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Effect of Concentration of Fenugreek (*Trigonella Foenum-Graecum L.*) on the Physicochemical, Microbial Stability and Sensory Quality of Stirred Yogurt

[1]Eze, Chinazom, M., [1]Amonyeze, Ahunna, O; [2]Okoye, Obiageli, I; [1]Okoronkwo; Ngozi, C.,, [1]Asogwa, Ifeyinwa S; [1]Uzoukwu, Precious, C

[1]Department of Food Science and Technology; [2]Department of Home Science and Management, University of Nigeria, Nsukka.

Corresponding Authors: chinazom.eze@unn.edu.ng & ngozi.okoronkwo@unn.edu.

Abstract

This study analyzed proximate composition, physicochemical properties, microbial stability, and sensory characteristics of stirred yogurt formulated with fenugreek (*Trigonella foenum-graecum* L.) extract and stored for four weeks. Yogurt was produced using standard methods by incorporating graded levels of fenugreek extract at 0.0, 0.1, 0.3, and 0.5% respectively. Weekly analyses were conducted using established standard procedures. Data were analyzed using mean, standard deviation and ANOVA at 0.05 level of significance. Results shows that moisture (83.74–84.85%), protein (11.61–12.06%), fat (0.31–1.79%), and carbohydrate (2.66–3.55%) contents increased as the concentration of fenugreek extract increased. No fiber was detected, while ash content ranged between 0.01–0.02%. The pH decreased steadily from 4.10 in week one to 3.85 in week four, with a corresponding rise in titratable acidity from 0.23 to 0.295% respectively. Lactic acid bacteria count ranged from 2.8–3.6 × 10^5 cfu/g at day zero to 2.1–3.9 × 10^4 cfu/g at week four, showing no significant difference ($p > 0.05$) initially. Total viable counts followed a similar pattern. Sensory evaluation indicated that fenugreek extract up to 0.5% produced acceptable yogurt in terms of flavor, texture, and overall quality, enhancing nutritional, microbial, and sensory attributes while maintaining good storage stability for four weeks at room temperature.

Keywords: Yogurt, Spices, Fenugreek, Physicochemical, Microbial Stability, Sensory, Characteristics.

Introduction

Yogurt, a fermented dairy product, offers significant nutritional benefits and functional benefits due to its high probiotic bacteria content (Meybodi *et al.*, 2020). It is a creamy, acidic food product, made by fermenting milk with lactobacillus strains or a mixed culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in a 1:1 ratio (Sansawal *et al.*, 2017). These organisms act on lactose and result in the production of lactic acid which increases the acidity of the yogurt, thereby forming gel. Like every other fermented food product, yogurt results from the activities of beneficial microorganisms in milk which may be obtained from cow, sheep, nut, soy, and several other creamy food substances. The health benefits of yogurts are well-known

and several yogurt-based products are consumed by people all over the world (El-Shibiny *et al*., 2018). Yogurt offers various textures, fat content, and flavors. A new approach is enhancing nutritional, antioxidant, and sensory properties by enriching milk-based fermentation mix with spices, fruits, and medicinal herbs (Ogunyemi *et al*., 2021).

