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Evaluation of Fenugreek Seed Extract, Beta-Chitosan, and Their Blends As Edible Coatings

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ABSTRACT

Fenugreek water extract and β-chitosan extract blends at ratios of 30:70 (A), 50:50 (C), and 70:30 (E) were evaluated as effective edible coatings for extending the shelf life of fresh chicken breasts. The study showed that the infrared spectral peaks of fenugreek seed powder and its water extract were observed at 3392.17cm^{-1} and 3428.81cm^{-1}, respectively, indicating the presence of -OH groups attributed to phenolic compounds. The fenugreek water extract exhibited the highest content of total phenols and flavonoids, with values of 61.00mg GAE/g, 34.04mg CAE/g, and 70.78% antioxidant activity, respectively. The results indicated that increasing the percentage of fenugreek in the blends led to higher levels of total phenols, flavonoids, antioxidant activity, and antimicrobial effectiveness against *Escherichia coli*, which showed the largest inhibition zone (48mm), while *Listeria monocytogenes* showed the smallest inhibition zone (9mm). Mechanical property analysis revealed that blend C (50% fenugreek + 50% chitosan) provided favorable mechanical characteristics, contributing to improved preservation of the coated chicken breast samples during storage. These findings were supported by sensory evaluation, which demonstrated that films made of β-chitosan, fenugreek, and their 50:50 blend delivered the best perfomance. Throughout the storage period, the control sample showed the highest increase in pH, reaching 7.40 by the end, while blend C (50% fenugreek water extract/50% β-chitosan extract) maintained the lowest pH. Additionally, blend C significantly reduced the rate of rancidity, with malonaldehyde levels reaching only 0.481mg/kg. In conclusion, blend C (50% fenugreek water extract/50% β-chitosan extract) was the most effective edible coating for improving the shelf life of fresh chicken breasts by enhancing antimicrobial, antioxidant, and mechanical properties, sensory quality, and by reducing rancidity.

1. Introduction

A recent development in packaging is the use of inexpensive, biodegradable edible films. Due to their biodegradability, edible films can significantly reduce the global reliance on plastics (Nazir and Wani, 2022). Millions of tons of food are lost annually due to spoilage and deterioration. Edible films and coatings offer an efficient and sustainable solution to minimize food waste. These films are typically prepared using polysaccharides, lipids, proteins, or their blends, along with other food-grade ingredients (Han, 2014). Meat deterioration during processing, distribution, and display presents major challenges to product quality and safety. It leads to undesirable changes and shortens the shelf life of products, negatively impacting both businesses and consumers. Therefore, edible films and coatings made from biopolymers such as proteins, lipids, and polysaccharides combined with active compounds represent a promising alternative. In general, various combinations of active edible films or coatings have proven beneficial in preserving chicken meat (Moura-Alves, 2023).

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Trigonella foenum-graecum, commonly known as fenugreek, is a leguminous plant that originated in Western Asia and has spread across Europe, the Mediterranean, and Asia (Kumar et al., 2009). Fenugreek seeds exhibit various biological activities, including antidiabetic, anticancer, antioxidant, anti-inflammatory, antibacterial, gastric stimulant (for anorexia), and hepatoprotective effects. They are widely used for their therapeutic and chemopreventive properties, such as antiulcer, anthelmintic, hypocholesterolemic, and hypoglycemic effects (Charles and Kehinde, 2014). Furthermore, fenugreek contains a variety of phytochemicals including steroids, alkaloids, and flavonoids which contribute to its wide range of medicinal benefits (Moradi Kor et al., 2013). Fenugreek seeds are rich in mucilage, a natural gummy substance that coats many seeds to aid hydration and germination (Aruna et al., 2018). Mucilage is a biocompatible, non-toxic, and cost-effective hydrocolloid, primarily composed of high molecular weight polysaccharides with excellent film-forming ability (Behbahani et al., 2018). Fenugreek gum, therefore, represents a promising biodegradable natural polymer with therapeutic significance. It is easily accessible, inexpensive, and non-toxic (Kumar and Sharma, 2022). Exploring the application of fenugreek seed mucilage in edible films is of significant interest, especially given its unique properties and potential advantages in the food industry. Fenugreek mucilage exhibits excellent emulsifying properties (Mohite and Chandel, 2020), highlighting its versatility—not only as a traditional culinary ingredient but also in innovative food technology applications. Nur et al. (2024) demonstrated that incorporating fenugreek into chicken meatballs enhances their quality. Specifically, using fenugreek paste at a 15% concentration significantly improved physical attributes, such as pH and cooking loss, as well as organoleptic characteristics, including aroma, taste, color, and tenderness. Increasing the concentration of fenugreek paste further enhanced both physical and sensory qualities. Chitosan is one of the most widely used renewable polymers in the medical, food, agricultural, and chemical industries due to its unique characteristics, including antioxidant and antimicrobial activities, film-forming ability, biodegradability, biocompatibility, and non-toxicity (Shahbazi, 2017). However, Baranenko et al. (2013) noted that chitosan films can lack sufficient strength and flexibility, resulting in brittleness and cracking, which reduces their protective effectiveness for meat products. They suggested that incorporating additives such as gelatin can improve the mechanical properties of chitosan-based coatings. Additionally, recent research has focused on incorporating natural antioxidant compounds into chitosan films to broaden their applications (Yaghoubi et al., 2021). This study examined the use of fenugreek extract and chitosan blends as edible coatings for chicken meat to enhance its taste, safety, and shelf life. The objective was to overcome the limitations of using fenugreek or chitosan individually by formulating composite coatings with superior mechanical properties, enhanced antioxidant and antibacterial activities, and improved overall consumer acceptability.

2. Materials and methods

Materials

Fenugreek (*Trigonella foenum-graecum* L.) was obtained from Medicinal and Aromatic Plants department, Horticulture Research Institute, Agricultural Research Center. β-Chitosan was extracted in the laboratory from calamari pen waste collected from El-Obour fish market in Cairo, as described by Abdou et al (2008). Glycerol was purchased from Adwik Company, Egypt. Folin-Ciocalteu reagent, methanol, and ethanol were obtained from E. Merck. Quercetin, gallic acid, 2,2-bi pyridyl, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sodium hydroxide (NaOH) and hydrochloric acid (HCl) were purchased from El-Nassr Pharmaceutical Chemical Co., Egypt. Fresh chicken meat was obtained immediately after slaughter from the local market in Giza, Egypt.

Methods

Extraction of β-Chitosan

β-Chitosan was extracted from 20g of calamari pens, which were crushed and treated according to the method described by Abdou et al. (2008).

A brief summary of the method is as follows:

- **Demineralization:** was carried out at room temperature using 1 M hydrochloric acid.
- **Deproteinization:** was performed using alkaline treatment with 1 M sodium hydroxide at 105–110 °C, followed by treatment with 40% NaOH to extract β-chitosan powder, yielding 10 g.
- **Chitosan characterization**: The degree of deacetylation (DDA%) was determined by potentiometric titration, and the molecular weight was calculated based on intrinsic viscosity measured using a Ubbelohde viscometer (Ravindra et al., 1998). The DDA% and molecular weight of the resulting chitosan were 95% and 6.8×10^5 g/mol, respectively. The chitosan powder was then used for further analysis.

