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Potential application of cumin, pepper and thyme spices on soft cheese quality

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ABSTRACT

White soft cheese is usually contaminated with pathogenic and spoilage microorganisms during manufacturing and storage. Fortunately, several spices contain natural antimicrobial and antioxidant components that control the safety of milk and milk products. So, this current work aimed to examine the inhibitory effect of cumin, pepper and thyme on total bacterial count, Coliforms, Staphylococcus aureus, yeast and mold count. Additionally, studying the impact on moisture content and fat percent The findings cleared that thyme, pepper and cumin have great antibacterial effect ($p \leq 0.05$) against total bacterial count, Coliforms, Staphylococcus aureus, mold and yeast count during cooling storage period (30 days) comparing with control. With regard that thyme acquired more antibacterial activity than pepper and cumin. Moreover, moisture percent decreased during the first two weeks from storage. The fat content of white cheese treatments was not significant affected by thyme, pepper and cumin until 10th days of storage then, fat percent increase in treated sample with thyme, paper and cumin, respectively. Overall, the pH values of cheese samples decreased while the acidity increased. The progress of acidity was faster in treated groups than control groups during storage. Sensory characters revealed that the highest total scores were given to groups preserved thyme. On conclusion, our findings recommend using thyme by a concentration 0.1% with cheese during manufacturing..

1. INTRODUCTION

White soft cheese is a good source for protein, minerals, calcium and vitamins, so it is viable for contamination by pathogenic and spoilage microorganisms which affect shelf life of dairy product(El-Sayed and El-Sayed, 2021). It has a great importance as it includes fatty acids, minerals, vitamins and volatile compounds (Cakmakci and Cakir, 2011).Poor hygienic practices in cheese processing plants may result in the contamination of cheeses with pathogens leading to prompting their decay, financial misfortunes, foodborne diseases in human and wellbeing hazard (Marjan et al., 2014).

Bacteriological and fungal growth on soft cheese as a result of typical pH range and moisture content considered the chemical and biological changes occurred during storage period (Costa et al., 2017).

Many types of Plants have been traditionally used in cheeses manufacturing, thus are mixed with the cheese curd at the beginning of ripening process. Usually used spices and herbs that added to cheeses include, peppers, thyme and cumin (Hayaloglu and Farkye, 2011) .

Thyme (Thymus vulgaris L), is used as flavoring agents in various dairy products, including cheese. It has antibacterial properties due to their thymol content (Burt , 2004).Pepper has antibacterial effect against several microorganisms as *E. coli* as it contains eighteen compounds having antibacterial activity Da Silva Dannenberget al. (2019). Cumin is effective in the inhibition of growth and reduction of food-borne bacteria, such as Salmonella spp., Escherichia coli O157: H7, and Listeria monocytogenes in addition to food-spoilage like Aspergillus flavus, Aspergillus niger, and Aspergillus nomius, thus safeguard the foods by preserve quality and increasing shelf life (El-Sayed and Ibrahim, 2021). Cumin is also well known for its antioxidative properties, and its methanol extract contains a total phenolic content of 9 mg/g dry weight (Thippeswamy and Naidu, 2005) .

As food spoilage recorded as one of the most significant problems encounter dairy industry and the emergence of resistance to most chemical antimicrobial agents necessitates the use of more effective natural preservatives as cumin, pepper and thyme used due to their effect on cheese organoleptic and microbiological quality of dairy products.

2. MATERIAL AND METHODS

2.1.*Natural spices*

Cumin, pepper and thyme spices used in this work were purchased from El Captain Company (CAP PHARM) .

2.2. *Preparation of soft cheese*

A total of 8 liters of fresh raw buffalo′s milk was obtained from the herd of Faculty of Agriculture, EL-Menofiya University. Soft cheese was manufactured according to the method described by Ahmed, (2016) as raw buffalo's milk were heated to 80 ◦C for 10 min (Laboratory pasteurization), cooled immediately using an ice bath. The

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amount of rennet used as described by the manufacture (50 ml of rennet per liter of milk). The rate of CaCl2 is 0.02% and NaCl is 1%. Milk was then divided into four groups the first group (control), second group (milk +0.1% cumin), third group (milk+0.1% pepper) and fourth group (milk+0.1thyme) thoroughly mixing for even distribution of natural spices according to El-Sayed and El-Sayed, (2021) and Katarzyna et al., (2022). Samples were examined at zero day, 5th day, 10th day, 15th day, 20th day, 25th day and 30th day during the storage period. The experiment was performed in triplicate.

