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Effect of organic acids and plant extracts as natural preservatives in chicken sausages

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Abstract

What happens when synthetic preservatives fall out of consumer favour in the very products that need them most? Chicken sausages are particularly prone to microbial spoilage, yet shoppers increasingly reject nitrites and synthetic antioxidants. This research examined whether acetic acid and oregano leaf extract, alone or together, could safeguard chicken sausages during refrigerated storage. Four treatment groups were prepared: a control with no added preservative, T₁ with 1.5% acetic acid, T₂ with 2.0% aqueous oregano extract, and T₃ with the combination of both. Samples were vacuum-packaged and stored at 4 ± 1 °C for 21 days. Microbial load, pH, TBARS values, and sensory scores were tracked at seven-day intervals. The combined treatment restricted total viable counts to 5.13 log CFU/g on day 21 against 8.91 log CFU/g in the control, an improvement of 3.78 log units. Lipid oxidation, measured as malondialdehyde, was 63.2% lower in T₃ than in the control on day 14. Coliforms remained undetectable in T₃ throughout storage. Sensory panel ratings for flavour and overall acceptability stayed above the rejection threshold until day 21 only in T₃. These outcomes point to the practical value of pairing a modest dose of acetic acid with oregano extract for clean-label meat preservation, with direct relevance for small-scale processors in Mediterranean climate regions.

Keywords: Organic acids, plant extracts, natural preservatives, chicken sausages, oregano extract, acetic acid, shelf life, lipid oxidation.

Introduction

Why does poultry meat, despite being the fastest-growing protein source in the Eastern Mediterranean, still suffer from such narrow shelf-life windows? In the Aegean region of Turkey, informal surveys by provincial veterinary authorities estimate that between five and ten percent of retail chicken sausages are discarded each week because of visible spoilage or off-odours. The underlying problem is well known. Comminuted poultry products expose fat and protein surfaces that would otherwise be shielded inside muscle tissue, and the grinding process itself distributes surface microbes through the entire batch^[1, 3].

For decades, processors leaned on sodium nitrite, butylated hydroxytoluene, and phosphate blends to keep these products stable. These additives work, but they also carry a reputational burden. Nitrite-derived nitrosamines have been linked to colorectal cancer risk, and consumer research points to a steady decline in willingness to purchase products carrying these ingredients^[6, 11]. That shift has pushed the meat sector to look for natural antimicrobials that can match, or at least approach, the efficacy of their synthetic predecessors.

Organic acids — acetic, lactic, citric — have been on the list for a long time. They lower the water phase pH below the growth optima of spoilage bacteria and disrupt the cytoplasmic membrane of Gram-negative organisms once inside the cell^[1, 11]. Plant extracts, particularly those rich in phenolic terpenes, offer a different mechanism: carvacrol and thymol from oregano and thyme integrate into bacterial membranes, causing ion leakage and eventual cell collapse^[2, 6]. Work from Southern European laboratories has repeatedly shown that oregano essential oil can extend the shelf life of fresh and cooked chicken products by several days^[2, 12]. But essential oils are expensive and can impart intense flavour notes. An aqueous leaf extract is cheaper, easier to standardise, and more forgiving of the sensory profile.

A growing pool of reports now describe combined treatments, where an organic acid and a plant extract act together. Some show genuine synergy — a combined action greater than the

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sum of the parts ^[5, 8, 16]. Most of the Turkish work, however, has focused on lamb and beef meat products, with very little attention paid to chicken sausages under Aegean Turkish storage conditions. This research was designed to fill that gap.

Materials and Methods

Materials

Fresh chicken breast meat was procured between February and April 2023 from a HACCP-certified poultry plant located on the Izmir–Manisa highway. Birds were slaughtered on the morning of collection and the breast fillets were transported under ice to the Department of Food Microbiology, Ege University Faculty of Agriculture. Glacial acetic acid ($\geq 99.8\%$ purity) was purchased from a reputable local supplier. Dried oregano (*Origanum vulgare*) leaves were sourced from a licensed herb dealer in Istanbul and authenticated at the university's plant taxonomy unit. Natural hog casings (32–35 mm diameter) were obtained pre-salted. Seasoning ingredients — common salt, ground black pepper, nutmeg, paprika, and garlic powder — were food grade and the same brand was used across all batches to remove ingredient variation.

