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Evaluation of edible film based on milk protein containing different polymers and essential oils for Edam cheese coating

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Abstract

The aim of this study was to evaluate the film based on total milk proteinates (TMP) mixed either with carboxy methyl cellulose (CMC) or palmitic acid as polymer containing some essential oils as dipping film on Edam cheese characteristics quality and safety during ripening. Eight Edam cheese samples were manufactured and dipped with different type of edible solution. Control Edam cheese samples were not dipped with any film, While, cheese samples (T1) were coated by VIWAX. Samples marked T2, T3 and T4 were dipped by solution total TMP based edible film made with CMC as polymer and containing thyme, cinnamon, and marjoram respectively. On the other hand, Samples marked T5, T6 and T7 were dipped by solution TMP based edible film made with palmitic acid as polymer and containing thyme, cinnamon, and marjoram respectively. All cheese treatments were kept at a temperature of 12 to 15 degrees Celsius and a relative humidity of 80 to 85%. Samples were taken when the cheese was green (3 days old) and every month for the first three months of storage.The results indicated that, significant differences were observed in chemical, microbiological, rheological, and sensory properties between uncoated cheese and all coated cheese samples. Edam cheese samples coated with solution containing palmitic acid as polymers exhibited the highest quality. As a conclusion, TMP based on edible film made with palmitic acid as polymer containing different essential oils (thyme, cinnamon, or marjoram) as preservatives could be used as dipping film in Edam cheese coating. Such edible films may be useful for preventing the moisture loss during ripening period besides improve its marketing quality and safety and produce high quality of Edam cheese.

Keywords: Total milk proteinates - Palmitic acid – Carboxy methyl cellulose – Thyme – Cinnamon - Marjoram

Introduction

Food packaging is now a crucial component of the food supply chain. It could protests the food products during processing, handling, transportation and packaging **[1]** and **[2]**. Edible packaging has been traditionally used to improve food appearance and preservation Food presentation and preservation have traditionally been enhanced by the use of edible packaging **[3]**. The primary role of edible film is for controlling the moisture loss and reducing the adverse chemical reaction rates to keep the quality and enhance safety of a wide range of processed as well as fresh foods The main function of edible film is to regulate moisture loss and lower the rates of harmful chemical reactions to maintain the quality and increase safety of a variety of processed as well as fresh meals **[4]**. Additionally, their uses are expanded by adding other food additives like antimicrobials, antioxidants, tastes, and colors to the edible film matrix **[5]**. An edible film or coating is generally defined as any layer of biomaterial added to food to extend its shelf life and that can be consumed along with the food with or without further peeling. By serving as a carrier for various chemicals, edible films can also preserve the look and nutritional value of food while delivering bioactives (antioxidants or antimicrobials) to increase shelf life **[6]**.

Composite packaging combines many edible packaging materials to enhance the qualities of the package as a whole. The gas barrier capabilities of polysaccharide and protein-based films are good, however the water vapor barrier is poor. Lipids, in contrast, have a strong gas barrier but a weak water barrier. In order to create composite films, lipid components are added to the polysaccharide or

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protein-based polymer matrix. Lipids may enhance the composite films' capabilities as water and oxygen barriers.