Dairy food products' limited bioactive compounds can however, diminish their value, prompting some authors to suggest adding plant or fruit-based additives to fortify yogurt (Caleja *et al*., 2016). Spicing yogurt is a recent modification in the dairy industry due to its antioxidant properties and nutritional benefits. Spices are natural plant or vegetable products used to impart flavor, aroma, and pungency (Chhteri *et al.,* 2019). Spices come from various plant components, including flowers, fruits, berries, seeds, rhizomes, roots, leaves, kernels, arils, bark, and bulbs. They can be found in various parts of plants like cloves, saffron, cardamom, chillies, black pepper, juniper, and more. Fenugreek (*Trigonella foenum-graecum* L.) is a medicinal plant with numerous health benefits due to its rich biogenic elements and favorable fatty acid and phytochemical composition. Its seeds, leaves, roots, and stems have been scientifically proven to possess antidiabetic, anticancer, antioxidant, and antimicrobial activities (Faisal *et al*., 2024; Almuzaini *et al*., 2024). Fortification of dairy products with fenugreek seed can increase mineral and micronutrient content and enhance functional properties of dairy products (Dhawi *et al*., 2020). Fenugreek, a medicinal herb and annual forage legume, has potent antimicrobial properties against foodborne pathogens, improved antioxidant and antibacterial activity thus, supporting its role in prolonging shelf-life and improving safety (Dhawi *et al*., 2020). Its phytochemicals include flavonoids, tannin, alkaloids, coumarin, anthocyanin, polyphenols, saponins, and soluble fiber (galactomannans) that provides specific mechanisms (radical scavenging, metal chelation, and microbe growth inhibition) that plausibly substitute for some synthetic antioxidants or preservatives in food formulations (Faisal *et al*., 2024; Bakhtiar *et al*., 2024). Globally, India is the principal producer of fenugreek and supplies the bulk of seed used for both culinary and medicinal purposes (Dhull, 2023). In West Africa and Nigeria in particular, fenugreek is present in the ethnobotanical and clinical landscape: it is used traditionally as galactagogue and medicinal herb and has appeared in recent Nigerian agricultural and animal-nutrition research, indicating local cultivation and applied use (Soyombo, 2024; Jimoh, 2024). Fenugreek though not widely consumed in everyday Nigerian cuisine as it is in South Asian diets, its documented traditional uses, growing experimental interest, such as its antioxidant and antimicrobial activities from the seed and leaf extract, which is known to delay lipid oxidation, inhibit spoilage of pathogenic microorganisms, thus, making them promising natural preservatives for foods with limited cold-chain stability (Faisal *et al*., 2024; Türker, 2024). In Nigeria, where poor or intermittent electricity and refrigeration are common, a low-cost, locally acceptable natural preservative that also

improves nutritive value is particularly attractive (Dhawi *et al*., 2020; Türker, 2024; Faisal *et al*., 2024). These pragmatic, nutritional, and safety rationales together justifies the choice of inclusion of fenugreek to yogurt in this study.

Objectives of the Study

The study evaluated physicochemical, microbial stability and sensory characteristics of formulated yogurt spiced with the fenugreek extract. Specifically, the study determined:

1. proximate composition of formulated yogurt spiced with fenugreek extract (moisture, protein, fat, ash, fiber carbohydrate)
2. physicochemical properties (pH and TTA) of yogurt spiced with fenugreek extract.
3. microbial stability (total viable count and lactic acid bacteria count) of spiced yogurt with the fenugreek extract.
4. sensory characteristics of yogurt spiced with fenugreek extract at graded levels.

Materials and Methods

Design of the Study: The design study used was an experimental research design.

***Procurement of Raw Materials*:** Materials used, whole milk, stabilizers (carboxyl methylcellulose), fenugreek seed, and sugar were purchased from Ogige main market in Nsukka. Starter culture (yoghurmet) was purchased from raw material Allied Market Onitsha. All the chemicals' reagents used for this study were of analytical grade and obtained from the Department of Food Science and Technology, the University of Nigeria Nsukka.

Processing of Samples: Fenugreek extract was extracted using ethanol from sorted fenugreek seeds. The extract was mixed with 95% ethyl alcohol and stored overnight. The solution was filtered, evaporated, dried to obtain the residue. The residue was then weighed before use.

Yogurt was produced through pasteurization, homogenization, fermentation, cooling, and storage. The yogurt was then inoculated with starter culture, fermented, homogenized, and divided into portions with varying concentrations of fenugreek. Samples were taken and analyzed at one-week intervals for four weeks.

