

Preservation Effect of Four Plant Extracts Used to Extending the Shelf-life of Mullet Fish Fillets During Cold Storage

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Abstract: The antioxidant and antimicrobial effects of blackcumin (*Nigella sativa*), laurel leaves (*Laurus nobilis*), cumin (*Cuminum cyminum*) and fenugreek (*Trigonella foenumgraecum*) extracts were studied as natural preservatives to extending the shelf-life of mullet fish fillets (*Mugil cephalus*). Skinless mullet fillets were treated with 1% of studied extract for 10 min at ambient temperature before storage at $4\pm 1^{\circ}\text{C}$. The chemical [pH, volatile basic nitrogen, trimethylamine nitrogen and thiobarbituric acid], microbiological [total viable bacteria, psychrophilic bacteria and yeast and mold counts] and sensory analysis were used to evaluate the preservative effect of this natural extracts during storage at $4\pm 1^{\circ}\text{C}$ for 16 days. Protein breakdown and lipid oxidation in mullet filets that treated with cumin or blackcumin were significantly suppressed during storage. The microbiological assays indicated that fillets treated with 1% cumin extract showed the lower counts of total viable bacteria, psychrophilic bacteria and yeasts and molds compared with other treatments. Treatment with cumin extract led to improve all studied sensory parameters followed by treatment with the extract of blackcumin, laurel leaves and fenugreek. Treatment with either cumin or blackcumin was suggested to extend the shelf-life of mullet fillets to 16 days compared with 8 days for the control samples during storage at $4\pm 1^{\circ}\text{C}$.

Key words: Antimicrobial • antioxidant • blackcumin • cumin • fenugreek seeds • laurel leaves

INTRODUCTION

Fish is a good source of protein, is low in total fat and has the added advantage of being high in types of fatty acid that provide protection against heart attack and also, to some extent, stroke. Oily fish, such as mullet contain up to eight times as much of these 'omega-3' and 'omega-6' fatty acids as lean fish. However, fish is highly susceptible to spoilage, which can be caused by both chemical reactions and microbial growth [1]. The development of lipid oxidation or microbial growth in fish during storage can be controlled by synthetic or natural preservatives but consumers are always concerned about the use of artificial preservatives in food, which may have potentially undesirable effects on human health [2]. The occurrence of such oxidative damage may be a significant causative factor in the development of many chronic disease such as cancer and cardiovascular diseases [3, 4]. Natural antioxidants have been suggested as safe alternative to synthetic antioxidants to retard oxidative processes and to improve the keeping quality of fish.

Spices and herbs played an important role in human diet. They have been used not only for favoring food but also for their medical and preservation properties. Twenty seven spices were recognized by the FDA as safe, they showed highest activities such as cancer preventive, anti-inflammatory agents and can be used as antiseptic, fungicide and bactericide [5]. Spices and their essential oils are the most efficient natural antioxidants and antimicrobial agents have long been used to preserve food [6].

Fenugreek was found to have high quantity of saponins which have antioxidant, anticarcinogenic and antimicrobial properties [7]. Blackcumin seeds have been widely consumed as a food spices and drug in Middle East since ancient time [8]. Also, El-Kayati [9] indicated that unsaponifiable matters of *Nigella sativa* have strongest effect among the tested sample as antimicrobial activity. In addition, they found that the extract from *Nigella sativa* should not cause undesirable side effects. The essential oils and various extracts of plants provoked interest as sources of natural products. They have been

screened for their potential uses as alternative remedies for the treatment of many infectious diseases and the preservation of foods from the toxic effects of oxidants [10]. The use of spices and herbs such as laurel leaves, licorice roots and thyme as antioxidants in food is a promising alternative to use of synthetic antioxidants [11]. El-Shrif and Ibrahim [12] reported that using of plant extracts (0.05%) improved both biochemical and sensory parameters of brined fish fillets. In the present study, therefore, the preservative effects of four plant extracts (blackcumin, laurel leaves, cumin and fenugreek), applied either alone have been investigated to evaluate their efficacy as a natural and safe preservative for mullet fillets during storage at 4°C.

MATERIALS AND METHODS

Materials: Mullet fish (*Mugil cephalus*) obtained from El-Serow station farm, National Institute of Oceanography and Fisheries. The average weight of studied fish was ranged between 300 and 400g. Fishes washed with tap water then transported in ice box to Fish Processing and Technology Laboratory, El-Kanater El-Khiria, National Institute of Oceanography and Fisheries.

Blackcumin (*Nigella sativa*), laurel leaves (*Laurus nobilis*), cumin (*Cuminum cyminum*) and fenugreek seeds (*Trigonella foenumgraecum*) were obtained from the local market.