The primary goals of cheese packaging are to prevent some degradation processes like oxidation and dehydration, guard against the development of harmful bacteria and outside contamination, and slow down or permit the ripening strains' metabolic processes to continue **[7]**. Material characteristics, such as a water vapor and gas barrier, as well as the shape and size of the box, are important when using packaging strategies to guarantee cheese quality and safety **[8]**. Edam cheese is known to be as the most popular semi-hard cheese in Egypt. It is normally marked after 40-45 days of ripening. The biggest issues facing the edam cheese industry are spoilage and pathogenic bacteria that can grow in cheese. These faults can impair the finished cheese's quality and shelf life **[9]**. When stored in ripening conditions, edam cheese is prone to the growth of yeasts and molds, which could cause a quality issue during storage and marketing **[10]**, **[11]** and **[12]**. While cheeses that have been contaminated with yeasts and mould develop unwanted organoleptic characteristics, such as an odd flavor and an unappealing look. Additionally, due to its high diffusivity into a cheese matrix and potential development of mycotoxins, particularly those belonging to the aflatoxins group, it may pose risks to the public's health **[13]** and **[14]**. In addition, fungal growth occurs during cheese ripening and later throughout the distribution chain, which is one of the main issues with semi-hard cheese in addition to moisture loss. Edible coatings containing preservation agents for some dairy product such as Edam cheese could be useful for keeping the cheese yield and controlling its shelf life by reducing oxygen penetration into the product, thus reducing microbiological and enzymatic activities during storage period. Therefore, the aim of this study was to evaluate the possibility of using total milk proteinates mixed whether with Carboxy Methyl Cellulose (CMC) or Palmitic acid as polymer and contained different essential oils (thyme, cinnamon, and marjoram) as preservatives film dipping for Edam cheese during ripening in relation to the cheese quality.

MATERIALS AND METHODS

1. Materials

Fresh cow's milk (3.25% fat and 11.5% total solids) used for milk protein preparation and cheese production was obtained from the herd belonging to the Faculty of Agriculture (Shalakan Farm), Ain Shams University. From CHR-Hansen's Lab in Denmark, calf rennet powder (Ha-La) with a strength of (10N) was obtained and it used for Edam manufacture. Glycerol used as plasticizer in this study was obtained from Algomhuria Co., Egypt. Carboxy methyl cellulose (CMC) (average molecular weight of 90,000 Dalton) used as polymer in this study was purchased from Sigma-Aldrich. VIWAX QSS ROJO/RED from Fecha de Fabricacion Co., Spain. Palmetic acid used as polymer in this study were obtained from Algomhuria Co., Egypt. Thyme, cinnamon, and marjoram used as antimicrobial agents in this study were obtained from the National Research Centre. Sodium chloride produced by EL-NASR SALINES Co., Alexandria, Egypt, was obtained from local market. Calcium chloride was purchased from EL-NASR Co. in Cairo, Egypt. ame from EL-NASR Co., Cairo, Egypt. The freeze-dried bacterial culture used in this study, commercially named FD-DVS CHN-11 consisting of *Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. lactis* and *Lactococcus lactis subsp. lactis biovar. diacetylactis* was obtained from Chr. Hansens Laboratiers, Denmark.

2. Experimental procedures

2.1. Preparation of total milk proteinates

Total milk proteinates (TMP), *i.e.* caseins and native whey proteins, were prepared utilizing the approach outlined by **[15]** as follows: Skim milk was adjusted to pH 10.0 and heated to 70±1°C to solubilize casein micelles. The pH was then adjusted to pH 3.5 after one hour at room temperature to complex the whey proteins and casein, using 1N HCl. Again, the pH was raised to pH 4.6 by 1N NaOH to precipitate the complexes proteins. The precipitate (TMP) was removed from the serum by using a cheese cloth. The curd was washed four times with equal volume to original whey by distilled warm water (37-40°C) and acidified to pH 4.6. The curd was pressed after washing thoroughly and blended to fine particles. Obtained precipitate was dried using air oven at 60°C for 6-8 h.

2.2. Preparation of edible films from total milk proteins and different polymers (polysaccharides and Lipid) using glycerol

Six different treatments of edible films-based on cow's TMP were prepared according to the method described by **[16]** with some modification as follow: In 100 ml-Standard flask, 3% TMP and 1.5% polymer carboxy methyl cellulose (as a polysaccharide) or palmitic acid (as a lipid) were mixed with 3%glycerol (as a plasticizer) in an amount of distilled water and the pH adjusted to 10 with 1M NaOH. Solutions were heated to 65.6 ±1.0°C for 45 min while being stirred continuously. Then 3 ml oil of thyme, cinnamon or marjoram was added at the room temperature and magnetic stirred for 5 min. The solution was completed to the mark using distilled water. Solutions were then filtered through a layer of cheesecloth and held at the room temperature (~25°C) for 4h to allow the foam to settle. The solutions were vacuum degassed for 30 min and hence be ready for dipping the Edam cheese samples.