Extraction of Fenugreek

Fenugreek seeds (100g) were ground into a fine powder using a laboratory mill and soaked in 1L of distilled water at 80°C for 24 hours, with a seed-to-water ratio of 1:10 (w/v). The pH of the mixture was unadjusted, ranging from 6.5 to 7.0. The resulting slurry was filtered through a double-layered cheesecloth (mesh size 100µm), and the filtrate was used immediately.

Fourier Transform Infrared (FTIR) spectroscopy of fenugreek seed powder and water extract

To identify the functional groups, the FTIR spectra of fenugreek seed powder and fenugreek water extract were measured using KBr pellets in transmission mode, within the range of 400-4000 cm^{-1}, using a Perkin-Elmer 2000 spectrophotometer.

Preparation of edible film from fenugreek water extract/β-chitosan extract and their Blend

A 1% chitosan solution (CS) was prepared by dissolving 1g of chitosan in a 2% (v/v) acetic acid solution, then blended with fenugreek extract (F) at different ratios using 1% glycerol as a plasticizer. The various blend formulations are presented in Table 1.

Table 1. Different formulations of fenugreek water extract /β-chitosan extract and their blend films

Sample	Fenugreek extract %	β-Chitosan solution %
CS	0	100
A	30	70
C	50	50
E	70	30
F	100	0

CS: β-chitosan extract, A: (30 % fenugreek water extract / 70% β-chitosan extract), C: (50 % fenugreek water extract / 50% β-chitosan extract), E: (70 % fenugreek water extract / 30% β-chitosan extract), F: fenugreek water extract

Film Formation of Fenugreek Water Extract/β-Chitosan Extract and Their Blends

The mixtures prepared as described in Table 1 were cast onto flat, leveled, non-stick Teflon plates (20cm×20cm). The plates were held at 50°C for 12 hours, undisturbed, and then cooled to ambient temperature before the films were peeled off.

Chemical analysis and antimicrobial activity

Determination of total phenol and flavonoid contents of fenugreek water extract/ β-chitosan extract and their blend films

Total phenol content of fenugreek seeds water extract, β-chitosan extract, and the fenugreek water extract/β-chitosan extract blends was measured using the Folin-Ciocalteu (FC) reagent method, as described by Hogan et al. (2009). Results were recorded as mg gallic acid equivalent (GAE)/g using a colorimetric technique at a wavelength of 735nm (Kenny et al., 2013). The total flavonoid content was determined as described by Yoo et al. (2008) and expressed as mg catechin equivalent (CAE)/g.

Antioxidant assay of edible film from fenugreek water extract/β-chitosan extract and their blend

The antioxidant activities of fenugreek water extract, β-chitosan extract, and their blends were evaluated using the free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH), as a reagent (Li et al., 2009). Absorbance was measured at 517nm using a UNICO spectrophotometer (Model: UV 2000,

UNICO spectrophotometer (Model: UV 2000, UNICO Instruments Co., Ltd.). The antioxidant capacity was expressed as a percentage of inhibition according to the following equation:

%DPPH (antioxidant activity)=((Ac-As)/Ac)×100%

Where, A_c Control sample absorbance and A_s Test sample absorbance.

Antimicrobial activity of fenugreek water extract/β-chitosan extract and their blend films

The agar well diffusion method was used to test the antimicrobial action of fenugreek extract, β-chitosan, and fenugreek extract/β-chitosan blends against Gram-negative bacteria (*E.coli* (ATCC25922), *Salmonella typhimurium (*ATCC140 28), as well as Gram-positive bacteria *Bacillus cereus* (ATCC14579), *Staphylococcus aureus* (ATCC 6538), and Listeria monocyto-genes (ATCC19111), and fungi *Aspergillus flavus* (ATCC9643). The antibacterial activity of fenugreek water extract/β-chitosan extract and their blend films was analyzed using the agar well diffusion method, as described by Amenu (2014). The minimum inhibitory concentration (MIC) of fenugeek, chitosan and their blend films were determined according to the method outlined by Alkhatib et al. (2023).

Texture characteristics

Thickness of fenugreek water extract/β-chitosan extract and their blend films

The edible films thickness was measured using a Palmer digital micrometer (Comecta, Barcelona, Spain) to the nearest 0.001 mm. Six to eight random positions on each film sample were measured.

Mechanical properties of fenugreek water extract/β-chitosan extract and their blend films

The mechanical properties of the films were measured using a Brookfield Texture Analyzer CT3 (USA) with a TA-AACC3 probe and a TA-FSF fixture. The measurement conditions included a 10g trigger value, a 20mm target, and a 0.5mm/s test speed.

Water vapour transmission rate (WVTR) of fenugreek water extract/β-chitosan extract their blend films

The WVTR was measured according to Abd El-Rehim et al. (2018) using the gravimetric method based on the ASTM E96 (2022) desiccant method. Dried films with dimensions of 5cm in diameter and an average thickness of 0.15mm (free from physical defects such as bubbles or cracks) were cut and placed as a cap over the mouth of a cup containing $CaCl_2$, with a diameter of approximately 5cm. Each cup was placed in a chamber for 10 days at a constant temperature of 25°C and 75% relative humidity (NaCl saturated solution). The cups were weighed daily, and the water vapor transmission rate was determined by plotting the weight increment against time. After dividing the data by the cross-sectional area, the water vapor transmission rate was calculated and expressed in g/cm²/day.

Water solubility of fenugreek water extract/β-chitosan extract their blend films

Edible Films water solubility was measured as described by Gontard et al. (2007) with some modifications. The test was performed in triplicate, and the films solubility was calculated using the following equation:

$$Solubility\ \% = \frac{Wi - wf}{wi} \times 100$$

Where W_i was the initial weight expressed as dry matter, and W_f was the weight of the undissolved film residue.

Chicken breast meat coating

Chicken breast meat samples were divided into six treatments. The first treatment involved dipping the meat in cold distilled water for 10 minutes to compensate for the possible physical removal of bacteria and moisture uptake, then draining and packing (control). The next three groups were dipped in blend solutions of (A), (C), and (E) with different ratios of fenugreek water extract/β-chitosan extract, as shown in Table 1. The last two treatments involved dipping in β-chitosan extract (CS) and fenugreek water extract (F). After dipping, all samples were drained well for 3 minutes. After

dipping, all samples were drained well for 3 minutes. Then, three pieces from each group were packed in foam trays, wrapped using stretch poly-ethylene films, chilled, and stored at 4 °C. Samples from each treatment were withdrawn for analysis every three days. Upon checking the samples on the 12th day, they were found to be corrupted. Then, three pieces from each group were packed in foam trays, wrapped using stretch polyethylene films, chilled, and stored at 4°C. Samples from each treatment were withdrawn for analysis every three days. Upon checking the samples on the 12th day, they were found to be corrupted.