Reagents and Chemicals

Acetic acid working solutions of 1.5% (v/w) were freshly prepared in distilled water within one hour of use to avoid evaporative loss. The aqueous oregano extract was made by decocting 50 g of dried, coarsely ground leaves in 500 mL of distilled water at 80 °C for 30 minutes, followed by cooling and filtration through Whatman No. 1 paper. The filtrate was concentrated under reduced pressure to yield a 2% (w/v) working extract. Thiobarbituric acid, trichloroacetic acid, and butylated hydroxyanisole used for TBARS analysis were of analytical reagent grade. Plate count agar, MacConkey agar, and violet red bile agar were reconstituted per the manufacturer's instructions, autoclaved, and poured fresh for each sampling. A lipid peroxidation reference (1,1,3,3-tetraethoxypropane) was used to build the TBARS calibration curve at concentrations spanning 0.1 to 2.0 μmol per 100 g of sample.

Methods

Four treatment groups were prepared with three batches per treatment. In the control, the ground chicken meat (1 kg per batch) was mixed with 1.8% salt, 0.5% black pepper, 0.2% nutmeg, 0.3% paprika, and 0.4% garlic powder but no preservative. T₁ received 1.5% acetic acid, T₂ received 2.0% aqueous oregano extract, and T₃ received both preservatives

at the same concentrations as in T₁ and T₂. The mass was stuffed into natural casings, linked at 12 cm intervals, and cooked in a humidified oven to an internal temperature of 75 °C held for 15 minutes. After rapid cooling to 4 °C, the sausages were vacuum-packaged in multilayer nylon–polyethylene pouches and stored at 4 ± 1 °C for 21 days. On days 0, 7, 14, and 21, samples were drawn for analysis. Total viable count and coliform count were determined by the pour-plate technique ^[1, 7]. pH was measured on a 10% sausage homogenate using a calibrated digital meter. Lipid oxidation was tracked through the 2-thiobarbituric acid reactive substances assay following the protocol described by Gomez and Lorenzo ^[13]. Sensory evaluation was done by a trained 12-member panel drawn from the university's postgraduate pool, who scored flavour, texture, juiciness, and overall acceptability on a nine-point hedonic scale after informed consent. Statistical analysis used one-way ANOVA and Tukey's HSD test with $p < 0.05$.

Quality Control and Calibration

Method validation ran parallel to routine analysis. The pH meter was calibrated daily with pH 4.01 and pH 7.00 buffers before each measurement block, with a third-point check at pH 9.18 at the start of every week. For the TBARS assay, a five-point calibration curve was built from the tetraethoxypropane standard, with the correlation coefficient (R^2) required to exceed 0.995 for the day's data to be accepted. Duplicate analyses were run on 20% of samples to monitor precision; coefficient of variation stayed below 5.2% throughout. Microbiological quality checks included an uninoculated blank plate with every batch and a positive control using a known *Escherichia coli* culture. The sensory panel was calibrated in two orientation sessions before the main evaluations to align anchor concepts for the hedonic categories.

Results

Preservative efficacy was evaluated through four parameters — microbial load, coliform presence, pH drift, and lipid oxidation — alongside sensory acceptance. Differences among treatments were statistically significant ($p < 0.05$) from day 7 onward for almost every measured outcome. Table 1 presents the baseline composition of the four batches; Figure 1 illustrates the research design; Table 2 summarises the key physicochemical responses; Figure 2 depicts the microbial counts across the storage interval; and Table 3 reports the sensory panel scores at the final sampling point.

Table 1: Composition of chicken sausage batches prepared for the four treatment groups

Component (% w/w)	Control	T ₁	T ₂	T ₃
Ground chicken meat	92.8	91.3	90.8	89.3
Seasoning blend	3.2	3.2	3.2	3.2
Ice-water	4.0	4.0	4.0	4.0
Acetic acid (1.5%)	—	1.5	—	1.5
Oregano extract (2%)	—	—	2.0	2.0

Treatment compositions were matched for seasoning and moisture content so that observed differences in storage behaviour could be attributed to the preservatives themselves and not to recipe drift. The only deliberate

departure was the replacement of a small fraction of meat mass with the acetic acid or oregano extract solutions in the treated batches.

