Preparation, Development and Evaluation of Whole Apple Matrix Cider Vinegar.

Abstract

Apple cider vinegar (ACV) is an ancient fermented functional food produced through a sequential two-stage microbial bioconversion of apple-derived sugars, first into ethanol and subsequently into acetic acid. This study was designed toprepare ACV from fresh apple pulp,develop three distinct flavored formulations — Mint, Green Chilli, and Apple Pulp with Honey and Lemon — and carry out proximate analysis, sensory evaluation, mineral profiling, and scanning electron microscopy (SEM) characterization of the prepared samples. Proximate analysis of the base ACV revealed moisture (98.76%), ash (0.22%), protein (0.20%), fat (0.001%), and carbohydrates (0.81%). Mineral analysis indicated the presence of Potassium (80 mg/100g), Phosphorus (10 mg/100g), Calcium (3.30 mg/100g), and Iron (0.51 mg/100g). Sensory evaluation conducted on a nine-point hedonic scale among 30 semi-trained panelists showed that Sample D (Apple Pulp with Honey and Lemon flavor) achieved the highest overall acceptability score (8.13 ± 0.82), followed by Sample A — plain ACV (7.70 ± 0.75), Sample C — Green Chilli (7.47 ± 1.04), and Sample B — Mint (7.40 ± 0.67). SEM micrographs at varying magnifications confirmed the presence of characteristic rod-shaped acetic acid bacterial cells, chain-forming aggregates, and early biofilm matrix structures, validating a biologically active fermentation product. These findings collectively position flavored ACV as a nutritionally viable and sensorially acceptable functional beverage with significant potential in nutraceutical applications.

Keywords: Apple cider vinegar, Fermentation, Proximate Analysis, Sensory Evaluation, Mineral Content, SEM, FlavoredVinegar, Acetic Acid Bacteria.

1. Introduction

Vinegar represents one of the oldest biochemically derived liquids in human history, documented across Babylonian, Egyptian, Greek, and Chinese civilizations for its dual role as a culinary

condiment and therapeutic agent. The term originates from the Old French *vin aigre* ("sour wine"), and the product is broadly defined as a liquid suitable for human consumption produced from suitable agricultural raw materials containing fermentable carbohydrates (Nilgun H. Budak et al., 2014). Apple cider vinegar (ACV) specifically is obtained through the controlled fermentation of *Malus domestica* apple juice or pulp via a characteristic two-stage process: an initial anaerobic alcoholic fermentation by *Saccharomyces cerevisiae*, converting fruit sugars into ethanol, followed by an obligate aerobic acetic acid fermentation by *Acetobacter* and *Komagataeibacter* species, oxidizing ethanol into acetic acid.

The bioactive profile of ACV extends well beyond simple acidity. Raw, unfiltered ACV retains a rich matrix of phenolic acids (chlorogenic, caffeic, gallic, p-coumaric acid), flavonoids (quercetin, catechin, epicatechin, phlorizin), organic acids (malic, citric, tartaric, lactic, succinic), trace vitamins (B1, B2, B6, C), and dietary minerals (Jeanelle Boyer & Rui Hai Liu, 2004). The presence of the "Mother of Vinegar" — a nanocellulose biofilm housing viable acetic acid bacteria — further amplifies the probiotic and health-promoting potential of the unfiltered product.

Contemporary public health challenges driven by metabolic syndrome, type 2 diabetes mellitus, obesity, and antimicrobial resistance have catalyzed renewed scientific interest in ACV as a low-cost, accessible functional food adjunct. Clinical investigations have documented ACV's capacity to improve insulin sensitivity by 19–34% in pre-diabetic individuals (Johnston et al., 2004), reduce serum total cholesterol by an average of -6.06 mg/dL (Hadi et al., 2021), and demonstrate antimicrobial efficacy against *Escherichia coli* O157:H7 and *Salmonella typhimurium*. However, the literature reveals a significant gap in empirical data on the physicochemical and sensory characteristics of flavored ACV formulations, particularly those incorporating traditional Indian botanicals such as mint and green chilli.

