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## THE USE OF SOME SELECTED NIGERIAN HONEY IN THE IMPROVING THE QUALITY OF YOGHURT PRODUCTION

Bilyaminu John Shaffa<sup>3</sup>, Fatimah Buba<sup>1&2</sup>, Mustafa Alhaji Is<sup>3</sup>, Ali Abdullahi Damasak<sup>2</sup>  
and Mohammed Adamu Milala<sup>2</sup>,

<sup>1</sup>School of Pharmacy, Faculty of Science and Engineering, University of Wolverhampton,  
Wolverhampton, WV1-1LY, U.K.

<sup>2</sup>Department of Biochemistry, Faculty of life Science, University of Maiduguri, Bama Road,  
Maiduguri, Nigeria

<sup>3</sup>Department of Microbiology, Faculty of life Science, University of Maiduguri, Bama Road,  
Maiduguri, Nigeria

\*Corresponding author: fatimahbuba@gmail.com

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### ABSTRACT

Maintaining viability of starter cultures, shelflife, physicochemical and sensory properties of many finished product like yoghurt constitute a major challenge to the food industries. Moreso, the use of synthetic antimicrobials, preservatives and non-nutritive sweeteners in many of such finished products may constitute potential health effect. Therefore; this work was aimed at using naturally occurring honey as a sweetener and preservative in yoghurt production owing to its rich bioactive constituents and sweetening effect. The bioactive constituents of honey screened had a total phenolic content of 8.0-86.0 mgGAE/g Extract, total flavonoid content of 3.15-16.7 mg QE/g extract, moisture content of 8.8-14.7, pH of 5.88-6.46, % acidity of 0.23-0.46 Meq/kg and DPPH value of 19.49-29.15(1C50). The different concentration of honey use in yoghurt production and stored at 4<sup>o</sup>c for 7,14 and 21 days, had no effect on the LAB counts, with all honey incorporated yoghurt samples remaining intact without rancidity during the whole storage period except for the control group. Sensory evaluation results showed that yoghurt produced using 15 and 20% honey had a high acceptability rate by the panelist in contrast to 25% and control group. Yoghurt incorporated with honey were liked and not reported rancid/spoiled after 21 days storage at 4<sup>o</sup>c,

**Keywords:** Yoghurt, Honey, DPPH, Sensory Evaluation, High Acidity and Viscosity.

### INTRODUCTION

Honey is a sweet natural organic substance obtained from nectar of flowers by *Apis mellifera*, which process and stores it as the main food source in beehive with a clear, golden amber colour (Al-Moussawi *et al.*, 2023). Honey is composed of water and sugars, primarily fructose (38%), glucose

(31%) and fructose-oligosaccharides (4-5%) which accounts for 95-99% of honey dry matter (Harnandez *et al.*, 2022). Other sugars identified in honey besides fructose and glucose includes; maltose, sucrose, maltulose, turanose, isomaltose, laminaribiose, nigerose, kojibiose and gentiobiose (Harnandez *et al.*, 2022). Bioactive substances identified in honey include polyphenols, organic acids, Maillard reaction products, carotenoid derivatives, vitamins, amino acids, and proteins. (Karapetkovska *et al.*, 2022). Honey also contains several vitamins like riboflavin, niacin, pantothenic acid, pyridoxine, folate, and vitamin c, minerals (calcium, iron, zinc, potassium, phosphorous, magnesium, selenium, chromium, and manganese), proteins (0.3%), fats, ash (0.2%) (El-Hawiet *et al.*, 2022). Enzymes like catalase, superoxide dismutase, reduced glutathione (Harakeh *et al.*, 2022), flavonoids like apigenin, pinocembrin, kaempferol, quercetin, galangin, chrysin and hesperetin (Matkovits *et al.*, 2023), phenolic acids like ellagic, caffeic, p-coumaric, and ferulic acids (Matkovits *et al.*, 2023), 1,2-dicarbonyl compounds, such as glyoxal (GO), 3-deoxyglucosulose (3-DG) and methylglyoxal (MGO) which all contributes to its biological effects(karapetkovska *et al.*, 2022).

Overall, the composition of honey varies depending on many factors such as the honeybee species, its floral source, seasonal and environmental factors etc which all influence its composition and its biological activity (Feknous *et al.*, 2022). Honey has been used as food source, natural preservative and sweetener, and for medicinal purposes in treatment of degenerative disease like cancer, inflammatory and cardiovascular diseases, diabetes, worm infestation, stomach pain, nasal congestion, mouth sores, and in cleaning of wounds (Nayik *et al.*, 2018). Honey's bioactive substances flavanoids, phenolic acids, carotenoid-like substance and oligosaccharides(non-digestible carbohydrate) in honey are selectively utilized by bacteria in the genera *Bifidobacteria* and *Lactobacillus* which have been found suitable candidate for use as probiotics (Sharma *et al.*, 2022) and extensively utilized as starter culture in yoghurt production (Chen *et al.*, 2022).

Yogurt is one of the most fermented dairy products accepted and consumed worldwide for its nutritious and numerous health benefits (Fiore *et al.*, 2022). yogurt is a rich source of milk proteins, carbohydrate, minerals such as calcium and phosphorous, and vitamins such as riboflavin (B2), thiamin (B1), cobalamin (B12), folate (B9), niacin (B3) and vitamin A (Rehman *et al.*, 2023). This study was aimed at producing bioactive yoghurt

with honey as a natural sweetener and preservative. The study assessed the suitability and efficacy of honey incorporated as sweeteners in producing functional bioactive yoghurt with prolonged shelflife and acceptable microbiological quality.

## **Materials and Methods**

### **Honey sample collection**

The method for honey sample collection was adopted from Tennokoon *et al.* (2023) and involved randomly collecting honey in 250 mL jars, which were sealed airtight and labeled with relevant sampling information, including the site and prevailing nectar sources. Samples were obtained from various locations in Nigeria and subsequently transported to the laboratory, where they were stored at room temperature for further evaluation

### **Preparation of honey samples**

The standard methods for *Apis mellifera* honey research as outlined by Bicudo *et al.*, (2020) were adopted in the preparation of the collected honey samples. The samples were homogenized by stirring with a spatula and subsequently filtered through a 0.5 mm stainless steel sieve to separate the honey from the honeycomb.

### **Determination of moisture content**

The moisture content was determined using the refractometric method as described by Mahmoud *et al.* (2023). The Wedmore equation was used to determine the moisture content (International Honey Commission, 2009).  
$$W = 1.73190 - \log(R.I.-1) \times 0.002243$$

Where  $W$  is the water content in g per 100g honey and R.I. is the refractive index

Moisture content was expressed as %water content g/100g

### **pH and Free Acidity**

The method adopted from Hassan *et al.* (2023) was used to determine the physicochemical properties of the samples, specifically pH and free acidity. Free acidity of the honey was expressed as milliequivalents or millimoles of acid per kilogram of honey (International Honey Commission, 2009).

### **Water activity**

Water activity of the honey sample was measured using a water activity meter (AQUALAB 3, Decagon, Pullman, WA, USA) at 25°C, as described by Oromokoma *et al.* (2023).

### **Quantification of bioactive components of honey samples**

#### **Total phenolic content**

The total phenolic content was determined using the Folin-Ciocalteu method as described by Singleton and co-workers (Ramnath *et al.*, 2012).

#### **Total flavanoids content**

The aluminum chloride (AlCl<sub>3</sub>) colorimetric assay was employed for the determination of total flavonoid content as previously described by Zhishen *et al.*, 1999 (Rebaya *et al.*, 2015).

#### **DPPH radical scavenging activity**

The DPPH radical scavenging activity of honey was determined using a spectrophotometer as previously reported by Zhang *et al.*, (2013). The DPPH radical scavenging activity was expressed as a percentage of inhibition using the formula:

$$\% \text{Inhibition} = \left( \frac{\text{Control Absorbance} - \text{Sample Absorbance}}{\text{Control Absorbance}} \right) \times 100\%$$

(International Honey Commission, 2009)

### **Isolation and characterization of Lactic acid bacteria**

#### **Preparation and Isolation of Bacteria for Yoghurt Production**

*Lactobacillus bulgaricus* and *Streptococcus thermophilus* used for the yoghurt production were isolated from commercial milk (fura).

#### **Media Preparation**

De Man Rogosa Sharpe (MRS) medium, supplemented with 100 mg of cycloheximide antibiotic, was used for the isolation of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* according to the manufacturer's instructions (Renschler *et al.*, 2023).

#### **Isolation Procedure**

An aliquot of 0.1 mL milk (fura) was placed on MRS agar plates and incubated at 35°C to 45°C for 72 hours to detect cocci and bacilli. Isolates were selected based on acid production, indicated by a clear zone around

the colony on MRS medium. Acid-producing isolates were subcultured on MRS agar and incubated again at 35°C to 45°C for 72 hours.

### **Yoghurt production**

Two hundred and fifty gram of powdered milk (250g) (full cream) was mixed with 75 ml of distilled water. This mixture was homogenized using a homogenizer or viscolizer to ensure uniform consistency. Subsequently, the homogenized mixture was heated to a temperature range of 80-85°C for 30 minutes to achieve pasteurization and optimize viscosity. Following pasteurization, the mixture was cooled to an incubation temperature of 34-36°C. Next, 15%, 20%, and 25% honey concentrations were incorporated into the cooled milk. Inoculation was performed using 0.1-0.2 ml of a 2% (v/v) concentration of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, mixed in a 1:1 ratio. The inoculated milk was allowed to remain undisturbed for 6-8 hours to facilitate yoghurt production. Once the yoghurt reached a pH of 4.5-4.6, it was blast-chilled and refrigerated at 4°C to halt the fermentation process and prevent further acid production (Salmazo *et al.*, 2023).