Proportions for the Production of Stirred Yogurt and Fenugreek Extract as follows:

Samples	C (%)(g)	SC (g)	SUG (g)	(CMC) (g)
Yoghurt control (YC)	0.0 %	10	10	10
Yoghurt + Fenugreek extract 1 (YFG $_1$)	0.1 (1.5 g)	10	10	10
Yoghurt + Fenugreek extract 2 (YFG $_2$)	0.3 (4.5 g)	10	10	10
Yoghurt + Fenugreek extract 3 (YFG $_3$)	0.5 (7.5 g)	10	10	10

C= Concentration; SC = Starter Culture; SUG = Sugar; CMC = Carboxyl Methylcellulose

Proximate Composition of Formulated Spiced Stirred Yogurt: Proximate composition analysis was carried out using American Association of Official Analytical Chemists (AOAC) (2010) for each content. It was carried out in duplicates using appropriate standard methods, as follows:

Moisture: Crucible was cleaned, dried, and weighed. then 2 ml of sample was added to the empty crucible, dried in a hot air oven, and cooled in a desiccator.

Crude Protein: Sample was dissolved in anhydrous sodium sulphate and hydrated copper sulphate, then digested with concentrated tetraoxosulphate acid, cooled, and collected for distillation. The ammonia was titrated with hydrochloric acid, and the end point was determined by observing the color change from green to pink

Crude Fiber: Sample was hydrolyzed with sulphuric acid (H_2SO4), boiled, filtered, and boiled again with NaOH and HCl acid. The residue was then dried, cooled, and burned in a muffle furnace at 500°C for five hours.

Crude Fat: Sample was placed in a Soxhlet apparatus and extracted for 4-6 hours. The oil was then dried, cooled, and weighed, and the fat content obtained percentage of raw materials.

Ash: A preheated and cooled crucible was weighed (W_1) and 2 ml was weighed into the preheated cooled crucible (W_2). The sample was charred. The charred sample in the crucible was transferred into a preheated muffle furnace at 550 ^oC for 2 hours until a white and light grey ash is obtained (W_3).

Carbohydrate content was determined as the nitrogen-free extraction calculated by difference.

PH Value: A standard pH meter (model 20 pH conductivity meter, Denver Instrument, United Nations Inventory Database) was standardized using buffer solutions of pH 4.0 and 9.0. The pH electrode was dipped into the yoghurt (10 %) and after a few minutes of equilibration, the pH of the yoghurt sample was taken (AOAC, 2010).

T**otal titratable acidity (TTA)** was determined using the AOAC (2010) method. The sample (1 g) at 25 ^oC was measured into a flask and diluted with 100ml distilled water. Phenolphthalein indicator (2 drops) was added to each yoghurt sample and titrated with 0.1 M NaOH to the first permanent pink colour. The acidity was reported as the percentage lactic acid by weight.

Total viable count was determined using pour plate method by Harrigan and McCance (1976) was used. One gram of the sample was macerated into 9 ml of Ringers solution and mixed thoroughly by shaking. This was further diluted to obtain 10^{-2} and 10^{-3} concentration. Then 0.1 ml dilution was transferred from each dilution bottle into the corresponding plate and 15 ml of sterile nutrient agar medium was poured and mixed thoroughly with the inoculum by rocking the plates. The plates were incubated at 38 ^oC for 24 hours after which the colonies formed, they were counted and expressed as colony forming units per gram (cfu/g).

Lactic acid bacteria (LAB) in the formulated yoghurt was determined using deMan Rogosa Sharpe (MRS) Agar (CM 361) as described by Oxoid manual (Oxoid, 1982). Samples were serially diluted in duplicates using the surface pour plate method. The plates were incubated under anaerobic conditions at 37 ^{o} C for 48 hours. After incubation, the number of colonies was counted and represented as colony forming unit per milliliter (cfu/ml).