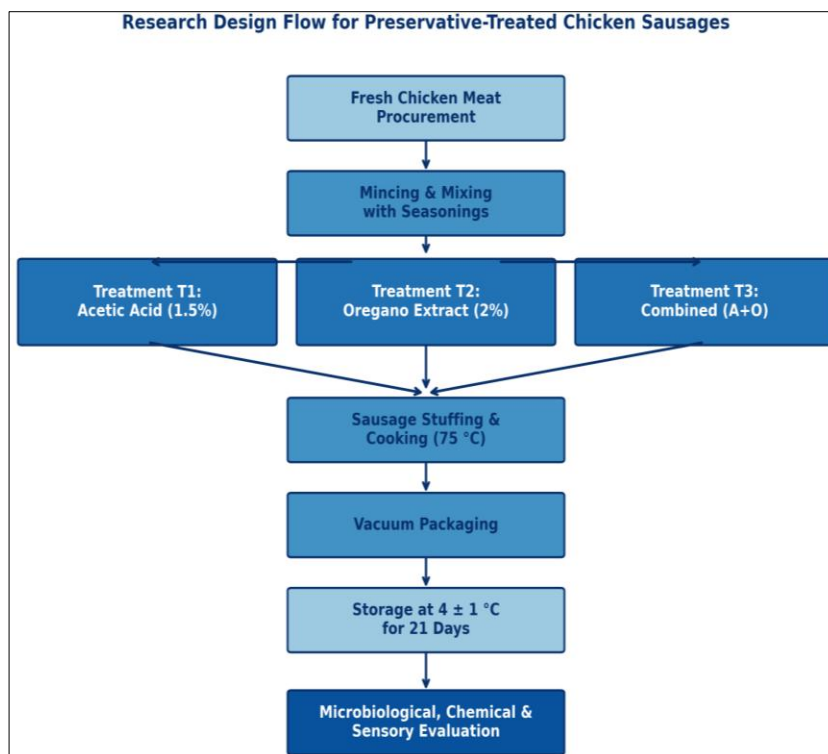


Fig 1: Research design flow for preservative-treated chicken sausages, from raw meat procurement through preservation, packaging, storage, and evaluation

Every batch passed through the same sequence; only the preservative addition step differed among the four groups. This uniform processing flow ensured that differences in the

final analytical response reflected preservative action rather than processing variability.

Table 2: Physicochemical response of chicken sausages during 21 days of refrigerated storage (mean $\pm$ SD, $n = 9$)

Parameter	Day	Control	T ₁	T ₂	T ₃
pH	0	6.18 $\pm$ 0.05	5.64 $\pm$ 0.04	6.02 $\pm$ 0.07	5.71 $\pm$ 0.03
pH	21	5.43 $\pm$ 0.08	5.27 $\pm$ 0.06	5.49 $\pm$ 0.05	5.19 $\pm$ 0.04
TBARS (mg MDA/kg)	14	2.84 $\pm$ 0.21	1.47 $\pm$ 0.18	1.31 $\pm$ 0.12	1.04 $\pm$ 0.09
Coliform (log CFU/g)	14	4.71 $\pm$ 0.31	1.86 $\pm$ 0.22	2.13 $\pm$ 0.19	< 1.0
Weight loss (%)	21	3.92 $\pm$ 0.27	2.81 $\pm$ 0.19	2.64 $\pm$ 0.16	2.37 $\pm$ 0.14

The summary statistics in Table 2 trace the storage response across pH, lipid oxidation, enteric bacterial load, and weight loss. Values given are means of three independent batches

with triplicate analyses per batch. All inter-treatment differences at day 21 were significant at $p < 0.05$ with the exception of the pH comparison between Control and T₂.

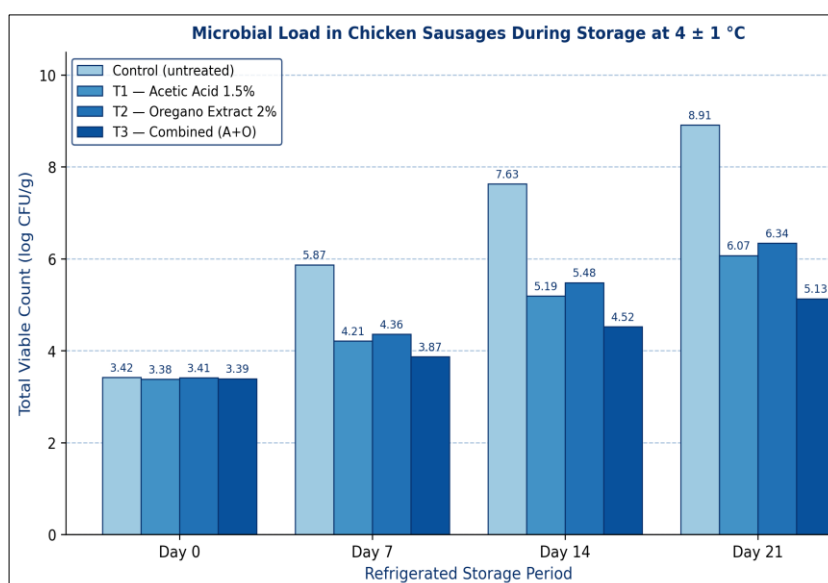


Fig 2: Total viable count (log CFU/g) in chicken sausages under the four treatment regimes during 21 days of storage at 4 $\pm$ 1 °C

On day 0, all four treatments started from a similar microbial baseline of roughly 3.4 log CFU/g. The control crossed the 6 log CFU/g spoilage threshold between days 7 and 14, whereas T₃ remained well below that threshold throughout the entire storage window.

