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Nano-armoured Vitamin C: A novel approach to micronutrient delivery and natural food preservation in integrated food systems

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Abstract

The present study highlights the development of nano-encapsulated ascorbic acid delivery systems to address challenges in food preservation and nutritional fortification. Ascorbic acid-producing *Acetobacter* strains isolated from natural sources were characterised and optimised for fermentation at pH 4–6 and 30°C, achieving 99% substrate conversion within 96 hours. Iron oxide nanoparticles synthesised via coprecipitation were functionalized with biosynthesised ascorbic acid through pH-controlled coating (pH 12.5, 80^0C, 1 hour). Scanning electron microscopy confirmed uniform coating architecture. Preservation efficacy was tested in fruit juices (apple, grape, orange, and banana) and dairy products (milk and curd), demonstrating that nano-encapsulated formulations achieved approximately 75% antimicrobial activity compared to commercial ascorbic acid. This reduction in microbial loads, from previously uncountable levels to 7–20 CFU at 10^ (-8) dilutions, was notable. The dual-functional vitamin C and iron fortification, and clean label compliance, are due to their biosynthetic origin. This integrated biotechnology-nanotechnology platform establishes proof of concept for next-generation multifunctional food additives that extend shelf life while enhancing nutritional value, providing viable solutions for food industries pursuing natural preservation strategies.

INTRODUCTION

The global food industry faces significant challenges in food safety, nutritional security, and adapting to evolving consumer preferences (Barrett, 2021). Microbial spoilage and lipid oxidation of food result in annual losses in billions of dollars and pose significant public health risks (Vesković, 2025). India's food processing sector, ranking sixth globally, exemplifies the transition from traditional preservation methods to advanced systems that incorporate sophisticated techniques and functional additives (Lisboa et al., 2024). Consumer demand has shifted dramatically toward natural, clean-label products, emphasising transparency and minimal processing over synthetic additives (Antony & Narayanaswamy, 2026). Current preservation strategies rely predominantly on antimicrobial agents (sulphuric acid, nitrates, nitrites) and synthetic antioxidants (BHA, BHT, TBHQ), which effectively extend shelf life but face increasing rejection due to health concerns, potential antimicrobial resistance, regulatory restrictions, and incompatibility with clean-label demands (Ahmed et al., 2022).

Ascorbic acid (vitamin C) is a multifunctional solution that meets both technical and consumer demands in food preservation (Tissera et al., 2025). As a potent antioxidant, it prevents lipid oxidation, pigment discolouration, and nutrient degradation while supporting collagen synthesis, immune function, and iron absorption (Alberts et al., 2025). Despite substantial health benefits, including protection against oxidative stress and chronic diseases, ascorbic acid faces critical limitations: instability under processing conditions (oxygen, elevated temperatures, pH variations, light, metal catalysts), compromising efficacy and nutritional value, plus averse effects from direct supplementation(gastrointestinal distress, nausea), limiting consumer acceptance (Malik et al., 2022). Nanotechnology offers transformative solutions through nanoencapsulation, enabling enhanced stability, controlled release, improved bioavailability, and targeted delivery (Mehta et al., 2025). Iron oxide

nanoparticles exhibit advantageous properties, including biocompatibility, superparamagnetic properties that enable controlled delivery and magnetic recovery, tunable surface chemistry for functionalization, and nutritional value as bioavailable iron supplements, thereby addressing micronutrient deficiencies (Waseem et al., 2025). This combination creates synergistic functionality in which ascorbic acid enhances iron absorption while iron oxide nanoparticles protect it from degradation, simultaneously addressing two prevalent global nutritional deficits (Kumari & Chauhan, 2022a). Biological production through microbial fermentation using *Acetobacter species, Gluconobacter oxydans*, and *Aspergillus niger*, offers sustainable alternatives to chemical synthesis, aligning with clean-label requirements while minimising environmental impacts (See et al., 2024).

Despite advances in nanoencapsulation and biological production methods, significant gaps remain in their combined use for food preservation (Anushree et al., 2025). Most studies have examined these technologies separately rather than together, testing nanoencapsulation in laboratory settings without verifying effectiveness in actual food products (Mehta et al., 2025). While some research has improved nutrient delivery or explored production methods, these studies have not demonstrated antimicrobial activity or addressed practical concerns, such as production costs, scaling up to industrial levels (Waseem et al., 2025). The food industry requires solutions that simultaneously enhance nutrition, prevent microbial spoilage, meet consumer demand for natural ingredients, and remain economically feasible; yet, few studies have connected laboratory-scale production with nanoparticle technology and testing in real food systems (Acharekar et al., 2025). Therefore, an integrated approach is needed that combines optimised microbial production, nano-encapsulation methods, and characterisation and testing across various food products to develop a practical, multipurpose preservation technology for commercial use (Lavanya et al., 2024).

This research addresses the integration gap by developing nano-encapsulated ascorbic acid delivery systems using iron oxide nanocarriers, combining microbial fermentation with *Acetobacter* strains, controlled synthesis and functionalization of iron oxide nanoparticles, and synthetic validation across diverse food matrices, including fruit juices and dairy products. The iron oxide nanoparticles serve dual functions: as protective carriers that shield ascorbic acid from degradation while enhancing its bioavailability, and as a source of iron supplementation to address micronutrient deficiency. This integrated methodology bridges the gap between fundamental science and industrial applications, offering solutions for natural food preservation while providing scalable platforms for micronutrient fortification. The specific objectives were to isolate and characterise ascorbic acid-producing microorganisms from environmental sources; to optimise fermentation parameters for maximum production; to synthesise iron oxide nanoparticles and encapsulate biosynthesised ascorbic acid within these matrices; to characterize the physicochemical and functional properties of nano encapsulated formulations; to evaluate antimicrobial efficacy and preservation performance; and to compare shelf life extension between nano encapsulated ascorbate, commercial ascorbic acid, and untreated controls across multiple food products.

METHODOLOGY

Isolation of ascorbic acid-producing bacteria: sample collection and enrichment

Acetic acid bacteria capable of direct ascorbic acid biosynthesis were isolated from naturally fermented substrates, including deteriorated fruits (apples and grapes), coconut toddy, and honey. These sources are selected for their high carbohydrate content and their established association with *Acetobacter* species. Samples were aseptically processed and subjected to selective enrichment using source-specific media formulations. For fruit-derived isolates, the enrichment medium contained glucose (1% w/v), ethanol (0.5% v/v), acetic acid (0.3% v/v), peptone (1.5%w/v), and yeast extract (0.8% w/v). Toddy samples were enriched in medium containing glucose (5% w/v), yeast extract (1% w/v), and cycloheximide (100 ppm) to suppress fungal contamination. All enrichment cultures were incubated at 30 $^{\circ}$C with continuous agitation (180 rpm) for 5 days to promote the proliferation of acetic acid bacteria (Rahman et al., 2024).

Selective isolation and culture characterisation

Following enrichment, cultures were transferred to differential media for isolation and preliminary identification. Fruit-derived isolates were plated on glucose yeast calcium carbonate (GYC) medium containing (10% w/v), yeast extract (1% w/v), calcium carbonate (2% w/v), and agar (1.5% w/v), adjusted to pH 6.8. The medium was supplemented with 100 mg/L of cycloheximide to inhibit non-target organisms. Plates were incubated aerobically at 30°C for 3-4 days (Lee et al., 2024).