### **Enumeration of LAB in yoghurt**

The plating and enumeration of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were conducted using the method described by Laurens-Hatting and Viljocu (2001). Briefly, 10 g of the produced yoghurt sample was weighed aseptically and transferred into sterile stomacher bags. The sample was then mixed with 90 mL of sterile Maximum Recovery Diluent (MRD) and homogenized for 1 minute. Serial dilutions (1:10) were prepared using MRD, extending to a dilution factor of 10<sup>-6</sup>. From each dilution, 100 µL was inoculated onto De Man, Rogosa, and Sharpe (MRS) agar plates. The results were expressed as colony-forming units per millilitre (CFU/mL) of lactic acid bacteria (LAB) (Atik *et al.*, 2023).

### **Sensory Evaluation**

Sensory evaluation of honey-incorporated yoghurt was conducted using five panellists after 21 days of storage at 4°C. A 9-point Hedonic scale assessed flavour, with scores ranging from 1 (poor quality) to 9 (excellent quality). Panellists were provided with descriptions of common yoghurt characteristics and defects, including overall sourness, bitterness, high acidity, and lack of flavouring, viscosity, lack of freshness, low sweetness, high sweetness, unnatural flavour, and uncleanliness. This enabled them to evaluate the flavour profile of each sample. Additionally, texture and colour

were assessed using a 5-point scale, where 1 represented poor quality and 5 indicated excellent quality (Basuny *et al.*, 2023).

### Statistical analysis

Analysis of variance (ANOVA) was employed to assess differences in the mean values of continuous variables. A two-sided p-value of less than 0.05 was considered statistically significant.

## RESULTS AND DISCUSSION

### Quantification of Bioactive Components of Nigerian Honey

The analysis of honey samples revealed the following quantification of bioactive components: the total phenolic content ranged from 8.0 to 86.0 mg GAE/g extract; the total flavonoid content ranged from 3.15 to 16.7 mg QE/g extract. The moisture content ranged from 8.8% to 14.7%; the pH ranged from 5.88 to 6.46; the percentage acidity ranged from 0.23 to 0.46 meq/kg; and the DPPH radical scavenging activity ( $IC_{50}$ ) ranged from 19.49 to 29.15  $\mu\text{g/mL}$ .

**Table 1:** Bioactive Components of Honey

Sample	Total phenolic content mgGAE/g Extract	Total flavanoid content mg QE/g extract	moisture content	PH	% acidity Meq/kg	DPP4 radical Scavenging activity( $IC_{50}$ )
HO1C	8.0 ± 0.5	16.7 ± 0.6	14.7±0.1	5.88±0.03	0.46±0.3	26.06±0.3
HO2T	86 ± 2.0	5.2 ± 0.03	13.6± 0.2	5.90± 0.07	0.46±0.02	28.43±0.20
HO3D	58 ± 2.0	3.66± 0.1	8.6± 0.3	5.97± 0.03	0.43±0.03	23.82±0.52
HO4H	30.3 ± 1.5	3.15 ± 0.03	10.2± 0.2	6.38 ±0.02	0.23±0.03	29.15±1.00
HO5Y	46± 2.0	4.75± 0.1	8.8± 0.2	6.46±0.04	0.25±0.02	19.49±0.95

Mean and Standard Deviation values (N=3)

The total flavanoid content of honey samples analyzed ranged between 3.15±0.03-16.7±0.6 mg QE/extract. These findings are in agreement to the total flavanoid content of 3.58-15.67 mgQE/extracts reported from Indonesian honey by (Jaya *et al.*, 2024). In addition, Hameed *et al.* (2024) also reported flavanoid content of 7.87-95.62 mgQE/extracts in Iraqi honey stating that the flavanoid contents contributes to the antioxidant and antibacterial property thus, enhances its therapeutic property. Itama honey from Thailand was reported by (Wongsa *et al.*, 2024) to have a mean total

flavanoid content of 43.15mgQE/extract ( $17.056 \pm 0.189$ - $58.19 \pm 1.068$  mgQE/extract). Exclusively flavanoids and phenolic content of honey are plant source. Hence, influences its aroma, antioxidant, antibacterial and overall quality which forms the basis for honey's identification.

Flavanoid content is affected by storage conditions which is in line with the findings of Sakac *et al.*, 2024 who showed that multifloral honey to have twice more total flavanoids content than Acacia honey samples (5.15 vs. 2.50) whose storage had a significant impact on the total flavanoid content. The differences in the flavanoid content of honey in this study, and total flavanoid content of honey from other studies could be a reflection of honeys different floral sources and storage conditions.

Moisture content of honey is affected greatly by climatic condition of higher temperature and humidity. Moisture content of honey is an important property of honey that guarantees its quality, stability, resistance to fermentation and granulation during storage (Krishnan *et al.*, 2021). Higher moisture content increases the likelihood of fermentation during the storage, while lower moisture content levels of less than 20% extends the shelf life of honey (Hameed *et al.*, 2024). Therefore, most food items that perishable are characterized by low temperature, pH, Moisture content and acidity with these qualities from the honey will improve the quality of the yoghurt produced.

However, the moisture content of honey in this study shows a ranged 8.6-14.7 with a mean value of  $11.2 \pm 0.2$ . Although various values were observed by Yegge *et al.*, 2022 to be 17.21%, and Wongsa *et al.*, 2023 (25.49-25.82%) to be found in fresh honey. The mean moisture content of 11.2 recorded in this study, is less than 20% thus aligns with the acceptable value of <20% moisture content standard for international honey commission (2002), Codex Alimentarius 1999 and commercially available honey (Hameed *et al.*, 2024). The mean pH values of the honey samples analyzed are 6.1 and ranged (5.88-6.46). This PH range falls within the acceptable PH standard limit of 3.4-6.1(Codex Alimentarius, 2014). PH values of 3.06-3.32 was reported by (Wongsa *et al.*, 2023) from honey samples collected in swamps forest which have slightly lower PH than those collected from tropical and subtropical regions. Similarly, PH values of 4.2-4.3 were also recorded by (Hameed *et al.*, 2024). The PH of honey is affected by carbohydrate fermentation to organic and inorganic acids, floral sources, processing and

storage conditions which contributes and affects its distinctive flavor, microbiological stability, structure and shelflife (Hameed *et al.*, 2024).

The percentage (%) free acidity of honey samples in this study ranged between 23-46%. The free acidity values obtained in this study are less than the maximum limit specified as satisfactory in the international standard of 50Meq/kg of honey thus, suggestive of the absence of undesirable fermentation in the honey samples. The free acidity of honey is an important parameter that determines honey quality as it influences honey taste, aroma and serves as important marker for fermentation. Free acidity of honey is influenced by organic acids, nectar or bees' secretions (Yadata, 2014), similarly, acid minerals and amino acids in honey, biophysical conditions, storage and processing methods, maturation and climatic conditions. Free acidity of honey can be increased during storage and ripening of honey, as well as during fermentation (Yadata, 2014). Free acidity of 11.0-47 Meq/kg has been reported by Guerzou *et al.* (2021). The mean DPPH radical scavenging activity of the honey analyzed is 25.39 ranged between (19.49-29.15). Wongsa *et al.* (2024) reported a DPPH values of 43.99-57 in raw honey from Thailand. Pontis *et al.*, 2014 also reported a DPPH value of 8.51-70.83mg/ml from Brazilian rainforest of the Amazon. Values of 42.87-131.26mg/ml were equally reported by Liu *et al.*, (2023) in Eurya honey Srividya *et al.*, (2023). A DPPH value of 58.98 mg ml<sup>-1</sup> was reported in Alfalfa honey by Fratianni *et al.*, (2024). Flavanoids and phenolic acids are important polyphenols in honey Flavanoids and phenolic acids are responsible for honeys bioactive properties including antiinflammatory, antioxidant, antimicrobial and anti cancer activities (Mokaya *et al.*, 2022).

### **Yoghurt production**

Two hundred and fifty gram of powdered milk (250g) 250 g of powdered milk (full cream) was mixed with 75 ml of distilled water. This mixture was homogenized using a homogenizer or viscolizer to ensure uniform consistency. Subsequently, the homogenized mixture was heated to a temperature range of 80-85°C for 30 minutes to achieve pasteurization and optimize viscosity. Following pasteurization, the mixture was cooled to an incubation temperature of 34-36°C. Honey concentrations of 15%, 20%, and 25% were incorporated into the cooled milk. Inoculation was performed using 0.1-0.2 ml of a 2% (v/v) concentration of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, mixed in a 1:1 ratio. The inoculated milk was allowed to remain undisturbed for 6-8 hours to



facilitate yoghurt production. At a pH of 4.5-4.6, it was blast-chilled and refrigerated at 4°C to halt the fermentation process and prevent further acid production (Salmazo *et al.*, 2023).

Days of Storage	Types of Honey	Concentration of Honey			Control	Mean Count (10 <sup>5</sup> ) (CFU/ml)	Std Dev	P-value
		15%	20%	25%				
7 DAYS	ILC	1656	1410	111	1900	1269.25	797.66	0.052577
	ILT	801	604	401	118	481.00	291.95	
	ILD	1674	1331	1209	1899	1528.25	315.98	
	ILH	1508	1219	956	1845	1382.00	382.23	
	ILY	1619	1288	839	1901	1411.75	456.68	
14 DAYS	ILC	1710	1501	1394	1945	1637.50	243.40	0.000206
	ILT	889	777	579	210	613.75	298.12	
	ILD	1800	1659	1501	1900	1715.00	173.57	
	ILH	1645	1681	1508	1905	1684.75	164.67	
	ILY	1695	1407	927	1898	1481.75	421.14	
21 DAYS	ILC	1532	1391	1017	1900	1460.00	365.05	0.016298
	ILT	729	558	411	118	454.00	258.96	
	ILD	1519	1268	874	1899	1390.00	430.84	
	ILH	1491	1131	685	1845	1288.00	496.56	
	ILY	1544	1277	868	1901	1397.50	435.84	

Table 2: LAB counts of isolates IL during 7, 14 and 21 days Storage

Statistical analysis was performed to assess the impact of varying honey concentrations (15%, 20%, and 25%) on lactic acid bacteria (LAB) counts during yoghurt storage. One-way ANOVA was used to analyze the mean bacterial counts after 7, 14, and 21 days of storage. After 7 days of storage, the LAB counts did not show a statistically significant difference among the different honey concentrations ( $p = 0.052577$ ). This indicates that the concentrations of honey (15%, 20%, and 25%) had no significant effect on LAB counts at this time point, as the p-value was greater than 0.05. In contrast, for storage periods of 14 days ( $p = 0.000206$ ) and 21 days ( $p = 0.016298$ ), the p-values were less than 0.05. This suggests that the honey concentrations significantly affected the LAB counts during these storage periods.