Preparation of plant extracts: The air-dried ground plants were extracted with 95% ethanol solution in Soxhlet apparatus for 6 h [13, 14]. The obtained extracts were evaporated at 70°C for 30 min and mixed with activated coal (2g /500ml), refrigerated, filtered through Whatman No, 1 and kept in brown bottles at 4±1°C until used.

pH value measurement: The pH of the mullet fish was measure on homogenized fillet samples diluted in distilled waster (1:10 v:v) with a pH meter (D-14, Horiba, Tokyo, Japan).

Treatment of fish fillets with plant extract: Fishes were beheaded, eviscerated, filleted and washed with tap water to remove blood traces. Fish fillets were divided immediately into 5 equal groups which were dipped for 10 min at ambient temperature (23±2°C) in the distilled water (control) or in a 1% solution of blackcumin, laurel leaves, cumin or fenugreek extract solutions. Treated fish fillets drained on sterilized stainless-steel grill for 2 min. After that, drained fillets were packaged in polyethylene

bags, each bag contained two fish fillets and stored in refrigerator at (4±1°C) for 16 days.

Determination of volatile basic nitrogen (VB-N): VB-N associated with fish spoilage was determined according to the method of [15].

Determination of Trimethylamine-nitrogen (TMA-N): Trimethylamine-nitrogen (TMA-N) was determined as described in [16].

Thiobarbituric Acid (TBA) test: TBA values mullet fish fillets were determined spectrophotometrically according to the procedure described by Siu and Draper [17]. Ten grams of mullet filet were homogenized in 25 ml of distilled water for 2 min and then mixed with 25 ml of 10% trichloroacetic acid (TCA). The mixture was mixed and filtered and then 1 ml of 0.06 M thiobarbituric acid was added to 4 ml aliquots of the filtrate and heated in a boiling water bath (10 min) for color development. The absorbance at 532 nm was measured using a UV/Visible spectrophotometer (6105-Jenway, U.K.). The TBA values of treated fillets were compared with those of control fillet. The TBA values were expressed in units of mg malonaldehyde/kg (mg MDA/kg) sample.

Total Viable Bacterial (TVB) count: TVB was enumerated as described by ICMSF [18]. Ten grams of mullet fillet were homogenized for 1 min at room temperature in 90 ml of sterilized 0.9% NaCl saline using a Stomacher 400 homogenizer (Seward, Basingstoke, England). Serial decimal dilutions were prepared in the saline solution. Pour plate technique was applied to determine TVB count on plate count agar (Difco). The cfu were enumerated after incubation at 30°C for 48 h. The experiment was carried out three times in duplicate.

Psychrophilic Count (PC): PC was enumerated as described by ICMSF [18] on plate count agar (Difco). The inoculated plates were incubated at 7°C for 7 days.

Yeasts and Molds Count (YMC): Yeasts and molds were enumerated described by ICMSF [18] on malt-extract agar medium (Oxoid) at 30°C for 72 h.

Sensory evaluation: Sensory properties including appearance, odor, texture and overall acceptability of chilled mullet fish fillets were evaluated using a panel taste of a point hedonic scale according to Fey and Regenstein [19].

Statistical analysis: For each treatment, data from three independent replicate trials were pooled and the mean values and standard deviations were determined. Differences between samples were determined by t-test and were considered to be significant when $p \leq 0.05$ [20].

RESULTS AND DISCUSSION

Changes in the pH of mullet fillets during storage at $4 \pm 1^\circ\text{C}$: Changes in the pH of mullet fillets during storage at 4°C are shown in Fig. 1. The initial pH values of the control and samples treated with blackcumin, laurel leaves, cumin and fenugreek extracts were 6.22, 6.18, 6.15, 6.20 and 6.17, respectively. During storage at 4°C , the pH value of the control samples increased rapidly to reach values of 6.85 compared with 6.33 and 6.35 of samples treated with cumin and blackcumin, respectively on the 16 day of storage. The increase of pH values at the end of storage period were 0.63, 0.13 and 0.17 for the control, cumin and blackcumin, respectively. There is a relationship between the increase of the pH value and the deterioration of food material due to microbial activity. Basic nitrogen compounds, such as ammonia and/or other basic nitrogenous compounds, which are produced as a result of the microbial activity, could cause the increase in pH [1, 12].

