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Effect of Thyme Extract on Antioxidant, Antimicrobial Properties and Nutritional Value of White Soft Cheese

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Abstract

Plant extracts have promising results due to their functional and therapeutic properties. This work aims to study to study the effect of Thyme extract (TE) as an antioxidant, antimicrobial on properties and quality of white soft cheese (WSC). Results indicated that the antioxidant activity (DPPH) of thyme extract was (17.05±0.15) mg TE/g, total phenols value was (25.18±0.05) mg GAE/g and total flavonoids value was (17.29±0.47) mg CE/g. And after studying the effect of different concentrations of thyme extract (10, 20, 50 mg/ml) as an antimicrobial, the results indicated that concentration (50 mg/ml) was most effective for all examined pathogens bacteria compared with other concentrations with significant differences ($P \leq 0.05$).*Bacillus cereus* and *Pseudomonas aeruginosa* were more effective than *E. coli* and *S. typhimurium* in examined pathogenic bacteria for all concentrations. The highest effect of thyme extract on examined fungi strains was for *Penicillium verrucosum*. The highest content of phenolic content was for caffeic acid (1868.19 µg/g) followed by protocatechuic acid (1096.49 µg/g). The chemical properties of white soft cheese were determined and results showed that TS and protein decreased with increasing concentration of TE with significant differences ($P \leq 0.05$) and it increase in all treatments during cold storage (30 days). Fat increased with increasing of TE and during storage period. DPPH of WSC which increased with significant differences ($P < 0.05$) with the addition of thyme extract. Aerobic mesophilic bacteria and psychrophilic bacteria count decreased significantly with increasing TE ratio. The sensory evaluation was acceptable to the arbitrators throughout the storage period; the flavor of cheese supplemented with thyme also received the highest score during the storage period.

Keywords: *Thymus vulgaris*, Extractions, Antioxidant, Antimicrobial, soft cheeses

1. Introduction

Thyme is an annual plant of the Lamiaceae family that is grown all over the world for its therapeutic, culinary, and cosmetic benefits. This herb's unique antispasmodic, antiseptic, expectorant, antioxidant, and antibacterial properties [1]. Thyme is an aromatic medicinal herb that is becoming increasingly important in Europe, North Africa, Asia and America. Thyme oil is one of the top ten essential oils[2]. In the last few years there has been an increase concern about the safety of synthetic food additives, including the potential toxicity of synthetic compounds used as antimicrobials and antioxidants. Therefore, the effort has been concentrated on using natural antibacterial and antioxidant chemicals to extend the shelf life of food products in the field of food processing [3-5]. Consumers are more attracted to natural food additives than they are to synthetic ones.*Thymus vulgaris*, a member of the Lamiaceae family. Thyme and their extracts had strong and successfully antimicrobial properties in cheese making with high acceptance with consumers[6-8]. Mokhtari et al. (2023)[9]studied the antimicrobial

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activity of thyme extract against four types of bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhomorium* and *Bacillus cereus*) using the agar well difusion method.[10] studied the antibacterial activity of *Thyme vulgaris* extracts (petroleum ether, chloroform, Methanol and Distell water) against four common bacteria, including Gram-positive *Bacillus subtilis* and Gram-negative *Salmonella typh*i and the results indicated that use of these crude extracts in the management of bacterial and fungi diseases. [1] stated that Thymus showed a variety of abilities, including antibacterial and antioxidant.Thyme (*Thymus vulgaris*. L) is used for flavor in variety of dairy products, including cheese. Because of the thymol content, it shows antimicrobial characteristics[3].

The dried leaves and flowering tops of *Thymus vulgaris* are used to make Thyme herb (Thyme herba), which includes triterpene components, flavonoids, tannins, and up to 2.5% essential oils.The main phenolic substances found in thyme extracts, particularly thymol and carvacrol, have greater antioxidant activity than the well-known BHT (butylated hydroxytoluene) and tocopherol antioxidants[11].One of the most popular dairy products is white soft cheese in Egypt and sold in large quantities and enjoyed by consumers everywhere[12 and 13]. White soft cheese is subject to contamination by pathogenic and spoilage bacteria because it includes protein, minerals, calcium, and vitamins, which reduce the shelf life of dairy products [14]. Soft cheese is a perishable product that can be infected by pathogenic and spoilage bacteria, reducing shelf life, deteriorating cheese quality, and posing health concerns to people[15 and 16]. Consumers prefer healthier and safer food that is free of synthetic preservatives, which are frequently regarded as hazardous and carcinogenic ingredients[17].

Thyme and its extracts are potent antibacterial compounds that have been successfully used in cheese manufacture with great customer acceptability [6, 7 and 8].The Thymus genus contains major medicinal herbs that come highly recommended because of the wide range of therapeutic characteristics of its essential oils, which are typically referred to as Thyme oil.

Thymol and carvacrol, the two primary ingredients, are responsible for its characteristics. All herbs except fresh thyme have the highest antioxidant content.Thyme extract had an antihyperglycemic effect on rabbits with hyperglycemia caused by alloxan, without changing body weights[18 and 19]. Thyme powder and its essential oil greatly reduced lipid peroxidation levels, preventing damage to liver tissue.

Treatment with thyme oil and powder helps reduce the liver irregularities and histological changes linked to obesity [20].In perspective with the foregoing, the present investigation was carried out to investigate the used of thyme extract in to produce functional white soft cheese. Therefore, to investigate the antioxidant and antimicrobial properties of thyme extract and its impact on improving quality of soft cheese to make them useful for industry and response to the rising demand for a variety of high-quality milk products globally.

2. MATERIAL AND METHODS

Fresh cow and buffalo milk obtained from the faculty of Agriculture, Tanta University, Rennet was obtained from Chr. Hansen' Lab., Denmark. *Thymus vulgaris* from the Ministry of Agriculture, Egypt. Salt: Commercial sodium chloride was obtained from El-Nasr Company, Alexandria, Egypt.

2.1. Preparation of thyme extract (TE)

After adding 350 ml of ethanol (80%) to 50g of dried thyme powder in an extraction machine (Soxhelt), the extraction process was carried out for 12 hours at 40°C using a vacuum rotary evaporator. Aqueous extract solutions were then made in the same manner[21].

2.2.Manufacture of white soft cheese

Fresh cow and buffalo milk blend (1:1) with 5% fat was pasteurized at 72°C for 15 seconds. The milk was mixed with rennet 0.4 %milk (w/v), and then incubated at 40 °C until full coagulation, scooped into cheese forms lined with cheese cloth, and allowed to drain whey then salt added (3 %).