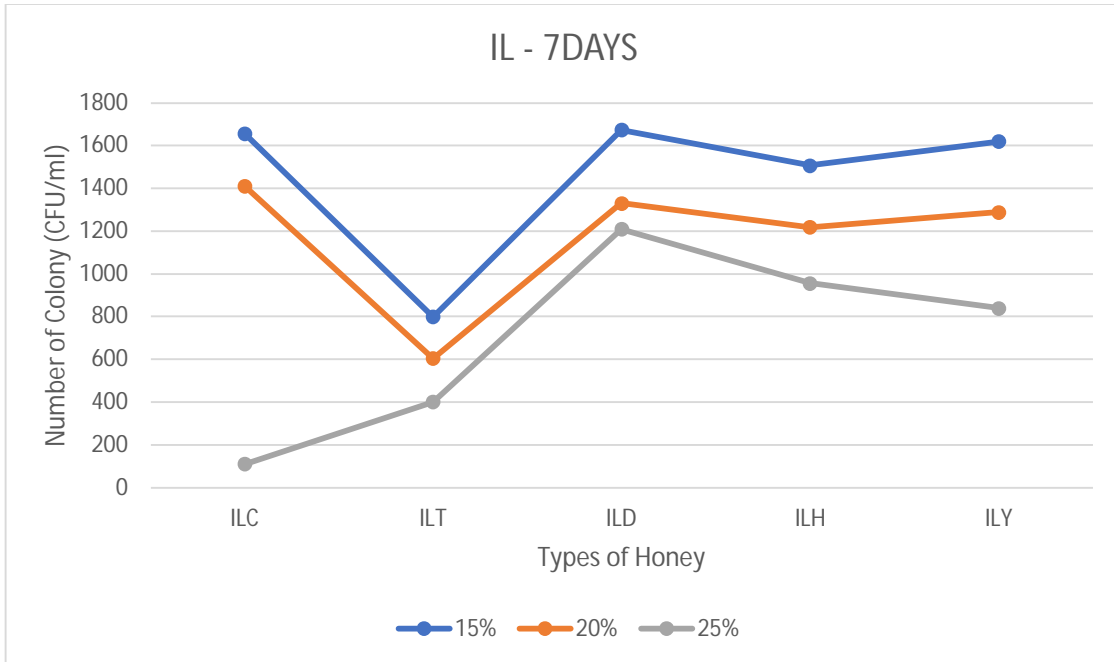


Figure 2: Trend of Growth of isolate 1L after 7 days storage at 4°C

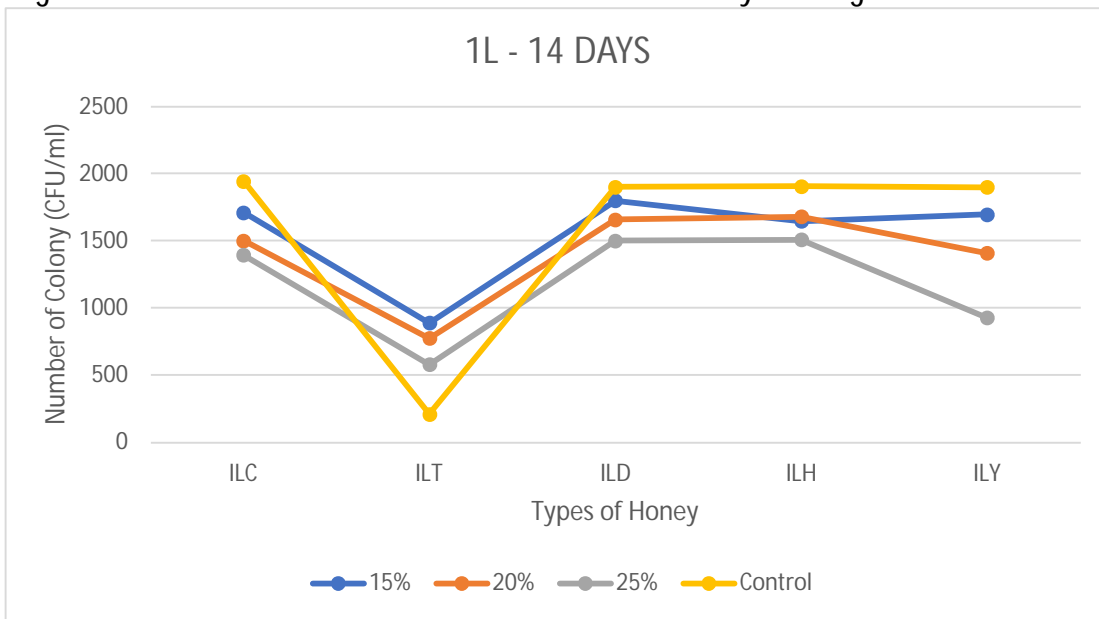


Figure 3 Trend of Growth of isolate 1L after 14 days storage at 4°C

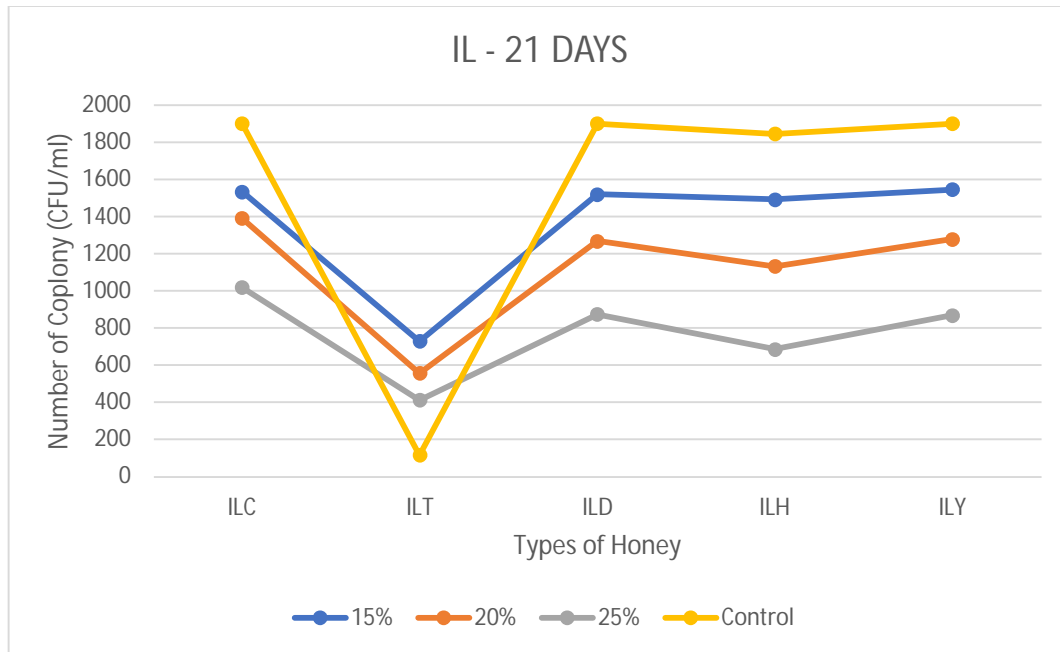


Figure 4 Trend of Growth of isolate 1L after 21 days storage at 4°C

	Types of Honey	Concentration of Honey			Control	Mean Count (10 <sup>3</sup> ) (CFU/ml)	Std Dev	P-value
		15%	20%	25%				
7 DAYS	2SC	409	318	295	1318	585.00	491.14	0.64148
	2ST	256	208	156	781	350.25	290.06	
	2SD	110	103	89	649	237.75	274.31	
	2SH	80	67	44	891	270.50	413.93	
	2SY	151	147	107	641	261.50	253.78	
14 DAYS	2SC	511	321	198	821	462.75	271.33	0.870815
	2ST	316	245	177	971	427.25	366.92	
	2SD	89	47	21	1209	341.50	579.01	
	2SH	101	97	55	1471	431.00	693.65	
	2SY	207	137	96	180	155.00	48.76	
21 DAYS	2SC	449	225	128	1178	495.00	474.76	0.998796
	2ST	287	221	151	1500	539.75	642.57	
	2SD	66	38	19	1910	508.25	934.70	
	2SH	73	54	36	1947	527.50	946.45	
	2SY	166	103	74	1245	397.00	566.64	

**Table 3: LAB counts of isolates 2S during 7, 14 and 21-days Storage**

The results show the impact of different honey concentrations (15%, 20%, and 25%) on the LAB counts in yoghurt stored at 4°C. A one-way ANOVA test was employed to analyze the mean bacterial counts after 7, 14, and 21 days of storage. As presented in Table 4.4, there was no statistically significant difference in bacterial counts among the different honey concentrations (15%, 20%, and 25%) at any of the storage intervals: 7 days ( $p = 0.64148$ ), 14 days ( $p = 0.870815$ ), and 21 days ( $p = 0.998796$ ). These  $p$ -values, all greater than 0.05, indicate that the honey concentrations used did not significantly affect the bacterial counts during yoghurt storage. The results are summarized in **Table 3** and are graphically represented in **Figures 5, 6 and 7**.