Ethanol tolerance and acid production characteristics were verified using Carr medium containing yeast extract (3% w/v), bromocresol green (0.2% w/v), ethanol (5-9% v/v), and agar (2% w/v) for toddy-derived. Isolates, Glucose Yeast Peptone (GYP) medium was employed, consisting of glucose (2% w/v), sodium acetate trihydrate (0.5% w/v), tryptone (0.5% w/v), yeast extract (0.5% w/v), potassium phosphate (0.1% w/v), Tween 80 (0.5% v/v), and agar (1.7% w/v) at pH 6.8. Plates were incubated at 37°C for 3-5 days (Sahoo et al., 2020).

Characterisation and identification of isolates

Isolates were characterised through Gram staining and comprehensive biochemical profiling, including enzyme assays (catalase, oxidase, and urease), IMVIC tests, triple sugar iron agar tests, and carbohydrate fermentation patterns, to establish morphological features and metabolic capabilities for taxonomic identification (L'Haridon et al., 2020).

Optimisation of growth conditions

Selected isolates were cultivated in brain heart infusion (BHI) broth to determine the optimal conditions for biomass accumulation and ascorbic acid production. Culture aliquots (200 µL) were inoculated into 15 mL BHI broth in triplicate. Temperature optimisation was conducted by incubating cultures at 4°C, 25°C, 30°C, 37°C, and 45°C for 7 days, while pH optimisation involved adjusting the fermentation medium to pH 4.0, 5.0, 6.0, and 7.0 using 0.1 N NaOH or 0.1 N HCl, with cultures incubated at the optimal temperature for 5 days. Growth was monitored by measuring optical density at 600nm with a UV-visible

spectrophotometer to identify conditions that yielded the maximum biomass and ascorbic acid production (Aswini et al., 2020).

Ascorbic acid production by fed-batch fermentation

Fed-batch fermentation was conducted using optimised *Acetobacter* strains with sorbitol as the primary carbon source. The production medium contained sorbitol (8% w/v), yeast extract (5% w/v), glycerol (0.05% v/v), magnesium sulfate (0.25% w/v), and calcium carbonate (1.5% w/v). Culture flasks were incubated at 30 °C with continuous agitation at 180 rpm for 96 hours. Following fermentation, the culture broth was centrifuged at 10,000 rpm for 15 minutes, and the cell-free supernatant was collected for ascorbic acid purification (Tucaliuc et al., 2022).

Identification and quantification of ascorbic acid

Thin-layer chromatography

Qualitative identification was performed using silica gel TLC plates with a mobile phase consisting of chloroform, ethanol, acetone, and ammonium hydroxide (2:2:2:1 v/v/v/v). The elution chamber was pre-saturated with solvent vapour for 10-15 minutes. Samples were spotted 6-8 mm from the edge of the plate using a micropipette. Elution continued until the solvent front advanced to 5-10 mm from the top edge. Plates were dried at 60 °C for 10 minutes and visualised under UV light (254 nm), where ascorbic acid exhibited characteristic blue fluorescence. Retention factor (Rf) values were calculated as :

$$\mathbf{Rf} = \mathbf{hx} / \mathbf{h_0}$$

Where hx is the distance from the origin to the spot centre, and h_0 is the distance from the origin to the solvent front. Blue fluorescent bands corresponding to ascorbic acid were scraped from the plate, dissolved in distilled water, and centrifuged at 10,000 rpm for 5 minutes to remove silica particles. The supernatant was filtered and used for quantitative analysis (Akasaka, 2013).

Iodometric titration

Ascorbic acid content was quantified by redox titration. TLC-purified samples (5 mL) were transferred to conical flasks containing 1 mL of starch indicator solution. The mixture was titrated against a standardised iodine solution (0.005 M) until the first persistent blue-black colouration appeared, indicating the formation of the starch-iodine complex. Titrations were performed in triplicate, and the results were recorded as concordant values (Belete et al., 2023).

2,6 – Dichlorophenolindophenol (DCPIP) assay

An alternative quantification method employed DCPIP dye reduction. Sample aliquots (5mL) were mixed with equal volumes of distilled water and titrated against standardised DCPIP solution. The endpoint

was marked by a persistent colour change from blue to pink, indicating complete oxidation of ascorbic acid. Titrations were performed in triplicate to ensure reproducibility (Nakamura et al., 2013).

Synthesis of iron oxide nanoparticles

Iron oxide nanoparticles were synthesised via the reverse co-precipitation method. Ferric chloride ($FeCl_3$, 0.8 g) and ferrous sulfate ($FeSO_4$, 2.75 g) were dissolved in 50 mL of deionised water under vigorous stirring. Sodium hydroxide (2.75 g) was added dropwise until the solution turned black, indicating the formation of magnetite (Fe_3O_4). The precipitate was collected by centrifugation at 10,000 rpm for 15 minutes and washed repeatedly with distilled water using a magnetic separation technique. The purified nanoparticles were dried in a hot-air oven at 80 °C (Mahmud et al., 2020).

Encapsulation of ascorbic acid onto iron oxide nanoparticles

The magnetic nanoparticle dispersion was adjusted to pH 12.5 with 10 M ammonium hydroxide to optimise the surface charge for ascorbic acid adsorption. Biosynthesised ascorbic acid (0.5 g) was added to the alkaline dispersion under continuous stirring. The mixture was maintained at 80 °C for 1 hour to facilitate stable coating formation through interaction between ascorbic acid functional groups and the hydroxylated iron oxide surface. The ascorbic acid-functionalised nanoparticles were recovered magnetically, washed with distilled water, and dried at 80 °C (Kumari & Chauhan, 2022b).

Characterisation of ascorbic acid-coated nanoparticles

Scanning electron microscopy (SEM)

Morphological characterisation was performed using scanning electron microscopy to visualise nanoparticle size, shape, and surface coating architecture. SEM analysis provides information on particle size distribution, surface topography, and the uniformity of ascorbic acid encapsulation at nanometre resolution (Mahmud et al., 2020).

Food preservation efficacy testing

The antimicrobial and preservative potential of ascorbic acid-coated nanoparticles was evaluated across multiple food matrices, including fruit juices (apple, grape, orange and banana) and dairy products (milk and curd) (Teshome et al., 2022). Three experimental groups were established for each food type:

1. **Control group**: Food products without preservatives.
2. **Commercial ascorbic acid group**: Products supplemented with commercially available ascorbic acid at standard concentrations.
3. **Nanoparticle group**: Products fortified with biosynthesised ascorbic acid coated iron oxide nanoparticles at equivalent ascorbic acid concentrations.

Samples were stored under standardised conditions, and microbial load was monitored using the viable plate count method. Serial dilutions (10^{-6} to 10^{-8}) were prepared, plated on nutrient agar, and incubated at 37 °C for 24 to 48 hours. Colony-forming units (CFU) were enumerated to assess antimicrobial efficacy and shelf-life extension across treatment groups.

RESULTS AND DISCUSSION

Isolation and characterisation of ascorbic acid-producing bacteria

Ascorbic acid-producing *Acetobacter* strains were isolated from deteriorated fruits and naturally fermented beverages. Samples were aseptically processed and enriched in a selective medium that promotes the growth of acetic acid bacteria, then transferred to GYC and CARR differential media for isolation and preliminary identification.