This study therefore addresses three specific objectives: (1) to prepare ACV from apple pulp through natural fermentation; (2) to develop three nutritious and sensorially distinct flavored variants; and (3) to conduct proximate analysis using AOAC methods (Ranganna, 2005) and evaluate sensory acceptability using a standardized hedonic scale, with supplementary mineral profiling and SEM characterization.

2. Materials and Methods

2.1 Locale of the Study

All experimental work was conducted in the Food Science Analysis Laboratory and Food Microbiology Laboratory, School of Home Science, Babasaheb Bhimrao Ambedkar University (BBAU), Lucknow, Uttar Pradesh, India, from September 2025 to May 2026.

2.2 Raw Materials

Fresh apples (*Malus domestica*) were procured from the local market near BBAU campus, Lucknow, using simple random sampling. Additional ingredients — granulated sugar, fresh mint leaves, green chillies, honey and lemon — were similarly sourced from local Lucknow markets. Glass jars and muslin cloth were used as primary fermentation and filtration vessels.

2.3 Preparation of Apple Cider Vinegar from Apple fruit

2.3.1 Ingredients

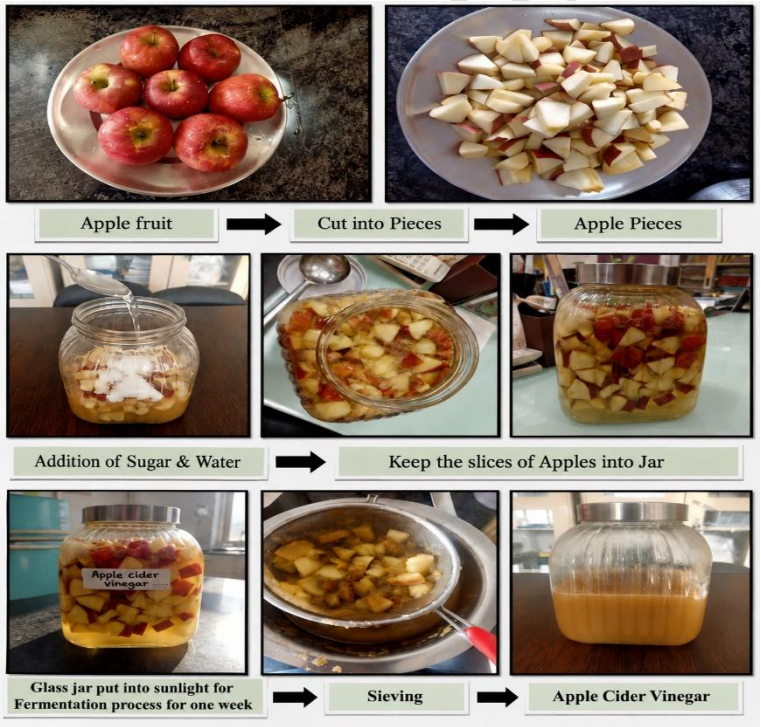
Ingredient	Quantity
Fresh apple fruit	1.5 kg
Granulated sugar	60 g
Potable water	2 liters

2.3.2 Procedure

Fresh, ripe apples were first sorted to remove any damaged or bruised fruit, then washed thoroughly under running water to eliminate surface contaminants. The cleaned apples were chopped into small uniform pieces, including the peel, to maximize the surface area available for fermentation and polyphenol extraction. The cut apple pieces were transferred into a clean glass jar along with the weighed quantities of sugar and water. The top of the glass jar was loosely covered with a swatch of breathable muslin cloth and secured with a rubber band, allowing the passage of air while preventing dust and insect contamination.

The prepared jar was placed under indirect sunlight for one full week to initiate and sustain natural fermentation. During this period, indigenous yeasts naturally present on the apple skin (*Saccharomyces cerevisiae* and related wild strains) converted the available sugars into ethanol under anaerobic conditions, producing alcoholic cider. As fermentation progressed, naturally occurring acetic acid bacteria (*Acetobacter* spp.) subsequently oxidized the ethanol into acetic acid under aerobic conditions at the liquid-air interface. The progression of fermentation was monitored daily for visible signs such as effervescence, characteristic color change from clear to amber-gold, and the gradual development of a pungent acidic aroma.