2.3. *Sensory evaluation*

Samples were carried out according to the scheme of Clark et al. (2009), a panel test of 3 panelists (each sample) of the staff members of Food Hygiene & Control Department, animal health research institute, Shebin el kom branch. The cheese samples were evaluated for flavor (40 points), Texture (40 points), color (10 points), salts (5 points) and appearance (5 points).

2.4. *Physicochemical examination*

2.4.1. *Moisture content*

Moisture content of the controlled and treated samples was measured though oven drying at 105 o C for 3 hr. (AOAC, 1990) .

2.4.2. *Fat content*

Fat was determined using extraction with hexane (AOAC Official Method 920.39) using a Soxhlet apparatus (Isolab GmbH, Eschau, Germany) (AOAC, 1995).

2.4.3.*pH*

The pH was determined using a pH meter (Mettler Toledo, Greifensee, Switzerland) at 20 °C. The cheese was mixed with distilled water (1:4 (w/v) ratio) (Lario et al., 2004)

2.4.4.*Titratable acidity*

According to AOAC (2000) titratable acidity was determined as lactic acid by the titration method with N/9 NaOH .

2.5. *Microbiological examination*

2.5.1. *Sample preparation*:

Twenty-five grams taken from each sample and blended aseptically in 0.1%peptone water (225 ml) for 2 min at 2500 rpm to obtain a dilution of 10-1, followed by making decimal serial dilutions (APHA, 2001).

2.5.2. *Determination of Total bacterial count*

Using surface plate method at 35°c: (Petran et al., 2015)

2.5.3. *Determination of coliforms count*

Using violet red bile agar (VRB) at 370c for 48 hr.(Feng et al., 2002)

2.5.4. *Determination total Staphylococcal count*

From each of previously prepared serial dilutions 100 µl was spread over duplicated plates of Baired Parker ager medium. The control and inoculated plates were incubated at 370C for 48 hours (MacFaddin, 2000).

2.5.5. *Determination of total mold and yeast count*

The isolation procedure of the yeasts and filamentous molds on Sabouraud dextrose agar media at 25oC for 5 days was performed according to ISO 21527-2.(2008)

2.6. *Statistical analysis*

Comparing the results of microbial evaluation, moisture and fat percent during the cooling storage period present in treated and control groups using one-way analysis of variance (ANOVA), Significance was set at $P \leq 0.05$.

3. RESULTS

3.1.*Physicochemical parameters of soft cheese*

Fig. 1 showed the sensory attributes (Flavor, Texture, Color, Salts, and appearance) of Kariesh soft cheese during 30 days of storage. The scores decrease with time as the flavor score decreased from 40 to 20, texture decreased from 40 to 19, colour also decreased from 10 to 3 while, salts and appearance decreased from 5 to 1 at the end of cooling storage period.

Moisture content decreased with prolonged shelf life of soft cheese along the cooling storage period 30day. Moisture % present in cheese during storage days was insignificantly ($p \leq 0.05$) differed between control and treated groups until day 20th of storage as shown in table.(1)

Table (2) showed that the fat content of control and treated groups were not significant affected by until 25 days of storage, while it significantly increased during storage time due to loss in moisture content in cheese.

The pH decreased with the storage time from 6.55 to 5.9 at the end of storage period while it decreased from 6.54 to 5.86, 5.86 and 5.85 for cumin, pepper and thyme, respectively as shown in Fig.(2) .

Moreover, data in table (3) showed that titratable acidity increased from 0.26 ± 0.01 to 0.45 ± 0.03 in control group also, treated group with cumin increased from 0.27 ± 0.01 to 0.52 ± 0.02 similar results obtained in group treated with pepper while thyme treated group significantly difference ($P \leq 0.05$) from other groups.