Cultural characterisation on GYC medium: Following 3-4 days of incubation at 37 °C, isolates exhibited characteristic colonial morphology on GYC medium. Colonies were large, mucoid, and slimy in texture, with milky-white to pale yellow colouration (Fig. 1). Clear halos formed around individual colonies due to the solubilization of calcium carbonate by metabolically produced organic acids. This zone of clearance indicated acid production, a characteristic metabolic feature of Acetobacter species, with observed morphology consistent with established profiles of acetic acid bacteria.

Cultural characterisation on CARR Medium: Complementary screening on CARR medium containing bromocresol green revealed that colonies initially appeared blue, transitioning to yellow as ethanol oxidation produced acetic acid (Fig.2). This colour change confirmed acid production and ethanol utilisation, characteristic metabolic features of *Acetobacter* species. Isolates tolerated ethanol concentrations of 5-9 % consistent with acetic acid bacteria.

Morphological and biochemical characteristics

Morphological characterisation through Gram staining and comprehensive biochemical profiling was performed to establish the taxonomic identity of the isolates. The results, including cell morphology, enzyme activities, metabolic patterns, and carbohydrate fermentation profiles, are presented in Table 1. The biochemical profile confirmed the isolates as *Acetobacter* species, particularly *Acetobacter aceti*, based on their characteristic aerobic respiratory metabolism and metabolic versatility, which is typical of acetic acid bacteria within the *Acetobacteraceae* family.

Table 1: Morphological and biochemical characteristics of the isolated strain

CHARACTERISTIC	RESULT
Colony morphology	**Large, mucoid, milky-white to pale yellow**
Gram reaction	**Gram-negative**
Cell shape	**Coccoid**
Catalase	**Positive**
Oxidase	**Positive**
Indole production	**Negative**
Methyl Red	**Negative**
Voges-Proskauer	**Positive**
Citrate utilization	**Positive**
Glucose fermentation	**Acid + Gas**
Lactose fermentation	**Acid + Gas**
Sucrose fermentation	**Acid + Gas**
Mannitol fermentation	**Acid + Gas**
Triple Sugar Iron	**Acid slant/Acid butt (A/A)**
Urease	**Negative**

Optimisation of culture conditions for ascorbic acid production

pH optimisation

The influence of pH on ascorbic acid biosynthesis was evaluated over a pH range of 4.0-7.0 (Fig. 3). Maximum production occurred at pH 4.0 and 6.0, representing optimal conditions for bacterial growth and enzymatic activity. Production declined substantially at pH values below 4.0 and above 7.0, reflecting the acidophilic nature of *Acetobacter* species and the optimal activity range of key biosynthetic enzymes.

Temperature optimisation

Temperature optimisation revealed maximum ascorbic acid production at 30 °C, with significantly lower yields at 4 °C and 40 °C (Fig. 4). This optimal temperature represents a balance between metabolic rate and enzyme stability characteristic of mesophilic *Acetobacter* species. Suboptimal temperatures (4 °C) reduced enzymatic activity, whereas 40 °C led to enzyme denaturation and cellular stress, limiting ascorbic acid biosynthesis

The isolation, characterisation, and optimisation studies established optimal conditions (pH 4.0-6.0, 30 °C) for ascorbic acid production by *Acetobacter* strains, providing parameters for subsequent fed-batch

fermentation and nanoparticle encapsulation applications.

Biosynthesis and analytical confirmation of ascorbic acid

Fed -batch fermentation production

Fed-batch fermentation using Acetobacter species with sorbitol as the carbon source achieved approximately 99 % substrate conversion after 96 hours. Ascorbic acid presence and concentration in the fermentation broth were confirmed through thin-layer chromatography, iodometric titration, and DCPIP dye reduction assay.

Qualitative identification by thin-layer chromatography

Thin-layer chromatography on silica gel using a chloroform-ethanol-acetone-ammonium hydroxide mobile phase confirmed the presence of ascorbic acid in the fermentation medium. The retention factor (Rf) was calculated using the standard formula:

$$R = hx/ho$$

Where hx represents the distance travelled by the substance from the origin (1.7 mm), and h_0 represents the distance travelled by the solvent front from the origin(2.5 mm)

Rf = 1.7 / 2.5 = 0.68

The calculated retention factor (Rf = 1.7/2.5 = 0.68) corresponded to ascorbic acid reference standards (Table 2). Under UV light at 254 nm, the separated compound exhibited characteristic blue fluorescence (Fig. 5), confirming the identification of ascorbic acid.

Table 2: Reference Retention Factors and UV Fluorescence of Vitamins

Vitamin	Common Name	Rf Value	Colour Under UV
B_1	Thiamine	0.40	Violet
B_2	Riboflavin	0.30	Yellow
B_3	Niacin (Nicotinic acid)	0.36	Violet
B_6	Pyridoxine	0.49	Blue
C	Ascorbic acid	<0.1*	Blue

*Reference value from standard vitamin chromatography protocols

Quantitative analysis by iodometric titration

Ascorbic acid content was determined by iodometric titration based on its oxidation to dehydroascorbic acid. The blue extract from TLC analysis (1g) was dissolved in 5mL of distilled water and titrated with standardised iodine solution in duplicate, showing reproducible results (Table 3).

Table 3: Iodometric Titration Results

S. No	Volume of the Sample	Burette reading (ml)		Volume of Iodine(mL)
		Initial	Final	
1	5 ml	0.0	1.25	1.25
2	5 ml	0.0	2.5	2.5

Iodometric titration of 5mL samples consumed an average of 1.9mL iodine solution (11.4 mL for 30 mL total volume). The endpoint was identified by the formation of a blue-black starch-iodine complex,

indicating complete oxidation of ascorbic acid and confirming its presence and reducing capacity in the fermentation medium.

Validation by 2,6-Dichlorophenolindophenol (DCPIP) assay

Ascorbic acid quantification employed DCPIP dye reduction, based on the conversion of blue DCPIP to its colourless form. Following standardisation with ascorbic acid in the fermentation media, the endpoint was determined by a stable pink colour (≥15 seconds). Titration of 5 mL samples consumed 2.8 mL DCPIP, corresponding to 16.8 mL for the complete 30 mL volume.

Three methods, TLC, iodometric titration, and DCPIP reduction, collectively confirmed ascorbic acid biosynthesis. TLC established identity via Rf values and UV response, while titrimetric approaches quantified concentration. Consistent findings across techniques validate the *Acetobacter*-mediated biotransformation of sorbitol to ascorbic acid as a sustainable production strategy, supporting future scale-up for nanoparticle encapsulation and food preservation applications.

Synthesis and characterisation of ascorbic acid-coated iron oxide nanoparticles

Iron oxide nanoparticle synthesis

Iron oxide nanoparticles were synthesised via coprecipitation using $FeCl_3$ and $FeSO_4$ as iron sources with ammonium hydroxide as the precipitating agent. This method produced black magnetite precipitates characteristic of Fe_3O_4 (Fig. 6), yielding magnetically responsive nanoparticles under ambient conditions for subsequent functionalization.