Changes in volatile basic nitrogen (VB-N) of mullet fillets during storage at $4 \pm 1^\circ\text{C}$: Measurement of VB-N is an important indicator of fish quality during storage. Changes in total volatile basic nitrogen (VB-N) of the mullet fillets during storage at 4°C are shown in Fig. 2. The VB-N of mullet fillets decreased insignificantly ($p > 0.05$) from 13.35 mg N/100 gm for the control sample to 12.55 mg N/100 gm immediately after treatment with blackcumin. During storage at 4°C , the VB-N of the control sample increased to reach the acceptable limit of fresh fish (30 mg N/100 g) on day 8. By contrast, the VB-N of fillets treated with blackcumin and cumin remained below the acceptable limit until the end of the storage period. Connel [21] reported that, the content of VB-N value is useful indicator of freshness of lean fish and suggested 30-40 mg N/100 gm and 60 mg N/100 gm sample (on fresh weight basis) as the upper limit for freshwater fish and marine fish respectively. Extension the shelf-life of fillets treated with cumin and blackcumin may be due to its inhibitory effects on microbial growth, which could delay the decomposition of mullet fillets protein as compared with control treatment. These results are in agreement with previous studies [12, 22, 23].

Changes in the Trimethylamine nitrogen (TMA-N) of mullet fillets during storage at $4 \pm 1^\circ\text{C}$: Changes in the Trimethylamine nitrogen (TMA-N) of mullet fillets during cold storage are shown in Fig 3. The TMA-N was significantly reduced by treatment with cumin compared with the control just after treatment. The TMA-N of all samples gradually increased as total microbial counts increased during storage. The TMA-N of control and cumin reached 13.25 and 4.62 mg on day 16 of storage. Maga [24] reported that perfectly fresh fish had 3.37 mg N/100 g sample of TMA-N, good grade fish showed 3.79-5.90 mg /100 gm, fair fish had 12.65-16.02 mg /100 g. These results are in agreement with previous studies [23, 25, 26].

Changes in the Thiobarbituric acid (TBA) of mullet fillets during storage at $4 \pm 1^\circ\text{C}$: Lipid peroxidation, corresponding to the oxidative deterioration of polyunsaturated fatty acids in fish muscle leads to the production of off-flavors and off-odors, thereby shortening the shelf-life of food [27]. The Peroxide Value (PV) and TBA value are both well-established methods for determining oxidation products in fats and oils [28]. Changes in the TBA of mullet fillets during storage at $4 \pm 1^\circ\text{C}$ are shown in Fig. 4. Immediately after treatment, there were no significant differences ($p > 0.05$) between the control and other treated sample. During storage at $4 \pm 1^\circ\text{C}$, the TBA values of the control and fillets treated with blackcumin extract increased from 0.88 and 0.75 to 4.03 and 1.22 mg Malonaldehyde (MDA) per Kg respectively, at the end of storage period. These results are in agreement with those of [26, 29, 30] who reported that the TBA values of carp fillets treated with a commercial antioxidant increased during storage at 5°C . On the other hand the obtained results indicated that the treatment of mullet fish fillets with blackcumin extract led to extension of their shelf-life comparing with the control fillets. This result is in accordance with those stated by Bonnell [31], who mentioned that fish and fish products of good quality will have TBA value less than 2 mg MDA/kg flesh, while poorer quality fish will have 3-27 mgMDA/kg. Therefore, fish had TBA values greater than 2 will probably smell and taste rancid.

Changes in the total bacterial count of mullet fillets during storage at $4 \pm 1^\circ\text{C}$: Microbial growth is the major cause of food spoilage. Changes in the total bacterial count of the mullet fillets during cold storage are shown in Fig. 5. Treatments, including blackcumin, laurel leaves and cumin showed significant reduction ($p=0.05$) in the