Four treatments of white soft cheese control (C1) without additives, (C2, C3 and C4) mixed with 0.5, 1.0 and 1.5 % thyme extract, respectively. [22] The method was followed whenmaking cheese. The resulting cheese was packaged in plastic bags at 5 ±2 °C for 30 days without brine. Three replicates were carried out from each treatment. The cheese was analyzed at fresh, 10, 20 and 30 days.

2.3.Phenolic acids profile

Phenolic acids of the Thyme (*Thymus vulgaris*. L) extract were identified according [23]. A Shimadzu HPLC system with an LC-10AD pump, SCTL 10A

system controller, and SPD 10A photodiode array detector is available from Shimadzu Corp. in Kyoto, Japan. Before being injected onto a prepacked LiChrospher 100RP-18 column (4 x 250 mm, 5 m; Merck, Darmstad, Germany), each sample was filtered through a 0.45 m nylon membrane. Water acetonitrile and acetic acid (88:10:2; v/v/v) made up the mobile phase. The flow rate was 1 ml/s.ms, and phenolic acid detection was seen at 320 nm.

2.4.Determination of total phenolic content

The Folin-Ciocalteu test was used to determine the total phenolic content of the extract according to[24]. The creation of a blue molybdenum tungsten compound in this colorimetric test phenolic content, which can be determined concerning Gallic acid Reagent Folin-Ciocalteu (1:10) was included into the extract, the benchmark, 5 minutes of incubation before adding 0.115 mg/ml of Na^2CO3. Using a spectrophotometer (Unicam, Helios Alpha, UK), absorbance was measured at 765 nm following a 2-hour incubation period. The calibration curve appears in the concentration range of 50-200 mg/L using gallic acid as the standard. Results were given in milligrams of gallic acid equivalents per gram of extract.

2.5.Total flavonoids content:

Flavonoids content of Thymus vulgaris extract were determined according to [25]. The extracts were dissolved in 80% methanol to produce solutions with a final concentration of 6 mg/ml. Each solution (5 ml) was combined with 0.3 ml of 5% aqueous NaNO2 (w/v) and left to stand at room temperature for 5 min before being mixed with 0.6 ml of 10% AlCl3 solution (w/v). 2 ml of 1M NaOH and 2.1 ml of water were added to the mixture after 6 minutes. A spectrophotometer (Unicam, Helios Alpha, UK) was used to measure the absorbance at 510 nm. For each 100 g of dry material, the results were represented as mg quercetin (Q) equivalents.

2.6. Antimicrobial activity of *Thymus Vulgaris* extracts (TE)

Antimicrobial activity was performed using the techniques described by [26]. For antibacterial characterization, the examined microorganisms were vaccination in Tryptic soy broth tubes, and it was storage in inculpated at 37 ℃ for 4 hr. the turbidity of these cultures was adjusted using 0.5 McFarland. On the surface of solid agar plates, Cotton swabs were taken were used to create a homogenous bacterial lawn. To make discs 6mm that were impregnated with concentrations of Thyme extract (10, 20 and 50 mg/mL), by using Whatman filter paper no. 1. The impregnated discs were placed on the surface of agar plates with streaks. The triplicate plates were inverted and incubated for 16 - 18 hr at 35 ℃

2.6.1.Antibacterial assay

Antibacterial assay was done against two gram-positive pathogenic bacteria (*Bacillus cereus* EMCC1080 and *Staphylococcus aureus* ATCC13565) and three Gram-negative bacteria (*Escherichia coli* O157-H7ATCC51659, *Salmonella typhi* ATCC15566, and *Pseudomonas auruginosa* NRRLB- 272) and *Candida albicans* as yeast. The strains were cultivated on nutrient agar dishes for 24 hours at a temperature of 37 ℃, then stored at a temperature of 4 ℃ until use.The experiment was carried out using the disc diffusion technique on nutrient agar. The examined microorganisms were added to tubes of Tryptic soy broth, and they were then cultured at 37 ℃ for four hours. These cultures' turbidity was adjusted with 0.5 Mc-Farland. On the surface of solid nutrition agar plates, sterile cotton swabs were used to create a homogenous bacterial lawn. To manufacture 6mm discs impregnated with*Thymus vulgaris* at varied concentrations (10, 20 and 50 mg/mL), Whatman filter paper no. 1 was utilized. On the surface of nutritional agar plates with streaks, the impregnated discs were placed. The triplicate plates were flipped over and incubated for 16–18 hours at 35 ℃ [26].

2.6.2.Antifungal assay

The antifungal assay was done against four fungi species including *Aspergillus flavus* NRRL 3357, *Aspergillus carbonarius*, *Pencillimverrecosum* ITEM10027, and *Fusarium proliferatum* MPVP328. They were grown on potato dextrose agar (PDA) dishes for 7 days at 28 °C.On Potato Dextrose Agar (PDA) media, the disc diffusion technique was used to perform the antifungal assay. A loopful of the growing, tested fungi was transferred to a test tube containing 10 mL of 0.01 % tween 80 solutions to create the spore suspension. Using a glass rod, 100 µl of the spore suspension was applied to the Potato Dextrose Agar plates that had solidified, and the plates were then left to dry for 30 minutes. The dry plates' surface was covered with the impregnated discs. The plates were turned over and left to sit at 28 ℃ for 24-48 hours. Following incubation, the inhibitory zones' dimensions, including the disc's diameter, were determined. A ruler held on the back of the inverted petri plates is used to measure zones to the nearest millimeter. The results of three times were given an average, and the results were expressed as mean ±SD according to [26].

2.7. Determination of physicochemicalcharacteristics of white soft cheese

The physicochemical characteristics of white soft cheese include TS, protein, fat, ash, pH and titratable acidity of the cheese samples was measured according to AOAC (2020)[27].

2.8. Radical DPPH scavenging activity

The extracts capacity to neutralize free radicals was assessed using the 2,2-diphenyl-2-picryl hydrazyl (DPPH) technique, which was described by [28] with some modifications. Briefly, 3.9 mL of a 0.2 mM DPPH methanolic solution and 0.1 mL of samples (100 g/mL in 80 % methanol) were combined. After stirring the reaction mixture, it was left in the dark at room temperature for 30 minutes. Using a spectrophotometer, the absorbance at 515 nm was used to calculate the amount of residual DPPH. (Unicam, Helios Alpha,UK). Ascorbic acid was used as a control and the following equation was used to calculate the percent inhibition of the DPPH radical:

$$\% \text{ Inhibition} = ((A \text{ control} - A \text{ sample}) / A \text{ control}) \times 100$$

Where: A is the absorbance at 515nm.