3.2. *Microbiological quality of the examined treated soft cheese*

The mean values of APC (log CFU/g) of control sample at day 10th of storage was 6.81while it was 5.69, 5.54 and 5.41 ($P \leq 0.05$) for cumin, pepper and thyme treated groups, respectively. Also, the coliforms count affected by adding natural spices as cumin, pepper and thyme as the coliforms count significantly decrease ($P \leq 0.05$) during the storage period unlike the control group. The count of control group was 2.53 (log CFU/g) at zero day then increase throughout the storage period to 3.85 (log CFU/g).Furthermore, the mean values of S. aureus count (log CFU/g) in control group were 4.97while it was 3.79, 3.70 and 3.63 groups treated with for cumin, pepper and thyme, respectively. Mold and yeast count in control group at zero day was 2.66 log CFU/g then increase gradually throughout the storage period that become 6.1 log CFU/g at the end on storage period. While mold and yeast count in treated groups at zero day was 2.53, 2.46 and 2.39 (log CFU/g) for cumin, pepper and thyme treated groups, respectively. At 25th day of storage mold and yeast not detected in sample treated with cumin, also it disappeared in groups treated with pepper and thyme at 20th day of storage.

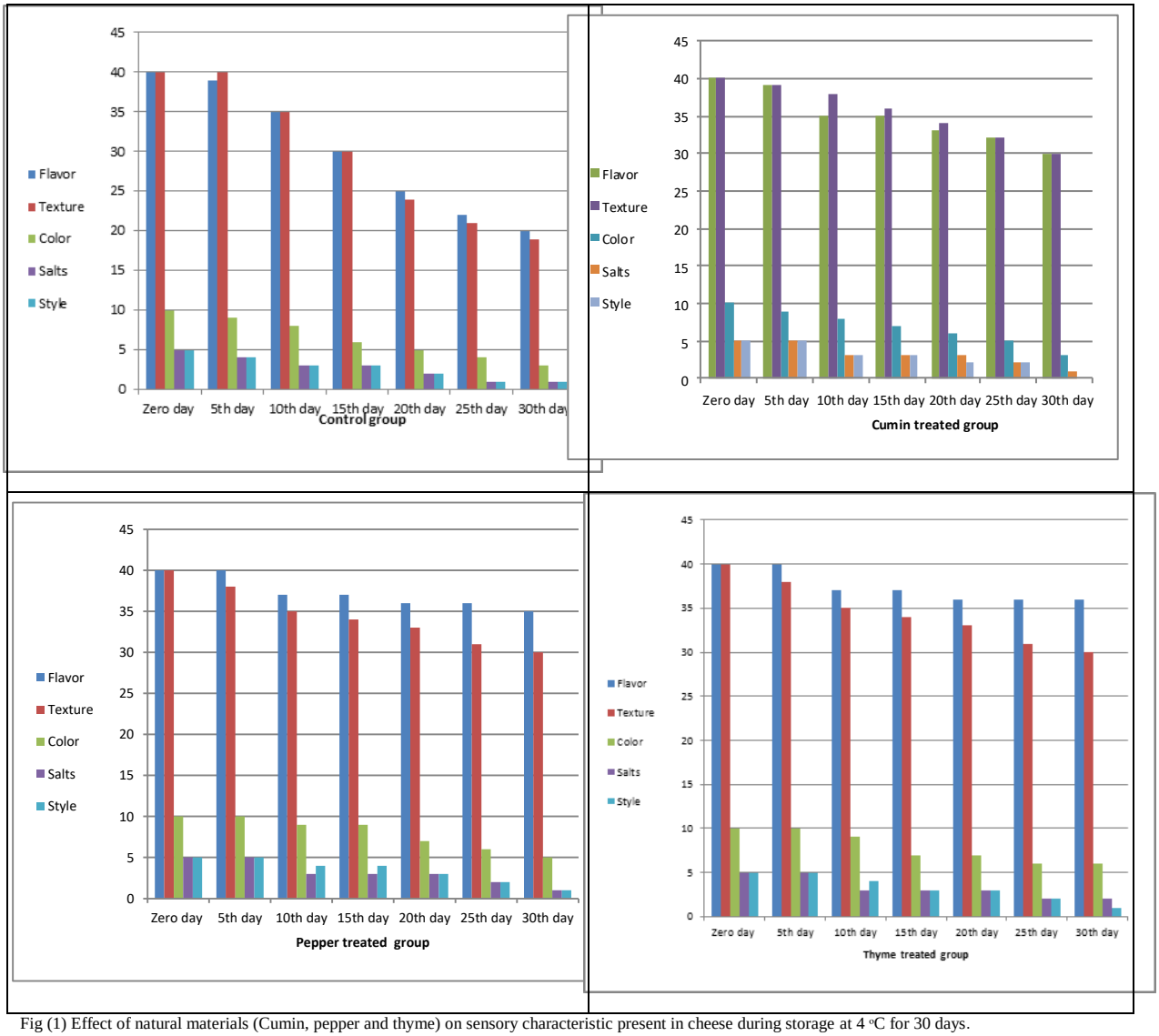

Fig (1) Effect of natural materials (Cumin, pepper and thyme) on sensory characteristic present in cheese during storage at 4 °C for 30 days.