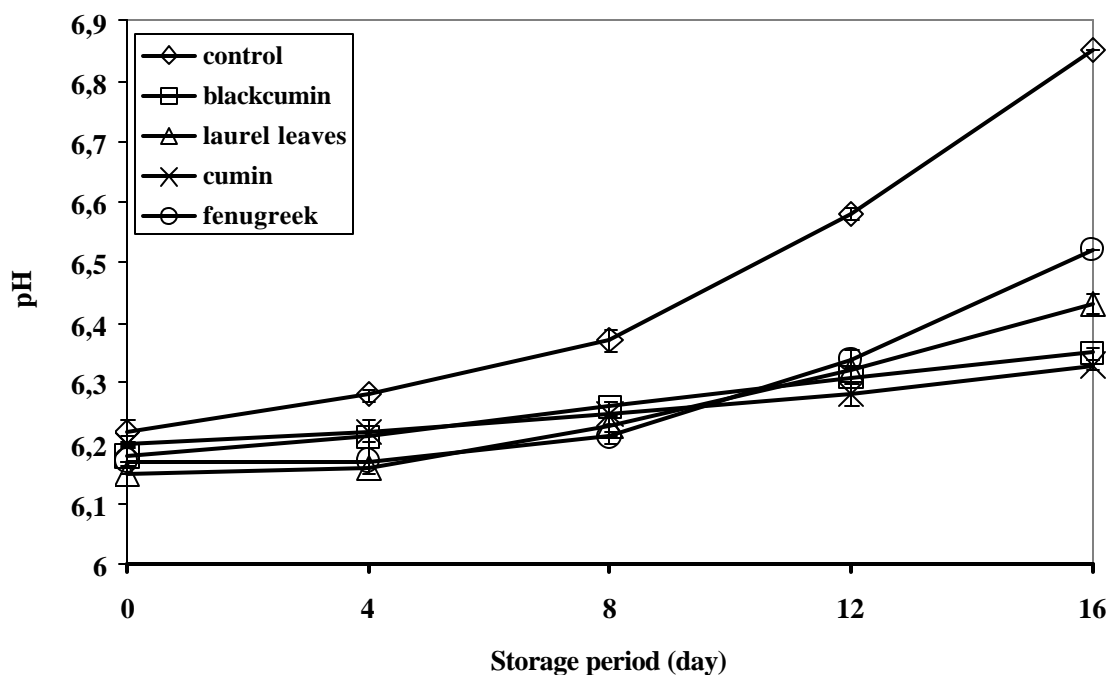


Fig. 1: Changes in the pH of mullet fillets during storage at 4±1°C. Mullet fillets were dipped before storage for 10 min in distilled water (control) or in a 1% solution of blackcumin, laurel leaves, cumin or fenugreek extract. Results are the means of three replicates ±SD

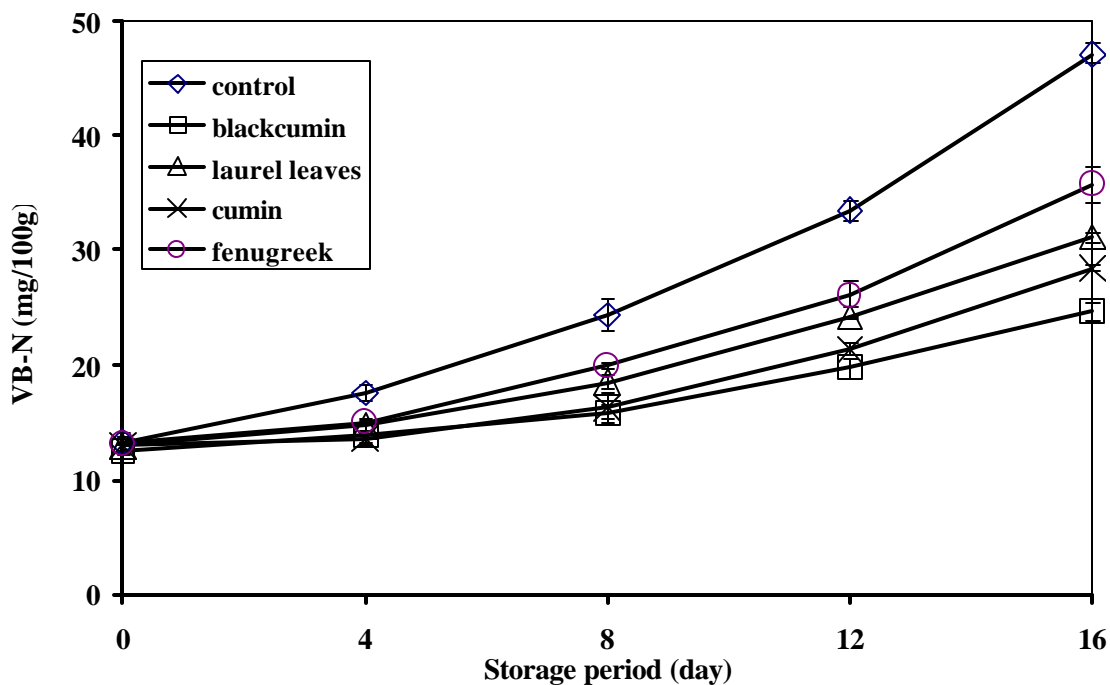


Fig. 2: Changes in the volatile basic nitrogen (VB-N) of mullet fillets during storage at 4±1°C. Mullet fillets were dipped before storage for 10 min in distilled water (control) or in a 1% solution of blackcumin, laurel leaves, cumin or fenugreek extract. Results are the means of three replicates ±SD