Ascorbic acid encapsulation

Iron oxide nanoparticles were surface-modified with ascorbic acid through pH and temperature-controlled encapsulation. The magnetic dispersion was adjusted to pH 12.5 with 10 N ammonia to optimise surface charge for ascorbic acid adsorption. Ascorbic acid (0.5 g) was added to the alkaline dispersion, and the mixture was heated at 80 °C with stirring for 1 hour to promote interaction between the ascorbic acid functional groups and the hydroxylated iron oxide surface. The coated nanoparticles were recovered by oven drying; the colour change indicated successful functionalization, reflecting the combined optical properties of the iron oxide core and the organic coating.

Morphological characterisation by SEM

SEM analysis revealed the nanoparticle morphology and coating structure at nanoscale resolution (Fig.8). The micrographs showed densely packed, spherical nanoparticles with a uniform size distribution. Ascorbic acid formed a conformal coating with complete surface coverage, demonstrating successful encapsulation without significant aggregation. The uniform distribution and spherical geometry confirmed the practicality of synthesis and encapsulation. This coating morphology provides structural protection for ascorbic acid while maintaining accessibility for functional applications.

Functional validation: Antioxidant activity retention

Ascorbic acid bioactivity post-encapsulation was verified using the methylene blue decolourisation assay. The addition of coated nanoparticles to the methylene blue solution resulted in rapid, complete decolourisation within minutes, as the dye reduced to its colourless leuco form. This transition confirmed that the encapsulated ascorbic acid retains its reducing capacity and antioxidant functionality, thereby maintaining both its nutritional value and chemical reactivity, which are essential for antimicrobial activity. The iron oxide core retained its magnetic properties, enabling efficient magnetic separation and recovery, a key advantage for industrial-scale applications. Characterisation results demonstrate successful synthesis of ascorbic acid-coated iron oxide nanoparticles with retained functionality, uniform morphology, and structural properties suitable for food preservation and nutritional fortification.

Antimicrobial efficacy and food preservation performance

Antimicrobial efficacy of biosynthesised ascorbic acid-coated iron oxide nanoparticles was evaluated across six food matrices: apple, orange, grape and banana juices, milk and curd. Viable plate count analysis compared untreated controls, nanoparticle-treated, and commercial ascorbic acid-treated samples (Fig.9, Table 4). Control samples exhibited uncontrolled microbial growth (TNTC) at 10^{-6} dilutions, confirming rapid spoilage. Commercial ascorbic acid reduced microbial loads to 2-12 CFU at 10^{-8} dilutions, while biosynthesised nanoparticles demonstrated substantial efficacy with 7-20 CFU at 10^{-8} dilutions across all tested matrices.

Table 4: Microbial Colony Counts in Treated and Untreated Food Samples

Sl. No	Food Sample	Dilution	Control (CFU)	Nano-encapsulated Ascorbic Acid (CFU)	Commercial Ascorbic Acid (CFU)	Remark
1	Apple juice	10^{-6}	TNTC	15	12	Highly effective
		10^{-7}	TNTC	13	7	Highly effective
		10^{-8}	TNTC	9	3	Highly effective
2	Grape juice	10^{-6}	TNTC	14	8	Highly effective
		10^{-7}	TNTC	11	5	Highly effective
		10^{-8}	TNTC	19	2	Highly effective
3	Orange juice	10^{-6}	TNTC	15	9	Highly effective
		10^{-7}	TNTC	13	7	Highly effective
		10^{-8}	TNTC	11	5	Highly effective
4	Banana juice	10^{-6}	TNTC	13	8	Highly effective
		10^{-7}	TNTC	12	6	Highly effective
		10^{-8}	TNTC	7	5	Highly effective
5	Milk	10^{-6}	TNTC	12	7	Highly effective
		10^{-7}	TNTC	10	6	Highly effective
		10^{-8}	TNTC	8	4	Highly effective
6	Curd	10^{-6}	TNTC	20	10	Highly effective
		10^{-7}	TNTC	15	7	Highly effective
		10^{-8}	TNTC	12	5	Highly effective

TNTC = Too Numerous To count. Nano-encapsulated ascorbic acid consistently reduced bacterial counts from TNTC to countable levels across all samples, demonstrating effective antimicrobial activity compared to the control (7-20 CFU).

Quantitative assessment of preservation efficacy

Comparative analysis revealed that biosynthesised ascorbic acid-coated nanoparticles achieved approximately 75% of the antimicrobial activity of commercial ascorbic acid across all tested food matrices, evidenced by consistently higher colony counts at equivalent dilutions. Despite this moderate reduction in potency, the nanoparticle formulation effectively suppressed microbial growth by several orders of magnitude compared with untreated controls, demonstrating its viability as a functional preservative. Antimicrobial performance varied across food matrices: banana juice exhibited the lowest colony count (7 CFU at 10^{-8}), while curd showed the highest (20 CFU at 10^{-6}; 12 CFU at 10^{-8}). These matrix-dependent differences reflect the influence of intrinsic food properties, including pH, nutrient composition, initial microbial load, and physicochemical characteristics, on preservation efficacy.

Dual functionality: Preservation and nutritional fortification

Beyond antimicrobial efficacy, the nanoparticle formulation demonstrated dual functional potential by simultaneously providing nutritional fortification. The iron oxide nanoparticle core serves as a bioavailable source of iron to address micronutrient deficiency, while the ascorbic acid coating provides vitamin C supplementation. This integrated approach delivers both preservation and nutritional enhancement within a single additive system, representing a significant advancement over conventional single-purpose preservatives. The controlled release characteristics imparted by nanoencapsulation offer additional functional advantages. The gradual liberation of ascorbic acid from the nanoparticle matrix potentially extends the effective preservation duration compared to free ascorbic acid, which may be rapidly consumed through oxidation or degradation reactions in food systems. Furthermore, the biosynthetic origin of the formulation aligns with consumer preferences for natural, clean-label ingredients while maintaining compatibility with existing food processing infrastructure.

Food matrix-specific performance

Analysis of preservation efficacy across different food categories revealed product-specific performance patterns. In fruit juices, acidic pH naturally complements the antimicrobial action of ascorbic acid, with all juice types showing effective microbial suppression. Orange and apple juices demonstrated intermediate colony counts (9-15 CFU at 10^{-6}), while banana juice exhibited the most effective suppression at higher dilutions. In dairy products, milk showed moderate colony counts (8-12 CFU across dilutions), while curd exhibited slightly elevated counts (12-20 CFU), potentially attributable to its inherent fermented nature and higher initial microbial populations. These results establish proof of concept for biosynthesised ascorbic acid-coated iron oxide nanoparticles as multifunctional food additives that extend shelf life while simultaneously enhancing nutritional value. The formulation

demonstrates practical applicability across diverse food matrices, with performance levels suitable for commercial food preservation applications.