Table 1 .Effect of natural material (Cumin, pepper and thyme) on moisture percent present in cheese during storage at 4 °C for 30 days.

Groups	Zero day	5th day	10th day	15th day	20th day	25th day	30th day
Control	58.71 ^{a}±0.2	58.49 ^{a} ± 0.1	58.42 ^{c} ±0.31	58.38 ^{a} ± 0.4	58.1 ^{f} ±0.12	57.10 ^{fg}±0.28	56.20 ^{f} ±0.12
Cumin	58.61 ^{a} ±0.15	58.4 ^{a} ±0.13	58.3 ^{c}±0.16	58.29 ^{cb}±0.15	57.49^{d}±0.4	56.43^{e}±0.28	55.15^{e}±0.15
Pepper	58.59 ^{a} ±0.23	58.37^{ab} ±0.8	58.23 ^{c} ±0.15	58.21 ^{cb} ±0.4	57.43^{d} ±0.4	56.22^{e} ±0.28	55.12^{e} ±0.11
Thyme	58.54 ^{a} ±0.12	58.43^{ab}±0.17	58.2 ^{c} ±0.16	58.18^{cb} ±0.1	57.35^{d}±0.4	56.15^{G}±0.28	55.08^{G}±0.20

The values are expressed as Mean ± standard error of three experiments. Means within a column followed by different letters are significantly different ($P \leq 0.05$).

Table 2.Effect of natural materials (Cumin, pepper and thyme) on fat percent present in cheese during storage at 4 °C for 30 days.

Groups	Zero day	5th day	10th day	15th day	20th day	25th day	30th day
Control	17.1 ^{a}±0.2	18.1 ^{a} ± 0.1	19.1 ^{a} ±0.31	20.1 ^{a} ± 0.4	21.25 ^{f} ±0.12	22.1 ^{fg}±0.28	23.08 ^{fg}±0.28
Cumin	17.30 ^{a} ±0.15	18.11 ^{a} ±0.13	19.15 ^{c}±0.16	20.19 ^{cb}±0.15	21.35^{b}±0.4	22.15^{fg}±0.28	23.11^{fg}±0.28
Pepper	17.35 ^{a} ±0.23	18.28^{a} ±0.8	19.35 ^{c} ±0.15	20.4 ^{cb} ±0.4	21.4^{cb}±0.4	22.26 ^{fg}±0.28	23.25 ^{fg}±0.28
Thyme	17.35 ^{a} ±0.12	18.55^{a} ±0.17	19.75 ^{c} ±0.16	20.78 ^{d}±0.1	21.54^{cb}±0.4	22.56 ^{fg}±0.28	23.39 ^{fg}±0.28

The values are expressed as Mean ± standard error of three experiments. Means within a column followed by different letters are significantly different ($P \leq 0.05$).

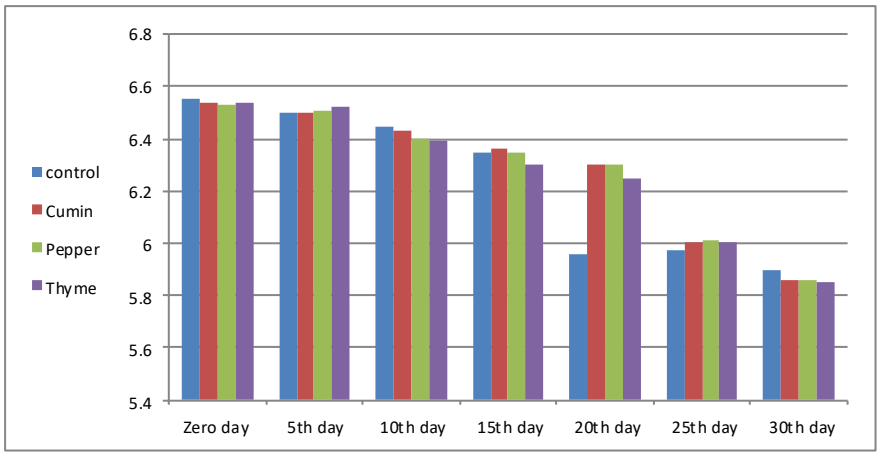

Fig 2.Effectof different natural material (Cumin, pepper and thyme) on pH present in cheese during storage at 4 °C for 30 days.