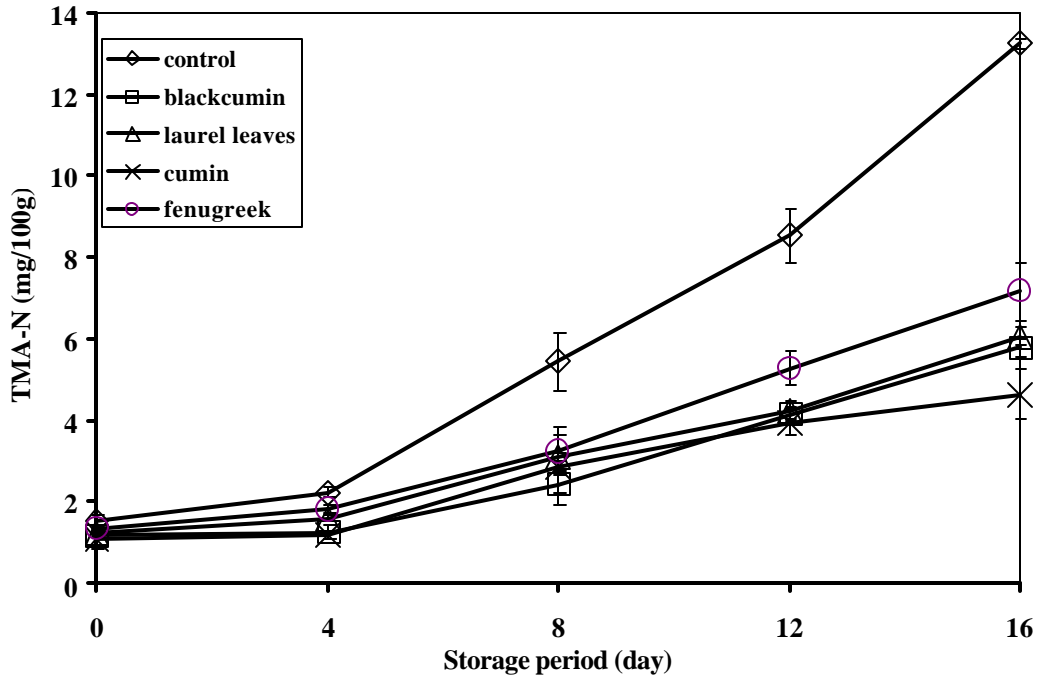


Fig. 3: Changes in the trimethylamine-nitrogen (TMA-N) of mullet fillets during storage at 4±1°C. Mullet fillets were dipped before storage for 10 min in distilled water (control) or in a 1% solution of blackcumin, laurel leaves, cumin or fenugreek extract. Results are the means of three replicates ±SD

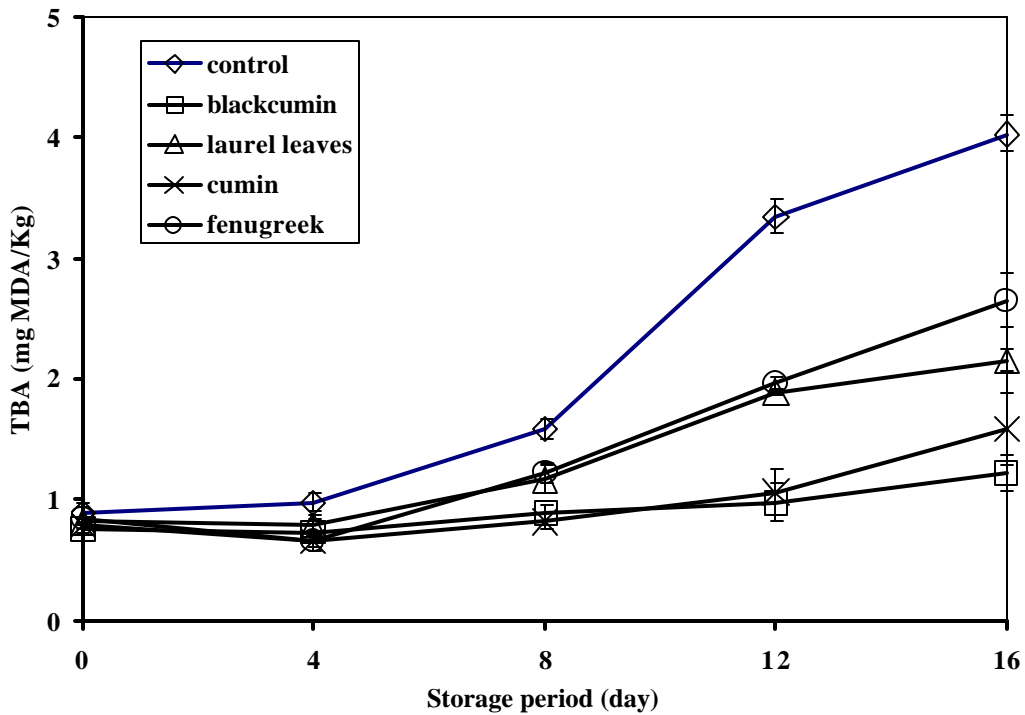


Fig. 4: Changes in the thiobarbituric acid (TBA) of mullet fillets during storage at 4±1°C. Mullet fillets were dipped before storage for 10 min in distilled water (control) or in a 1% solution of blackcumin, laurel leaves, cumin or fenugreek extract. TBA values were expressed in units of mg malonaldehyde/kg (mg MDA/kg) sample. Results are the means of three replicates ±SD