Sensory evaluation was conducted to assess various attributes of the formulated spiced yoghurt including their taste,

consistency, mouth-feel, flavor, aftertaste, appearance, and overall acceptability. The panel consisted of a 20-man semi-trained panelist comprising of students and staff members from the Department of Food Science and Technology, University of Nigeria, Nsukka. These panelists were selected for their ability to provide informed opinions on food products based on prior training and familiarity with basic sensory evaluation techniques. To get the sensory responses, a structured questionnaire was developed using a nine-point Hedonic scale coded as extremely, 9; like very much, 8; like moderately, 7; like; slightly, 6; neither like or dislike, 5; dislike slightly, 4; dislike moderately, 3; dislike very much, 2; dislike extremely. This scale is commonly used in sensory testing to quantify the level of preference or acceptance for each attribute.

***Data Analysis Technique*:** The data obtained was expressed as mean ± standard deviation. Results of two replicates were used. Analysis of variance (ANOVA) was used to determine statistically significant differences between the means. Level of significance was at 5% level of probability ($P<0.05$).

Findings of the study

Table 2: Proximate Composition of Yogurt Spiced with Fenugreek.

Sample	Moisture	Protein	Fat	Fibre	Ash	Carbohydrate
YFG_1	83.74^{a}±0.08	11.61^{a}±0.09	1.10^{b}±0.00	ND	0.02^{a}±0.01	3.55^{b}±0.01
YFG_2	84.28^{b}±0.07	11.84^{ab}±0.08	1.20^{b}±0.01	ND	0.02^{a}±0.01	2.66^{a}±0.03
YFG_3	83.95^{a}±0.07	11.60^{a}±0.14	1.79^{c}±0.15	ND	0.01^{a}±0.00	2.66^{a}±0.36
YC	84.85^{c}±0.19	12.06^{b}±0.07	0.31^{a}±0.04	ND	0.02^{a}±0.01	2.77^{a}±0.32

YC = Yogurt control, Yoghurt + Fenugreek extract 1 (YFG1), Yoghurt + Fenugreek extract 2 (YFG 2), Yoghurt + Fenugreek extract 3 (YFG 3), ND = Not detected

Values are mean ± standard deviation of duplicate readings. Means on the same column with different superscripts are statistically different ($p < 0.05$).

Table 2 reveals the composition of yoghurt spiced with fenugreek. The control sample had the highest moisture content, protein content, and fat content. The least protein content was found in sample YFG3 (0.5%). Fiber was not detected in any of the samples, yogurts have poor fiber level because they are milk and water-based products and the extract added has no fiber. Ash content did not show significant differences. The carbohydrate content of the yogurt samples was low. Carbohydrate (lactose, the major constituents of milk) is converted to lactic acid during yogurt (fermentation) production. So, conversion of lactose to lactic acid in fermentation accounts for the low content of carbohydrate of yogurt.

Total titratable acidity of the Fenugreek spiced yogurt

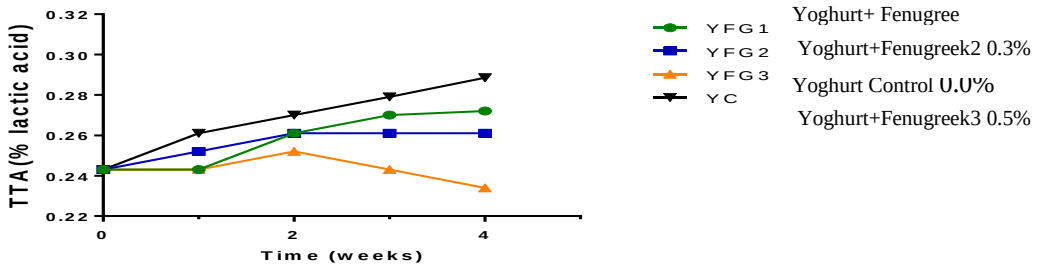

Figure 1: Total titratable acidity of the spiced yogurt

Figure 1 reveals the changes in titratable acidity of stored yoghurt samples. There was regular increase in titratable acidity of the unspiced yoghurt sample (YC) during storage (0.23-0.295% from the zero day to the fourth week) which could be due to fermentation (activity of lactic acid bacteria). However, the spiced yoghurt samples were observed to maintain a relatively stable titratable acidity. This indicated that fenugreek could have an antimicrobial property that could inhibit the activity of the probiotics, thus less lactic acid formed during storage. The high titratable acidity of the unspiced yoghurt sample compared with the spiced samples, this could be an indication of the positive effect of fenugreek on the preservation of yoghurt.