Upon completion of the fermentation cycle at one week, the fermented material was strained through a double layer of muslin cloth to separate the liquid ACV from the spent apple pulp. The clarified vinegar was collected into sterile glass bottles, sealed, and stored at room temperature in a cool, dark place pending analysis.



Flow Chart — Preparation of Apple Cider Vinegar from Apple Pulp:

Fresh Apple Selection & Washing

↓

Cutting into Small Pieces

↓

Loading into Glass Jar (with sugar + water)

↓

Covering with Muslin Cloth

↓

Fermentation Under Sunlight (7 Days)

Stage 1: Alcoholic Fermentation (Yeast → Ethanol + CO_2)

Stage 2: Acetic Acid Fermentation (Acetobacter → Acetic Acid)

↓

Straining Through Muslin Cloth

↓

Collection of Apple Cider Vinegar

↓

Storage in Sterile Glass Bottles

2.4 Preparation of Different Flavored ACV Variants

Three distinct flavored ACV formulations were developed to enhance palatability, nutritional density, and sensory appeal of the base vinegar.

2.4.1 Sample B — Mint Flavor ACV

Ingredient	Volume
Apple cider vinegar	60 ml
Water	60 ml
Mint puree	30 ml

Lemon juice	20 ml
Honey	30 ml

Procedure: All measured ingredients were combined in a large jug. The mixture was vigorously whisked until the honey dissolved completely and all components were uniformly incorporated. The formulation was served fresh or chilled in a refrigerator before serving.

Flow Chart — Mint Flavor ACV:

Measure ACV (60 ml) + Water (60 ml) + Mint Puree (30 ml) + Lemon Juice (20 ml) + Honey (30 ml)

↓

Combine in Large Jug

↓

Whisk Until Honey Dissolves and Mixture is Uniform

↓

Serve Fresh or Refrigerate Before Serving

2.4.2 Sample C — Green Chilli Flavor ACV

Ingredient	**Volume**
Apple cider vinegar	60 ml
Water	70 ml
Green chilli puree	20 ml
Lemon juice	20 ml

Honey	30 ml

Procedure: All measured ingredients were combined and mixed well until the preparation was uniform. The formulation was served immediately or chilled for a few hours to allow flavor infusion before consumption.

Flow Chart — Green Chilli Flavor ACV:

Measure ACV (60 ml) + Water (70 ml) + Green Chilli Puree (20 ml) + Lemon Juice (20 ml) + Honey (30 ml)

↓

Mix Well to Combine

↓

Serve Immediately or Chill for 2 Hours

2.4.3 Sample D — Apple Pulp with Honey and Lemon Flavor ACV

Ingredient	**Volume**
Apple cider vinegar	60 ml
Apple pulp	60 ml
Water	40 ml
Lemon juice	10 ml
Honey	30 ml

Procedure: All ingredients were measured and added to a mixing vessel. The mixture was stirred and then whisked vigorously for approximately 30 seconds until all components were thoroughly incorporated into a uniform, slightly viscous formulation.

Flow Chart — Apple Pulp with Honey and Lemon Flavor ACV:

Measure ACV (60 ml) + Apple Pulp (60 ml) + Water (40 ml) + Lemon Juice (10 ml) + Honey (30 ml)

↓

Stir and Whisk for 30 Seconds

↓

Serve Immediately

3.5 Proximate Analysis and Sensory Evaluation

3.5.1 Proximate Analysis

All proximate analyses were performed on the plain ACV (Sample A) as the primary test sample using standard AOAC methods described in Ranganna (2005).

a) Moisture Content

Moisture content was determined gravimetrically in accordance with IS 12711 (1989) RA 2005. Exactly 5 g of each sample was accurately weighed into pre-weighed, pre-dried petri dishes. The dishes were placed in a hot air oven maintained at 105 °C for 4 hours. After drying, the dishes were transferred to a desiccator containing anhydrous silica gel, cooled to room temperature, and weighed. The moisture content was calculated as:

$$\% \text{ Moisture} = \frac{W_1 - W_2}{W_1} \times 100$$

where W_1 = weight of sample before drying, and W_2 = weight of sample after drying.