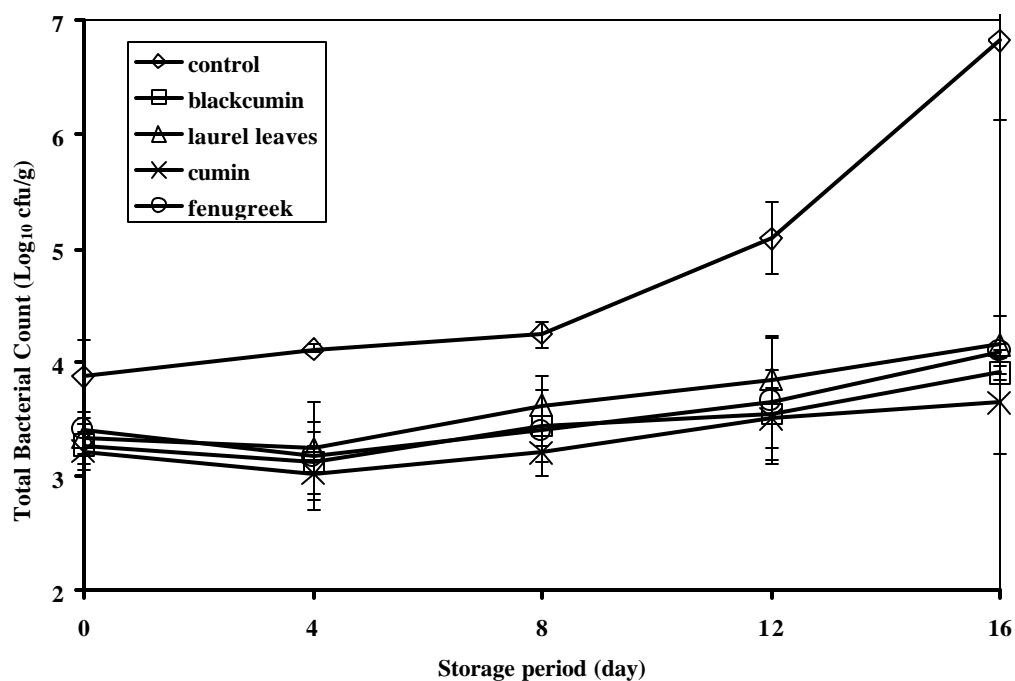


Fig. 5: Changes in the total bacterial count of mullet fillets during storage at 4±1°C. Mullet fillets were dipped before storage for 10 min in distilled water (control) or in a 1% solution of blackcumin, laurel leaves, cumin or fenugreek extract. Results are the means of three replicates ±SD