pH value for the Fenugreek spiced yogurt

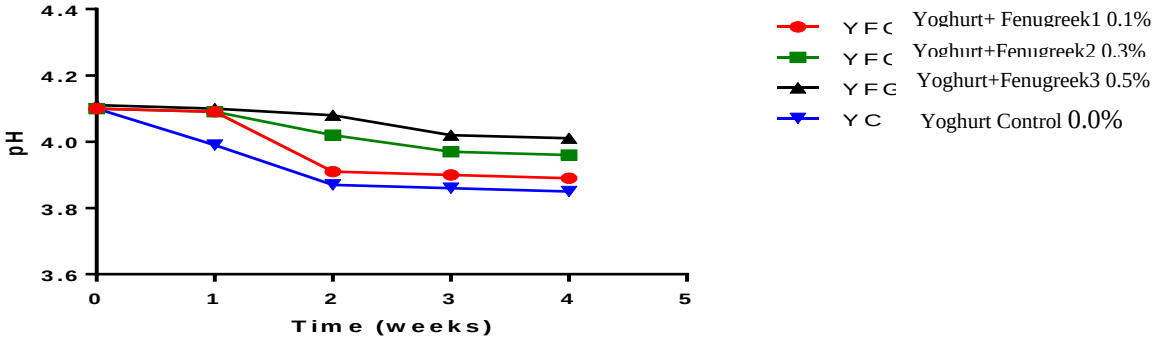

Figure 2: pH value of the spiced yogurt

Figure 2 shows a slight drop in pH of yoghurt spiced with fenugreek, slightly increasing (4.1% - 3.85%), with storage time, indicating an inverse relationship with titratable acidity. The changes in pH values obtained were not significant from the beginning of storage for the unspiced yogurt sample (4.1%), a significant drop in pH was noticed (4.0%). The unchanged pH from the 2nd week of storage (3.85%) could be due to the insignificant activity of lactic acid bacteria. During storage, activity of lactic acid bacteria continued with production of lactic acid leading to

drop in pH value. The unspiced yogurt showed the lowest pH value (3.82%) by the end of the four (4) weeks storage period.

Table 3: Microbial Composition of Fenugreek Spiced Yogurt at 0 day and 4th Week

Samples	TVC(cfu/g) zero day	Fourth week	LAB (cfu/g) zero day	Fourth week
YC	3.0×10^3	1.4×10^3	3.8×10^5	6.5×10^4
YFG_1	2.4×10^3	1.1×10^3	2.1×10^5	4.8×10^4
YFG_2	1.7×10^3	5.6×10^2	1.3×10^5	3.6×10^4
YFG_3	1.6×10^3	3.3×10^2	3.7×10^5	5.2×10^4

YC = 0.0% fenugreek or control, YFG_1= 0.1% fenugreek, YFG_2 = 0.3% fenugreek, YFG_3 = 0.5% fenugreek

The Total viable count showed significant difference ($p < 0.05$) between the samples this could be as a result of the anti-microbial effect of the fenugreek spice, since herbs and spices are known to retard microbial spoilage in dairy products (Abdel- Kader *et al*., 2001). as a result of the anti-microbial effect of the fenugreek spice, since herbs and spices are known to retard microbial spoilage in dairy products (Abdel- Kader *et al*., 2001).