b) Ash Content

Ash content was estimated by the dry ashing technique using a muffle furnace. Pre-dried sample residues from moisture determination were transferred to pre-weighed silica crucibles and

subjected to ignition in a muffle furnace at 550 °C for approximately 5–6 hours until a constant, grey-white, carbon-free ash residue was obtained. The crucibles were cooled in a desiccator and weighed. Ash percentage was calculated based on the weight of the residue relative to the dry weight of the original sample.

$$\% \text{ Ash} = \frac{W_{\text{ash}}}{W_{\text{dry sample}}} \times 100$$

c) Crude Protein Content

Crude protein was determined using the standard macro-Kjeldahl method. A 5 g sample was subjected to acid digestion in concentrated sulphuric acid with a selenium-based catalyst mixture (K_2SO_4: $CuSO_4$: Se = 100:10:1) in the digestion unit. The resultant ammonium sulphate in the digest was distilled in the Kjeldahl distillation unit after the addition of excess 40% NaOH. The liberated ammonia was collected in 2% boric acid and titrated against 0.1N HCl using mixed indicator (methyl red + bromocresol green). Crude protein was calculated by multiplying total nitrogen by the factor 6.25:

$$\% \text{ Crude Protein} = \%N \times 6.25$$

d) Crude Fat Content

Crude fat was determined by continuous solvent extraction using the Soxhlet apparatus. Approximately 5 g of moisture-free sample was wrapped in pre-weighed Whatman filter paper and placed in the Soxhlet thimble. Petroleum ether (boiling range 40–60 °C) was used as the extraction solvent over a 6–8-hour reflux cycle. After extraction, the solvent was evaporated, and the flask containing the extracted fat was dried in an oven at 105 °C for 30 minutes, cooled, and weighed. Fat content was expressed as the percentage weight of ether extract:

$$\% \text{ Crude Fat} = \frac{W_{\text{fat extracted}}}{W_{\text{sample}}} \times 100$$

e) Carbohydrate Content (By Difference)

Total carbohydrates were calculated by difference, subtracting the sum of moisture, ash, crude protein, and crude fat percentages from 100:

$$\% \text{ Carbohydrates} = 100 - (\%\text{Moisture} + \%\text{Ash} + \%\text{Protein} + \%\text{Fat})$$

3.5.2 Mineral Content Analysis

Mineral analysis (Iron, Calcium, Phosphorus, and Potassium) was performed on the ACV sample using standard Atomic Absorption Spectrophotometry (AAS) following wet acid digestion of the sample. Results were expressed as mg per 100 g of sample.

3.5.3 Scanning Electron Microscopy (SEM)

The morphological ultrastructure of the ACV sample was characterized using a Scanning Electron Microscope. ACV samples were fixed, dehydrated through a graded ethanol series, critical-point dried, gold-sputter coated, and examined at various magnifications (×550, ×1000, ×3000, and ×5000) to visualize the bacterial populations, biofilm matrix structures, and particulate components present within the fermented matrix.

3.5.4 Sensory Evaluation

Sensory evaluation was conducted using a standardized 9-point hedonic scale (1 = Dislike extremely; 9 = Like extremely) in accordance with the protocol of Kiran Bala & Reena Gupta (2020). A semi-trained panel of 30 volunteers was randomly selected from the university campus population. Each panelist evaluated all four samples — Sample A (plain ACV), Sample B (Mint flavor), Sample C (Green Chilli flavor), and Sample D (Apple Pulp with Honey and Lemon flavor) — under uniform lighting conditions and at room temperature. The samples were presented in coded, opaque cups in a balanced order to minimize carry-over effects. Panelists assessed five organoleptic attributes: appearance, taste, smell, mouthfeel, and overall acceptability. Statistical analysis was performed to determine mean scores and standard deviations (±SD) for each attribute and sample.

4. Results and Discussion

4.1 Proximate Analysis of Apple Cider Vinegar

The proximate composition of the prepared ACV (Sample A) was determined using AOAC methods (Ranganna, 2005). The results are presented in Table 1.