2.3. Manufacture of Edam cheese

Edam cheese was manufactured according to the method described by **[17]** with some modifications as follows: Fresh cow's milk (3.25% fat) was heated to 72ºC for 15 sec then cooled immediately to 32 ºC. The milk was placed in cheese vat and inoculated with freshly activated FD-DVS CHN-11 starter culture (1.00%) and left for 30 min to increase the titratable acidity by 0.01%. Then, 0.02% $CaCl_2$ was added, and milk was renneted at the rate of 3g rennet (dissolved in 75 ml of distilled water) for each100 kg milk. The complete coagulation was occurred within 40 min. The curd was cut and settled for 10 min without agitation. Scalding started and continued for 30 min reaching 37ºC. The whey was drained off and the curd was washed with warm water. The curd was filled into the classic ball forms and pressed. After removing the curd balls from the forms, salting was carried out by immersing them into the brine salted 16 % NaCl for 48 h at 12ºC. The salted balls were extracted from the brine and left for the dripping stage until dry at the refrigerator for 48 h. Each salted curd ball (~ 1.8 Kg) was divided vertically into eight equal eighths, coated for 10 min at 40ºC using the different experimental film treatments namely, VIWAX (T1), TMP+CMC with thyme (T2), TMP+CMC with cinnamon (T3), TMP+CMC with marjoram (T4), TMP+ palmitic acid with thyme (T5), TMP+ palmitic acid with cinnamon (T6), TMP+ palmitic acid with marjoram (T7) or without coating (the control, C). After holding at 12 - 15ºC for 3 days, at when cheese is considered in the green case, samples were taken periodically for physicochemical, microbiological, rheological and sensory evaluations, whether when green or after 30, 60 or 90 days of ripening at 12 - 15ºC and relative humidity of 80 – 85% . Three replicates were done for every treatment.

3. Analytical methods

3.1. Physiochemical Analysis

Dry matter, total protein and soluble nitrogen contents were determined in Edam cheese samples according to the method described in **[18]**. Fat content was determined using the conventional Gerber's technique as outlined by **[19]**. Ash content of Edam cheese was determined according to the method described by **[20]**. Titratable acidity (TA) for different Edam cheese samples was determined as given by **[19]**. The results were expressed as lactic acid percentages. The pH of the cheese was determined using a Genway pH meter type 3510 with a combination glass electrode (Electric Instruments Limited). pH values were reported to within 0.01 units.

The loss of moisture in different Edam cheese samples was calculated as the following equation:

$$\text{Loss of moisture} = \frac{\text{Initial moisture (\%)} - \text{recent moisture (\%)}}{\text{Initial moisture (\%) of cheese}} \times 100.$$

The moisture content of films was determined according to the method of **[21]**.Film thickness was determined using a digital micrometer (Mitutoyo Co., Japan) with 0.001mm sensitivity. The thickness measurements were obtained from eight different areas of each film. The mean value of measurements for individual sample was utilized **[22].**The permeability of films for air was measured according to the manometric principle in compliance as described by **[23].**Water vapor permeability of films was measured according to **[24].** The cups method was used to determine water vapor permeability as described by**[25].**

3.2. Microbiological Analyses

Lactic acid bacterial count in different Edam cheese samples were enumerated using MRS agar according to **[26]**. The plates were incubated for 48 hours at 30°C. Coliform bacterial counts were carried out to the method of **[27]** using Violet red bile lactose agar media**.** The plates were incubated for 24 hours at 37°C. Yeast and molds were assessed using the DG18 Agar medium supplemented with glycerol according to **[28]**. The plates were incubated for three days at 30°C. *Salmonella spp.* detection in different Edam cheese samples was carried out according to the method of **[29]**. Staphylococci were counted on Baird Parker Agar medium according to the method described by **[30]**. The plates were incubated for 48 hours at 37°C.