FUTURE SCOPE

The development of biosynthesised ascorbic acid-coated oxide nanoparticles provides a foundation for future commercial translation. Priority research areas include optimising encapsulation through advanced characterisation (TEM, XRD, FTIR), scaling production via continuous fermentation and automated reactors, and establishing stability profiles under storage and processing conditions. Food matrix-specific formulations require investigation across beverages, dairy, high-fat products, and bakery applications to maximise functionality while minimising sensory impacts. Rigorous toxicological evaluation, following guidelines from the FDA, EFSA, and FSSAI, including acute, chronic, genotoxicity, and reproductive toxicity studies, is essential for regulatory approval, alongside sensory evaluation and consumer acceptance testing. The nano-encapsulation platform offers opportunities to extend the shelf life of other unstable bioactives, such as B-complex vitamins, vitamin E, polyphenols, omega-3 fatty acids, and natural antimicrobials. Stimuli–responsive coatings triggered by pH, temperature, or microbial presence could enable intelligent preservation systems. Human bioavailability studies would validate the nutritional benefits, particularly the synergistic effects of combined vitamin C and iron supplementation in populations prone to deficiency. Life cycle analysis, waste-stream behaviour assessment, and the development of biodegradable alternatives would address environmental stability. Integration with active packaging technologies could create comprehensive preservation systems, transforming this proof of concept into a commercially viable, regulatory-compliant solution for the industry.

CONCLUSION

This study developed an integrated bioprocess for producing nano-encapsulated ascorbic acid, a dual-functional food additive for preservation and nutritional fortification. Ascorbic acid-producing *Acetobacter* strains isolated from natural sources were characterised through morphological and biochemical analyses. Fermentation optimisation identified pH 4.0–6.0 and 30°C as optimal conditions, achieving 99% substrate conversion within 96 hours. Biosynthesis was validated via thin-layer chromatography (Rf 0.68), iodometric titration, and DCPIP assay. Iron oxide nanoparticles synthesised by co-precipitation were functionalised with biosynthesised ascorbic acid, producing a uniform coating architecture, as confirmed by SEM. Preservation testing across fruit juices and dairy products showed nano-encapsulated formulations achieved approximately 75% antimicrobial activity relative to commercial ascorbic acid, reducing microbial loads from uncountable levels to 7–20 CFU at 10^{-8} dilutions while providing vitamin C and iron fortification. The approach offers advantages in sustainable biological production, enhanced stability through nanoencapsulation, dual micronutrient fortification, and multimodal antimicrobial action. Successful translation from the laboratory to functional food applications demonstrates commercial viability through scalable fermentation and straightforward nanoparticle synthesis, applicable across diverse food matrices. Future work should optimise encapsulation efficacy, assess long-term stability and safety, evaluate sensory properties, and develop

industrial-scale protocols. This proof of fortification agent aligns with clean-label trends and sustainability requirements.

Abbreviations

% Percentage

A/A Acid slant and Acid butt

BHA Butylated Hydroxyanisole

BHT Butylated Hydroxytoluene

CFU Colony Forming Unit

DCPIP 2,6 – Dichlorophenolindophenol

DNA Deoxyribonucleic Acid

EFSA European Food Safety Authority

et al. et alii (and others)

FAO Food and Agriculture Organisation

FDA Food and Drug Administration

FSSAI Food Safety and Standards Authority of India

FTIR Fourier Transform Infrared spectroscopy

GYC Glucose Yeast Calcium carbonate

GYP Glucose Yeast Peptone

IMViC **I**ndole, **M**ethyl Red, **V**oges-Proskauer, and **C**itrate

nm Nanometres

pH Potential of Hydrogen

Rf Retention factor

rpm Rotation per Minute

SEM Scanning Electron Microscopy

TBHQ Tert-butylhydroquinone

TEM Transmission Electron Microscopy

TLC Thin-Layer Chromatography

TNTC Too Numerous To Count

U.S. United States

UV Ultraviolet

w/v weight/volume

w/w weight/weight

XRD X-ray diffraction

Declarations

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CONTRIBUTION OF AUTHORS

Catherine Antony: Conceptualisation, Methodology, Experimental design, Data handling, Writing - original draft and editing

Dr Krishnaveni N: Validation, supervision and critical review

CONFLICT OF INTEREST

The authors declare no competing interests.

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DATA AVAILABILITY

The data generated and analysed during the study are included in the manuscript.

