for up to 10 days before culture to improve microbial identification. However, our data imply that early lactic acid accumulation, a spoiling proxy, was already detectable by Day 5-6 at the same temperature. This suggests that the incubation duration for quality control in acidified canned goods should be optimized to increase speed without sacrificing accuracy. (USDA F., 2023) advocating short, mild-temperature CS protocols for acidified canned foods.

These results are consistent with those who demonstrated that chemical spoilage indicators can precede microbial detection (Choskit *et al.*, 2023). Moreover, similar conclusions have been drawn in UHT milk and juice studies using lactic acid and pH drops as proxies for shelf-life stability (Karlsson *et al.*, 2019).

5. Conclusions

This study demonstrated that chemical indicators, particularly lactic acid accumulation, can serve as early markers of tomato paste quality changes during the CS test. The results highlight the possibility that chemical alterations may occur prior to microbial spoilage, which may be utilized to enhance early quality control interventions. Notably, the most distinct chemical trends were obtained after 5–6 days of incubation at 37°C without the need for extreme temperature stress. This suggests that a shorter, moderate incubation protocol may be more effective and practicable than the traditional 10-day incubation time for identifying early degradation in acidified canned foods.

6. Recommendations

To improve the efficiency of commercial sterility testing for canned tomato paste, it is recommended to optimize the pre-culture incubation conditions by incubating samples at approximately 37°C for 5 to 6 days. This approach allows for early detection without relying on prolonged incubation. Additionally, the use of a rapid enzymatic assay helps detect spoilage metabolites before any visible microbial growth begins. Implementing these strategies in quality control protocols can improve the efficiency of spoilage detection while maintaining food and product safety.

Conflict of Interest

The authors declare no conflict of interest.

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