3.3. Texture profile analyses

The hardness (N), cohesiveness (~), springiness (mm), gumminess (N) and chewiness (N.mm) of Edam cheese samples were analyzed with a Texture Analyzer (Model CT310K Texture Analyzer, USA) at room temperature (20°C) in accordance with **[31]**.

3.4. Sensory evaluation

A panel test was used to evaluate the sensory qualities of cheese samples when they were green as well as after 30, 60, and 90 days of ripening. It was carried out by twenty members who's selected among the staff of Food science Department, Faculty of Agriculture, Ain shams university, Egypt. The panelists were requested to give the cheese a score for: appearance (10 points), flavor (50 points) and Body & Texture (40 points) according to the score card suggested by **[32]**.

3.5. Statistical Analysis:

The obtained results were statistically analyzed according to **[33]** considering the primary effect of treatments using a general linear model (GLM). To distinguish between the three replicates at P ≤ 0.05, Duncan's multiple range was utilized.

RESULTS AND DISCUSSION

1. Physio-chemical properties

1.1. Specifications of couting film

Regarding the specification data given in **Table (1)**, in comparison with VIWAX, TMP helped the films to hold their moisture content. Moreover, the moisture content of all TMP films lowered due to the mixing with polymer, especially palmitic acid but stilled higher than that of VIWAX. Nevertheless, the moisture holding of TMP films was stronged when CMC was involved. That is, indeed, reflecting the relative higher flexibility possessed all TMP films regardless the kind of polymer used for the formula. Therefore, This actually allowed the film to be even thinner as could be observed regarding the true thickness property.

Table (1): Specification of total milk proteinate film as a function of formulas

Specification	VIWAX	Total milk proteinate mixed using						
		Pure	Polymer type					
			Carboxy methyl cellulose			Palmitic acid		
			with Thyme (T1)	with Cinnamon (T2)	with Marjoram (T3)	with Thyme (T4)	with Cinnamon (T5)	with Marjoram (T6)
Moisture (%)	24.49	38.73	32.42	33.24	32.41	30.11	31.42	30.98
True thickness (mm)	0.26	0.14	0.08	0.09	0.12	0.10	0.13	0.17
Air permeability (g/m2.day)	22.34	21.95	9.24	9.23	9.25	21.33	21.31	21.34
Water vapor permeability (g.mm/day.m2.kpa)	22.28	58.01	28.55	28.51	28.53	21.88	21.84	21.87

Concerning the air permeability of edible films, it could remarke that the CMC containing films were characterized with the lowest ability to permeate air *versus* those polymerized with palmitic acid or not and even VIWAX itself. That may explained with regard to the difference in the polarity between the tow polymers used in film making, where CMC is polare and a voracious moisture absorber and swells, reducing the film pores capacity and thus their permeability. While both of VIWAX and palmitic acid is hydrophobic, which reduces the water vapor permeability of the membrane. Needless to add that, the absence of the polymers, as in the case of pure TMP, led to obtain the highest water vapor permeability (Table, 1).