Sensory evaluation of coated chicken breast meat

For the sensory evaluation of chicken breast meat, ten experienced panellists were chosen from the Food Technology Research Institute staff. Chicken meat from each formula was broiled at 200 °C and kept warm in the oven until testing within 3-8 minutes (Fernandez-Lopez et al., 2006). Each panellist evaluated three replicates of all samples in a randomized order and was asked to assign a numerical value ranging from 1 to 10, representing "dislike extremely" to "like extremely." The sensory attributes evaluated included color, taste, flavor, tenderness, and overall acceptability (Ramadhan et al., 2011). Based on the previous data, three coating blends (A, C, and E) were selected for storage.

Characteristics of chicken breast meat samples during storage

Weight loss of chicken breast meat samples during storage coated with fenugreek water extract/β-chitosan extract and their blends

Weight loss of chicken meat samples was determined as described by Garavito et al. (2020) by weighing the chicken samples using a semi-analytical balance (Gehaka, model BG400) with 0.001 g readability. The analysis was performed on the processing day and every 3 days, using the following equation to calculate the weight loss percentage:

$$weight\ loss\ \% = \frac{W_i - W_t}{W_i} \times 100$$

Where Wi is the initial weight and Wt is the sample weight at day t.

pH measurement of chicken breast meat samples during storage coated with fenugreek water extract/β-chitosan extract and their blends

The pH value was recorded using a pH meter (Lovibond, Model Sensodirect 150). The instrument was standardized at pH 4 and pH 7. For the analysis, 5g of each sample (at zero time, 3, 6, 9, and 12 days) were homogenized for 30 seconds at 13,500 rpm in Falcon 50mL tubes with 10mL of distilled water to measure the pH (Pearson, 2006).

Thio-barbituric acid (TBA) of chicken breast meat samples during storage coated with fenugreek water extract/β-chitosan extract and their blends

According to the procedure performed by Kirk and Sawyer (1991), the Thio-barbituric acid (TBA) value was calculated spectrophotometrically. The TBA values were expressed as mg malonaldehyde/ kg.

Microbiological evaluation of chicken breast meat samples during storage coated with fenugreek water extract/β-chitosan extract and their blends

Total bacterial counts (TBC), yeast, mold and counts, and psychrophilic bacteria of chicken samples were determined in plate count agar using the pour-plate method (AOAC, 2019). One gram of chicken breast meat was aseptically weighed and homogenized with 10mL of sterilized water for 1 minute. The homogenized samples were serially diluted (1:10), and then 1mL of the dilution was further diluted until 1:1000. One milliliter of the 1:100 and 1:1000 serial dilutions was plated onto plate count agar and incubated at 35-37°C for 48 hours.

Statistical Analysis

The experiment used a completely randomized design (CRD) with three replications. The data from this study were subjected to analysis of variance and the Fisher's least significant difference test (SAS software version 9.3) to compare the mean values of

the investigated parameters at significance levels of $P \geq 0.05$.

3. Results and Discussion

Fourier transform infrared (FTIR) spectroscopy of fenugreek seed powder and fenugreek water extract

Fourier Transform Infrared (FTIR) spectroscopy is an analytical technique used to identify and characterize the chemical composition of substances by measuring the vibration and absorption of functional groups through infrared radiation. Fenugreek seed powder and the obtained water extract were characterized using FTIR spectroscopy. Figure 1 shows that the IR spectrum peaks for fenugreek seed powder and its water extract were 3392.17cm^{-1} and 428.81cm^{-1}, respectively. Comparing these peaks with the IR spectrum absorption tables indicates the presence of the -OH group, which can be attributed to phenolic groups. The results also show that the IR spectrum peaks were 2925.68cm^{-1} and 2920.66cm^{-1} for fenugreek seed powder and its water extract respectively. According to Keshari et al. (2018), these results could be due to the stretching vibrations of -CH3 and -CH2, corresponding to aldehyde, carboxyl groups, glycosides, and aromatic rings. Additionally, the peaks revealed that -C-O and -C-OH groups appear at 1061.37cm^{-1} and 1074.23cm^{-1} for fenugreek seed powder and its water extract respectively, suggesting the presence of phenols, glycosides, and terpenes (Qi et al., 2017).

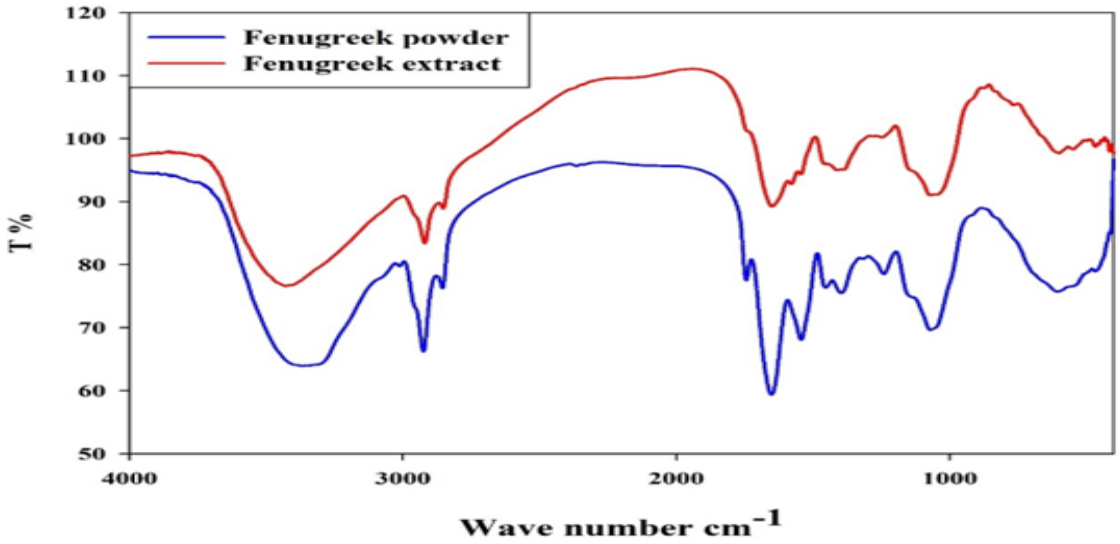

Figure 1. Fourier transform infrared (FTIR) spectroscopy of fenugreek seed powder and extract

Chemical analysis and antimicrobial activity of films

Total phenols, flavonoids contents, and antioxidants of fenugreek water extract, β-chitosan extract, and their blend films