ETHICS STATEMENT

Not Applicable

CONCENT TO PARTICIPATE

Not Applicable

CONCENT TO PUBLISH

Not Applicable

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Figures

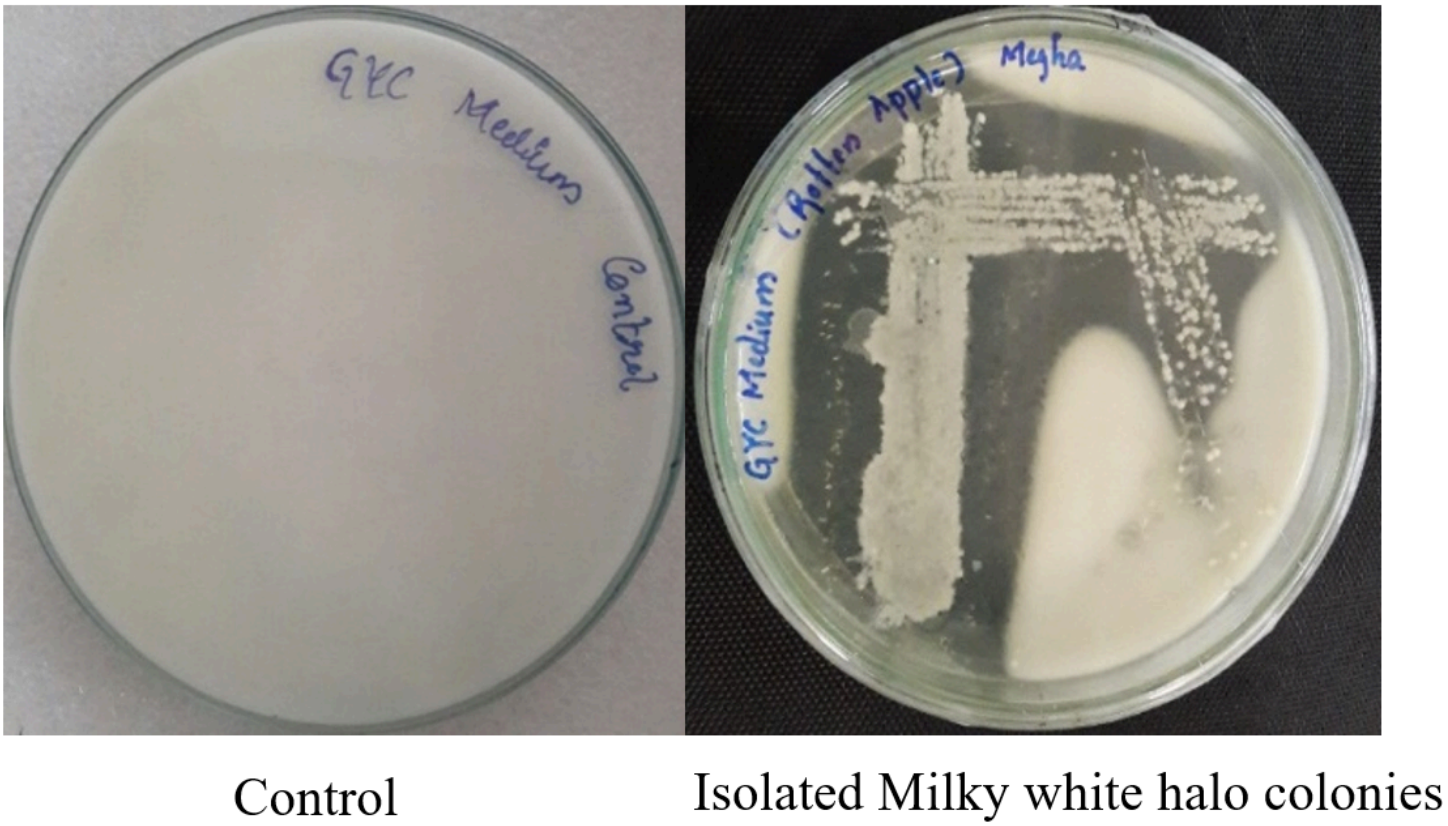


Figure 1

Isolated *Acetobacter* from fruit juice

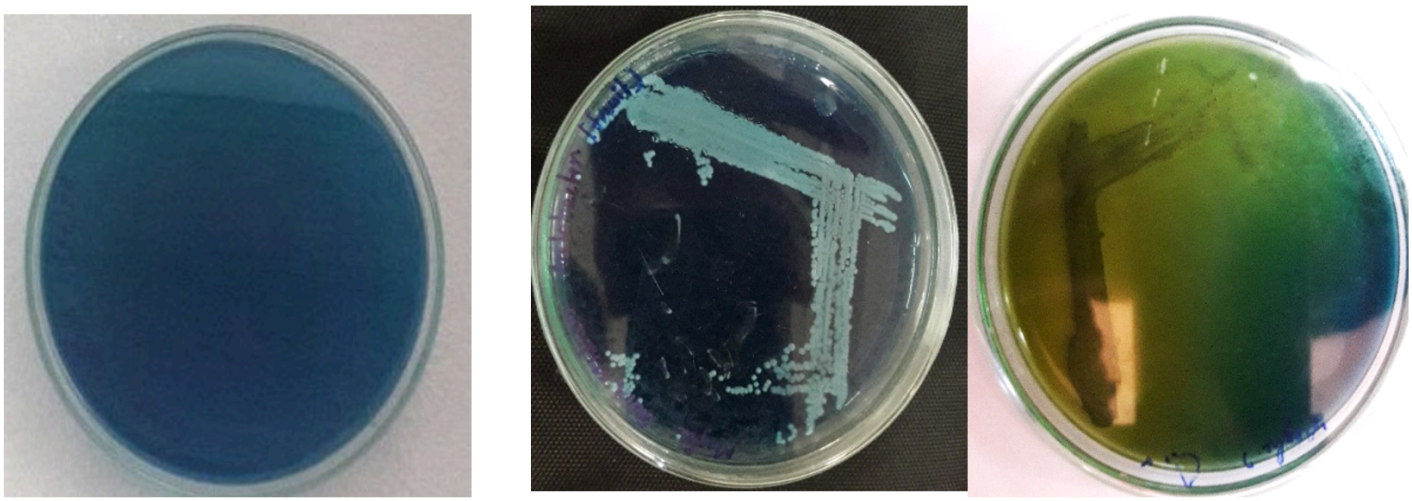


Figure 2