1.2. Cheese moisture loss

As shown in Table (2), the data indicated that, there were significant differences in the % of moisture loss between uncoated cheese samples and cheese coated with VIWAX (T1) or all cheese samples dipped with different TMP edible films along storage period. The uncoated cheese showed the highest loss of moisture content among all treatments, followed by Edam cheese samples coated with edible film made with CMC as polymer. During ripening period, cheese shrinkage can be caused adverse effects on properties of different dairy products such as appearance and nutritional quality **[34]**. The results indicated also that, dipping Edam cheese with different TMP edible film containing different essential oils caused a significant decrease in cheese moisture loss *versus* uncoated cheese. This phenomenon may be due to the applied TMP dipping possessed a good water vapor permeability value, and hence lowered the water loss the cheese matrix. Moreover, the enhanced moisture retention in TMP-treated cheese during proceeding maturation leading **[35]** to suggest that milk protein could serve as a barrier and resistance against water vapor permeability. The water vapor barrier of VIWAX (T1) and edible film made with palmitic and fortified with cinnamon coating (T6) had the best effect in minimizing moisture evaporation, followed by (T5) edible film made with palmitic and fortified with Thyme among all edible film cheese treatments. **[35]** and **[36]** reported that, despite the fact of that milk proteins had such limited moisture barrier properties; they were possible that hydrophilic milk proteins may interact with hydrophobic cheese matrix substrates at the surface, thereby enhancing the moisture barrier capacity. A similar result was observed by **[37],** who found that the moisture content of coated Saloio cheese (semi-hard cheese) before storage to be significantly higher than that of uncoated cheese samples. **[38]** mentioned that, loss of moisture and weight loss values were lower in chitosan coated Ras cheese during storage period compared with uncoated Ras cheese samples. **[39]** As alginate, gellan, and k-carrageenan were employed as coating materials on semi-hard cheeses, they were proven to have a lesser weight loss (about 2.0%) as compared to uncoated cheese samples. **[40]** showed that, the presence of the coating on ‘‘Regional” cheese is significant factor in the weight loss. It could be seen that, there were a significant difference in loss of moisture values between Edam cheese dipped on VIWAX and Edam cheese with (TMP) based on edible film made with palmitic acid as polymer and containing different essential oils (thyme, cinnamon, and marjoram) as preservatives than the other treatments (uncoated edam cheese and Edam cheese with TMP based on edible film made with CMC as polymer and containing different essential oils as preservatives) during ripening during ripening. The results are in agreement with the results obtained by **[41],** who showed that the coating process of semi hard Kashar cheese when compared to the control, casein, casein/natamycin, and natamycin solution may have delayed moisture losses.

Table (2): Moisture loss % of Edam cheese dipped using different coating formulas during ripening

Ripening Period (day)	Edam cheese dipped with coating formula							
	C	T1	T2	T3	T4	T5	T6	T7
30	5.03 ^{Ac}	1.56 ^{Cc}	2.14 ^{Bc}	2.16 ^{Bc}	2.09 ^{Bc}	2.05 ^{Bc}	2.07 ^{Bc}	2.09 ^{Bc}
60	12.20 ^{Ab}	2.48 ^{Db}	3.45 ^{Bb}	2.73 ^{Bb}	2.78 ^{Bb}	2.71 ^{Bb}	2.69 ^{Bb}	2.76 ^{Bb}
90	13.00 ^{Aa}	3.86 ^{Da}	5.28 ^{Ca}	5.03 ^{Ca}	5.51 ^{Ca}	4.09 ^{Ba}	4.02 ^{Ba}	4.20 ^{Ba}

C: Edam cheese not coated (control), **T1**: Edam cheese coated by VIWAX, **T2**: Edam cheese dipping on TMP+CMC with Thyme, **T3**: Edam cheese dipping on TMP+CMC with Cinnamon, **T4**: Edam cheese dipping on TMP+CMC with Marjoram, **T5**: Edam cheese dipping on TMP+ Palmitic acid with Thyme, **T6**: Edam cheese dipping on TMP+ Palmitic acid with Cinnamon,**T7**: Edam cheese dipping on TMP+ Palmitic acid with Marjoram
A, B, C: Means with same letter among treatments in the same storage period are not significantly differed.
a, b, c: Means with same letter for same treatment during storage period are not significantly differed.