Table 3.Effect of natural materials (Cumin, pepper and thyme) on titratable acidity present in cheese during storage at 4 °C for 30 days.

Groups	Zero day	5th day	10th day	15th day	20th day	25th day	30th day
Control	0.26±0.01^{a}	0.3±0.02^{a}	0.35±0.04^{ab}	0.37±0.03^{b}	0.39±0.04^{b}	0.42±0.02^{c}	0.45±0.03^{c}
Cumin	0.27±0.01^{a}	0.32±0.03^{a}	0.37±0.01^{ab}	0.39±0.01^{b}	0.45±0.01^{c}	0.5±0.03^{d}	0.52±0.02^{d}
Pepper	0.27±0.02^{a}	0.32±0.01^{a}	0.37±0.02^{b}	0.39±0.01^{b}	0.45±0.02^{c}	0.5±0.01^{d}	0.52±0.02^{d}
Thyme	0.27±0.01^{a}	0.34±0.02^{a}	0.38±0.02^{b}	0.42±0.03^{c}	0.42±0.01^{c}	0.51±0.02^{d}	0.55±0.01^{e}

The values are expressed as Mean ± standard error of three experiments. Means within a column followed by different letters are significantly different (P ≤ 0.05).

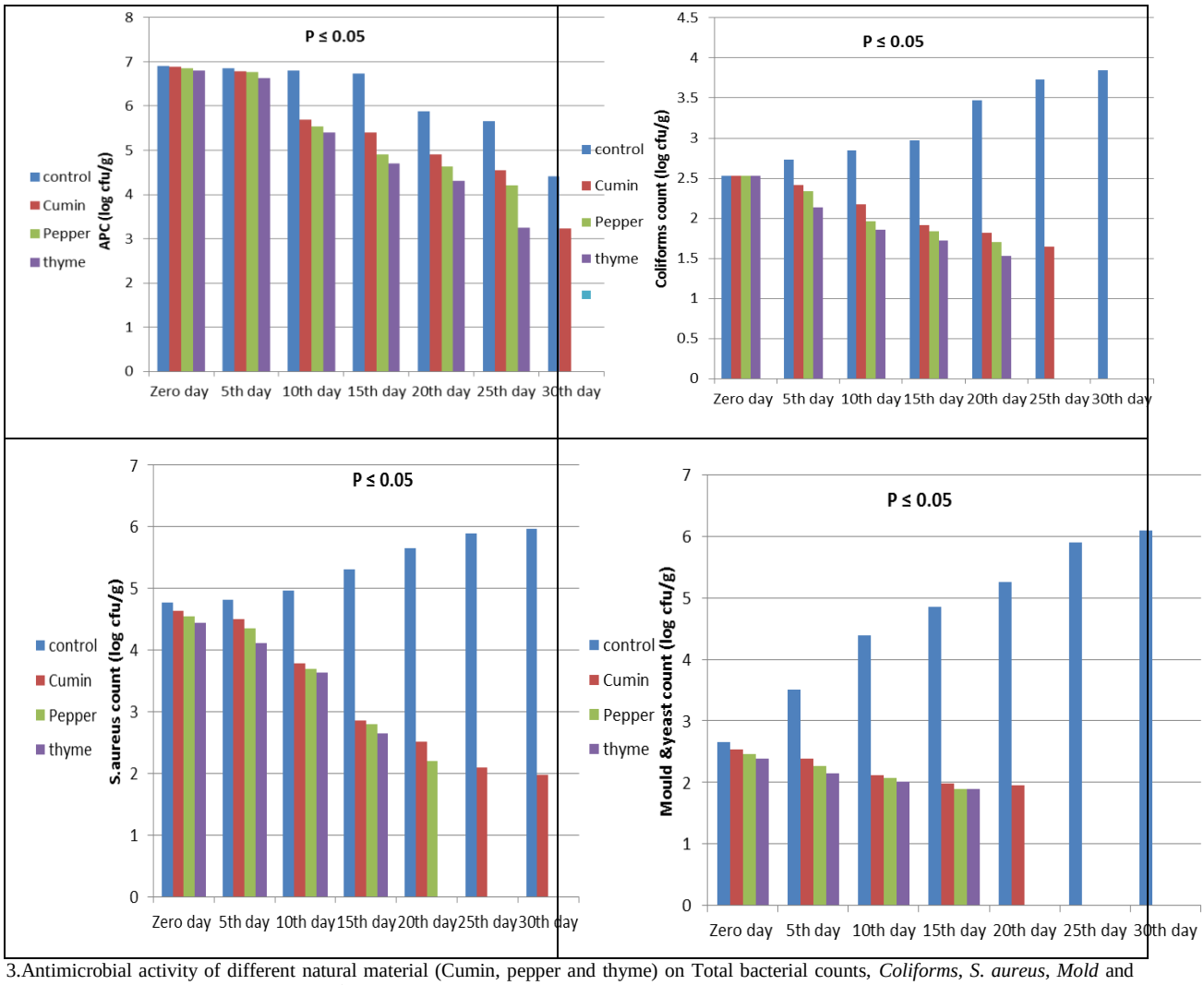

Fig 3.Antimicrobial activity of different natural material (Cumin, pepper and thyme) on Total bacterial counts, *Coliforms*, *S. aureus*, *Mold* and *Yeast* present in cheese during storage at 4 °C for 30 days.

4. DISCUSSION

The sensory attributes as texture, color, salts and appearance in Fig. (1) showed great improvement due to the usage of cumin, pepper and thyme as they delayed spoilage during the storage period. Flavor is the sensation that a substance gives off when it is consumed. It is primarily detected by the senses of taste and smell, but it can also be felt by the mouth's general pain, touch, and temperature receptors.

Additionally, flavour refers to the combination of the properties of the substance that causes that sense. Similar results obtained by Hassanien, (2014) who reported that while using black cumin the sensory evaluation of cheese behaved the same trend in all cheese treatments, as gradual enhancement was noticed during the storage. However, continuous production of lactic acid and other organic acids lead to fragile cheese showing a gradual decrease in body and texture and appearance scores recorded for all cheese treatments up till the end of storage period. Also the color scores decrease throughout storage period this decrease can be attributed to moisture content and proteolysis in cheese proteins this results disagreed with Govaris et al.(2011) .

