MICROBIOLOGICAL AND PROXIMATE ANALYSIS OF NATURALLY FERMENTED APPLE

ABSTRACT

Aim: This study aims to produce apple cider and analyze the proximate content of the naturally fermented cider.

Place and study duration: Department of Microbiology, between June and August 2023.

Methodology: The purchased apples were weighed, cut into dice, cleaned, and incorporated into distilled water. Afterward, they were allowed to ferment spontaneously at room temperature in a 500 mL conical flask. The sample was inoculated onto sterilized glucose yeast carbonate agar and subsequently incubated at °C for 48 hours. The colony characteristics of the isolates on glucose yeast carbonate agar were observed. Gram's staining and other standard biochemical tests were performed for the identification of the isolates. During the fermentation period, the pH of the sample was determined. The proximate content of the apple cider was also analyzed, this includes moisture, protein, crude fat, and carbohydrate content.

Results: Microscopy revealed the isolates to be Gram-negative bacteria with rod-shaped morphology. The growth characteristics of the isolates on selective media and the results of biochemical tests suggested the presence of *Acetobacter* sp. The pH of the sample tends toward 4.0, suggesting the production of acidic compound. The apple cider produced showed a high concentration of moisture (78.04%), and protein content was 20.04%. The results showed low concentrations of ash content (0.49%), carbohydrate (0.44%), and lipid (0.99%).

Conclusion: The presence of macro and micronutrients in apple cider has been associated with some positive health effects which are enhanced through the process of fermentation, making them a valuable option for health-conscious consumers.

Keywords: Apple cider, *Acetobacter*sp, Acetic acid bacteria, fermentation

1. INTRODUCTION

Apples (*Malus sylvestris*) are a significant economic fruit species belonging to the Rosaceae family, widely consumed globally for their appealing taste, juiciness, vibrant color, pleasing texture, and nutritional value(FAO, 2018). Apples, being easily preserved, remain available year-round at affordable prices, and their versatility allows for the production of various apple products through different processing technologies (Bílková, 2020; Li *et al*., 2020). These products include juices, dehydrated, canned, or purées, as well as fermented items like cider and fermented apple juices, all utilizing apple pomace as industrial by-products (Madrera*et al*., 2017).Fermentation, a transformative process facilitated by microorganisms under specific conditions, is pivotal in the production of fermented apple products. The

success of fermentation is influenced by factors including the microorganism(s) utilized, culture medium, processing conditions, and stages of product recovery(García *et al.,* 2019; Guiné*et al*., 2021). Control of pH, temperature, humidity, aeration, medium bed thickness, and agitation speed is essential for effective fermentation (Peris, 2013; Reihani and Khosravi-Darani, 2019).Apples are recognized for their high content of bioactive compounds, particularly flavonoids like quercetin and its derivatives, with antioxidant, anti-inflammatory, antimicrobial, anti-depressive, and anticarcinogenic properties. Fermentation impacts the properties of apple products, inducing changes in composition, nutritional value, and organoleptic characteristics. According to findings from the study conducted by Peng *et al*. (2017), certain fermented apple products undergo considerable changes in chemical composition, sensory profiles, and bacterial counts. These observations imply their potential to serve as functional, non-dairy probiotic beverages (Roberts and Spadafora., 2020). The concentration and variety of bioactive molecules in apples may vary depending on factors such as species, cultivar, climatic conditions, agronomic practices, and processing methods. Fermentation further enhances the health benefits of apple products, making them a valuable option for health-conscious consumers.This study aims at the bench-scale production of apple cider and the proximate analysis of the naturally fermented cider.

2. MATERIAL AND METHOD

Apples were purchased from the Erekesan market in Ado-Ekiti, Ekiti state, Nigeria. Glucose yeast carbonate (GYC) agar, formulated with glucose 4%, yeast extract 1%, CaCO3 1%, and agar 1.5%, was prepared according to Ezemba*et al*. (2021).

2.1 Preparation of Apple Cider

Twenty grams (20 g) of the apple were weighed, diced, washed, and soaked in distilled water. Subsequently,they were left to undergo natural fermentation at room temperature within a 500 mL conical flask.Additionally, at each stage, 1 ml of the sample was serially diluted and cultured in GYC agar medium for further analysis.

2.2 Isolation of Acetic Acid Bacteria from Naturally Produced Vinegar

Aseptically, 0.1 ml of diluted samples were transferred and spread onto GYC agar plates using a sterile bent rod and incubated at 30°C for 48 hours. Well-formed colonies with a single morphology were isolated and sub-cultured using the streak plate technique on GYC agar plates and then incubated at 30°C for 48 hours.