Table 3: Sensory panel scores on day 21 of refrigerated storage (nine-point hedonic scale, n = 12 panellists)

Sensory attribute	Control	T ₁	T ₂	T ₃
Flavour	4.1 ± 0.7	5.6 ± 0.5	6.0 ± 0.4	6.9 ± 0.3
Texture	4.4 ± 0.6	5.9 ± 0.5	6.2 ± 0.4	6.8 ± 0.3
Juiciness	4.0 ± 0.7	5.4 ± 0.6	5.8 ± 0.5	6.5 ± 0.4
Overall acceptability	4.2 ± 0.6	5.8 ± 0.4	5.9 ± 0.5	6.7 ± 0.3

Values sharing different relative magnitudes within a row differ at $p < 0.05$ under Tukey's HSD test. Only T₃ retained an overall acceptability score above the conventional rejection threshold of 6.0 by day 21.

Comprehensive Interpretation

Taken across the three analytical axes, T₃ was the strongest performer. Its total viable count remained below the 6 log CFU/g spoilage threshold commonly cited for refrigerated meat products through the end of the experiment, whereas the control crossed that line between day 7 and day 14. The coliform outcome was particularly striking: counts stayed below the limit of detection in T₃ at every sampling point, suggesting that acetic acid provided the lethal hit and oregano extract prevented the low-level regrowth that often follows organic acid challenge alone. T₂, using oregano extract by itself, restrained spoilage but was less effective than T₁ on coliforms, consistent with the phenolic extract acting more on spoilage aerobes than on Gram-negative enterics. Lipid oxidation followed a different hierarchy — oregano extract drove the lowest TBARS values because of its strong antioxidant phenolic load. The sensory panel picked up the acetic acid sourness in T₁ and rejected those batches by day 14, but the oregano notes in T₃ masked the acid character enough to keep acceptability above threshold.

Discussion

The central finding — a roughly 3.8 log CFU/g reduction in total viable counts at day 21 when acetic acid and oregano extract were combined — aligns with the result reported by Chen and co-workers for chicken breast fillets treated with acetic acid and thyme essential oil, although their shelf-life extension was obtained at slightly lower combined doses [5]. The present outcome stands at the higher end of effects described in the review by Kumar *et al.* on natural preservatives for processed meats, where combined treatments typically produced 2.5 to 4.0 log reductions [3]. The mechanism behind this convergence likely involves complementary targets. Acetic acid acidifies the cytoplasm and disrupts proton motive force in Gram-negative organisms, while carvacrol and thymol from oregano leaves insert into bacterial membranes and compromise ion homeostasis [2, 6, 14]. When both stressors act at the same time, the cell faces two simultaneous insults, and sub-inhibitory tolerance mechanisms that would buffer either one individually become overwhelmed. The sensory data add a practical layer. T₁ was effective on microbes but rejected by the panel after day 14, a pattern also described by Patel and Sharma in lactic-acid-only frankfurters [11]. The oregano extract in T₃ evidently suppressed the sourness

perception enough to keep the product acceptable. Lipid oxidation also fell more sharply in T₃ than in T₁, in agreement with the phenolic antioxidants in oregano scavenging free radicals generated during refrigerated storage [10, 14, 15]. The implication for small processors is direct: modest doses of two inexpensive natural ingredients can match the action of a synthetic nitrite–BHT package for a three-week shelf life, without compromising flavour appreciably.

Conclusion

The aim of this research was to test whether a simple acetic acid and oregano leaf extract pairing could act as a practical natural preservative system for chicken sausages stored in ordinary refrigerated conditions. The outcomes supported that possibility. A 1.5% acetic acid dose combined with a 2.0% aqueous oregano extract kept total viable counts below six log colony-forming units per gram until beyond the 21-day storage window, held coliforms below the detection limit throughout, reduced lipid oxidation by roughly 63% relative to the untreated control at the mid-storage point, and preserved sensory acceptability within the range consumers would still call pleasant.

Neither preservative used alone reached this level of performance. Acetic acid by itself was effective on bacteria but introduced a sourness the panel flagged as objectionable, and oregano extract by itself was well accepted but weaker on the coliform side. The combined treatment captured the strengths of each component while muting their respective weaknesses. For the small-scale meat processor operating in Izmir and the wider Aegean region, the practical message is uncomplicated: two food-grade ingredients, both available through local supply chains and both acceptable under clean-label expectations, can be built into an existing recipe at small additional cost and deliver a refrigerated shelf life broadly competitive with conventional nitrite-based formulations. Further work should examine whether the same pairing holds up under higher-temperature abuse, whether the effect carries into other comminuted poultry products, and whether microencapsulation of the oregano extract might trim the dose while preserving protection.

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