2.9.Test microorganisms:

2.9.1.Aerobic mesophilic Count

The plate count agar medium was determined to count the total viable bacteria as recommended by [29] Dilutions were produced and then transferred to sterile plates. (1 mL per plate approximately). The plates were examined for total count (CFU/gram) after two days of aerobic incubation at 35 ℃.

2.9.2. Mold and Yeast Count

Potato dextrose agar was used to test for mould and yeast. Dilutions of samples, then transfer to sterilized plates (1 mL per plate approximately). After 3 days at 30 ℃ of incubation, the plates were examined for the count of yeast and other fungi as (cfu/gram) [30].

2.10. Sensory evaluation of white soft cheese with Thymus Vulgaris extract

Sensory evaluation of white soft cheese was evaluated by method described by [31]. Panelists judged the white soft cheese samples using the following points: appearance (10 points), Body and texture (40 points), flavor (50 points), and over all acceptability (100).

2.11.Statistical analysis

The data was evaluated using SPSS 20 for Microsoft Windows[32]. The statistical data was evaluated by Duncan's multiple-range test at level of significant ($P < (0.05)$).

3. RESULT AND DISSECTION

3.1. Phytochemical properties of thyme extract (TE)

Table (1) showed phytochemical properties of thyme extract include antioxidant activity (DPPH), total phenol and total flavonoids. Results indicated that antioxidant activity (DPPH) of thyme extract was (17.05±0.15) mg TE/g, total phenols value was (25.18±0.05) mg GAE/g and total flavonoids value was (17.29±0.47) mg CE/g. These results were in agreement with [33].

Table (1) Phytochemical properties of thyme extract

Antioxidant activity DPPH (mg TE/g)	Total phenol (mg GAE/g)	Total Flavonoids (mg CE/g)
17.05±0.15	25.18±0.05	17.29±0.47

TE = Trolox Equivalent. GAE=Gallic acid equivalent.CE= Catechin equivalent

3.2.Antimicrobial activity of Thymus vulgaris L. extract

Table (2) shows the antimicrobial effect of *Thymus vulgaris* L. extract against gram-positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginos*), fungi (*Aspergillus flavus*, *Aspergillus carbonarius*,*Fusariumproliferatum* and *Penicillium verrucosum*) and yeast (*Candida albicans*). The effect of different concentrations of *Thyme vulgaris* extract (10, 20 and 50 mg/mL) was studied as an antimicrobial against gram-positive bacteria, gram-negative bacteria, fungi and yeast. The results indicated that concentration (50 mg/mL) was most effective for all examined pathogens bacteria and the value was the highest compared with other concentrations with significant differences ($P \leq 0.05$). The antimicrobial effect of *Thymus vulgaris* L. extract was due to the presence of hydroxyl groups (-OH-), which form hydrogen bonds between hydroxyl groups in these compounds and thymol, flavonoids, tannins, and some phenolic compounds, alcohol extract has an inhibitory effect on many bacteria

groups. These results were in agreement with [34] who studied the antibacterial effect of thyme against *Escherichia coli* and *Staphylococcus aureus*. Results indicated that *Bacillus cereus* and *Pseudomonas aeruginosa* were more effective than *E. coli* and *S. typhimurium* inexamined pathogenic bacteria for all concentrations. These results were in agreement with [9] who found that *E. coli* and *S. typhimurium* were less sensitive to the inhibitory action of the thyme extract. The highest effect of thyme extract on examined fungi strains was for *Penicillium verrucosum*.

Table (2) Antimicrobial activity of *Thymus vulgarisL.* extract

Type of pathogenic microbes	Tested organisms	Inhibition zone in mm developed by different concentration of *Thyme vulgaris* extract(Mean ± SD)		
		10 mg/ml	20 mg/ml	50 mg/ml
Gram-positive bacteria	*Bacillus cereus*	9.67^{c}± 0.58	10.67^{b}± 0.58	12.67^{a}± 0.58
	Staphylococcus aureus	8.00^{c}± 0.00	9.00^{b}± 1.00	10.67^{a}± 0.58
Gram-negative bacteria	*Escherichia coli*	8.67^{c}± 0.58	9.33^{b}± 0.58	10.67^{a}± 0.58
	Salmonella typhi	9.33^{c}± 0.58	10.00^{b}± 0.00	11.00^{a}± 1.0
	Pseudomonas aeruginosa	9.33^{c}± 0.58	10.33^{b}± 0.58	12.33^{a}± 0.58
Fungi	*Aspergillus flavus*	9.67^{c}± 0.58	10.67^{b}± 1.15	12.00^{a}± 1.00
	Aspergillus carbonarius	9.33^{c}± 0.58	10.67^{b}± 0.58	12.30^{a}± 0.58
	Fusariumproliferatum	9.33^{c}± 0.58	11.67^{b}± 0.58	12.30^{a}± 0.58
	Penicillium verrucosum	9.67^{c} ± 0.58	11.67^{b}± 0.58	12.70^{a} ± 0.58
Yeast	*Candida albicans*	10.33^{c}± 0.58	11.33^{b}± 0.58	12.67^{a}± 0.58

[a-c] The different superscript letter have a significant difference in the same raw. (Duncan's test P<0.05)

3.3. Phenolic compounds of *Thyme vulgaris L.*Extractby HPLC

Table (3) shows phenolic content of *Thyme vulgaris* L. extract by HPLC. The highest content of phenolic content was for caffeic acid (1868.19µg/g) followed by protocatechuic acid (1096.49µg/g).[20] resulted that thyme powder contains phenolic components such as gallic acid, 4-amino benzoic acid, protocatchuic acid, chlorogenic acid, catechol, vanillic acid, p-coumaric acid, isoferulic acid, coumaric acid, 3, 4, 5 methoxycinammic acid, coumarin, and cinammic acid in concentrations ranging from 10 to 100 ppm. In general, the phenolic content of different thyme herbs varies depending on the environment in which they grow.[35-38] reported pyrogallol, caffeic acid, ferulic acid, and benzoic acid were among the typical thyme components.