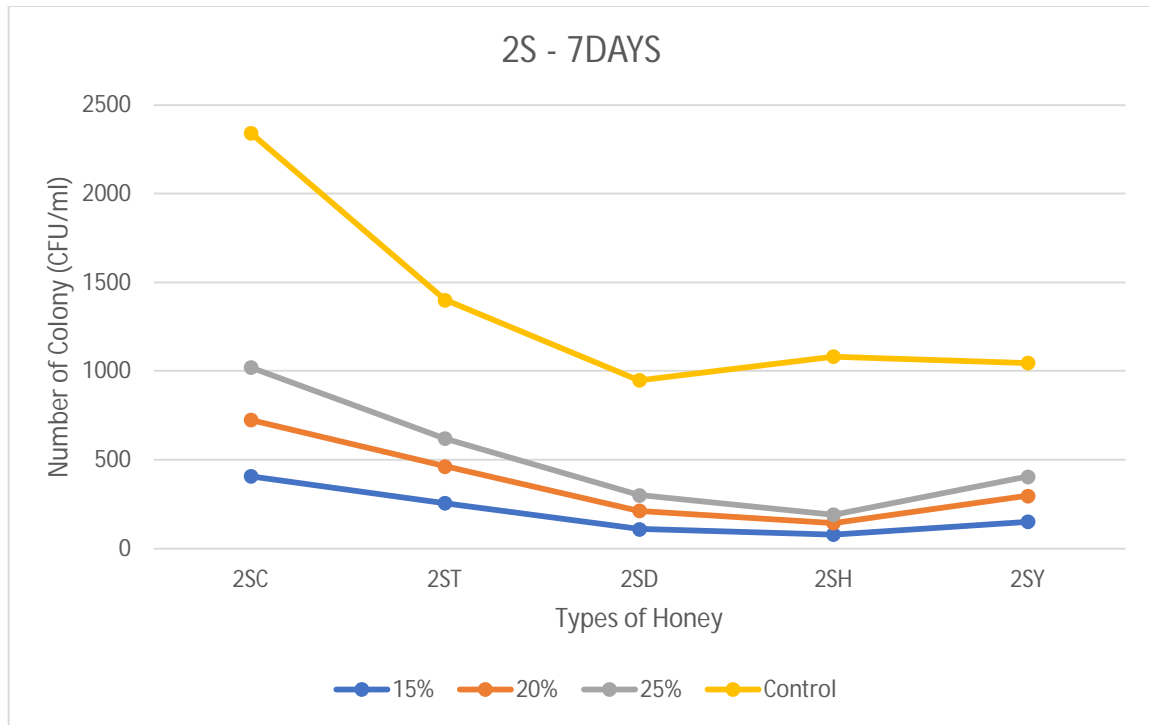


Figure 5: Trend of Growth of isolate 2S after 7 days storage at 4°C

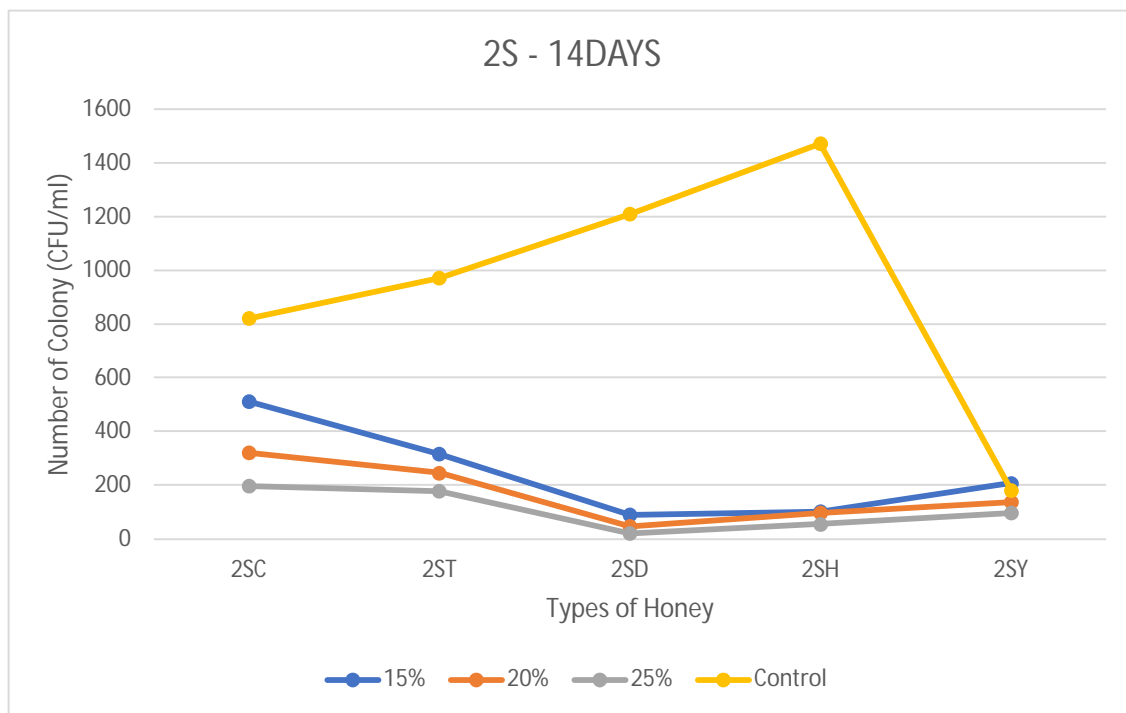


Figure 6: Trend of Growth of isolate 2S after 14 days storage at 4°C

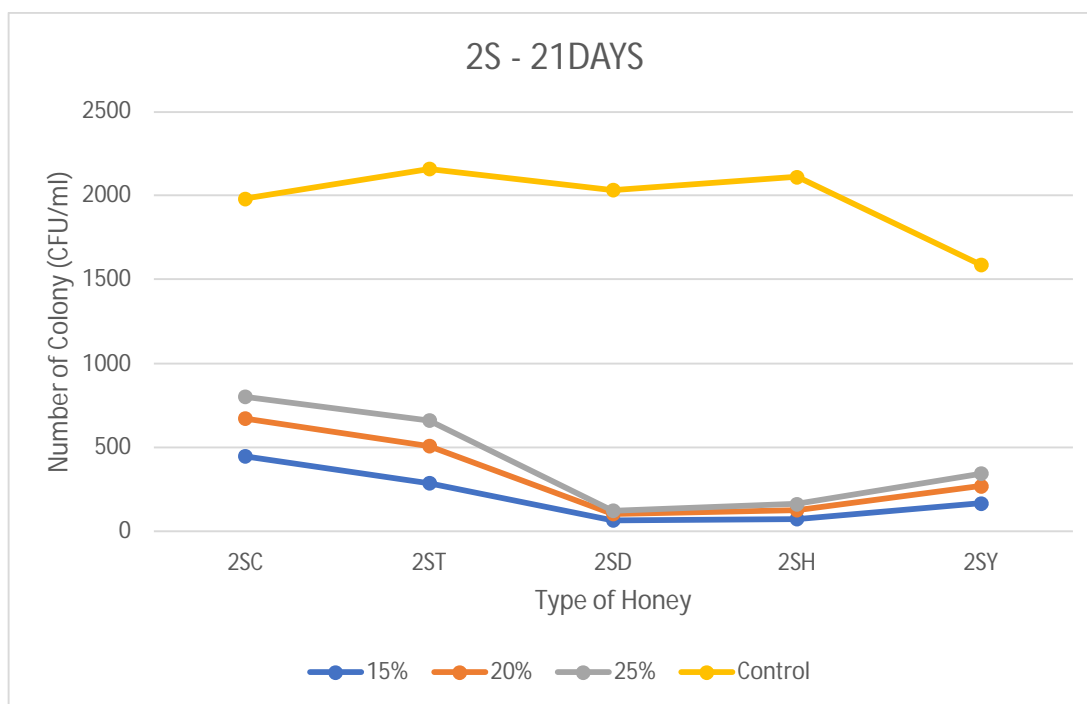


Figure 7: Trend of Growth of isolate 2S after 21 days storage at 4<sup>0</sup>

**Table 4: LAB counts of isolates 3S during 7, 14 and 21 days Storage**

Days of Storage	Types of Honey	Concentration of Honey				Control	Mean Count (105) (CFU/ml)	Std Dev	P-value
		15%	20%	25%					
7 DAYS	3SC	571	487	317	923	574.50	255.23	0.308705	
	3ST	215	143	108	518	246.00	186.72		
	3SD	309	262	148	731	362.50	254.80		
	3SH	464	371	229	841	476.25	261.66		
	3SY	572	456	318	918	566.00	256.61		
14 DAYS	3SC	695	362	261	1314	658.00	475.02	0.798606	
	3ST	271	161	87	918	359.25	380.09		
	3SD	377	296	201	1310	546.00	514.39		
	3SH	568	415	392	1570	736.25	561.30		
	3SY	680	511	352	1121	666.00	331.58		
21 DAYS	3SC	234	228	187	1900	637.25	842.09	0.803635	
	3ST	174	178	109	118	144.75	36.31		
	3SD	238	108	56	1899	575.25	885.81		
	3SH	444	317	253	1845	714.75	757.67		

Statistical analysis was conducted to determine the effect of varying honey concentrations (15%, 20%, and 25%) on the microbiological quality of yoghurt during storage. A one-way ANOVA test was employed to analyze the mean bacterial counts after 7, 14, and 21 days of storage.

After 7, 14, and 21 days of storage, no statistically significant differences were observed in bacterial counts among the different honey concentrations, with p-values of 0.308705, 0.798606, and 0.803635, respectively. These p-values, all greater than 0.05, indicate that the honey concentrations used did not significantly affect the microbiological quality of the yoghurt during storage.