Table 1. Proximate Composition of Prepared Apple Cider Vinegar (Sample A)

Parameter	Result (%)
Moisture	98.76
Crude Protein	0.20
Crude Fat	0.001
Ash	0.22
Carbohydrates	0.81

The moisture content of 98.76% is characteristic of a fermented liquid matrix and is consistent with values reported in literature, where Faznira Zakaria et al. (2014) documented 98.49% moisture in comparable apple vinegar samples. The extremely low-fat content (0.001%) confirms the negligible lipid contribution of fruit vinegars, while the modest ash content (0.22%) reflects the mineral residue contributed by the apple substrate and fermentation by-products. The crude protein value of 0.20% is largely attributable to the residual acetic acid bacteria and yeast biomass retained in the unfiltered matrix. Total carbohydrates (0.81%) represent the residual fermentable and non-fermentable sugars not consumed during the acetification process.

These results collectively confirm the successful production of a standard-quality ACV and are well within the reference ranges established for artisanal fruit vinegars (Osualao Oluchi Judith et al., 2021; Faznira Zakaria et al., 2014).

4.2 Sensory Evaluation

A panel of 30 semi-trained assessors evaluated all four samples on a 9-point hedonic scale.

4.3 Mean and Standard Deviation of Sensory Attributes

The aggregate mean scores and standard deviations for all four samples across all five evaluated attributes are summarized in Table 6.

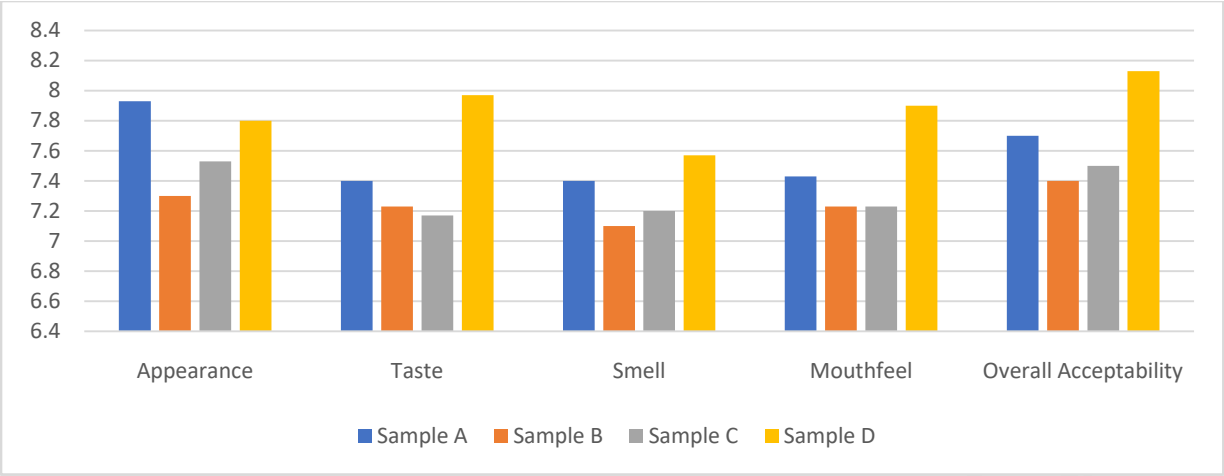
Table 6. Mean ± Standard Deviation of Sensory Attributes across All ACV Samples

Attribute	Sample A (Plain ACV)	Sample B (Mint)	Sample C (Green Chilli)	Sample D (Apple Pulp + Honey + Lemon)
Appearance	7.93 ± 0.78	7.30 ± 0.75	7.53 ± 0.86	7.80 ± 0.76
Taste	7.40 ± 0.72	7.23 ± 0.86	7.17 ± 0.91	7.97 ± 0.96
Smell	7.40 ± 0.77	7.10 ± 0.84	7.20 ± 0.81	7.57 ± 0.90
Mouthfeel	7.43 ± 0.86	7.23 ± 0.73	7.23 ± 0.97	7.90 ± 0.71
Overall Acceptability	7.70 ± 0.75	7.40 ± 0.67	7.50 ± 1.04	8.13 ± 0.82