3.4. Chemical composition of white soft cheese with *Thyme vulgarise*extract during cold storage

Results in Table (4) showed the effect of thyme extract (TE) on white soft cheese with different treatments C_1 (Control), C_2 (0.5% TE), C_3 (1% TE) and C_4 (1.5% TE) on the chemical composition of cheese samples (TS, protein, fat and ash) during cold storage period for 30 days. It was observed that TS decreased with increasing of TE with significant differencescompared to the control sample. This could be attributed to the antibacterial action of TE on acid formation in cheese, which is connected to whey expulsion during storage[39 - 41]. TS content of cheese from all treatments gradually increased during storage period (30 days). This may be due to loss of moisture during storage period (30 days). Also, results showed that protein (%) of cheesesamples decreased with increasing of TE ratios and increased gradually during 30 days of storage period.

Table (3)Phenolic compounds of *Thyme vulgaris L.*Extractby HPLC

Compound	Result (concentration µg/g)
Protocatechuic acid	1096.49
p-hydroxybenzoic acid	48.58
Catechin	69.52
Chlorogenic acid	14.06
Caffeic acid	1868.19
Syringic acid	196.75
Vanillic acid	10.60
Ferulic acid	629.91
Sinapic acid	36.40
p-coumaric acid	10.39
Rutin	115.91
rosmarinic acid	1141.15
apegnin-7-glycoside	233.55
Cinnamic acid	15.07
Quercetin	34.69
Kaempferol	7.79
Chrysin	10.34
Gallic acid	ND

ND= Not detected

Fat content increased with increasing of TE ratio. Fresh C_4 treatment had the highest value of fat content (25.03%) and fresh C_1 control treatment had the lowest fat value (24.70%).

Fat content increased during storage period for all treatments. These results were in agreement with [42] who studied effect of moringa leaves extract on white soft cheese. Ash content decreased with increasing TE ratios and increased during storage period for all treatments and there were no significant differences between treatments during storage period (30 days).

These results were in agreement with [43] who studied white soft cheese with natural cold pressed oils.

Table (4). Chemical composition of white soft cheese with thyme extract during cold storage.

Composition (%)	Treatments				
	C_1	C_2	C_3	C_4	SE
0 time					
TS	44.85^{a} ±0.44	44.67^{b}±0.36	44.55^{c}±0.30	44.49^{d}±0.22	0.08
Protein	16.95^{a}±0.01	16.77^{b}±0.01	16.58^{c}±0.01	16.39^{d}±0.01	0.07
Fat	24.70^{d}±0.05	24.81^{c}±0.05	24.92^{b}±0.04	25.03^{a}±0.05	0.05
Ash	2.15^{a}±0.05	2.13^{b}±0.01	2.07^{c}±0.01	2.01^{d} ±0.05	0.06
10 Days					
TS	44.98^{a}±0.51	44.79^{b}±0.37	44.70^{c}±0.30	44.66^{d}±0.01	0.04
Protein	16.99^{a}±0.18	16.81^{b}±0.17	16.67^{c}±0.16	16.44^{d}±0.05	0.07
Fat	24.72^{d}±0.05	24.83^{c}±0.05	25.12^{b}±0.04	25.33^{a}±0.05	0.08
Ash	2.16^{a}±0.05	2.14^{a}±0.05	2.12^{a} ±0.01	2.11^{a} ±0.05	0.05
20 Days					
TS	45.13^{a}±0.01	44.89^{b}±0.05	44.81^{c}±0.01	44.78^{d}±0.04	0.05
Protein	17.04^{a}±0.05	16.86^{b}±0.05	16.72^{c}±0.06	16.59^{d}±0.05	0.03
Fat	24.75^{d}±0.05	24.86^{c}±0.06	25.25^{b}±0.05	25.45^{a}±0.05	0.07
Ash	2.17^{a}±0.05	2.15^{a}±0.05	2.13^{a}±0.01	2.12^{a}±0.05	0.05
30 Days					
TS	45.25^{a} ±0.05	45.04^{b}±0.04	44.98^{c}±0.05	44.94^{d}±0.03	0.07
Protein	17.10^{a}±0.11	16.97^{b} ±0.02	16.84^{c} ±0.02	16.71^{d}±0.03	0.08
Fat	24.79^{d}±0.05	24.98^{c}±0.05	25.32^{b}±0.04	25.54^{a}±0.04	0.05
Ash	2.18^{a} ±0.05	2.16^{a}±0.05	2.15^{a} ±0.01	2.14^{a} ±0.01	0.04

C_1= Control. C_2= White soft cheese with 0.5% thyme extract. C_3= White soft cheese with 1% thyme extract. C_4= White soft cheese with 1.5% thyme extract.

$^{a-d}$ The different superscript letter have a significant difference in the same row. (Duncan's test P≤0.05)

3.5. Effect of thyme extract on titratable acidity and pH of white soft cheese during cold storage

Fig.1 showed titratable acidity values which increased significantly until the end of the storage period (P ≤ 0.05). The cheese samples with TE had lower titratable acidity than the control cheese samples (P ≤ 0.05). This may be due to the effect of TE concentration on the activities of total bacteria [44]. This could be attributed to the antibacterial action of TE on acid formation in cheese

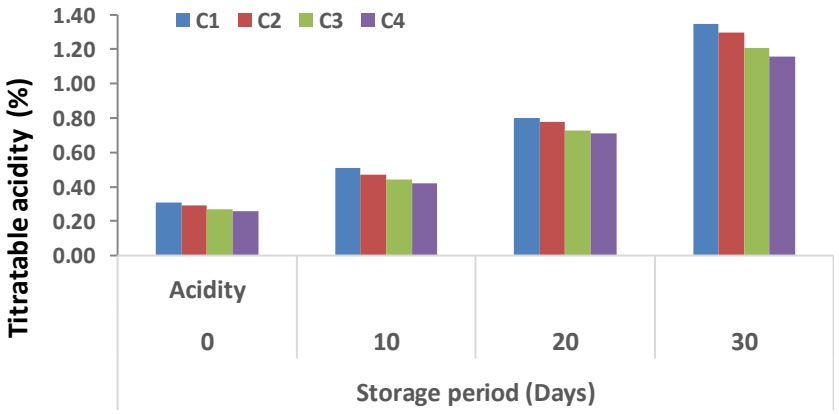

Fig.1 Acidity percentage of white soft treated with different concentration of thyme extract during storage.
C1= Control. C2= White soft cheese with 0.5% thyme extract. C3= White soft cheese with 1% thyme extract. C4= White soft cheese with 1.5% thyme extract.