Isolated Acetobacter colonies appeared blue initially and turned yellow

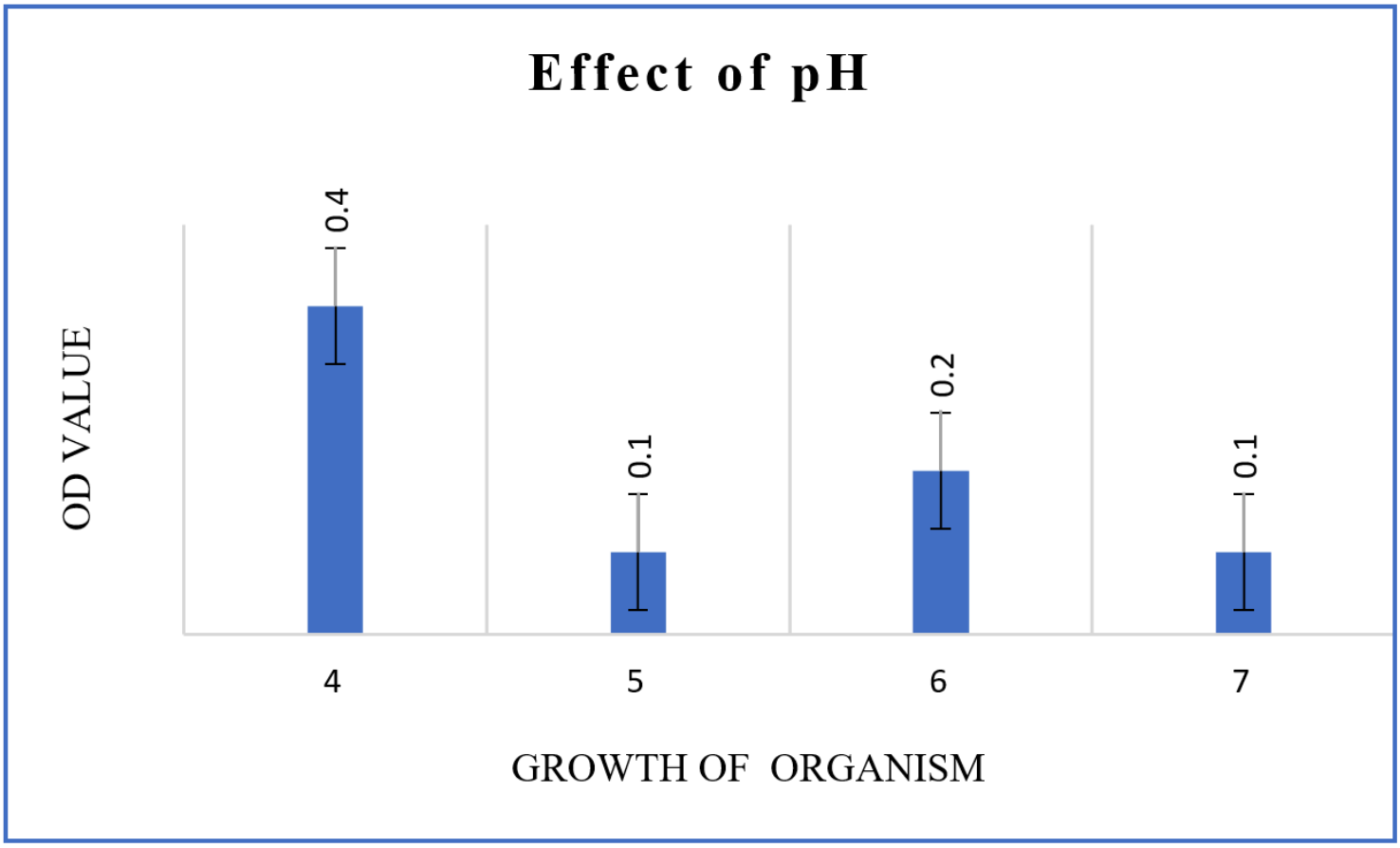


Figure 3

pH Optimisation

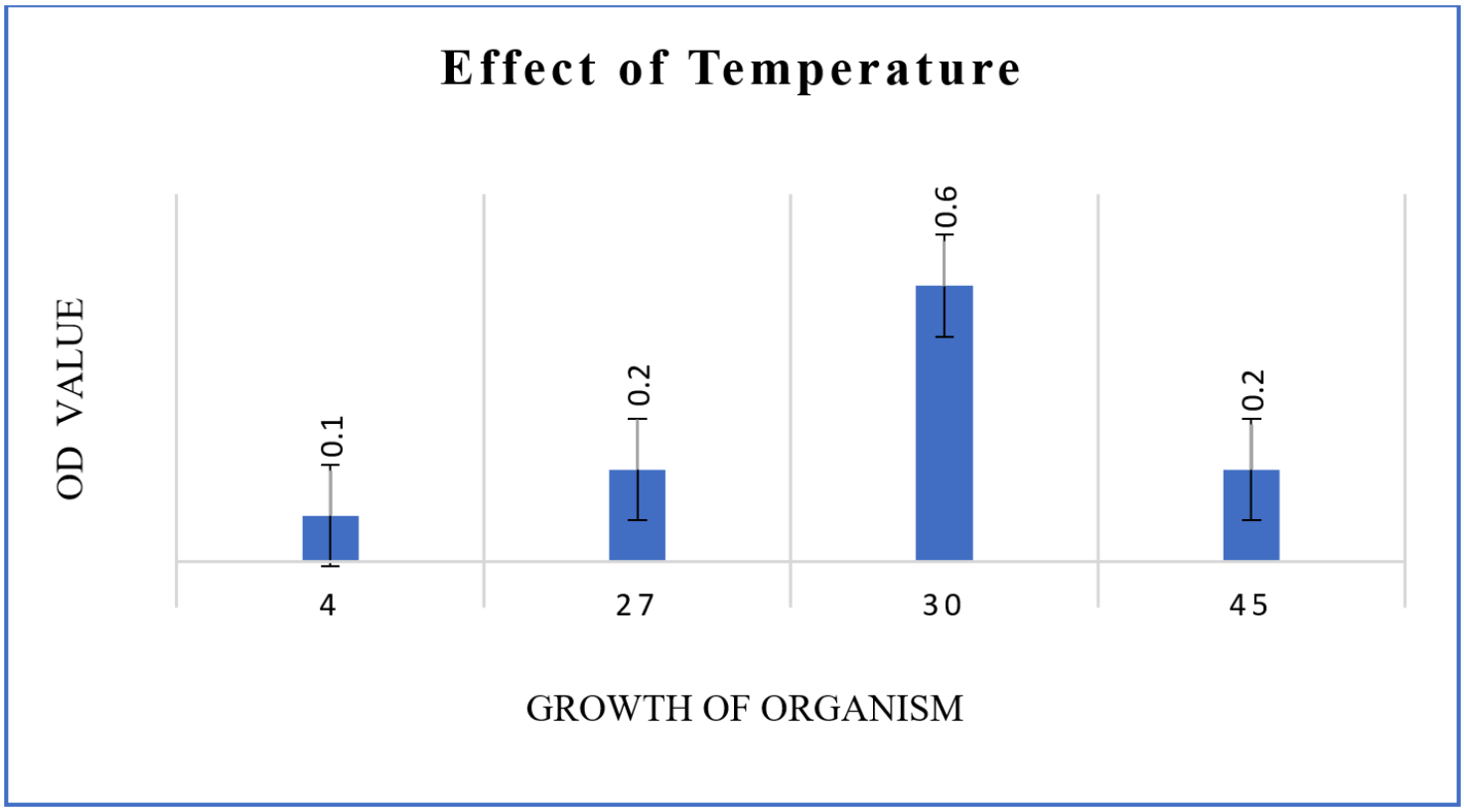


Figure 4

Temperature Optimisation

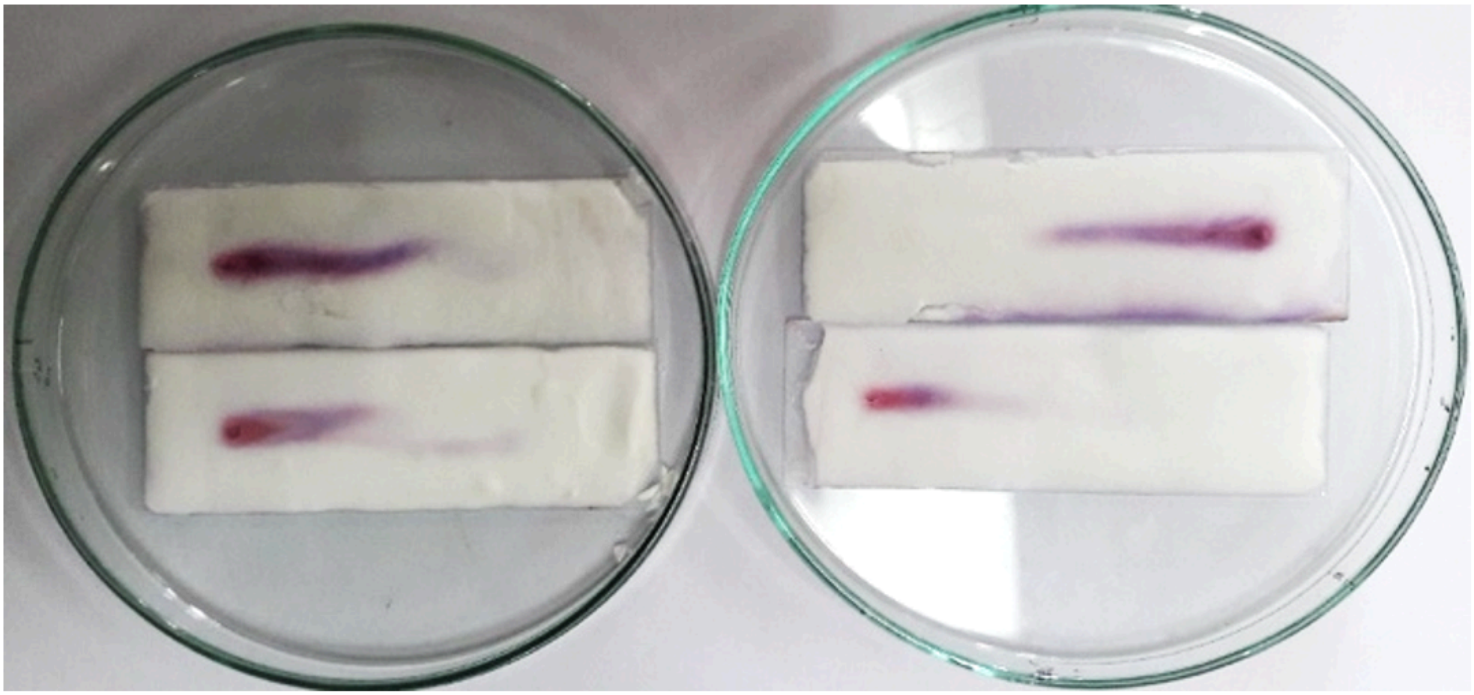


Figure 5

Thin-layer chromatography



Figure 6