Total phenolic, flavonoid, and antioxidant activity were determined in fenugreek water extract, β-chitosan extract, and their blend films, with the findings presented in Table 2. The results indicated that the fenugreek water extract film exhibited higher total phenolic and flavonoid content, as well as antioxidant activity, with values of 61.00mg GAE/g, 34.04mg CAE/g, and 70.78%, respectively, compared to the β-chitosan extract, which had values of 0.051 mg GAE/g, 0.04mg CAE/g, and 20.32%, respectively. The results showed that the (E) blend film (70% fenugreek and 30% β-chitosan) significantly exhibited the highest content of phenolics, flavonoids, and antioxidant activity (44.72mg GAE/g, 24.32mg CAE/g, and 64.91%, respectively), followed by the (C) blend film, which recorded moderate values of 30.5mg GAE/g, 17.30mg CAE/g, and 46.92% for phenolics, flavonoids, and antioxidant activity, respectively. In contrast, the (A) blend film (30% fenugreek and 70% β-chitosan) showed the lowest values for phenolics, flavonoids, and antioxidant activity (19.14mg GAE/g, 10.56mg CAE/g, and 35.24%, respectively). These results align with those of Dua et al. (2013), who mentioned that

various active phytochemicals contribute to fenugreek's antioxidant properties. According to Ali et al. (2015), the total phenols of different varieties of fenugreek seeds ranged from 127.8 to 139.2mg GAE/100g. Naidu et al. (2012) found a significant relationship between the polyphenolic components present in fenugreek and its antioxidant activity, suggesting that these compounds play a crucial role in the plant's ability to neutralize free radicals and reduce oxidative stress. Premanath et al. (2011) found that flavonoids are good free radical scavengers. β-Chitosan showed the lowest total phenolic and flavonoid content, as well as antioxidant activity, which may be due to chitosan being a positively charged linear polysaccharide (Das et al., 2023). Although β-chitosan is a weak antioxidant, it can still scavenge free radicals. The primary functional groups in chitosan that are responsible for its antioxidant action are the hydroxyl (-OH) and amino (-NH2) groups. Numerous mechanisms regarding the antioxidant effect of chitosan have been hypothesized, as evidenced by the experimental findings of Rajalakshmi et al. (2013), who reported that chitosan exhibited good DPPH radical-scavenging activities.

Table 2. Total phenols and flavonoids as well as antioxidant activity % free radical scavenging activities in fenugreek water extract, β-chitosan extract, and their blend films

Samples	Total phenol, mg GAE/g	Total flavonoids, mg CAE/g	antioxidant activity %
CS	0.051^{e} ±0.004	0.04^{e} ±0.02	20.32^{e} ±0.31
A	19.14^{d} ±3.62	10.56^{d} ±1.36	35.24^{d} ±4.59
C	30.50^{c} ±0.40	17.30^{c} ±0.30	46.92^{c} ±1.00
E	44.72^{b} ±4.00	24.32^{b} ±2.17	64.91^{b} ±1.03
F	61.11^{a} ±4.01	34.04^{a} ±0.70	70.78^{a} ±0.6

Values (means ±SD) Means having different letters in the same columns are statistically significantly various at ($P \leq 0.05$).
CS: β-chitosan extract, A: (30 % fenugreek water extract / 70% β-chitosan extract), C: (50 % fenugreek water extract / 50% β-chitosan extract), E: (70 % fenugreek water extract / 30% β-chitosan extract) F: fenugreek water extract

Antimicrobial activities of fenugreek water extract, β-chitosan extract, and their blend films

The antimicrobial capacity of edible coatings holds promise for improving food safety (Singh et al., 2022). The antimicrobial activity (expressed as inhibition zone, mm) of fenugreek extract, β-chitosan, and their blends against pathogenic Gram-positive and Gram-negative bacteria, as well as fungi, is presented in Table 3. The results show that blend C (50% fenugreek extract/50% β-chitosan), followed by blend E (70% fenugreek extract/30% β-chitosan) and F (fenugreek extract), had a significant effect on Gram-positive bacteria, Gram-negative bacteria, and fungi compared to Ciprofloxacin and Amphotericin as commercial antibiotics for all the studied microorganisms. Blend C exhibited the highest inhibition zone for all the studied microorganisms, recording 48 mm for *E. coli*, followed by *S. aureus* (41 mm), *B. cereus* (33 mm), *S. typhimurium* (22 mm), *A. flavus* (19 mm), and *L. monocytogenes* (9 mm). The observed antimicrobial activity may be due to the synergistic effect of β-chitosan and fenugreek seed extract, both of which contain a high level of bioactive components known to possess antimicrobial properties. Fenugreek contains various metabolites, such as tannins, alkaloids, flavonoids, terpenoids, and glycosides, which significantly inhibit microbial growth (Abdalla et al., 2023). Notably, fenugreek flavonoids have been reported as potent antibacterial compounds (Premanath et al., 2011), and studies have shown that increasing the concentration of fenugreek extract in coating blends enhances the zone of inhibition against various microorganisms (Mohite & Chandel, 2020). Likewise, β-chitosan possesses potent antimicrobial activity, mainly attributed to the positively charged amino groups ($-NH_2$) and hydroxyl groups (-OH), as these groups destabilize microbial cell membranes (Ke et al., 2021 and Confederat et al., 2021). Additionally, the concurrent use of β-chitosan and natural extracts, such as fenugreek, has significantly improved antimicrobial effectiveness (Muñoz-Tebar et al., 2023).

Table 3. Antimicrobial activities (inhibition zone, mm) of fenugreek extract, β-chitosan, and their blends versus tested bacteria

Samples	Inhibition zone (mm)					
	Bacteria Gram-negative		Bacteria Gram-positive			Fungi
	Escherichia coli (ATCC25922)	Salmonella typhimurium (ATCC14028)	Bacillus cereus (ATCC14579)	Staphylococcus aureus (ATCC6538)	Listeria monocytogenes (ATCC19111)	Aspergillus flavus (ATCC9643)
CS	20.0	10.0	15.0	18.0	5.0	9.0
A	12.0	6.0	8.0	10.0	ND	10.0
C	48.0	22.0	33.0	41.0	9.0	19.0
E	36.0	20.0	29.0	33.0	7.0	17.0
F	30.0	15.0	22.0	25.0	6.0	13.0
Control			Ciprofloxacin			Amphotericin
(100µg/ml)	8.0	4.0	4.0	8.0	3.0	9.0

CS: β-chitosan extract, A: (30 % fenugreek water extract / 70% β-chitosan extract), C: (50 % fenugreek water extract / 50% β-chitosan extract), E: (70 % fenugreek water extract / 30% β-chitosan extract) F: fenugreek water extract
ND: No inhibition zone was detected

Texture Characteristics of films

Thickness of fenugreek water extract, β-chitosan extract, and their blend films

Thickness can impact changes in the film structure, and controlling thickness is crucial for the barrier and mechanical properties of the final film (Maran et al., 2013). The thickness results of films conditioned at room temperature are presented in Table 4. The β-chitosan film (CS) was the thinnest, with a thickness of about 0.104 mm, while the fenugreek extract film (F) had a thickness of 0.115 mm. For fenugreek extract/β-chitosan coating blends A, C, and E, the thickness increased to 0.24, 0.26, and 0.25mm, respectively, with no significant difference, which gives edible fenugreek film good properties. The establishment of intermolecular hydrogen bonds between β-chitosan and fenugreek, the amount of dry matter, and the contact between polysaccharides are all thought to be the causes of the thickness increase (Prakash Maran et al., 2013).