1.2. Cheese gross composition

chemical composition content (%) in Edam cheese samples, dipped with (TMP) based on edible film made with CMC or palmitic acid as polymer and containing different essential oils (thyme, cinnamon, and marjoram) as preservatives during ripening are shown in Table (3). The results refer that, dry matter content of different fresh Edam cheese samples ranged between 56.47 and 56.49, while, the contents of dry matter ranged from 58.24 to 62.13 % in different Edam cheese samples after 90 days of ripening period. There were no significant differences in the dry content of different fresh cheese samples and control. On the other hand, at the end of ripening period, there were significant differences in the dry matter content between control cheese and all other dipped cheese. Non-significant differences were observed among all TMP treated Edam cheese samples. The dry matter content of Edam cheese significantly affected with TMP treatment dipping and progression of ripening. The increase in dry matter was more pronounced in the uncoated sample (control) compared to VIWAX and TMP dipped Edam cheese. At 90 days of ripening, Edam cheese coated by dipping on TMP+ Palmitic acid with Cinnamon (T6) had the lowest dry matter content, while uncoated cheese (C) showed the greatest. These results agree with **[38]** who found that, moisture content of Ras cheese chitosan coating treatment had a substantial impact on ripening progression. Also, **[41]** found that, dry matter values of Kashar cheese samples coated with casein-natamycin edible film significantly increased during ripening period and this increase was higher in the cheese samples without coating.

Results of Table (3) indicated also that, the highest protein content was determined in control samples during storage, followed by samples coated by (TMP) based on edible film made with CMC and containing different essential oils (thyme and marjoram) (T2 and T4).

Both fat and ash contents of control samples were significantly higher than TMP-dipped Edam cheeses in all stages of ripening. This could be explained by the fact that the control sample had a high dry matter concentration. As expected, this was due to the control samples not being coated with any packaging material, which also resulted in a larger loss of moisture. The protein, fat and ash contents increased in all samples during storage in parallel with the increment of dry matter. Naturally, these increases were higher in uncoated cheeses. Similar trandings were also reported by **[41]** and **[42],** who found that, the control of semi hard Kashar cheese had significantly higher dry matter, protein, fat and ash contents than those of cheese sample coated with beeswax or casein edible film. However, by delaying moisture loss, the coating decreased the creation of a thick crust layer.

Table (3): Gross composition of Edam cheese dipped using different coating formulas during ripening

Component %	Ripening Period (day)	Edam cheese dipped with coating formula							
		C	T1	T2	T3	T4	T5	T6	T7
Dry matter	Green	56.47 Ac	56.48 Ab	56.48 Ab	56.48 Ab	56.48 Ab	56.49 Ab	56.49 Ab	56.48 Ab
	30	58.66 Ab	57.16 Bb	57.41 Bab	57.42 Bab	57.39 Bab	57.38 Bab	57.39 Bab	57.39 Bab
	60	61.78 Aa	57.56 Ba	57.98 Ba	57.67 Ba	57.69 Ba	57.67 Ba	57.66 Ba	57.68 Ba
	90	62.13	58.16	58.78	58.67	58.88	58.27	58.24	58.31
Protein (Total nitrogen X 6.38)	Green	23.42 Ac	23.41 Ad	23.42 Ad	23.44 Ad	23.42 Ad	23.43 Ad	23.44 Ad	23.42 Ad
	30	23.88 Ab	23.71 Ac	23.74 Ac	23.76 Ac	23.75 Ac	23.73 Ac	23.72 Ac	23.73 Ac
	60	24.67 Aa	24.37 Ab	24.42 Ab	24.43 Ab	24.41 Ab	24.38 Ab	24.36 Ab	24.37 Ab
	90	24.94 Aa	24.67 Aa	24.74 Aa	24.73 Aa	24.74 Aa	24.69 Aa	24.71 Aa	24.69 Aa
Fat	Fresh	23.22 Ac	23.24 Ac	23.22 Ac	23.23 Ac	23.22 Ac	23.24 Ac	23.24 Ac	23.23 Ac
	30	24.19 Ab	24.14 Ab	24.21 Ab	24.22 Ab	24.21 Ab	24.18 Ab	24.17 Ab	24.18 Ab
	60	25.26 Aa	25.16 Aa	25.22 Aa	25.21 Aa	25.22 Aa	25.19 Aa	25.18 Aa	25.19 Aa
	90	25.72 Aa	25.22 Aa	25.22 Aa	25.24 Aa	25.28 Aa	25.26 Aa	25.24 Aa	25.26 Aa
Ash	Fresh	2.98 Ac	3.01 Ab	2.99 Ac	3.02 Ac	3.01 Ac	3.01 Ac	3.02 Ac	2. 99 Ad
	30	3.19 Ab	3.11 Ab	3.17 Ab	3.16 Ab	3.17 Ab	3.13 Ab	3.14 Ab	3.13 Ac
	60	3.26 Aab	3.16 Aab	3.22 Aab	3.23Aa	3.22 Aab	3.19 Aab	3.18 Aab	3.19 Ab
	90	3.38 Aa	3.21 Ba	3.33 ABa	3.30 ABa	3.32 ABa	3.27Ba	3.26 Ba	3.27Ba