Fig.2 showed pH values and indicated that the fresh control cheese had lowest pH value, while the fresh C_4 treatment had the highest value. The pH values of all cheeses reduced during a period of storage; since some lactose converted to lactic acid during storage, it is possible that a decrease in pH values and increase in acidity over the storage period[45]**.** These results were in agreement with [46] who studied the effect of potatoes peels on white soft cheese during storage period.

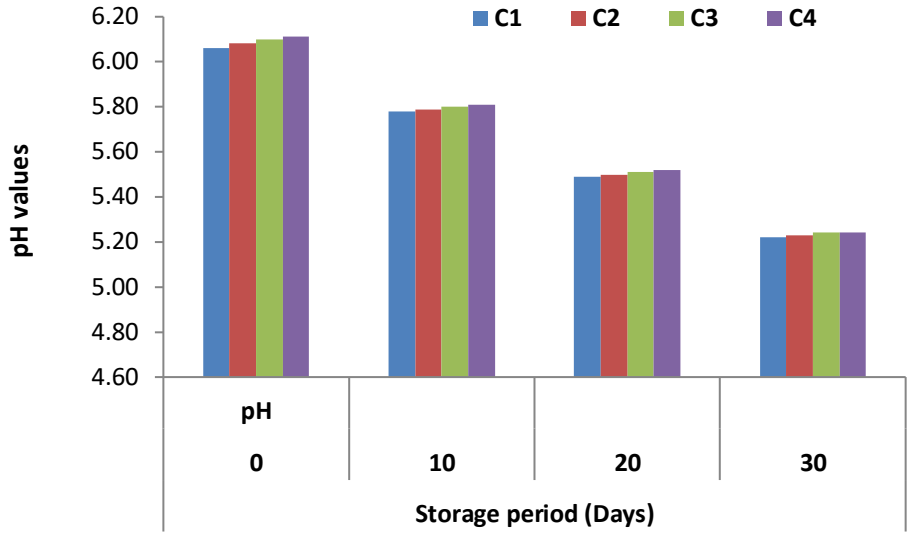

Fig.2. pH values of white soft treated with differentconcentration of thyme extract during storage.

C_1= Control without thyme extract. C_2, C3 and C4= White soft cheese with 0.5, 1, 1.5 % thyme extract respectively.

3.6. DPPH content of white soft cheese with TE during cold storage

Fig.3 shows the DPPH content of white soft cheese samples C_1 (Control), C_2 (0.5 % thyme extract), C_3 (1 % thyme extract) and C_4 (1.5 % thyme extract).

Fig. 3 showed the DPPH of white soft cheese which increased significantly ($P \leq 0.05$) with the addition of thyme extract (TE) compared to the control (white soft cheese prepared without TE). The DPPH value was lowest in the fresh control sample (0.21 mg TE/g cheese) in comparison to white soft cheese samples made with different TE concentrations.

Fresh C_4 sample prepared with 1.5 % TE had the highest DPPH value (0.71 mg TE/g cheese). DPPH values decreased during the storage period (30 days) for all treatments.

Similar results were in agreement with [47] who resulted increasing of phenol content of white soft cheese with an increase in thyme.

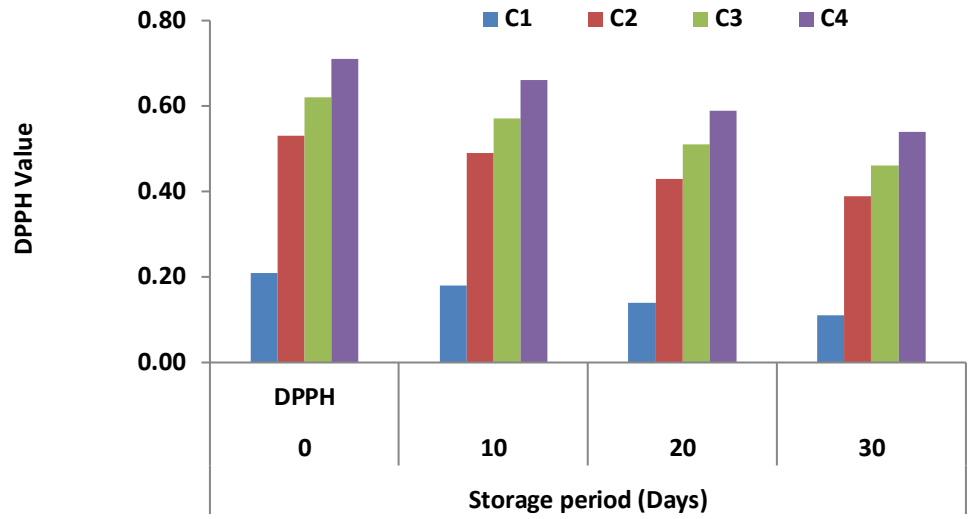

Fig. 3. DPPH of white soft treated with different concentration of thyme extract during storage.
C_1= Control without thyme extract. C_2, C3 and C4= White soft cheese with 0.5, 1, 1.5 % thyme extract respectively.

3.7.Effect of thyme extract (TE) concentration (%) on microbiological properties of white soft cheese during cold storage.

Table (5) shows themicrobiological (Aerobic mesophilic, Psychrophilic, Mold & Yeast) effect of TE on white soft cheese. Aerobic mesophilic bacteria and psychrophilic bacteria count decreased significantly with increasing TE ratio and this is may be due to the antimicrobial effect of TE.

Table (5): Effect of thyme extract concentration(%) on microbiological quality of white soft cheese (log CFU/g) during cold storage.