Synthesis of iron oxide nanoparticle



Figure 7

Iron oxide nanoparticle encapsulated with Ascorbic acid

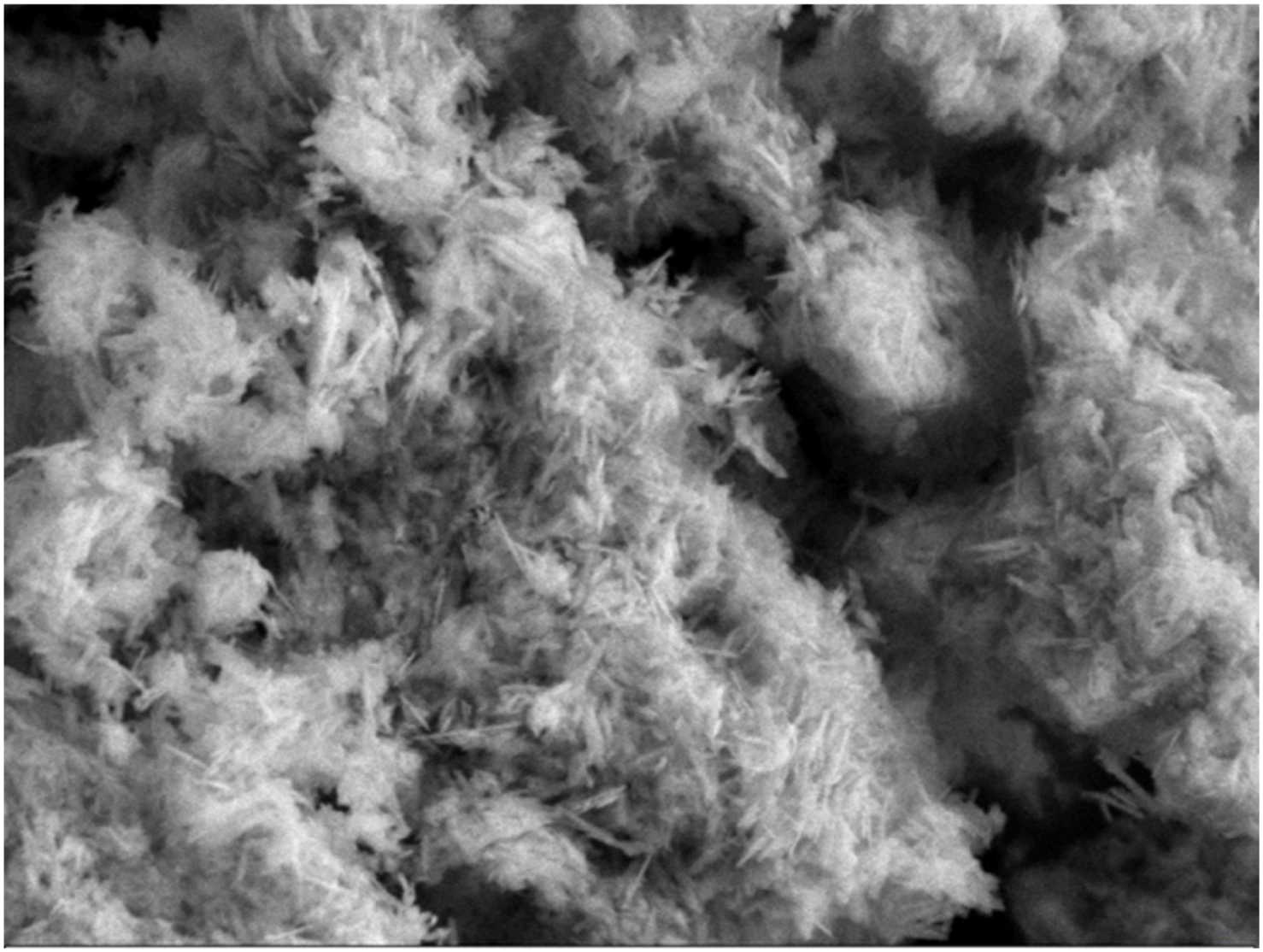


Figure 8

Scanning Electron Microscopic view of iron oxide encapsulated ascorbic acid

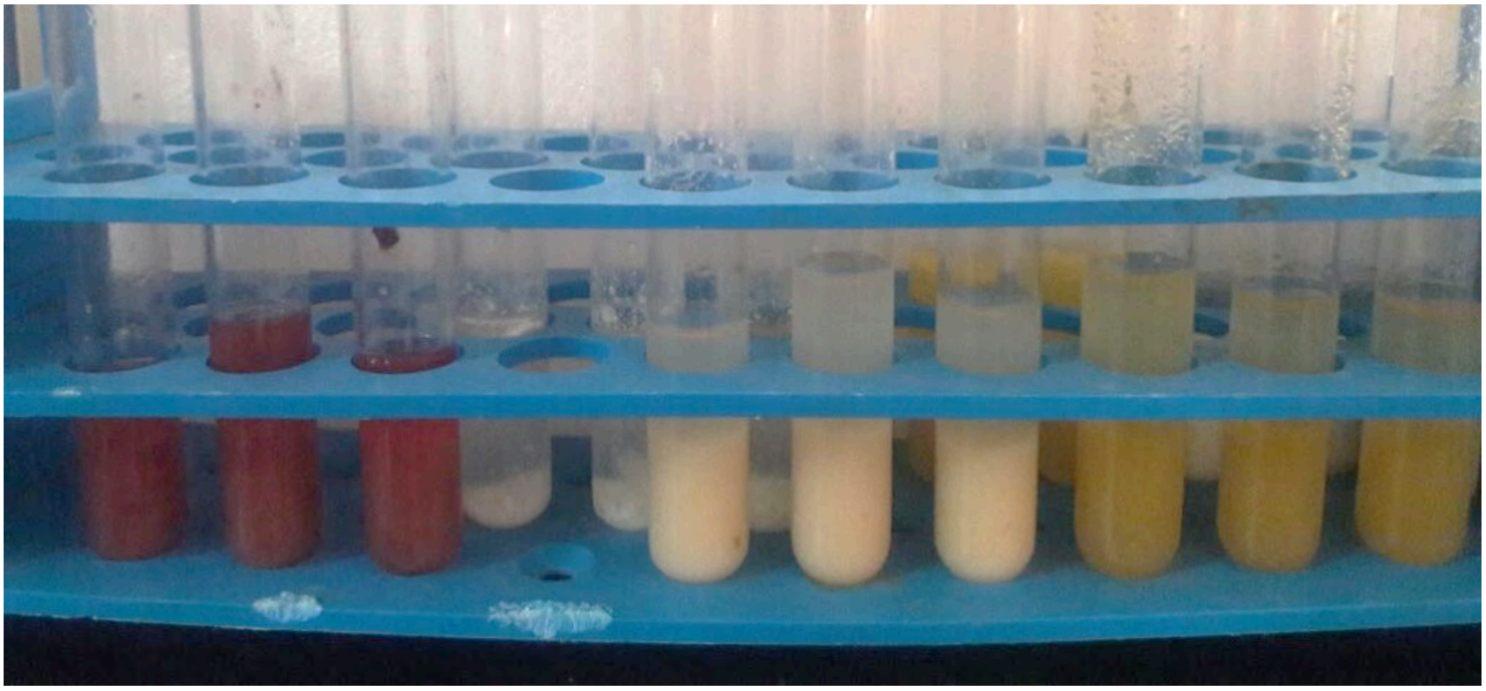


Figure 9

Comparative Preservation Activity Across Food Matrix