2.3 Microscopic and Biochemical Characterization of Acetic Acid Bacteria

The colony characteristics of the isolates on GYC agar were observed. Gram staining was performed for morphological characteristics of isolated strains. For the characterization of isolates, Gram staining, sugar fermentation tests, catalase tests, and motility tests were conducted.

2.4 Determination of pH

The pH was determined according to methods of Association of Analytical Chemists (2000). 1 mL of the vinegar was introduced on pH paper and the colour change was compared with the standard. pH measurements were taken at intervals of 0, 24, 48, and 72 hours, as well as on day 5, week 1, week 3, week 4, and week 5.

2.5 Proximate Analysis of Natural Fermented Apple Vinegar

The proximate components of the vinegar, including protein, moisture, crude fiber, crude fat, and ash, were analyzed using standard analytical procedures. Specifically, the protein content was determined following AOAC (2000) method 960.52, while ash, moisture content, and total fat were determined according to AOAC (2000) methods 923.03, 934.01, and 963.15, respectively.The percentage of carbohydrateswas calculated as follows:

$$\%\text{Carbohydrate} = 100 - (\%\text{protein} + \%\text{moisture} + \%\text{crude fat} + \%\text{ash})$$

3.0 RESULTS

3.1 Cultural and Microscopic characterization of isolates

Five colonies were chosen from the culture plate based on their cultural characteristics, designated as FAC1, FAC2, FAC3, FAC4, and FAC5. The cultural characteristics of isolated strains are described and represented in Table 1. FAC1, FAC2, FAC3, and FAC4 were filamentous whereasFAC5 was irregular in shape. All isolates exhibited a creamy colony color and a viscid consistency. he isolates were identified as Gram-negative or Gram-variable (isolates FAC3 and FAC5) small rods, exhibiting straight or slightly curved shapes.

Table 1. Cultural and microscopic characteristics of isolates

Isolates	Shape	Consistency	Colony color	Gram Stain	Shape	Motility
FAC 1	Filamentous	Viscid	Creamy	-ve	Small rods	+
FAC 2	Filamentous	Viscid	Creamy	-ve	Rods	+
FAC 3	Filamentous	Viscid	Creamy	-ve	Curved rod	+
FAC 4	Filamentous	Viscid	Creamy	-ve	Rods	+
FAC 5	Irregular	Viscid	Creamy	-ve	Rods	+

3.2 Biochemical Characterization

The selected isolates tested positive for catalase and were initially identified as Acetic acid bacteria following the standard guidelines outlined in Bergey's Manual of Determinative Bacteriology (De Ley, 1984).Table 2 also illustrates the utilization of selected carbohydrates by the five isolates presumed to be acetic acid bacteria (AAB). All isolates demonstrated the ability to ferment glucose with acid production, whereasnone utilizedsucrose.

Table 2: Biochemical characterization of fermented apple cider isolates

Isolates	Catalase	Utilization of sugar			Organisms
		Glucose	Lactose	Sucrose	
FAC 1	+	+	-	Acid and gas produced	*Acetobacter*sp
FAC 2	+	+	-	Acid and gas produced	*Acetobacter*sp
FAC 3	+	+	-	Acid and gas produced	*Acetobacter*sp
FAC 4	+	+	-	Acid and gas produced	*Acetobacter*sp
FAC 5	+	+	-	Acid and gas produced	*Acetobacter*sp

3.3 pH Value During Fermentation

The pH decreases from 7.0 to 5.0 after day 3, retaining this value on till week 4to become 4.0 as shown in Fig. 1. The pH value of 4.0 was retainedat week 5

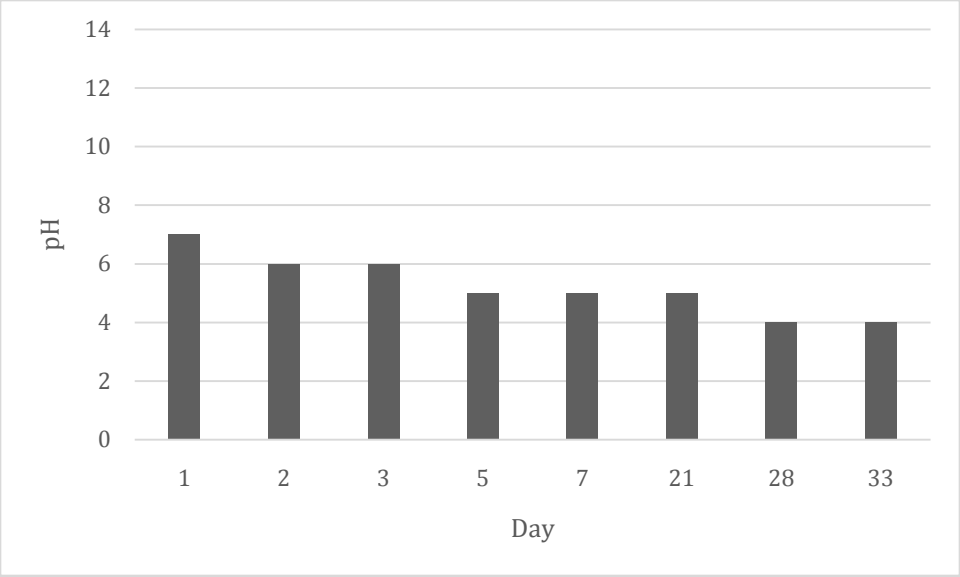

Figure 1:pH of fermented apple cider at different days of fermentation.