Mechanical Properties of Fenugreek Water Extract, β-Chitosan Extract, and Their Blend Films

The mechanical properties of films are required to ensure the integrity of packaging materials (Wang et al., 2009). Tensile strength and elongation % at break were used to test the mechanical properties of edible films. The mechanical properties of all films prepared from coating blends are shown in Table 4. The results indicated that the β-chitosan film had the highest tensile strength at 32.2 MPa, followed by blend A at 10.1 MPa, while the fenugreek extract film had the lowest tensile strength at 2.52 MPa. A strong and cohesive film matrix cannot be produced from fenugreek extract, as its molecular interaction is weak due to the content of bioactive substances like sugars, galactomannan, saponins, and fatty acids (Gavahian et al., 2023 and Alu'datt et al., 2024), which act as plasticizers, enhancing flexibility at the cost of rigidity and tensile strength. Furthermore, strength loss is due to high galactose substitution in fenugreek galactomannan that disrupts polymer interaction through steric hindrance and enhances molecular mobility. Additionally, the hydrophilic character of fenugreek facilitates water absorption and swelling, further reducing the mechanical strength of the film. Fenugreek extract improves flexural performance but reduces tensile strength by disrupting polymer cohesion and increasing sensitivity to moisture (Senarathna et al., 2023). Therefore, blending fenugreek extract with β-chitosan leads to stronger edible films with increasing β-chitosan percentage as follows:
A > C > E. Since both are polysaccharides with hydrophilic properties, to overcome their drawbacks,

polysaccharides are used in combination with other polysaccharides, proteins, or fats (Zhang et al., 2014). Also, Baranenko et al. (2013) indicate that incorporating additional components, such as gelatin, can enhance the mechanical properties of chitosan-based coatings. As a measure of the film's elasticity, the elongation % at break refers to the most significant change in film length before breaking (Nandane and Jain, 2014). The elongation % at break of all prepared films varied from 3.4% for β-chitosan to 40.0% for fenugreek extract. In the case of fenugreek extract/β-chitosan blend films, elongation at break % increases with increasing fenugreek extract %, which gives the fenugreek-blended coating good properties due to its high content of galactomannan (Cerqueira et al., 2011). Memis et al. (2017) reported that fenugreek seed gum edible film had the highest elongation % at break compared with nano clay-reinforced films. Additionally, higher elongation percentages suggest greater flexibility, allowing the film to accommodate the shapes of different food items without tearing (Yanti et al., 2021). A film with high elongation at break can conform to the contours of various food items, minimizing exposure to environmental factors that contribute to deterioration (Kumari et al., 2021).

Water vapour transmission rate (WVTR) of fenugreek water extract, β-chitosan extract, and their blend films

The WVTR results are illustrated in Table 4 for all prepared films. β-Chitosan has the lowest water vapor transmission rate (22.9×10^{-3} g/cm²/day), while WVTR was elevated in fenugreek to 36.4×10^{-3} g/cm²/day. For all prepared blend films, A, C, and E, WVTR increased gradually and significantly with increasing fenugreek extract %, with values of 24.9×10^{-3}, 25.1×10^{-3}, and 33.2×10^{-3} g/cm²/day, respectively. Likely, the fenugreek peptide and β-chitosan could not be fully cross-linked when the amount of fenugreek peptides was elevated, leading to increased water vapor permeability (Bumbudsanpharoke et al., 2022). Hydrophilic polysaccharides have a poor water vapor barrier, which limits their commercial application in edible coatings (Hassan et al., 2018). Additionally, under acidic conditions, chitosan's positively charged amino groups ($-NH_3^+$) bind with negatively charged molecules and surfaces, enabling it to bind and create films with negatively charged molecules like fats, cholesterol, and proteins (Riaz Rajoka et al., 2019).

Solubility of Fenugreek Water Extract, β-Chitosan Extract, and Their Blend Films

Table 4 also demonstrates the solubility of fenugreek extract/β-chitosan films in water. β-Chitosan has lower solubility (20.1%) than fenugreek extract (75.5%). Increasing the fenugreek extract % in A, C, and E blends leads to a gradual increase in solubility, with values of 47.65%, 50.0%, and 67.92%, respectively. Chitosan's low solubility is due to its crystalline structure and deacetylated units (free $-NH_2$ groups form strong hydrogen bonds). In contrast, fenugreek's high solubility comes from galactomannan's hydrophilic –OH groups and amorphous structure (Gavahian et al., 2023). This result agrees with Ramesh et al. (2001), who found that fenugreek gum has higher water solubility due to its greater galactose content.

Table 4. Mechanical and WVTR of fenugreek water extract and β-chitosan extract and their blend films

Sample	Thickness mm	Tensile strength MPa	Elongation %	WVTR g/cm²/day	Solubility %
CS	0.104^{a} ±0.003	32.200^{a} ±0.64	3.400^{c} ±0.12	22.9×10^{-3d} ±0.15	20.100^{d} ±0.60
A	0.240^{a} ±0.01	10.100^{b} ±0.5	4.940^{c} ±0.04	24.9×10^{-3c} ±0.02	47.650^{c} ±0.01
C	0.260^{a} ±0.01	5.560^{c} ±0.1	16.500^{b} ±0.2	25.1×10^{-3c}±0.02	50.000^{c} ±0.05
E	0.250^{a} ±0.004	3.680^{d} ±0.001	17.740^{b} ±1.00	33.2×10^{-3b} ±0.01	67.920^{b} ±0.02
F	0.115^{a} ±0.003	2.520^{e}±0.02	40.000^{a} ±0.05	36.4×10^{-3a} ±0.05	75.500^{a} ±0.03

Values (means ±SD) Means having different letters in the same columns are statistically significantly various at ($P \leq 0.05$).

CS: β-chitosan extract, A: (30 % fenugreek water extract / 70% β-chitosan extract), C: (50 % fenugreek water extract / 50% β-chitosan extract), E: (70 % fenugreek water extract / 30% β-chitosan extract) F: fenugreek water extract, Water vapour transmission rate (WVTR)

Sensory evaluation

The sensory analysis of different coated chicken meat samples is presented in Table 5. It can be observed that the control sample without coating recorded the lowest mean values for sensory scores. On the other hand, using the coating films, which consists of fenugreek and β-chitosan at various levels, resulted in improvements in sensory properties, depending on the percentage of fenugreek, β-chitosan, or both. The best result was recorded in the sample coated with an equal percentage of fenugreek and β-chitosan (50:50), as compared to the other samples, with significant differences ($P < 0.05$), followed by the sample coated with 30% fenugreek and 70% β-chitosan. It was also found that the addition of fenugreek increased tenderness. Based on the obtained results, using fenugreek extract in coating films (50%) can improve the chicken meat's color, flavor, taste, and tenderness. Nursetiani and Herdiana (2018) revealed that fenugreek has a fragrant aroma that improved the physical quality of meatballs. Adding fenugreek improved the product's color due to its high content of antioxidants. The good samples were easy to bite when eaten, indicating better tenderness. Hegazy (2011) concluded that using fenugreek seed flour in beef burger patties, instead of soybean flour, enhanced the nutritional, physicochemical, and sensory quality criteria.