Composition (%)	Treatments				SE
	C_1	C_2	C_3	C_4	
0 Time					
Aerobicmesophilic	4.50^{a}±0.09	4.41^{b}±0.18	4.37^{c}±0.08	4.19^{d}±0.32	0.005
Psychrophilic	2.75^{a}±0.04	2.69^{b} ±0.05	2.64^{c}±0.06	2.58^{d}±0.04	0.006
Mold & Yeast	ND	ND	ND	ND	
10 Days					
Aerobic mesophilic	5.02^{a}±0.08	4.91^{b}±0.04	4.82^{c}±0.05	4.72^{d}±0.04	0.007
Psychrophilic	3.06^{a}±0.02	2.99^{b}±0.03	2.93^{c}±0.04	2.87^{d}±0.06	0.008
Mold & Yeast	ND	ND	ND	ND	
20 Days					
Aerobic mesophilic	6.11^{a}±0.08	5.98^{b}±0.05	5.86^{c}±0.04	5.75^{d}±0.07	0.009
Psychrophilic	3.40^{a}±0.04	3.33^{b}±0.07	3.26^{c}±0.06	3.20^{d}±0.06	0.007
Mold & Yeast	2.61^{a}±0.05	2.55^{b}±0.05	2.51^{c}±0.07	2.46^{d}±0.04	0.008
30 Days					
Aerobic mesophilic	5.71^{a}±0.05	5.59^{b}±0.04	5.47^{c}±0.06	5.37^{d}±0.05	0.006
Psychrophilic	3.77^{a}±0.06	3.69^{b}±0.05	3.62^{c}±0.04	3.55^{d}±0.04	0.004
Mold & Yeast	3.04^{a}±0.05	2.97^{b}±0.03	2.91^{c}±0.06	2.86^{d}±0.05	0.005

C_1= Control without thyme extract. C_2, C3 and C4= White soft cheese with 0.5, 1, 1.5 % thyme extract respectively. $^{a-d}$ The different superscript letter have a significant difference in the same row.
(Duncan's test P<0.05). ND= not detected.

The highest value of aerobic mesophilic bacteria was for fresh control treatment (4.50 log CFU/g) and decreased to (4.19 log CFU/g) for fresh C_4 treatment. The count of psychrophilic bacteria was (2.75 log Aerobic mesophilic bacteria increased with significant differences during the storage period (30 days). The count of aerobic mesophilic bacteria was (5.71 log CFU/g) for control treatment at the end of the storage period and decreased to (5.37 log CFU/g) for C_4 treatment at the end of the storage period (30 days). These results were in agreement with [48] who studied the effect of thyme, paprika, cumin and turmeric on tallaga - like cheese during storage period.

Mold and yeast were not found at first 10 days for all treatments and this is may be due to sanitary conditions for cheese making then appeared on the 20 days and the count increased to 30 days. It may be due to lower pH values that may support the growth of yeast and molds. These results were in agreement with [46]who studied the effect of natural antioxidants on white soft cheese.

CFU/g) for fresh control treatment and decreased to (2.58 log CFU/g) for fresh C_4 treatment.

3.8. Sensory evaluation of white soft cheese with TE during cold storage

Table (6) shows sensory evaluation (Appearance, Body & Texture, flavor and over all acceptability) of white soft cheese with TE during cold storage.

Table (6): Effect of thyme extract concentration (%) on sensory evaluation of white soft cheese during cold storage

Parameter	Treatments				
	C1	C2	C3	C4	SE
0 Time					
Appearance (10)	7.13^{a}	6.93^{b}	6.16^{c}	6.03^{d}	0.06
Body & Texture (40)	33.13^{a}	32.98^{b}	31.52^{c}	30.52^{d}	0.04
Flavor (50)	42.73^{d}	42.94^{c}	43.57^{a}	43.06^{b}	0.09
Over all acceptability (100)	82.99^{a}	82.85^{b}	81.25^{c}	79.61^{d}	0.08
10 Days					
Appearance (10)	7.27^{a}	7.58^{b}	7.02^{c}	6.97^{d}	0.04
Body & Texture (40)	34.48^{a}	33.75^{b}	32.67^{c}	31.65^{d}	0.03
Flavor (50)	44.21^{d}	44.32^{c}	44.94^{a}	44.62^{b}	0.07
Over all acceptability (100)	85.96^{a}	85.65^{b}	84.63^{c}	83.24^{d}	0.09
20 Days					
Appearance (10)	7.51^{d}	7.68^{c}	8.08^{b}	8.49^{a}	0.08
Body & Texture (40)	34.97^{d}	35.83^{c}	36.17^{b}	36.87^{a}	0.05
Flavor (50)	45.02^{d}	45.94^{c}	46.14^{b}	46.24^{a}	0.06
Over all acceptability (100)	87.50^{d}	89.45^{c}	90.39^{b}	91.60^{a}	0.07
30 Days					
Appearance (10)	7.81^{d}	7.98^{c}	8.81^{b}	9.04^{a}	0.05
Body & Texture (40)	37.16^{d}	37.95^{c}	38.25^{b}	38.71^{a}	0.06
Flavor (50)	48.01^{d}	48.29^{c}	48.47^{b}	48.92^{a}	0.04
Over all acceptability (100)	92.98^{d}	94.22^{c}	95.53^{b}	96.67^{a}	0.05

C_1= Control without thyme extract. C_2, C3 and C4= White soft cheese with 0.5, 1, 1.5 % thyme extract respectively. $^{a-d}$ The different superscript letter have a significant difference in the same row. (Duncan's test $P \leq 0.05$).

For appearance, results indicated that the control sample had the highest score during first ten days, while treatments with TE improved appearance during 20 and 30 days.Values of appearance increased during the storage period for all treatments. It may be due to forming flavor components. For Body & Texture, the control treatment had the highest values for fresh samples and during first days from storage period (30 days) then treatments with TE improved body and texture for white soft cheese.It may be due to antioxidant and antimicrobial properties of TE.These results were in agreement with [42] who studies the effect of natural food additives on white soft cheese.For flavor TE enhanced flavor of white cheese. The value of flavor for C_3 treatment was the highest during first 10 days, while after that treatment C4 value was the highest in flavor.Over all acceptability value of control treatment was the highest for first 10 days then treatments with TE improved over all acceptability. C4 treatment with 1.5% TE had the highest value of overall acceptability after 30 days. These results were in agreement with [43] who studied the effect of natural food additives on white soft cheese.

4. Conclusions

It is deduced from the present study that thyme extracts with deferent concentrations had the antioxidant and antimicrobial good properties. And when added at a concentration of 1.5 % it gives best properties antioxidant and antimicrobial properties with good flavor to white soft cheese compared with other different concentrations from thyme extract. Further work is in progress to create to possibly use thyme extract for the production of other types of cheeses to work to produce functional white soft cheese with medical properties. Therefore it is recommended to use Thyme extract as a natural herb for production of healthy white soft cheese.

5.Competing interests

The authors declare that they have no competing interests.

6. Funding

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7. Ethics approval

The topic of the research doesn't require ethic

8.Consent for publication

Not applicable

9 .Consent to participate

All authors of the manuscript are aware from submission of this manuscript.

10.Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

11. Code availability

Not applicable.