Data revealed that moisture percent significantly ($P \leq 0.05$) differed between control and treated groups due to the metabolites of microorganisms that present in control group but, moisture percent decrease in treated one as bacterial count and there metabolites decrease .unlike results recorded by Al-Moghazy et al.(2021) who reported that moisture not affected by application of thyme .While Masmoudi et al. (2020) reported decrease in moisture content of cheese during storage period while using natural preservatives. Similar results also reported with by Giroux et al. (2013) as they reported that using materials that have antioxidant activity (phenolic compound) not affect moisture while it decreases with storage time. Moreover, the fat content of control and treated groups were not significant affected by until 25 days of storage, while it significantly increased during storage time due to loss in moisture content in cheese. Similar results were reported by Abd-El-Salam et al. (2011). As, Cumin is known for its antioxidant activity, also considered as a preserver's agent as a results of their antioxidant, that enhance the shelf life of foods (Singh et al., 2005). Moreover, Govaris et al. (2011) reported that using thyme not affect the fat percent in cheese during storage period.

Treated groups tend to be significantly different ($P \leq 0.05$) with less pH values. Similar results obtained by Al-Moghazy et al. (2021) and El-Sayed and El-Sayed (2021a). Also, El-Sayed and El-Sayed (2021a) reported that total acidity increase during storage with treated groups. Unlike, Al-Moghazy et al. (2021) who reported that total acidity insignificantly difference ($P \leq 0.05$) between treated and control groups.

Bacteria, yeasts and mold are the main reason for cheese spoilage, among of these pathogens *S. aureus* as well as E. coli are the most commonly isolated from cheese and are accompanied with foodborne illnesses (Khorshidian et al., 2018).