Table 5. Sensory properties of broiled coated chicken breast

Samples	Colour	Taste	Flavour	Tenderness	Overall acceptability
Control	$5.23^{f} \pm 0.06$	$5.19^{f} \pm 0.23$	$5.43^{f} \pm 0.14$	$5.67^{f} \pm 0.19$	$5.36^{f} \pm 0.31$
CS	$6.95^{e} \pm 0.20$	$7.23^{e} \pm 0.001$	$7.41^{e} \pm 0.25$	$6.45^{e} \pm 0.04$	$6.94^{e} \pm 0.50$
A	$8.61^{b} \pm 0.12$	$8.89^{b} \pm 0.31$	$8.79^{b} \pm 0.22$	$8.68^{b} \pm 0.37$	$8.74^{b} \pm 0.10$
C	$8.89^{a} \pm 0.21$	$9.22^{a} \pm 0.25$	$9.07^{a} \pm 0.06$	$9.12^{a} \pm 0.32$	$9.08^{a} \pm 0.30$
E	$7.31^{d} \pm 0.04$	$7.55^{d} \pm 0.18$	$7.68^{d} \pm 0.05$	$7.89^{c} \pm 0.28$	$7.33^{d} \pm 0.30$
F	$7.56^{c} \pm 0.09$	$7.90^{c} \pm 0.28$	$7.96^{c} \pm 0.18$	$7.33^{d} \pm 0.41$	$7.67^{c} \pm 0.50$

Values (means ±SD) in the columns are statistically significantly various at ($P \leq 0.05$).

CS: β-chitosan extract, A: (30 % fenugreek water extract / 70% β-chitosan extract), C: (50 % fenugreek water extract / 50% β-chitosan extract), E: (70 % fenugreek water extract / 30% β-chitosan extract) F: fenugreek water extract

Characteristics of coated chicken breast meat samples during storage.

Weight loss

The weight loss percentages of both coated and uncoated chicken breast meat for all samples are clearly presented in Table 6. The data demonstrates that the weight loss percentage increased significantly during the storage period. Notably, a marked difference between the control and coated samples was evident throughout the storage period. The control group displayed a high weight loss percentage, reaching 15.55% by the end of the storage period. On the other hand, the weight loss in the coated samples was considerably lower. Blend C exhibited the lowest weight loss (2.56%), followed by chitosan and fenugreek extracts at the end of the storage period. The weight loss observed in the (C) coating blend remained moderate. This reduction in weight loss can be attributed to edible coatings acting as physical barriers to the migration of moisture. Chitosan-based coatings, which have superior film-forming characteristics and low water vapor permeability, combined with fenugreek extract, increased moisture retention. Although Blend C presented an intermediate weight loss compared to the other coatings, it still ensured better protection against water evaporation, indicating the efficiency of edible coatings in maintaining the quality of meat during storage (Muñoz-Tebar et al., 2023).

pH measurement of coated chicken breast meat during storage period

The initial pH values of coated and uncoated breast samples ranged from 5.40 to 5.79. The resultsin Table 7 demonstrate that after a 12-day

storage period at 4°C, significant gradual increases in pH were observed for all samples. This may be attributed to endogenous enzymes such as lipase, protease, or bacteria utilizing amino acids (Manju et al., 2007). The highest increase in pH was recorded for the control sample, which reached 7.40 by the end of the storage period. In contrast, the C blend (50% fenugreek water extract/50% β-chitosan extract) exhibited the lowest increase in pH. Furthermore, on the ninth day of storage, the pH of the C blend decreased; this result may be due to fenugreek's antioxidant and antibacterial activity. The reduction in pH may be related to decreased microbial growth and the inhibition of endogenous proteases (Fan et al., 2009). These findings align with those obtained by Surmei and Usturoi (2012), who observed that the pH of poultry meat increased from 5.87 on the first day post-slaughter to 6.38 by the tenth day of storage under refrigeration conditions.

Table 6. Weight loss % of chicken meat samples during storage period

Samples	Storage period (12 days)				
	Zero time	3	6	9	12
Control	--	3.70^{aD} ±0.25	6.00^{aC} ±0.36	9.34^{aB} ±0.28	15.550^{aA} ±0.25
CS		1.64^{cD} ±0.10	2.17^{cC} ±0.05	3.00^{cB} ±0.19	3.63^{cA} ±0.09
C	--	1.00^{dD} ±0.40	1.29^{dC} ±0.02	1.85^{dB} ±0.25	2.56^{dA} ±0.08
F	--	2.51^{bD} ±0.32	3.25^{bC} ±0.15	4.00^{bB} ±0.18	4.67^{bA} ±0.22

Values (means ± SD) in the columns are statistically significantly different at ($P \leq 0.05$). Capital letters indicate significant differences for storage time, while lowercase letters indicate significant differences between samples.
CS: β-chitosan extract, C: (50 % fenugreek water extract / 50% β-chitosan extract), F: fenugreek water extract.

Table 7. pH Measurements of coated chicken meat samples during storage period (12 days)

Samples	0	3	6	9	12
Control	5.40^{bE} ±0.03	5.77^{aD} ±0.03	6.27^{aC} ±0.04	7.28^{aB} ±0.02	7.40^{aA} ±0.01
CS	5.79^{aD} ±0.04	5.70^{bE} ±0.07	6.12^{bC} ±0.01	6.20^{bB} ±0.01	6.51^{bA} ±0.04
C	5.29^{cB} ±0.03	5.25^{dB} ±0.01	5.26^{dB} ±0.03	5.23^{dC} ±0.01	5.31^{dA} ±0.03
F	5.26^{dE} ±0.03	5.55^{cC} ±0.05	5.44^{cD} ±0.05	6.12^{cB} ±0.03	6.20^{cA}±0.03

Values (means ± SD) in the columns are statistically significantly different at ($P \leq 0.05$). Capital letters indicate significant differences for storage time, while lowercase letters indicate significant differences between samples.
CS: β-chitosan extract, C: (50 % fenugreek water extract / 50% β-chitosan extract), F: fenugreek water extract

Thio-barbituric acid (TBA) of coated chicken breast meat during storage period

Thio-barbituric acid (TBA) values were measured to estimate the effect of coating on the lipid oxidation of chicken during storage. Table (8) displays the effects of fenugreek, β-chitosan, and their mixture coating on the extent of lipid oxidation in chicken breast meat samples (TBA value) during the storage period. The malonaldehyde concentration at zero time ranged from 0.220 to 0.291 mg/kg. Throughout the storage period, the TBA values of each chicken sample under analysis gradually increased. Data showed that after 12 days of storage, the TBA value of the control chicken samples increased from 0.233mg malonaldehyde/kg at the beginning of storage to 1.58mg malonaldehyde/kg. β-Chitosan coating slowed the deterioration reaction by about 50% compared to the control sample, with a concentration of 0.836mg/kg after 12 days of storage. On the other hand, applying fenugreek extract (C) substantially reduced the rate of rancidity reaction, with a concentration of malonaldehyde reaching 0.481 mg/kg. The use of fenugreek extract/β-chitosan mixture solutions in coating chicken breast achieved lower malonaldehyde concentrations, with increasing fenugreek extract ratio in the coating mixtures to blend C (50% fenugreek water extract / 50% β-chitosan extract), which recorded the lowest TBA value at the end of the storage period.