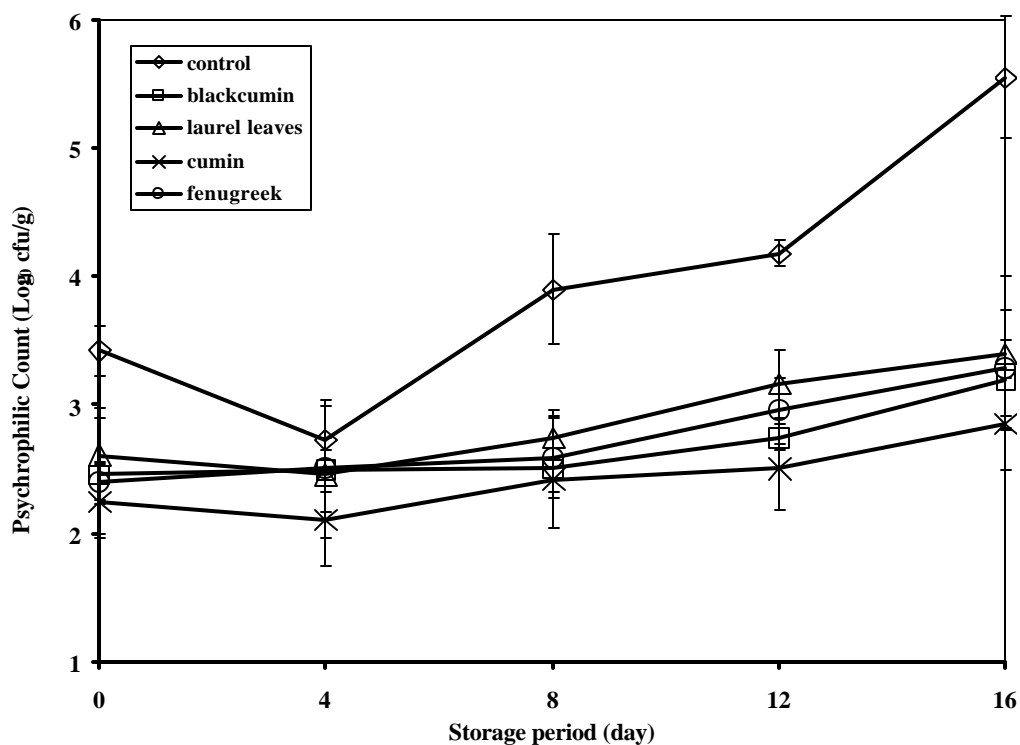


Fig. 6: Changes in the psychrophilic bacteria of mullet fillets during storage at 4±1°C. Mullet fillets were dipped before storage for 10 min in distilled water (control) or in a 1% solution of blackcumin, laurel leaves, cumin or fenugreek extract. Results are the means of three replicates ±SD

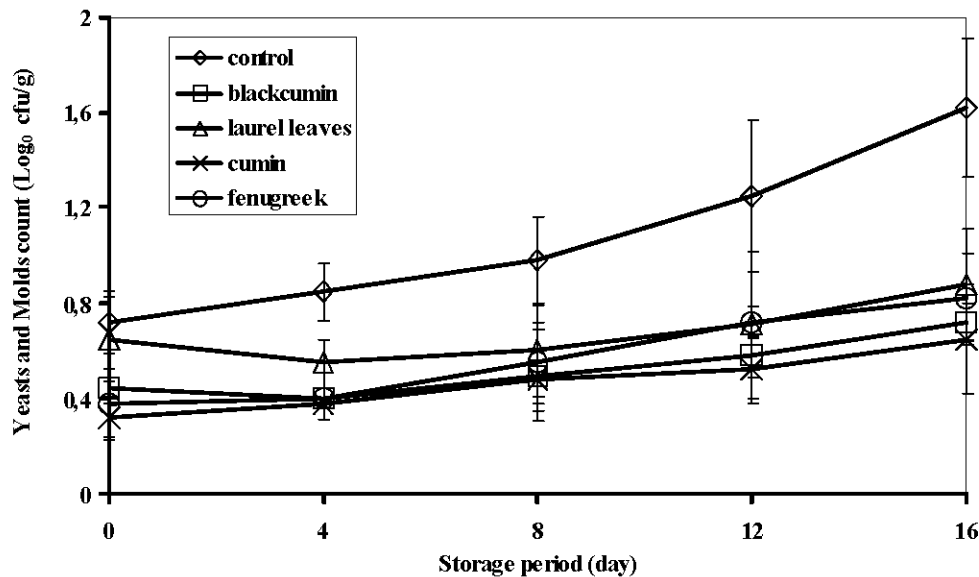


Fig. 7: Changes in the Yeasts and Molds of mullet during storage at $4\pm 1^\circ\text{C}$. Mullet fillets were dipped before storage for 10 min in distilled water (control) or in a 1% solution of blackcumin, laurel leaves, cumin or fenugreek extract. Results are the means of three replicates \pm

total microbial count immediately after treatment compared with that treatment with the control solution. The reduction caused by treatment with cumin was about $0.66 \log_{10} \text{cfu/g}$. Our results are in agreement with those of [12, 32]. Shen [33] found that the total plate count of was less than $4 \log_{10} \text{cfu/g}$ sample for fresh fish, ranged between 4 and $6 \log_{10} \text{cfu/g}$ for sub fresh and more than $6 \log_{10} \text{cfu/g}$ for deteriorated fish. The total bacterial count of the mullet fillets increased during storage at 4°C . The total microbial count in samples treated with cumin and blackcumin increased slowly comparing with the other samples. A considerable decrease of TBC that noticed in stored fish samples treated with plant extracts at the 4th day storage is due to the effect of plant extracts used as antimicrobial agents [34]. The chemical composition of the oils that give rise to inhibitory effects could be due to the presence of an aromatic nucleus containing a polar functional group [35].