The results are summarized in **Table 4** and are graphically represented in **Figures 8,9 and 10**

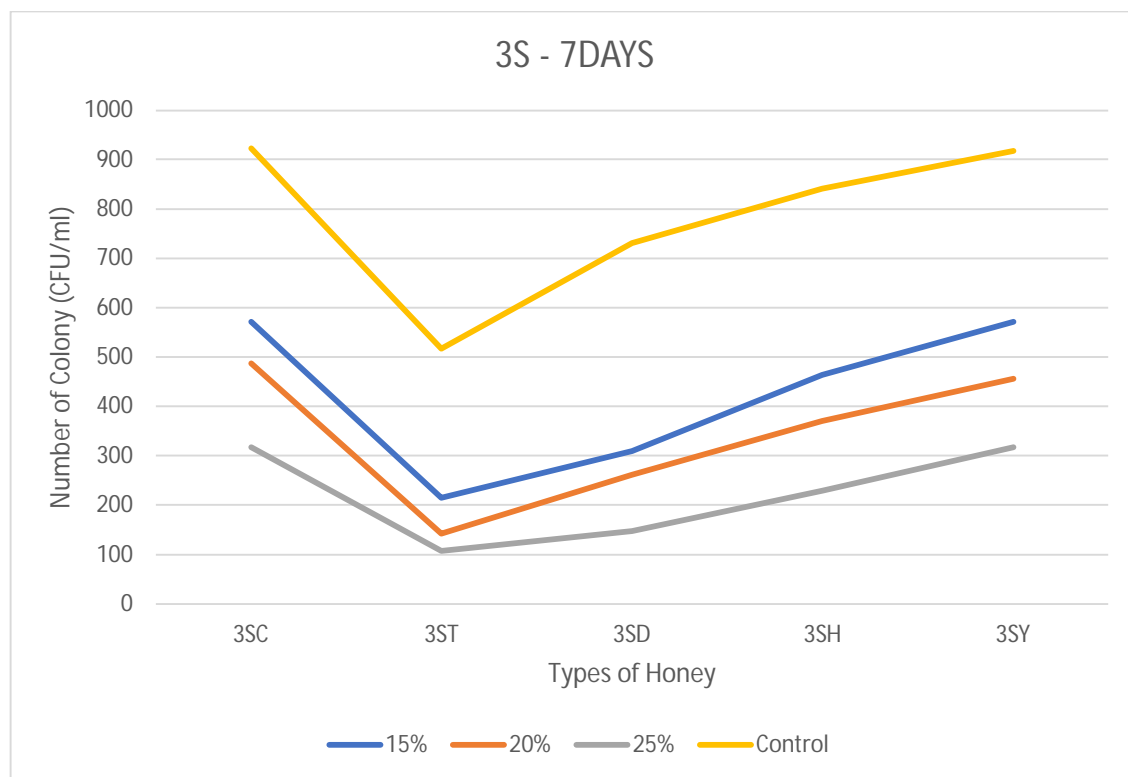


Figure 8: Trend of Growth of isolate 3S after 7 days storage at 4°C



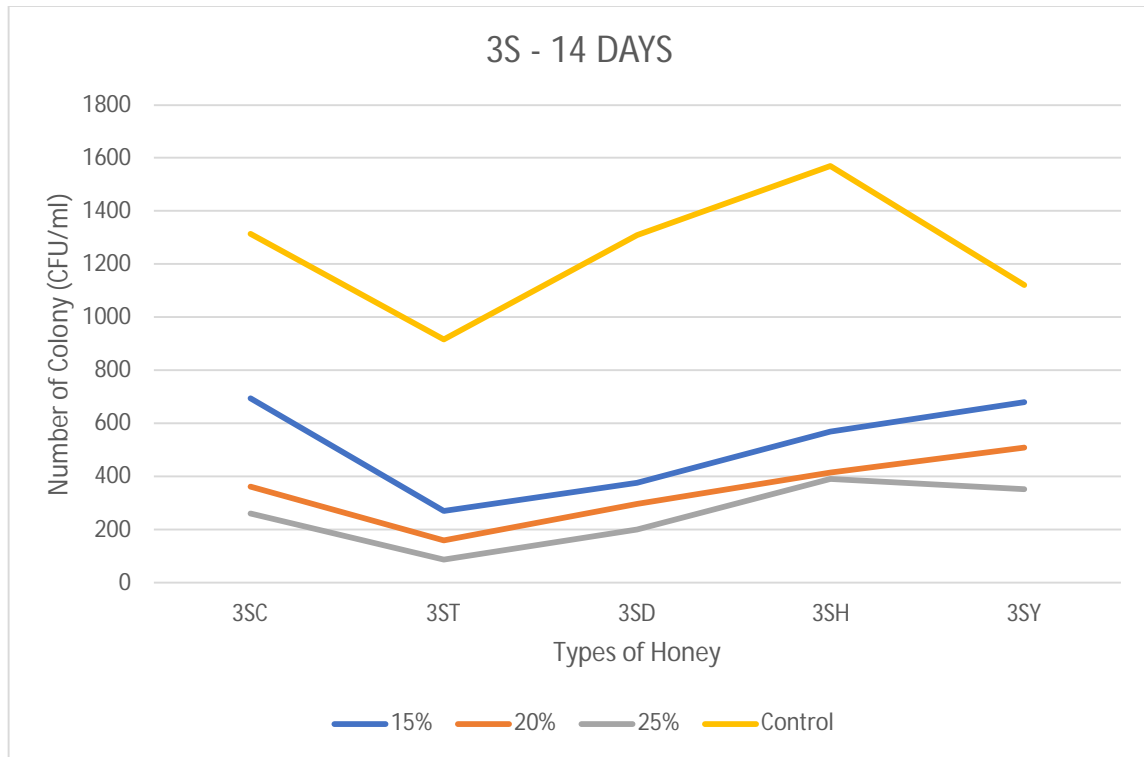


Figure 9: Trend of Growth of isolate 3S after 14 days storage at 4°C

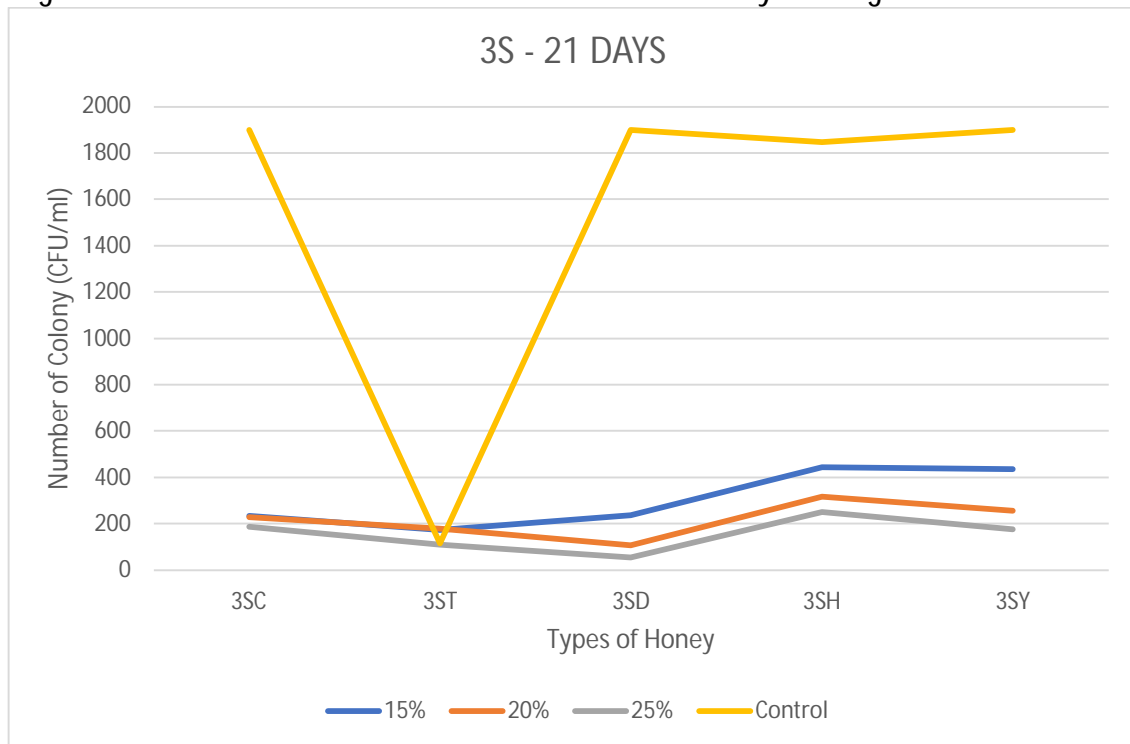


Figure 10: Trend of Growth of isolate 3S after 21 days storage at 4°C

A standard lactic acid bacterium count of  $6 \log_{10} \text{cfu/ml}$ - $7 \log_{10} \text{cfu/ml}$  is required to be present in finished food at the point of consumption to promote health benefits (Mulawet *et al.*, 2019). Higher counts of  $7.3 \log_{10} \text{cfu/ml}$  was reported by (Mohan *et al.*, 2020) in yoghurt produced by *Lactobacillus reuteri* DPC16 and maintained throughout the storage period. Bacterial count of  $8.5 \log_{10} \text{cfu/ml}$  after 7 days storage,  $7.5 \log_{10} \text{cfu/ml}$  and  $7.2 \log_{10} \text{cfu/ml}$  after 14<sup>th</sup> and 21 days of storage was reported by (Machado *et al.*, 2017) in yoghurt containing added stingless bee honey. (Coastaet *et al.*, 2014) reported high lactic acid bacterial count in goat yohurt after fermentation using *Streptococcus thermophilus* ( $8.99 \log_{10} \text{cfu/ml}$ ), *Lactobacillus delbruekii* ( $7.8 \log_{10} \text{cfu/ml}$ ) and *Lactobacillus acidophilus* ( $9.49 \log_{10} \text{cfu/ml}$ ). Findings of (Bathazar *et al.*, 2016) and (Varga *et al.*, 2014) showed that at the end of the storage period (28 days), the counts of starter bacteria groups were closely approximated to be  $6.5 \log_{10} \text{cfu/ml}$  in all yoghurt formulations regardless of the addition of stingless bee honey. Therefore, decrease (1-2 log cycle) in starter bacteria counts has been an expected behavior during yoghurt storage (Bathazar *et al.*, 2016, Varga *et al.*, 2014).