The impacts of the flavonoids and phenolic compounds in fenugreek, as well as β-chitosan and naturally occurring antioxidant components, may be responsible for the decrease in TBA readings. These results are supported by Qureshi et al. (2018), who noted that incorporating fenugreek seed powder (FSP) into spent hen meat patties improved their functional value and increased antioxidant activity, which can be inferred to potentially reduce lipid oxidation and thus lower TBA values. Also, extracted β-chitosan has strong antioxidant properties, referring to the existence of functional groups in chitosan that are responsible for its antioxidant action, namely the hydroxyl (-OH) and amino ($-NH_2$) groups (Rajalakshmi et al., 2013), as previously reported in this study. However, (TBA) levels in chicken meat were within the allowed upper limit of 0.9 mg malonaldehyde/kg sample (Egyptian Standard, 2005), except for the control sample, which exceeded the limit.

Table 8. TBA (mg/kg) for coated chicken breast meat during storage period (12 days)

Samples	Zero time	3	6	9	12
Control	0.233^{dE} ±0.001	0.616^{aD} ±0.004	0.860^{aC} ±0.006	0.956^{aB} ±0.004	1.580^{aA} ±0.006
CS	0.291^{aE} ±0.004	0.590^{bD} ±0.002	0.615^{bC} ±0.002	0.719^{bB} ±0.001	0.836^{bA} ±0.004
C	0.222^{cE} ±0.003	0.345^{dD} ±0.001	0.403^{dC} ±0.001	0.446^{dB} ±0.004	0.481^{dA} ±0.003
F	0.220^{bE} ±0.001	0.470^{cD} ±0.002	0.591^{cC} ±0.002	0.652^{cB} ±0.003	0.775^{cA}±0.001

Values (means ± SD) in the columns are statistically significantly different at ($P \leq 0.05$). Capital letters indicate significant differences for storage time, while lowercase letters indicate significant differences between samples.
CS: β-chitosan extract, C: (50 % fenugreek water extract / 50% β-chitosan extract), F: fenugreek water extract

Microbiological evaluation of coated chicken breast meat during storage

The effects of fenugreek water extract, β-chitosan extract, and their blend on the microbial count of chicken breast during storage are illustrated in Figures 2 and 3. Several factors influence meat deterioration, including storage temperature, oxygen content, moisture levels, light exposure, endogenous enzymes, and microbial activity (Odeyemi et al., 2020). Microbiological degradation is the primary cause of meat spoilage (Iulietto et al., 2015). Additionally, the initial microbiota and the conditions under which the meat is prepared may contribute to a reduced shelf life (Wójcik et al., 2015). The initial bacterial count of both control and coated chicken breast samples ranged from 3.3 to 3.6 logcfu.g^{-1}. The uncoated sample (control) demonstrated rapid bacterial growth, with the colony-forming unit (cfu) count reaching 9.51 log cfu.g^{-1}. Applying a pure β-chitosan coating was insufficient to inhibit bacterial growth, as the bacterial count reached 8.74 log cfu.g^{-1} by the end of the 12 day storage period. In contrast, the fenugreek extract coating was more effective at reducing bacterial growth, with total bacterial counts of 7.07 log-cfu.g^{-1}, while blend C (50% fenugreek extract and 50% β-chitosan) recorded the lowest bacterial count (4.9 log cfu.g^{-1}) at the end of the storage period. These results may be attributed to the high concentration of phytochemicals in fenugreek, combined with hydroxyl (-OH) and amino ($-NH_2$) groups, which impart higher antioxidant activity to the blend. Furthermore, Bumbudsanpharoke et al. (2022) ob-served that the water barrier properties of the coated blends improved as the percentage of β-chitosan increased in packaged bread. After 12 days of storage, all samples exceeded the microbial limits established by the Egyptian standard (2005), which is 5 logcfu.g^{-1}.

No yeast or molds were detected in the chicken breast samples, both uncoated and coated, until the third day of storage (Figure 3). Then, the yeast and mold count gradually increased, recording the highest count for uncoated chicken breast (4.23 log cfu.g^{-1}) at the end of the storage period due to the lack of a protective barrier, which allowed microbial proliferation. Meanwhile, blend C (50% fenugreek extract and 50% β-chitosan) displayed the lowest count (1.8 log cfu.g^{-1}) compared to pure chitosan and fenugreek extract, which

(recorded 3.91 and 2.0 log cfu.g^{-1}, respectively. The high efficacy observed is due to the synergistic antimicrobial effect of β-chitosan and fenugreek extract, showing higher inhibition activity against fungal growth compared to pure chitosan (3.91 log-cfu.g^{-1}) or individual fenugreek extract (2.0 log cfu.g^{-1}).

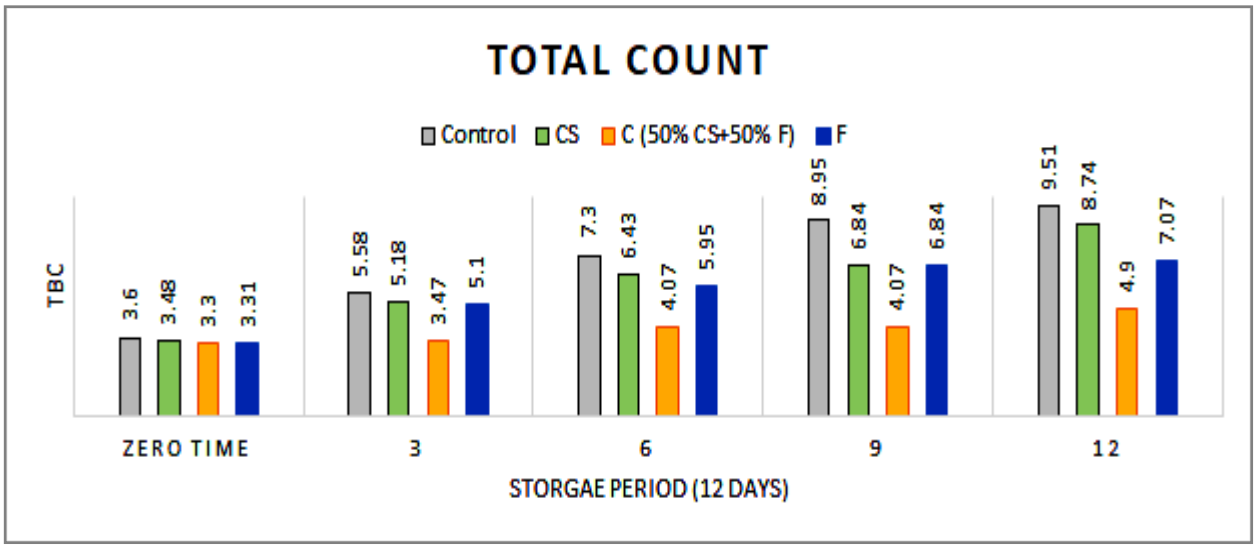

Figure 2. Change in total bacterial count (TBC) log (cfu.g-1) of chicken breast samples during storage

CS: β-chitosan extract, C: (50 % fenugreek water extract / 50% β-chitosan extract), F: fenugreek water extract.