Sample D (Apple Pulp with Honey and Lemon flavor ACV) recorded the highest overall acceptability score of 8.13 ± 0.82, followed by Sample A (plain ACV) at 7.70 ± 0.75, Sample C (Green Chilli) at 7.50 ± 1.04, and Sample B (Mint) at 7.40 ± 0.67. These findings are consistent with those of Rakesh Sharma et al. (2020) and Akanksha Yadav & Sunita Mishra (2023), who demonstrated that incorporation of honey into ACV formulations substantially improves color depth, textural viscosity, and overall palatability, as honey functions as a natural humectant and flavorstabilizer. The relatively lower scores for Sample B (Mint) are likely attributable to the dominant volatile menthol aroma partially suppressing the perception of the acetic acid base rather than enhancing it in certain panelists, consistent with observations by Kiran Bala & Reena Gupta (2020).

The graphical representation of mean sensory scores across all samples is illustrated below:

Graph — Mean Sensory Score Comparison (All Samples):



4.4 Mineral Content of Apple Cider Vinegar

Elemental analysis of the prepared ACV sample was conducted using standard Atomic Absorption Spectrophotometry, and the mineral concentrations are reported in Table 7.

Table 7. Mineral Content in Prepared Apple Cider Vinegar

Mineral	Concentration (mg/100g)
Iron	0.51
Calcium	3.30
Phosphorus	10.00
Potassium	80.00

Potassium was the most abundant mineral detected at 80 mg/100g, which is consistent with the established literature for fruit-based vinegars, where Faznira Zakaria et al. (2014) reported Potassium concentrations ranging from 66.65 to 205 mg/100g in apple vinegar using atomic absorption spectroscopy. Phosphorus was the second most concentrated mineral at 10 mg/100g, contributing to the vinegar's role as a dietary electrolyte source. Calcium (3.30 mg/100g) and Iron (0.51 mg/100g) were present at comparatively lower concentrations, reflecting their typical distribution in apple-derived fermented liquids. These mineral values confirm that ACV, despite its predominantly aqueous composition, provides a modest but physiologically relevant mineral

contribution. Potassium in particular supports cardiovascular function and electrolyte balance, further reinforcing the nutraceutical value of the product.

4.5 Scanning Electron Microscopy (SEM) Characterization

Scanning electron microscopy was employed to characterize the morphological ultrastructure of the ACV sample at multiple magnifications. The SEM analysis provided visual evidence of the living microbial community and structural matrices within the product.

SEM Observations:

- **At ×3,000 magnification (Figure 1):**Rod-shaped bacterial cells arranged in characteristic chain-like aggregates were observed, alongside irregularly shaped particulate matter and scattered smaller debris, consistent with the morphology of acetic acid bacteria (*Acetobacter* spp.).

- **At ×3,000 magnification (Figure 2):**Large, flattened, lobulated aggregate structures were visible, surrounded by densely distributed ellipsoidal bacterial cells. This architecture is characteristic of extracellular polymeric substance (EPS) secretion, indicating active biofilm development within the ACV matrix.

- **At ×1,000 magnification (Figure 3):**Mixed populations of rod-shaped bacterial cells and spherical-to-ovoid aggregates were identified, with clear evidence of early biofilm chain formation and extracellular matrix deposition — structures associated with the "Mother of Vinegar."

- **At ×5,000 magnification (Figure 4):**High-resolution imaging revealed long chain-forming rod-shaped cells, a central dense aggregate mass with irregular surface topography, and evidence of cellular adhesion and incipient biofilm matrix formation.

- **At ×550 magnification (Figure 5):**A low-magnification overview displayed dispersed bacillary microorganisms, micro-aggregate clusters, and a heterogeneous particulate background representative of the raw unfiltered ACV matrix.

These SEM findings collectively confirm the biological activity of the prepared ACV, validating the presence of a viable acetic acid bacterial community and an intact extracellular nanocellulose biofilm matrix. The biofilm-associated structures observed are consistent with the bacterial cellulose "Mother of Vinegar" described extensively in the literature, composed of β-1,4-linked glucopyranosyl nanofibers capable of retaining up to 200 times their dry mass in water and providing a protective microenvironment for AAB cells (Nilgun H. Budak et al., 2014).