12. Authors' contributions

AAK-A, DMM, and AMMK contributed to the study conception and design. AAK-A, DMM, and AMMK prepared materials and collected data. AMMK prepared the first draft of the manuscript and revised the manuscript. All authors read and approved the final manuscript.

13.REFERENCES

[1] Li, X., He, T., Wang, X., Shen, M., Yan, X., Fan, S., Wang, L., Wang, X., Xu, X., Sui, H.& She, G. (2019). Traditional uses, chemicalconstituents and biological activities of plantsfrom the genus thymus. Chemistry anbiodiversity, 16(9),1900254. DOI:10.1002/cbd.201900254.

[2] Cetin, B., Cakmakci, S. &Cakmakci, R. (2011). The investigation of antimicrobial activity ofthyme and oregano essential oils. Turk. J.Agric. For., 35: 145-154.

[3] Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods ea review. International Journal of FoodMicrobiology, 94, 223-253.

[4] Sacchetti, G., Maietti, S., Muzzoli, M.,Scaglianti, M., Manfredini, S., Radice,M&Bruni, R. (2005). Comparative evaluationof 11 essential oils of different origin asfunctional antioxidants, antiradicals andantimicrobials in foods. Food Chem, 91(4):621-632.

[5] Santoyo, S., Lloria, R., Jaime, L., Ibanez, E., Senorans, F.J &Reglero, G. (2006). Supercritical fluid extraction of antioxidantand antimicrobial compounds from Laurusnobilis L. chemical and functionalcharacterization. Eur. Food Res. Technol,222(5-6): 565-571.

[6] Hayaloglu, A. A. & P. F. Fox. (2008). Cheesesof Turkey: 3. Varieties containing herbs orspices. Dairy Sci. Technol. 88:245-256.

[7] Leuschner, R. G. K. &Ielsch, V. (2003)Antimicrobial effects of garlic, clove and redhotchilli on Listeria monocytogenes in brothmodel systems and soft cheese. Int. J. FoodSci. Nutr. 54:127-133.

[8] Gammariello, D., DI Giulio, S., Conte, A. &Del Nobile, M. A. (2008). Effects of naturalcompounds on microbial safety and sensoryquality of fior di latte cheese, a typical Italiancheese. J. Dairy Sci. 91:4138-4146.

[9] Mokhtari, R., Fard,M., Rezaei, M., lMoftakharzadeh, S. & Mohseni, A. (2023).Antioxidant, Antimicrobial Activities, andCharacterization of Phenolic Compounds ofThyme (Thymus vulgaris L.), Sage (Salviaofficinalis L.), and Thyme–Sage MixtureExtracts. Hindawi Journal of Food QualityVolume 2023, Article ID 2602454, 9 pages. https://doi.org/10.1155/2023/2602454.

[10] Almostafa, Wafaa & Adam, M. (2019). Phytochemical Screening and Antimicrobial Activity of *Thyme vulgaris*. Acta Scientific Microbiology 2.10 : 74-78.

[11] Olmedo, R. H., Nepote, V. & Grosso N.R. (2013). Preservation of sensory and chemical properties in flavoured cheese prepared with cream cheese base using oregano androsemary essential oils, LWT Food Science and Technology, 53(2), 409.

[12] Alizadeh, M., Hamedi, M., &Khosroshahi,A.(2006). Modeling of proteolysis and lipolysis in Iranian white brine cheese. FoodChemistry,97, 294–301.https://doi.org/10.1016/J..foodchem.2005.05.009.

[13] Mahgoub, S.A., Ramadan, M.F & El-Zahar, K.M. (2013). Cold Pressed Nigella Sativa Oil Inhibits the Growthof Foodborne Pathogens and Improves the Quality of Domiati Cheese. Journal of Food Safety, 33,470–480.DOI: 10.1111/jfs.12078.

[14] El-Sayed, S.M. & El-Sayed, H.S. (2021). Antimicrobial nanoemulsion formulation basedon Thyme Thymus vulgaris essential oil for UFLabneh preservation. J. Mater. Res. Technol. 10, 1029–1041.

[15] Hassanien, M.F.R., Mahgoub, S.A. & El-Zahar,K.M.C. (2014). Soft cheese supplemented withblack cumin oil: Impact on food borne pathogensand quality during storage. Saudi J.Biol.Sci.,21,280288. https:// doi.org/ 10.1016/j.sjbs. 10.005.

[16] Abd El-Salam, B.A. & Saad, M.F. (2016). Influence of selected essential oils on somepathogenic microorganisms in white soft cheese.International Journal of Chem. Tech. Research, 9,214-220. https:// sphinxsai.com/ 2016/ch_vol9_no12/ 1/ (214-220) V9N12CT.

[17] Moro, A., Librán, C., Berruga, M.I., Zalacain,A., & Carmona, M. (2015). Dairy matrix effecton the transference of rosemary (Rosmarinusofficinalis) essential oil compounds during cheesemaking. Journal Sci. Food Agric., 95, 1507–1513. https://onlinelibrary.wiley.com/doi/10.1002/jsfa.6853.

[18] Marrif, H.I., Ali, B.H & Hassan, K.M (1995).Some pharmacological studies on Artemisiaherba –alba (Asso) in rabbits and mice. J.Ethnopharmacol. 49: 51-55.https://doi.org/10.1016/0378- 8741(95)01302-4.

[19] Alamgeer, Mushtaq M.N., Bashir S, RashidM.,Malik M.N.H., Ghumman, S.A., Irfan, H.M.,Akram, M., Khan, A.Q & Rashid HU (2012).Hypoglycemic and hematological effects ofaqueous extract of Thymus serpyllum Linn. Inalloxan-induced diabetic rabbits. Afri. J. Pharm.Pharmacol. 6(40): 2845-50.

[20] Sobhy, H., Hassanen, Naglaa & Ahmed, M.(2020) . Hepatoprotective activities of thyme(Thymus vulgaris L.) in rats suffering fromobesity. Egypt. J. Chem. Vol. 63, No. 12 pp.50875101.

[21] Ba - Angood, S. A (1996). Azadirachtin ontent of Yemeni neem seed kernels (Azadirachta) indiaA.(Juss) and its effect on the Development of theMexican bean beetle Epilachnavarivestsmuls.Univ.of Aden J. of Natural and APL. Sci. 1:pp13-25.

[22] Fahmy, A. H., & Sharara, H. A. (1950). Studies on Egyptian Domiati cheese. Journal Dairy Research 17: 312-321.