Similarly, reports of (Varga 2006), Showed that the addition of 1%, 5% acacia honey to yoghurt did not significantly affects the growth of *Streptococcus thermophilus* during storage. These findings are in agreements with the trends of count in this study. The findings suggest that minimal amount of available sugar was available to promote the growth of starter culture onwards beyond 14-21 days storage period. Honeys addition to the yoghurt denotes a potential nutritional advantage of probiotic proliferation effect. The counts of *Streptococcus thermophilus* range from  $1.44$ - $7.36 \log_{10} \text{cfu/ml}$ ,  $2.37$ - $5.85 \log_{10} \text{cfu/ml}$  and  $4.81$ - $17.15 \log_{10} \text{cfu/ml}$  during the storage period.

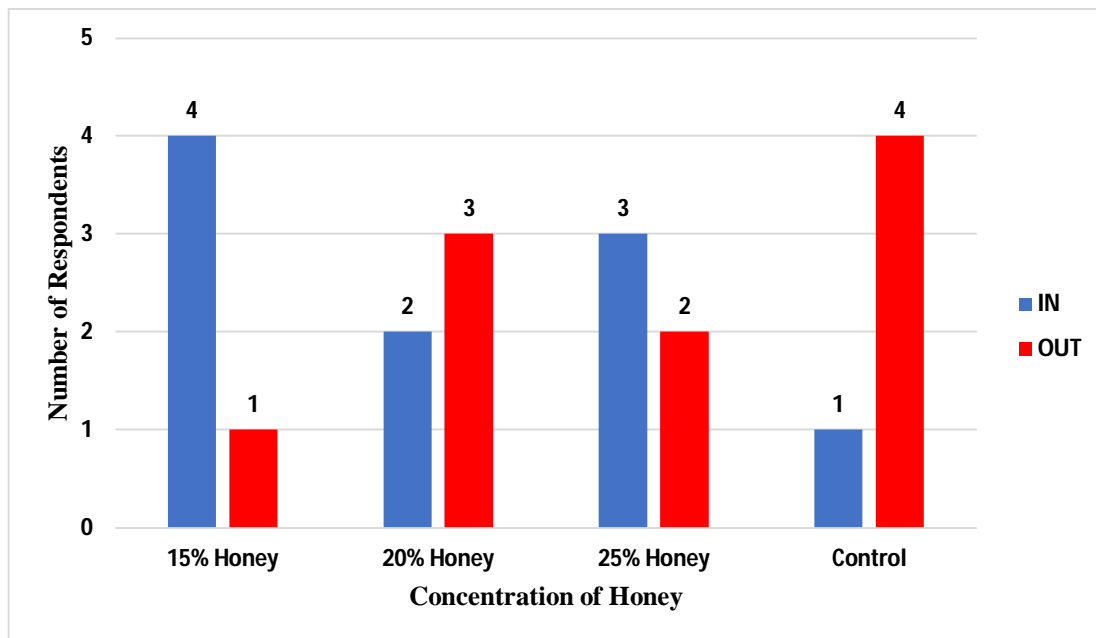
### **Sensory Evaluation of yoghurt produced with isolate 1L**

A sensory evaluation was conducted using different concentrations of honey (15%, 20%, and 25%) on yoghurt samples, assessed by five different panelists. The results of the sensory evaluation revealed that all honey concentration samples, including the control sample, were rejected by the panelists. The rejection was based on deficiencies in appearance/color, aroma,

mouthfeel/texture, sweetness, and freshness as shown in **Table 5** and **Figure 10**.

**Table 5:** Sensory Evaluation of 1solate 1L

Conc of Honey	In	Out	Comment
15%	4 (80%)	1 (20%)	Rejected
20%	2 (40%)	3 (60%)	Rejected
25%	3 (60%)	2 (40%)	Rejected
Control	1 (20%)	4 (80%)	Rejected



**Figure 11:** Sensory evaluation of yoghurt with 15%, 20% and 25%

**Key: In:** Acceptability of Appearance/color, Aroma flavor, Mouth feel/Texture, Sweetness and Freshness

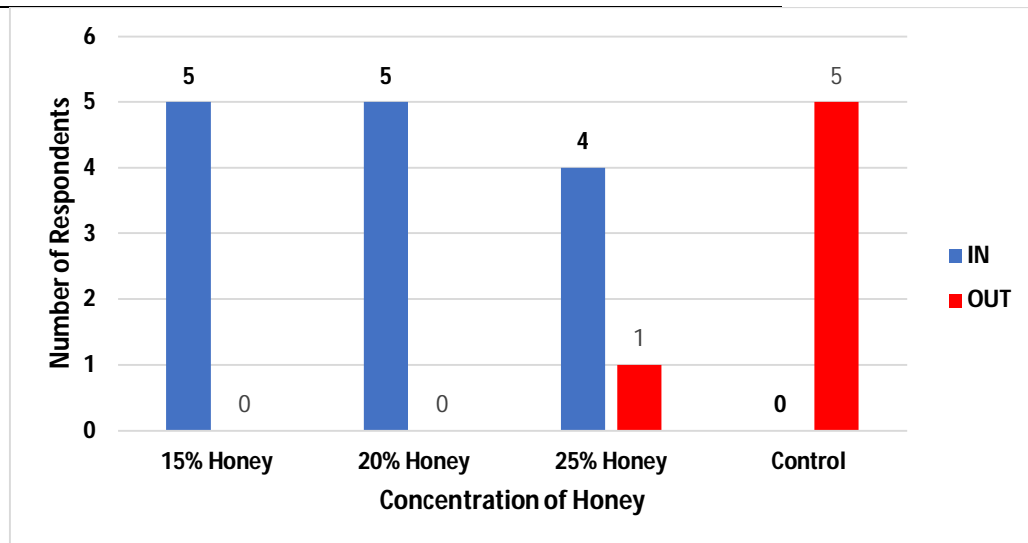
**Out:** Rejected due Appearance/color, Aroma/flavor, Mouth feel/Texture, Sweetness and Freshness

### Sensory Evaluation of Yoghurt Produced with Isolate 2S

**Table 6 and Figure 12** presents the results of the sensory evaluation of yoghurt produced with isolate 2S using different concentrations of honey (15%, 20%, and 25%). The sensory evaluation was conducted by five panelists. The results revealed that the yoghurt samples with honey concentrations of 15% and 20% were accepted by the panelists. However, the samples with 25% honey concentration and the control sample were rejected as shown in table 6 and figure 12.

**Table 6:** Sensory Evaluation of 1solate 2S

Conc of Honey	In	Out	Comment
15%	5 (100%)	0 (0%)	Accepted
20%	5 (100%)	0 (0%)	Accepted
25%	4 (80%)	1 (20%)	Rejected
Control	0 (0%)	5 (100%)	Rejected



**Figure 12:** Sensory evaluation of yoghurt with 15%, 20% and 25%

**Key: In:** Acceptability of Appearance/color, Aroma flavor, Mouth feel/Texture, Sweetness and Freshness

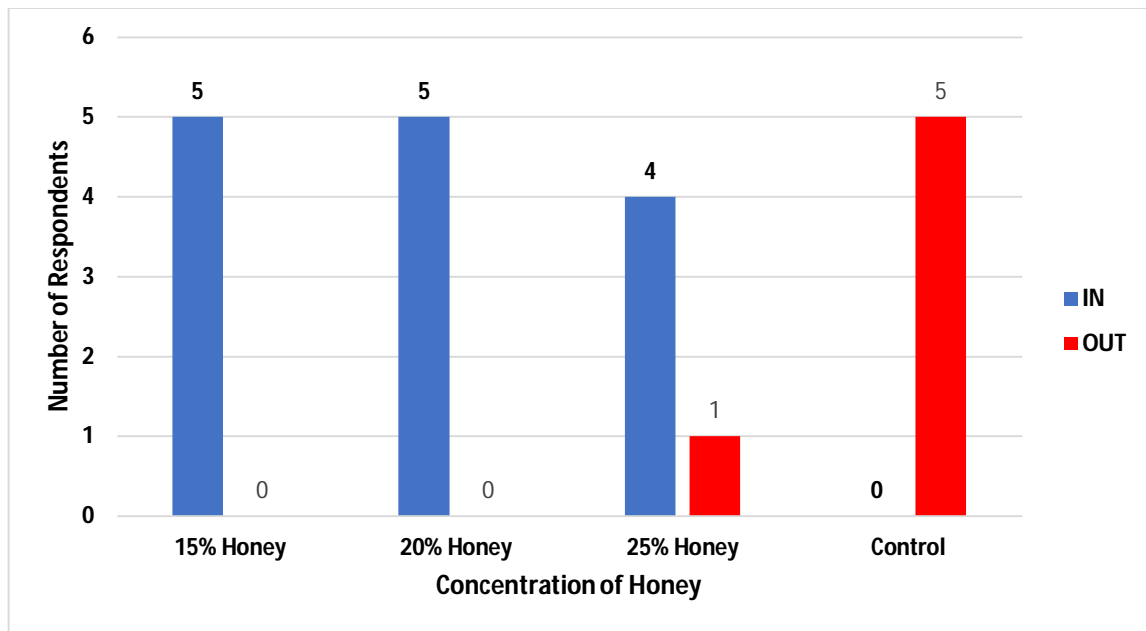
**Out:** Rejected due Appearance/color, Aroma/flavor, Mouth feel/Texture, Sweetness and Freshness

**Sensory Evaluation**

Sensory evaluation was carried out on the different concentration (15%, 20% and 25%) of honey samples on five different panelists. Sensory result revealed that the honey concentration of (15% and 20%) samples were accepted while 25% and the control samples were rejected by the panellist as shown in **Table 7** and **Figure 13**

**Table 7:** Sensory Evaluation

Conc of Honey	In	Out	Comment
15%	5 (100%)	0 (0%)	Accepted
20%	5 (100%)	0 (0%)	Accepted
25%	4 (80%)	1 (20%)	Rejected
Control	0 (0%)	5 (100%)	Rejected