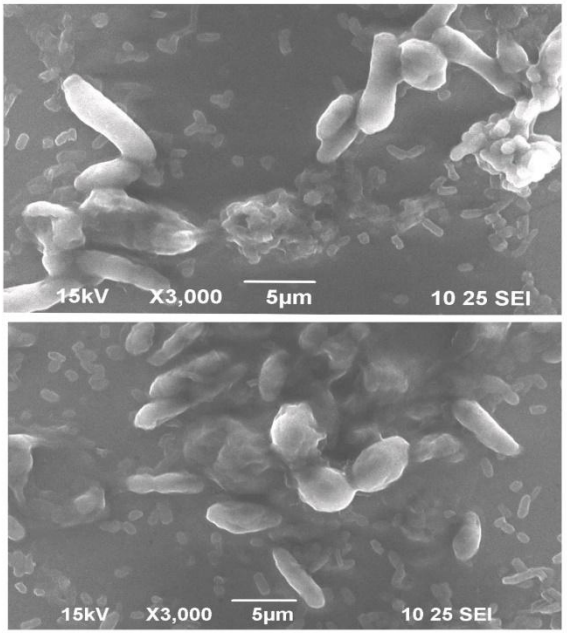


Fig 1 & Fig 2

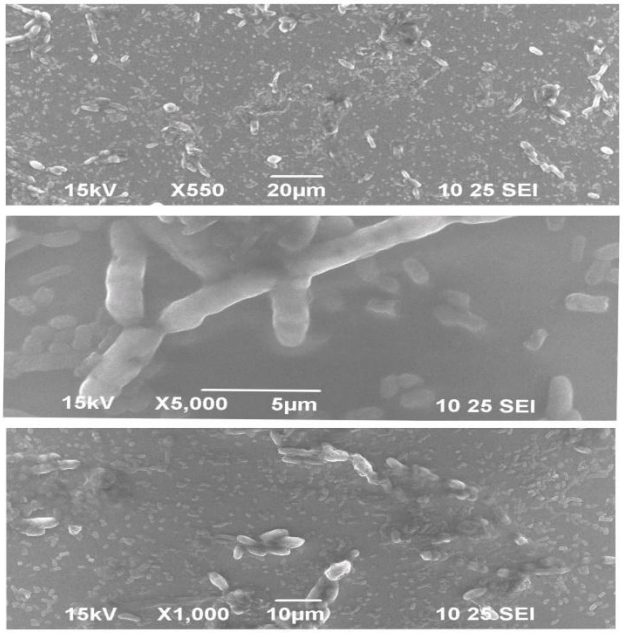


Fig 3, Fig 4 & Fig 5

5. Conclusion

This investigation demonstrates that high-quality Apple Cider Vinegar can be effectively prepared from apple pulp through controlled natural fermentation and can be further transformed into nutritionally diverse, sensorially appealing flavored variants. The proximate analysis confirmed the expected macronutrient profile of ACV: a predominantly aqueous matrix with low fat and protein contents typical of fermented fruit liquids. The mineral profile highlighted Potassium (80 mg/100g) as the principal dietary mineral, with secondary contributions from Phosphorus, Calcium, and Iron.

Among the four formulations evaluated, Sample D (Apple Pulp with Honey and Lemon) demonstrated superior sensory acceptability across all five attributes — appearance, taste, smell,

mouthfeel, and overall acceptability (8.13 ± 0.82) — followed by plain ACV (7.70 ± 0.75), Green Chilli ACV (7.50 ± 1.04), and Mint ACV (7.40 ± 0.67). SEM characterization confirmed the biological viability of the product, revealing characteristic bacterial morphologies and biofilm matrix structures indicative of an active, probiotic-rich fermented matrix.

Future research should explore the use of standardized starter cultures to improve batch consistency, longer fermentation and maturation periods, and clinical evaluation of flavored ACV formulations for specific therapeutic endpoints such as glycemic control and lipid modulation in human subjects.

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