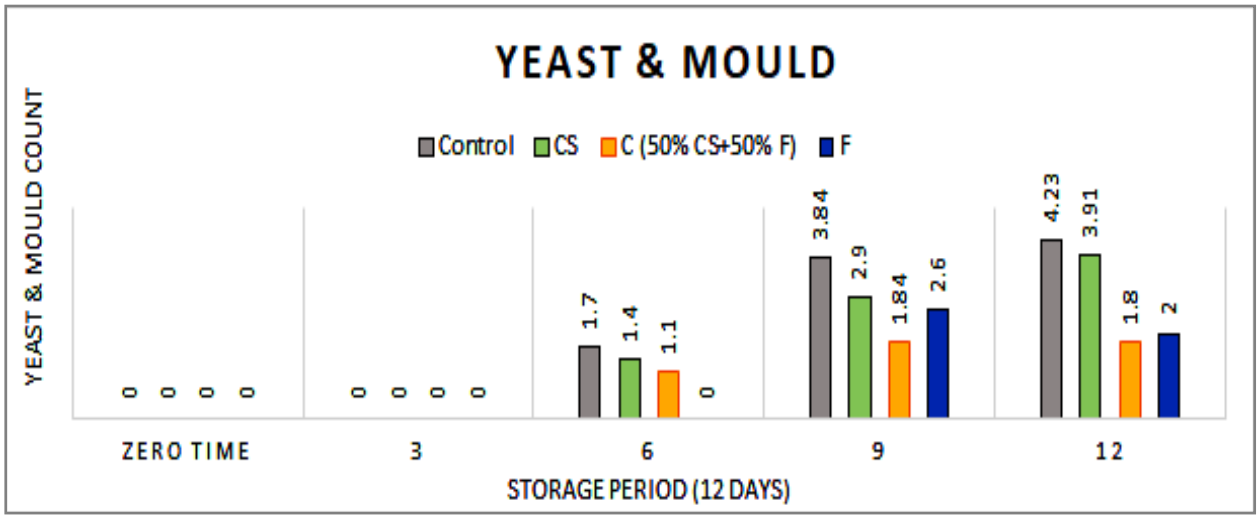

Figure 3. Change in yeast and mold count (log cfu.g-1) of chicken breast samples during storage

CS: β-chitosan extract, C: (50 % fenugreek water extract / 50% β-chitosan extract), F: fenugreek water extract

Psychrophilic counts have long been used as a general indicator of the potential shelf life of chicken (Capita et al., 2001 and Wong et al., 2023). The effects of changes in psychrophilic bacteria (log cfu.g^{-1}) in chicken breast samples during storage are illustrated in Figure 4. Typically, no psychrophilic bacteria are present in chicken breast samples at zero time, and they remain absent after three and six days. However, after nine days, the psychro-philic bacteria counts in different chicken breast samples ranged from 0.7 log cfu.g^{-1} to a maximum of 1.83 log cfu.g^{-1} for C blend and control respectively. Furthermore, the C blend, which consists of 50% fenugreek extract and 50% β-chitosan, proved to be the most effective at reducing psychrophilic bacteria, achieving a count of 1 log cfu.g^{-1} by the end of the 12-day storage period. Therefore, using a 1:1 ratio of fenugreek water extract and β-chitosan

extract as edible coatings can extend the shelf life of chicken breast meat from the 6 days specified by the Egyptian standard (2005) to 12 days. This method improves microbial safety during refrigerated storage and helps maintain certain quality parameters.

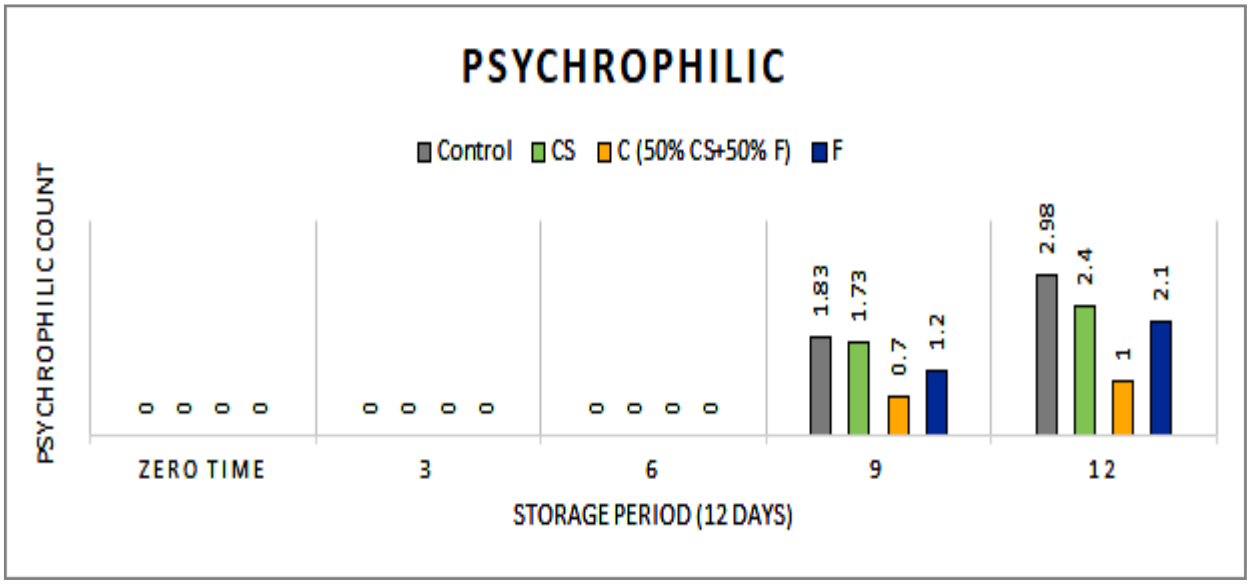

Figure 4. Change in *psychrophilic* (log cfu.g-1) of chicken breast samples during storage

CS: β-chitosan extract, C: (50 % fenugreek water extract / 50% β-chitosan extract), F: fenugreek water extract

4. Conclusion

Coated chicken breast meat with fenugreek, β-chitosan, and their blends demonstrated significant antimicrobial and antioxidant properties, enhancing overall sensory qualities and consumer appeal. The best results were observed with the 1:1 blend of fenugreek and β-chitosan, which exhibited remarkable antimicrobial activity, effectively preventing microbial growth and extending the shelf life of chicken meat up to 12 days. Using 50% β-chitosan extract and 50% fenugreek water extract mixture played a crucial role in maintaining the sensory attributes of the chicken breast, including its color, texture, and flavor. Based on these findings, it can be concluded that fenugreek, β-chitosan, and their 1:1 combination show promise as effective and versatile ingredients in food packaging materials. They can enhance the physicochemical, antioxidant, and antimicrobial properties of food, thereby improving food quality and extending shelf life.

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