The reduction in total bacterial count resulted from using some spices as cumin that contain a total of sixteen major compounds has antimicrobial effect (El-Sayed and El-Sayed, 2021) also they reported that using 0.5% of cumin decrease the total bacterial count (log CFU/g) to 5.61 at 30th day of storage. At 25th day of storage the group that treated with thyme significantly differs from group treated with cumin and pepper as it was 3.25 for thyme while it was 4.55 and 4.20 for cumin and pepper, respectively. Similar results obtained by Al-Moghazy et al. (2021) as they reported that using thyme improve the microbiological status of cheese as it decrease the total bacterial count throughout the storage period and reported that the counts decreased significantly ($P \leq 0.05$) to 3.6 log CFU/g at the end of 4 weeks of storage period at 4 ◦C. Moreover, El-Sayed and El-Sayed (2021) reported that total bacterial count of soft cheese significantly decrease during storage while using cumin. Also Ahmed et al. (2015) revealed that using pepper decrease the total bacterial counts.

Coliforms count affected by adding natural spices as cumin, pepper and thyme as it significantly decrease ($P \leq 0.05$) during the storage period unlike the control group. Results indicated that the examined samples satisfied according to Egyptian organization standard (2005). During ripening, changes in physico-chemical conditions, such as pH increase, might be more favorable for the growth of microorganisms including pathogenic ones. At 20th day of storage the coliforms count in group treated with pepper and thyme significantly affected as it was 1.70 and 1.53 (log CFU/g), respectively, While it was 1.82 log CFU/g for group treated with cumin. Among pathogens, Gram-negative coliforms are the most commonly found in cheese and are associated with foodborne illnesses (Khorshidian et al., 2018).Coliforms determined in cheese are considered as a sign for unsatisfactory sanitation and possible enteric contamination (Frazier and Westhoff, 1978). Similar results reported by Ahmed et al. (2021) as they reported that thyme affect coliforms especially E. coli. Also, Ahmed et al. (2015) proved the antibacterial effect of pepper against coliforms. Hamid (2014) added 1% and 2% cumin to white cheese and proved that it not only affected the chemical properties and color of cheese (during storage to 14 days at room temperature) but also enhanced the flavor and the taste of cheese than control. Similarly, Algarni (2016) indicated that the addition of 2% thyme and cumin improve the microbiological status of soft cheese.

Moreover, there were significant differences ($P \leq 0.05$) in *S. aureus* count among treated and non-treated samples. *S. aureus* is a facultative anaerobe that produces a highly resistant enterotoxin, able to withstanding the harsh conditions without the presence of viable cells. It can be present in soft and hard cheeses and has the affinity to survive for a long period (Khorshidian et al., 2018). *S. aureus* presence in soft cheese may arise from skin, nose or mouth of workers handling the cheese so, *S. aureus* is a good indicator of the personal hygiene of workers (Kamat et al., 1991). Similar results obtained by El-Sayed and El-Sayed(2021) as the recorded significant antimicrobial activity of cumin against *S. aureus* in treated cheese sample. Moreover, Wahba et al. (2010) examined the antibacterial activity of pepper against *S. aureus* and reported their antibacterial activity. Also El-Sayed and El-Sayed (2021) proved the antibacterial activity of thyme against *S. aureus* present in soft cheese.

Cumin is effective in the inhibition of growth of food-spoilage microbes as Aspergillus spp. thus safeguard the foods by preserve quality and increasing shelf life (El-Sayed and Ibrahim, 2021).

The most common spoilage in cheese is caused by molds belonging to the genera Penicillium, Aspergillus, as well as yeasts of the genera Candida spp. Intense microbial growth is associated with undesirable sensory changes. In extreme cases, some species of the genus Aspergillus can produce mycotoxins, which penetrate the cheese mass and consumption of the contaminated cheese can pose a serious health threat to consumers (Khorshidian et al., 2018).

5. CONCLUSION

The current study showed that using some spices in cheese manufacturing have valuable profit as they have antimicrobial and antioxidant effects. Thyme showed great antimicrobial effects more that pepper and cumin. High percentage of phenolic compounds, as thymol present in spices possess the strongest antimicrobial properties against food-borne and spoilage pathogens. Also, their antioxidant activity protects the cheese moisture and fat percent content.

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