Changes in the psychrophilic bacteria of mullet fillets during storage at $4\pm 1^\circ\text{C}$: Changes in the psychrophilic bacteria of mullet fillets during storage at 4°C are shown in Fig. 6. All treatment, except laurel leaves, gave a significant reduction ($p = 0.05$) in the psychrophilic bacteria immediately after treatment as compared with control treatment. The reduction caused by treatment with cumin was about $1.17 \log_{10} \text{cfu/g}$ as compared with control. Subsequently, the psychrophilic bacteria of mullet fillets

decreased through the first 4th days of storage then gradually increased during storage at 4°C until the end of storage. Additionally, the growth pattern of psychrophilic bacteria showed same behavior as that of total bacterial counts. These results are in accordance with that of Abou-Taleb [36].

Changes in the Yeasts and Molds of mullet fillets during storage at $4\pm 1^\circ\text{C}$: Changes in the yeasts and molds of mullet fillets during storage at 4°C are shown in Fig. 7. Treatment with cumin resulted in a significant reduction ($p=0.05$) in the initial yeast and mold counts as compared with the control. The reduction caused by treatment with cumin was about $0.4 \log_{10} \text{cfu/g}$ compared with control. These results are in agreement with those reported by Yasin [26] And Badr *et al.* [23]. Microbiological load of total viable bacteria, yeasts and molds counts were 3.21 and $0.60 \log_{10} \text{cfu/gm}$ sample. There were no significant differences in the yeast and mold counts among samples treated with blackcumin, laurel leaves, fenugreek seeds on day 0. The yeast and mold counts of the mullet fillets increased during storage at 4°C until reached to $1.62 \log_{10} \text{cfu/g}$ in the control sample and $0.65 \log_{10} \text{cfu/g}$ in fillets treated with cumin on day 16.

Changes in organoleptic properties of mullet fillets during storage at $4\pm 1^\circ\text{C}$: Table 1 summarized the sensory properties of mullet fish fillets during storage at 4°C .

Table 1: Changes in sensory properties of mullet fillets treated with plant extracts during storage at 4±1°C

Storage period (day)	Treatment					L.S.D. 0.05%
	Control	blackcumin	Laurel leaves	cumin	fenugreek	
Appearance						
0	8.8	8.5	8.3	8.7	8.5	0.83
4	7.6	8.2	8.0	8.5	7.8	1.00
8	6.3	8.0	7.8	8.0	7.5	1.19
12	5.8	7.6	7.3	7.7	7.2	1.02
16	4.6	7.0	6.5	7.2	6.0	1.48
L.S.D. 0.05%	1.06	0.81	0.81	0.76	0.93	
Odor						
0	8.5	9.0	9.0	9.2	9.0	0.93
4	7.6	8.6	8.4	9.0	8.5	0.83
8	6.4	8.2	8.0	8.5	8.4	0.89
12	5.5	7.5	7.0	7.8	6.5	1.04
16	4.0	6.2	5.5	6.5	5.8	1.31
L.S.D. 0.05%	1.31	1.17	1.25	0.87	0.97	
Texture						
0	9.0	8.8	8.5	9.0	8.5	0.78
4	7.5	8.5	8.2	8.5	8.0	1.08
8	6.5	8.0	7.5	8.2	7.2	0.64
12	5.8	7.2	7.0	7.5	6.5	0.49
16	4.2	6.5	6.0	7.0	5.5	1.42
L.S.D. 0.05%	0.83	0.78	0.91	1.00	0.96	
Overall acceptability						
0	8.8	8.8	8.6	9.0	8.6	0.74
4	7.5	8.4	8.2	8.7	8.1	0.81
8	6.7	8.1	7.7	8.2	7.7	0.97
12	5.6	7.4	7.1	7.6	6.7	1.08
16	4.2	6.6	6.0	7.0	5.8	1.04
L.S.D. 0.05%	0.68	0.78	0.98	0.89	0.89	

No significant difference was detected between control fillets and other treated mullet fillets before storage in all sensory properties. During storage at 4°C of mullet fillets a gradual decrease for all the parameters of sensory evaluation were observed. On day 4, no significant difference between control fillets and other treated mullet fish fillets at 4°C in appearance and texture properties. From day 8 onwards there was significant differences ($p \leq 0.05$) between control fillets and other treated mullet fillets in appearance, odor, texture and overall acceptability properties and control fillets were reached the unacceptable score, while the fillets samples treated by cumin and samples treated with blackcumin were not

reached to unacceptable score until the end of storage (16 days). The onset of spoilage was easily detected by undesirable odor (off-odor) that due to the formation of TMA-N, ammonia, indol and other amines [37] as metabolites induced by the action of spoilage bacteria on fish tissues during storage [38].

In conclusion, the results obtained in the present study, provide useful information for improving the quality of mullet fish fillets during cold storage through pre-treatment with the alcoholic extract of one of the studied plants.

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