Table 4: Sensory Scores of the Yogurt Spiced with Fenugreek Extract

Samples	Appearance	Consistency	Mouthfeel	Flavor	Taste	AfterTaste	Overall acceptability
YFG1	7.30^{bc}±1.32	7.30^{b}±1.38	5.40^{ab}±1.57	4.45^{b}±1.57	4.10^{a}±1.55	5.85^{b}±1.92	5.60^{b}±2.11
YFG2	7.65^{c}±1.42	6.75^{ab}±1.59	6.40^{b}±1.88	5.25^{ab}±1.8	4.70^{ab}±1.52	5.00^{ab}±1.38	5.50^{b}±1.64
YFG3	6.40^{b}±1.96	6.60^{ab}±1.50	5.90^{ab}±1.68	5.45^{b}±2.01	5.75^{b}±1.97	5.05^{ab}±2.26	5.00^{ab}±1.90
YC*	5.15^{a}±1.73	5.70^{a}±1.87	5.20^{a}±1.74	4.15^{a}±1.57	4.60^{ab}±2.35	4.25^{a}±2.20	4.25^{a}±1.83

Values are presented in mean ± standard deviation. Sample means with the different superscripts on the same column are significantly ($p<0.05$) different YC = 0.0% fenugreek or control, YFG_1= 0.1% fenugreek, YFG_2 = 0.3% fenugreek, YFG_3 = 0.5% fenugreek

Table 4 shows that sample YFG2(0.3%) scored highest in appearance, consistency, and mouthfeel, with the control sample having the lowest rating. Sample YFG3(0.5 %) had the highest flavor and aftertaste score, followed by YFG2(0.3%) and YC (0.0%), with the control (YC) (0.0%) scoring the least. The yoghurt samples with fenugreek flavor were analyzed, with samples YFG1 and YFG2 (0.3%) exhibiting the highest acceptability, followed by YFG3(0.5 %) and the control YC (0.0%). Yogurt spiced with fenugreek was acceptable for its sensory characteristics, thus, the addition of fenugreek seed extract did not overlap with the yogurt's overall acceptability.

Discussion of Findings

The moisture content of the spiced yoghurt as reported by Ndife, 2014 was maintained within the range of most yoghurt available in the market (80-86%). According to Ihemeje *et al*. (2015) and Bakhtiar *et al*., (2024), the high moisture content of the formulated product could be as a result of the dilution effect (reconstitution) of the milk prior to fermentation.

The spiced yogurt samples contained a moderate protein content (11.60-12.06%),

meeting the National Yogurt Association's (2000) commercial standard of 11-18% protein in commercial yogurt. Ihemeje *et al*. (2015) found a 10.03% protein content in this study, possibly due to the use of skimmed milk, a common source for fortified yogurt. Spiced yoghurt may have enhanced protein content through fortification with plant proteins, thereby enhancing its quality and nutritional value (Dabija *et al*., 2018). Fermented dairy products' increased protein content enhances satiety, especially beneficial for those following specific diets and health reasons (Dabija *et al*., 2018). Adding fenugreek seed flour typically increases the protein and ash content of the yogurt in proportion to the fortification rate because seed flour contributes additional protein and mineral residues (Dhawi, 2020).

The addition of fenugreek extract to yogurt samples increased fat content, with sample YFG3(0.5% conc. fenugreek) having the highest value (1.79%), attributed to its lipid form and low oil content of the milk used for production. Fenugreek addition usually does not markedly raise yogurt fat; if defatted flours or aqueous extracts are used, its fat effects are negligible (Faisal *et al*., 2024). Ihemeje *et al*. (2015) found yogurt fat content varies by milk oil content, with skimmed milk having low fat and full cream having higher fat content and ginger-spiced yogurt has the highest fat content.

The study reveals that yogurts typically have low fiber content due to their milk and water-based nature. The addition of fenugreek extract did not significantly alter the ash content, attributed to factors like milk composition, nature, and ingredient quantity (Bakhtiar *et al*., 2024).