**Figure 13:** Sensory evaluation of yoghurt with 15%,20% and 25%

**Key: In:** Acceptability of Appearance/color, Aroma flavor, Mouth feel/Texture, Sweetness and Freshness

**Out:** Rejected due Appearance/color, Aroma/flavor, Mouth feel/Texture, Sweetness and Freshness

Organoleptic property evaluation of finished products like yoghurt is an important tool to measure consumer's preference and offers wide range of options to assist in marketing and promotion. Yoghurt produced using the first isolate, across the 15, 20 and 25% of honey added, serve sheets of the questionnaire showed that most respondents slightly disliked the yoghurt due to bitter after taste across all the honey concentration -including the control. Possible reasons for these rejections could be linked to the very high count observed during the storage period of the isolate in the yoghurt samples. Overall, yoghurt produced with this isolate was rejected. Yoghurt produced with the other two isolates of *Streptococcus thermophilus*, had 100 and 80% acceptance with yoghurt produced using 15 and 20% honey having the highest preferences of likes in terms of appearance, color, aroma, mouth feel/texture, freshness and sweetness. Yoghurt produced with 25% honey was list preferred as its color was not appealing to the respondents and, was too sweet which makes most respondents slightly disliked it. Across all the yoghurt produced, the control yoghurt samples had the highest acceptance in terms of appearance, However, it is the sourest with lowest sweetness and the only sample reported to be rancid after 21 days storage. Varga, 2006, studied 1, 3 and 5% of acacia honey in yoghurt during storage at 4°C and found that the samples of yoghurt containing 3% honey had the optimum sweetness. Samples with 1% were weak in flavor and sweetness, were the yoghurt with 5% were too sweet and strong in flavor. This is in agreements in our results of yoghurt with 15 and 20% honey having the optimum aroma and sweetness, in contrast to that with 25% having too strong flavor and sweetness. Sert *et al.* (2010) studied the sensory effect of sunflower honey addition at 2%, 4% and 6% on the property of yoghurt during storage at 40c yoghurt with 2% honey had similar flavor and sweetness intensity as the control. Optimum flavor and sweetness intensity was observed at 4% honey addition however, the yoghurt with 6% honey had the highest flavor and sweetness intensity.

#### **pH values of yoghurt over 7, 14 and 21days storage**

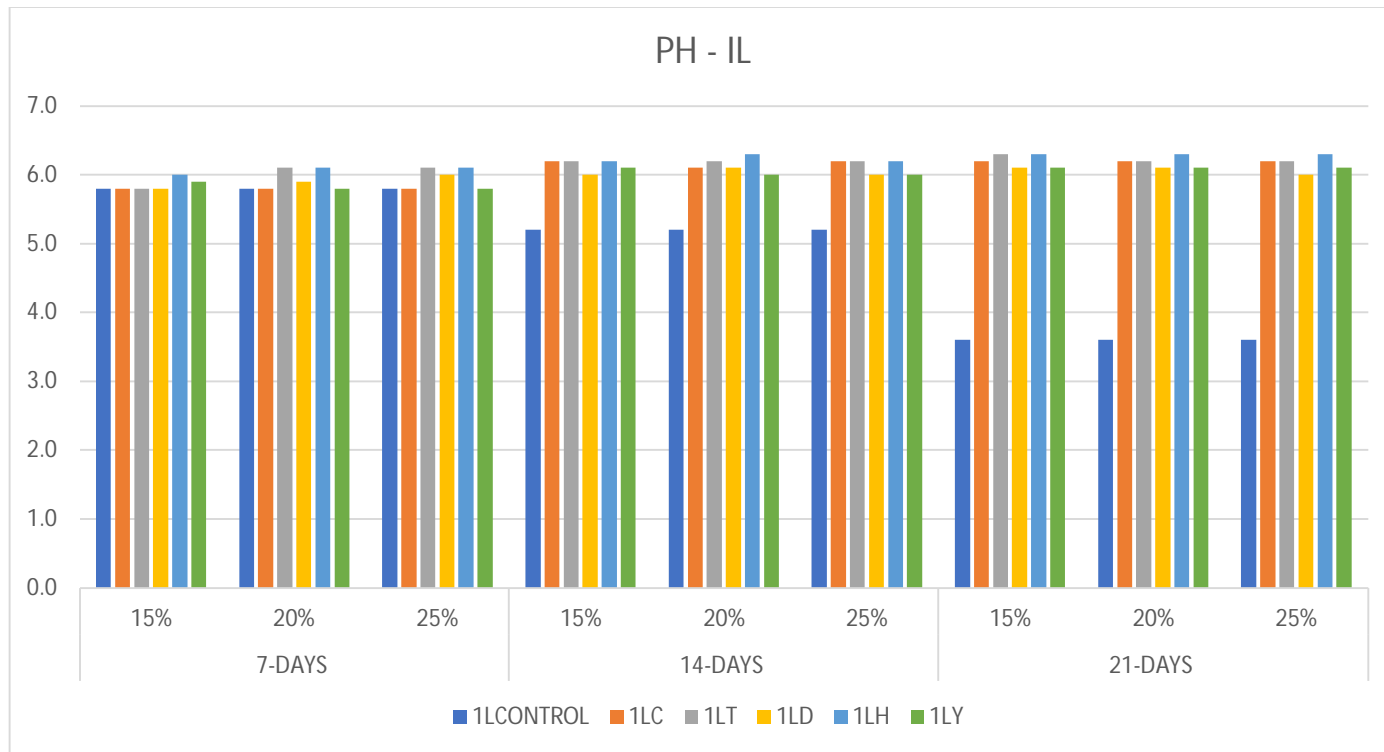
pH values of yoghurt over 7, 14 and 21days storage. After 7 days, the control samples (1LCONTROL, 2SCONTROL, 3SCONTROL) displayed consistent values ( $5.8 \pm 0.00$ ,  $5.5 \pm 0.00$ ,  $5.5 \pm 0.00$ , respectively). Minor variations were observed in other samples, such as 1LT ( $6.0 \pm 0.17$ ), 2SC ( $5.8 \pm 0.06$ ), and 3SC ( $5.8 \pm 0.06$ ). After 14 days, control samples (1LCONTROL,

2SCONTROL, 3SCONTROL) showed slight decreases ( $5.2 \pm 0.00$ ,  $4.8 \pm 0.00$ ,  $4.3 \pm 0.00$ ), while other samples exhibited minor variations, such as 1LT ( $6.2 \pm 0.00$ ), 2ST ( $6.2 \pm 0.06$ ), and 3SY ( $6.2 \pm 0.06$ ). After 21 days, control samples (1LCONTROL, 2SCONTROL, 3SCONTROL) further decreased ( $3.6 \pm 0.00$ ,  $4.1 \pm 0.00$ ,  $4.0 \pm 0.00$ ), whereas other samples maintained higher values with minor variations, such as 1LC ( $6.2 \pm 0.00$ ), 2ST ( $6.2 \pm 0.06$ ), and 3SH ( $6.3 \pm 0.06$ ). These results indicate stability in the control samples over time while our samples exhibited variations, reflecting the impact of storage intervals on different pH as shown in **Table 9** and **Figure 14,15 and 16**

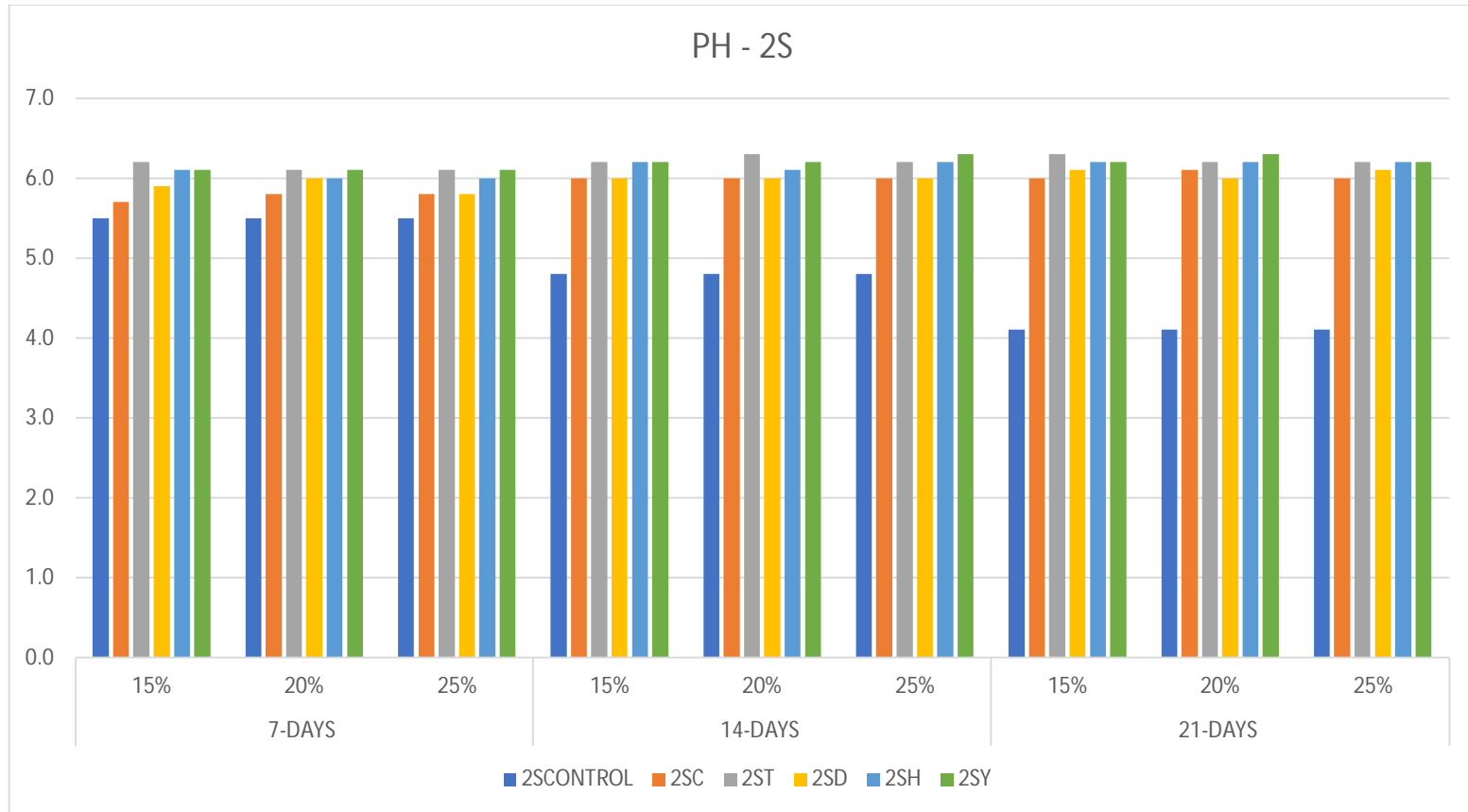
**Table 8:** The pH values of the honey incorporated yoghurt