[23] Janas, K.M., Amarowicz, R., Zielinska-Tomaszewska, J., Kosinska, A &Posmyk, M.M (2009). Induction ofphenoliccompoundsintwo dark-grown lentil cultivarswith different tolerance to copper ions . ActaPhysiol Plant ., 31: 587–595.

[24] Singleton, V.L., Orthofer, R &Lamuela-Raventós, R.S (1999). Analysis of totalphenols and other oxidation substrates andantioxidants by means of Folin- CiocalteauReagent. Methods in Enzymol 299:152–178.

[25] Zhishen, H., Mengcheng, T & Jianmin, W.(1999). The determination of flavonoidcontents in mulberry and their

scavengingeffects on superoxide radicals. Food Chem;64:555-559.

[26] Bauer, A.W., Kirby, W.M., Sherris, J.C. & Turck, M. (1996). Antibiotic susceptibility testing by standardized single method. Am. J. Clin. Pathol, 45, 493-496 (1996). https://doi.org/10.1093/ajcp/45.4_ts.493.

[27] AOAC (2020). Official Methods of Analysis of Association of Official Analytical Chemists. Published by the A.O.A.C international 17th Ed. Gaithersburg, MD. USA.

[28] Yang, J., Martinson, T. E & Liu, R.H(2009). Photochemical profiles and antioxidantactivities of wine grapes, Food Chem. 116,332–339.

[29] Petran, R. L., Grieme, L. E. & Foong-Cunningham, S. (2015). Culture Methods forEnumeration of Microorganisms. InCompendium of Methods for theMicrobiological Examination of Foods.

[30] Atlas, R & Parks, L.C (1997). Handbook of microbiological media. 2nd ed., CRC press,1706.

[31] Scott, R. (1981). Cheese making practice. Applied Sci. Publisher Ltd. London.

[32] IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.

[33] Aldosarya, S., El-Rahman, S., Al-Jameel, S &Alromihi, N. (2023). Antioxidant andantimicrobial activities of Thymus vulgaris essential oil contained and synthesis thymus (Vulgaris) silver nanoparticles. BrazilianJournalof Biology, vol. 83, e244675 | https://doi.org/10.1590/1519-6984.244675.

[34] Fayad, N., AL- Obaidi, O., Al-Noor, T &Ezzat, M.(2013). Water and alcohol extractionof Thyme plant (Thymus vulgaris) and activitystudy against bacteria, tumors and used as antioxidantin margarine manufacture. InnovativeSystems Design and Engineering. Vol.4, No.1.

[35] Varga, E., Bardocz, A., Belák, A., Maráz, A., Boros, B., Felinger, A., Böszörményi, A. & Horváth G. (2015). Antimicrobial activity and chemical composition of thyme essential oils and the polyphenolic content of different Thymus extracts. Farmacia, 6(3), 357–361.

[36] Köksal, E., Bursal, E., Gülçin, I., Korkmaz, M., Çağlayan, C., Gören, A.C. & Alwasel S.H. (2016). Antioxidant activity and polyphenol content of Turkish thyme (Thymus vulgaris) monitored by liquid chromatography and tandem mass spectrometry. International Journal of Food Properties, 20(3), 514–525(2016).

[37] El-Newary S.A., Shaffie N.M. & Omer E.A. (2017). The protection of Thymus vulgaris leaves alcoholic extract against hepatotoxicity of alcohol in rats. Asian Pacific Journal of Tropical Medicine, 10 (4): 361–371.

[38] Rtibi, K., Selmi, S., Wannes, D., Jridi, M., Marzouki, L. & Sebai, H. (2019). The potential of thymus vulgaris aqueous extract to protect against delayed gastric emptying and colonic constipation in rats. RSC Advances, 9(36), 20593– 20602.

[39] Monagas, M., Garrido, I., Lebron-Aguilar, R., Gomez-Cordoves, M.C., Rybarczyk, A., Amarowicz, R. & Bartolome, B. (2009). Comparative flavan-3-ol profile and antioxidant capacity of roasted peanut, hazelnut, and almond skins. J. Agric. Food Chem., 57: 10590−10599.

[40] Moongngarm, A. & Saetung, N. (2010). Comparison of chemical compositions and bioactive compounds of germinated rough rice and brown rice. Food Chem., 122 : 782–8.

[41] Andreia, M., Patricia, F., Franciele, B., Alexander, T.R., Rogerio, J.C. & Cansian, L. (2015). Inffuence of the addition of natural antioxidant from mate leaves (Ilex paraguariesis SL. Hill) on the chemical, microbiological and sensory characteristics of different formulations of prato cheese. J. Food Sci. Technol., 52 (3): 1516-1594.

[42] El-Siddig, E., Abdelgadir,W., Kabeir,B., Koko, M. & Ibrahim, R. (2018). Quality of White Cheese Made Using Moringa oleifera Leaf Extract. Journal of Academia and Industrial Research (JAIR) Volume 7, Issue 1.

[43] Khider, Manal., Ahmed, A. & Hassaan, M. (2022). Enhancement of ultrafiltrated white soft cheese with cold pressed oils as natural food additives for extending shelf life. Egypt. J. Food. Sci. Vol. 50, No. 2, pp. 251-268.

[44] Côté, J., Caillet, S., Doyon, G., Dussault, D., Sylvain, J. & Lacroix, M. (2011). Antimicrobial effect of cranberry juice and extracts. Food Control 22: 1413-1418.

[45] Hassan, A & Amjad, I. (2010). Nutritional evaluation of yoghurt prepared by different starter cultures and their physiochemical analysis during storage. African Journal of Biotechnology, 9,2913-2917. https://doi.org/10.5897/AJB2010.000-3121.

[46] Abd El-Galeel, A. & El-Zahar, K. (2018). Production of white soft cheese fortified with natural antioxidants as a functional dairy food. Zagazig J. Agric. Res., Vol. 45 No. (2).

[47] Alsirrag, M (2019). Estimation of biological properties in processed white soft cheese supported with Thymus vulgaris seed oil extract. J. Pharm. Sci. & Res. Vol. 11(1).251-253.

[48] Hammad, M., Taha, E & Mohamed, W. (2015). Effect of Fortification Palm Oil with Some Egyptian Spices on Physico-Chemical Composition, Microbiological Analysis, Sensory Evaluation and Economic Study of Tallaga-like Cheese. World Journal of Dairy & Food Sciences 10 (2): 181-192. DOI: 10.5829/idosi.wjdfs.2015.10.2.10228.