The yogurt samples showed low carbohydrate content, likely due to the conversion of lactose to lactic acid during fermentation. This is consistent with previous reports by Ihemeje *et al*., (2015), making yogurt an ideal food for lactose intolerant sufferers. Unspiced yogurts showed increased titratable acidity during storage, possibly due to lactic acid bacteria fermentation. Similar results were also observed by Madhubasani *et al*., (2020). Cinnamon-added yogurts had a pH between 3.98 and 4.20 and a TTA between 0.74% and 1.97% (Gunes and Bilgin, 2019). According to Felfoul, (2017) the pH was found to decrease from 4.62 (control) to 4.44 on the addition of 2.5% ginger powder. Spiced yogurts maintained stable acidity, with pH changes minimal. Sample YFG3(0.5% conc of fenugrek) yogurt treatment showed the highest pH value (4.19) on the 4th week, attributed to high levels of fenugreek seed extract with strong bacteria activity. This increase in titratable acidity during storage may be due to lactic acid production (Joung *et al.,* 2016). The pH of yogurts with plant extracts slightly decreased due to fenugreek seed extract's antimicrobial activity thus, indirectly stabilizing pH over storage (Türker, 2024). The stable pH and TTA value in spiced yogurts may be due to low fermentation levels, contributing to yogurt preservation (El-Abd *et al.,* 2018). Fenugreek seed flour significantly increased the viable counts of bacteria in yogurt compared to control and moringa-fortified yogurt (Dhawi, 2020). The total viable count ranged from 1.6×10^3 - 3.0×10^3 for the zero day, and 1.4×10^3 - 3.3×10^2 after the fourth week.

The Lactic Acid Bacteria count ranged from 1.3 x 10^5-3.8 x 10^5 for the zero day and 3.6 x10^4-5.2 x 10^4 for the fourth week. This could be due to the anti-microbial potential of fenugreek spice which increase as concentration increases (Türker, 2024; Faisal *et al*., 2024).

The study found that lactic acid bacteria in fenugreek spiced yogurt ranged from 1.3-3.8 CFU/mL for the first week, and increased with increased concentration of fenugreek extract (Faisal *et al*., 2024). The storage time significantly affected the total lactic acid bacteria count, as longer storage led to higher organic acid production, causing a decrease in pH and ultimately causing the bacteria's death (Dhawi, 2020; Faisal *et al*., 2024; Türker, 2024).

The overall acceptability was appreciated at 0.3 percent concentration of fenugreek extract. Sensory studies with fenugreek-fortified yogurt show similar findings that fortification at 0.1–0.2% seed flour produced yogurts with acceptable sensory scores (Dhawi, 2020). The findings also align with those of Kumar *et al*. (2013) who prepared yoghurt concentrate supplemented with crushed leaves mint at ratios 2, 4 and 6. Also, the yoghurt spread shelf life was 10 days at 5^{o}C. This finding also aligns with previous studies indicating that adding spices like cinnamon, cardamom, and fenugreek powder to goat's milk curd enhances the flavor and odour of the cheese (Hamid and Abdelrahman, 2012). The addition of fenugreek seed extract did not overlap with the overall acceptability of the yogurt, thus making the spiced yoghurt acceptable to the panelists.

Conclusion

The study found that fenugreek seed extract significantly improved yogurt's chemical properties, including pH, titratable acidity, microbiological content, and sensory qualities. It stabilized yogurt samples for up to four weeks without refrigeration, preserving quality metrics and consistency. The proximate, physicochemical, microbial and sensory evaluation of yogurt without plant extracts revealed that it cannot be stored beyond four weeks at ambient temperature, due to high acidity and other quality parameters. Sensory evaluation indicated that fenugreek extract up to 0.5 percent produced acceptable yogurt in terms of flavor, texture, and overall acceptability with improved microbial stability. This therefore, suggests that fenugreek extract could be used as a natural food preservative for dairy products.

Recommendations

Based on the findings of the study, the following recommendations were made:

1. Food scientists should organize nutrition education and programmes to provide consumers with information on benefits and nutritional values of fenugreek extracts., particularly in dairy products
2. Further studies should be carried out to evaluate other properties of fenugreek that could promote its practical applications as food additive.

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