S/N	7 DAYS STORAGE INTERVAL			14 DAYS STORAGE INTERVAL				21DAYS STORAGE INTERVAL				
	15%	20%	25%	Mean ± Std Dev	15%	20%	25%	Mean ± Std Dev	15%	20%	25%	Mean ± Std Dev
1LCONTROL	5.8	5.8	5.8	5.8 ± 0.00	5.2	5.2	5.2	5.2 ± 0.00	3.6	3.6	3.6	3.6 ± 0.00
1LC	5.8	5.8	5.8	5.8 ± 0.00	6.2	6.1	6.2	6.2 ± 0.06	6.2	6.2	6.2	6.2 ± 0.00
1LT	5.8	6.1	6.1	6.0 ± 0.17	6.2	6.2	6.2	6.2 ± 0.00	6.3	6.2	6.2	6.2 ± 0.06
1LD	5.8	5.9	6.0	5.9 ± 0.10	6.0	6.1	6.0	6.0 ± 0.06	6.1	6.1	6.0	6.1 ± 0.06
1LH	6.0	6.1	6.1	6.1 ± 0.06	6.2	6.3	6.2	6.2 ± 0.06	6.3	6.3	6.3	6.3 ± 0.00
1LY	5.9	5.8	5.8	5.8 ± 0.06	6.1	6.0	6.0	6.0 ± 0.06	6.1	6.1	6.1	6.1 ± 0.00
2SCONTROL	5.5	5.5	5.5	5.5 ± 0.00	4.8	4.8	4.8	4.8 ± 0.00	4.1	4.1	4.1	4.1 ± 0.00
2SC	5.7	5.8	5.8	5.8 ± 0.06	6.0	6.0	6.0	6.0 ± 0.00	6.0	6.1	6.0	6.0 ± 0.06
2ST	6.2	6.1	6.1	6.1 ± 0.06	6.2	6.3	6.2	6.2 ± 0.06	6.3	6.2	6.2	6.2 ± 0.06
2SD	5.9	6.0	5.8	5.9 ± 0.10	6.0	6.0	6.0	6.0 ± 0.00	6.1	6.0	6.1	6.1 ± 0.06
2SH	6.1	6.0	6.0	6.0 ± 0.06	6.2	6.1	6.2	6.2 ± 0.06	6.2	6.2	6.2	6.2 ± 0.00
2SY	6.1	6.1	6.1	6.1 ± 0.06	6.2	6.2	6.3	6.2 ± 0.06	6.2	6.3	6.2	6.2 ± 0.06
3SCONTROL	5.5	5.5	5.5	5.5 ± 0.00	4.3	4.3	4.3	4.3 ± 0.00	4.0	4.0	4.0	4.0 ± 0.00
3SC	5.7	5.8	5.8	5.8 ± 0.06	6.0	5.9	5.1	5.7 ± 0.49	6.0	6.1	6.1	6.1 ± 0.06
3ST	6.0	6.0	6.0	6.0 ± 0.00	6.1	6.1	6.1	6.1 ± 0.00	6.2	6.2	6.2	6.2 ± 0.00
3SD	5.9	6.0	6.0	6.0 ± 0.06	6.2	6.3	6.2	6.2 ± 0.06	6.3	6.2	6.2	6.2 ± 0.06
3SH	6.0	6.1	6.0	6.0 ± 0.06	6.3	6.2	6.2	6.2 ± 0.06	6.4	6.3	6.3	6.3 ± 0.06
3SY	6.1	6.1	6.1	6.1 ± 0.00	6.2	6.1	6.2	6.2 ± 0.06	6.3	6.3	6.3	6.3 ± 0.06





**Figure 14:** pH value of isolate 1L during 7, 14 and 21 days storage



**Figure 15:** pH value of isolate 1L during 7-, 14- and 21-days storage

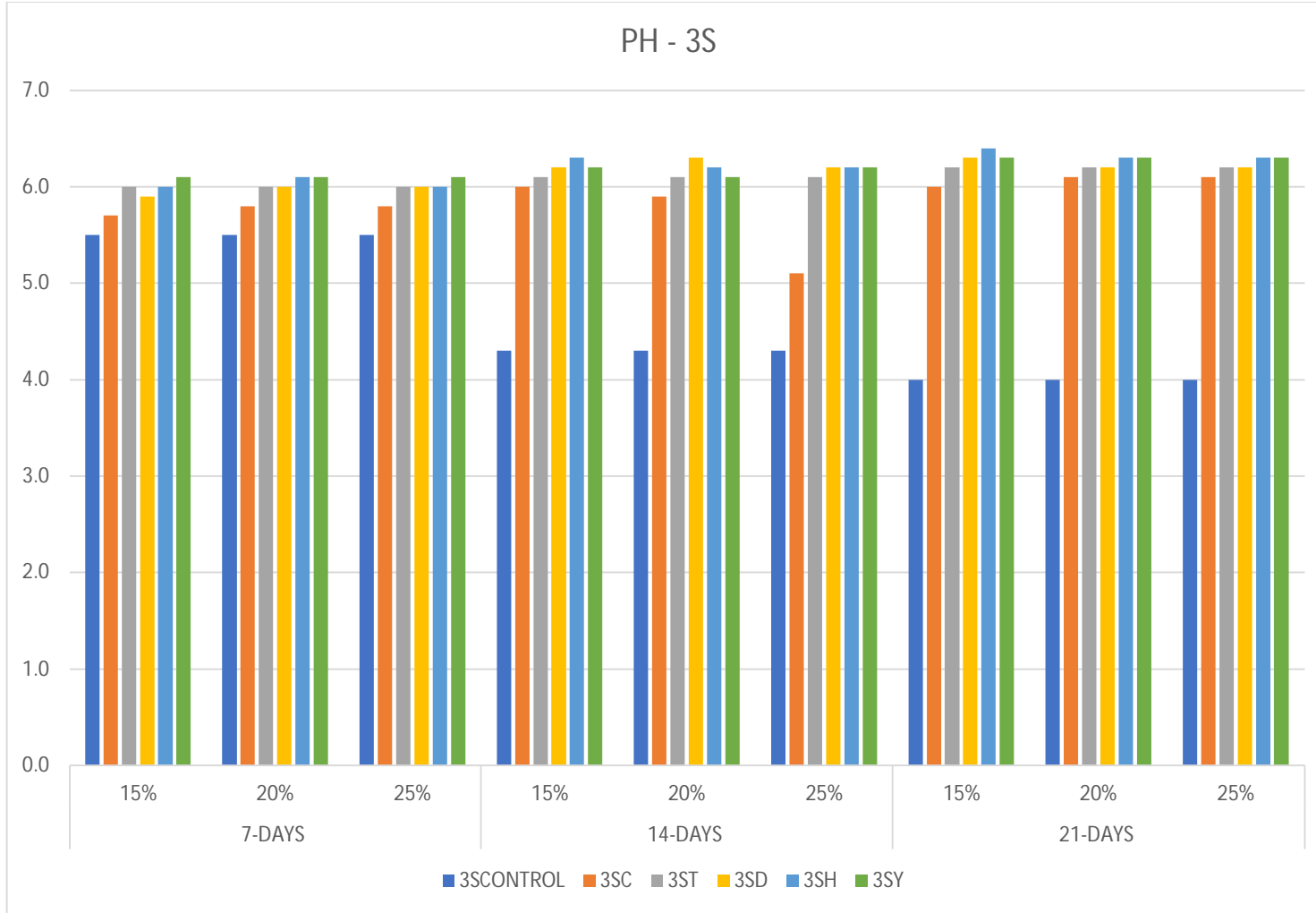


Figure 16: pH value of isolate 1L during 7, 14 and 21 days storage

The relatively stable count in this study is in agreements with findings of (Rizal *et al.*,2024) that states; a strong correlation exist between lactic acid bacteria count and pH in symbiotic bioactive yoghurt, when the lactic acid bacteria count increases, the pH of the product decreases. This is also in conformity to the pH values of the control yoghurt samples were pH decreases further as the lactic acid bacteria count increases. Similar findings in pH decrease in dairy products as a result of increase in counts during storage were reported by (Martharini and indratiningsih 2017, Purnomo and Muslimin 2012). Therefore, adding honey as a natural sweetener makes it possible to increase the nutritional, energy value and sensory ranking of the yoghurt produced.

## CONCLUSION

The honey samples analyzed, confirmed the presence and acceptable ranges of the bioactive components screened, to be in line with international honey standards requirements and previously documented literatures. This is suggestive of the suitability of the honey for use in food production. Honey has shown promising in regards to its acceptability, sensory and preservative effect. Yoghurt incorporated with honey were liked and not reported rancid/spoiled after 21 days storage at 4°C, in contrast to the control samples that were dislikes based on the sensory perception, reduced pH values and rancidity after 21 days storage at 4°C. Based on the sensory evaluation results, preferably honey as natural sweetener and preservative outstand when to compared to commercially synthesized on nutritive sweeteners used in yoghurt production.

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