3.4 Proximate Composition of the Apple Cider

The apple cider produced showed a high concentration of moisture (78.04%). The protein content was 20.04%. The results showed low concentrations of ash content (0.49%), carbohydrate (0.44%), lipid (0.99%),and no fiber content as outlined in Table 3.

Table 3: Proximate composition of fermented apple cider in percentage

Proximate	%
Moisture	78.04
Crude fat	0.99
Protein	20.04
Ash	0.49
Carbohydrate	0.44

Discussion

The microscopic and biochemical characterization revealedthat the isolates are Acetic acid bacteria (AAB) and identified as *Acetobacter* spp.The main species responsible for apple cider production belong to the genera *Acetobacter*, *Gluconacetobacter*, *Gluconobacter*, and *Komagataeibacter*, owing to their

capacity to oxidize ethanol and their tolerance to acetic acid released during fermentation (Matthew *et al*., 2019). The pH is a crucial parameter in fermentation processes as it affects the growth and activity of microorganisms. The gradual increase in the acidic condition of the fermented apple suggests the production of acidic compounds. The acidity of cider primarily stems from the production of organic acids, including acetic acid and tartaric acid, by the fermenting microorganisms (Ahmad, *et al.,* 2020). These fastidious organisms and strainstend to lose their capacity to produce high concentrations of acetic acid when sub-cultured(Mas *et al*., 2014). This can be attributed to the reduction in the oxygen concentration available and decrease in pH due to continuous acetic acid production, a primary metabolite (Matthew *et al*., 2019).

The analysis of the proximate content of the apple cider as shown in Table 3, suggests that apple cider contains high moisture content, this aligns with the findings of Esselen and Fellers, (2016). High moisture content is indeed a significant factor that affects the flavor of juices and can result in reduced shelf stability (Ekanem and Ekanem, 2019; Guiné *et al*., 2021).Ash content signifies the mineral content of the food sample, as minerals do not combust and are left behind in the form of ash. Therefore, by measuring the ash content, one can gain insights into the types and quantities of minerals present in the food product (Ekanem and Ekanem, 2019). Theash content of ciderproduced in this study shows similar results to that of Khan*et al.*, (2017) who recorded the ash content of a given apple juice sample in the range of 0.045-0.17%. The lower ash content also indicates the minimized quantity of apple fruitused in the preparation of this cider. Long storage at different temperatures also affects the quality parameters of the juices (Rai and Saxena, 2018).

Carbohydrate was recorded to be low with a value of 0.44%. Carbohydrates account for the principal food value of apples. They are starches, sugars, pectin, cellulose, and hemicelluloses (Hayes and Haddad, 2016). The study of Yilmaz *et al.*, (2016) also reported (3.6%) total sugar in apple juices. Pectin is a component of carbohydrates in apple cider that has shown additional health benefits on digestive systems (Esselen and Fellers, 2016). Differences in results obtained from this study and those of other researchers may be attributed to variations in pectin content. The pectin content of different apple cultivars grown in diverse locations influences the carbohydrate composition of cider and other apple juices (Bray, 2014).The lipid content in the apple cider sample was low which is common for fruits.The elevated protein content in the cider could stem from the presence of apple pomace. Apple pomace serves as a source from which valuable components like pectin, aroma compounds, edible fibers, and antioxidant polyphenols can be extracted. It can also be utilized to obtain protein-enriched feeds, synthesize pectolytic enzymes, or produce natural aroma compounds through fermentation processes (Guiné*et al*., 2021).

4.0 CONCLUSION

Acetobacter sp. have been associated with the fermentation of apples based on their cultural and biochemical characteristics. The pH of the sample tends toward 4.0, suggesting the production of acidic compound. The apple cider proximate contents are moisture (78.04%), protein content 20.04%, ash content (0.49%), carbohydrate (0.44%), and lipid (0.99%). The presence of these macro and micronutrients have been linked to the positive health effects of apple fermented products therefore, further research is needed to explore the specific mechanisms behind the health benefits of cider and to establish optimal consumption recommendations.

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