***See Table (1) for details.**

1.3. Ripening indices

Table (4) showed that, at the beginning of storage period, there were non-significant differences in titratable acidity values among all treatments. Along ripening period, the titratable acidity values increased gradually with increasing of ripening period. At the end of ripening period, all Edam cheese samples dipped with different TMP based on edible film made with CMC or palmitic acid as polymer and containing different essential oils (thyme, cinnamon, and marjoram) as preservatives and Edam cheese samples coated by VIWAX exhibited significantly higher acidity values than that of control cheese. This may be due to the aerobic condition in dipped Edam cheese samples may be encourage the growth of lactic acid bacteria in dipped Edam cheese samples. The results agree with those obtained with **[38],** who found that, the change in acidity was significantly higher in chitosan-coated Ras cheeses compared with uncoated Ras cheese samples. Likewise, **[43]** found that, all samples of Edam cheese had titratable acidity that rose as they aged and stated that, the cheese flora's fermentation of the leftover lactose, which confers the cheese's acidity values, may be the cause of the increase in acidity. Additionally, these results concurred with **[44],** who demonstrated that the progressive rise in cheese acidity during the two months of ripening was caused by lactic acid bacteria fermenting lactose. From the data, it could be seen that, there were non-significant differences in titratable acidity between Edam cheese dipped with different TMP based on edible film made with CMC as polymer and containing different essential oils (thyme, cinnamon, and marjoram) as preservatives and Edam cheese dipped with different TMP based on edible film made with palmitic acid as polymer and containing different essential oils (thyme, cinnamon, and marjoram) as preservatives. This may be due to the use of different polymers with TMP to solution for dipped Edam cheese had no effect on the growth of lactic acid bacteria in cheese sample. The same results were observed by **[41]** in semi hard Kashar coated with casein and casein containing natamycin edible film. Also, **[45]** showed that significant differences in titratable acidity values between uncoated Cheddar cheese (control cheese) and milk protein film-wrapped cheese samples for all storage intervals tested.

The changes in pH values among all cheese treatments and during ripening periods (Table, 4) behaved trending opposite to those of titratable acidity values. This means that the pH values of Edam cheese treatments dipped by different TMP based on edible film made with CMC or palmitic acid edible films were lower than uncoated Edam cheese along the ripening period.

Results in Table (4) showed also that, along ripening period, the SN/TN % of all Edam cheese samples took trending like those exhibited by the titratable acidity %, where the proteolysis rate and hence SN/TN % increased as the cheese matrix kept its moisture content, that was obviously achived when the edible film was polymerized with the palmitic acid, regardless the kind of essential oils (thyme, cinnamon or marjoram) used as preservative. The relative higher SN/TN % possessed coated Edam cheese during ripening leads to suggest that the activity of the rennet and the internally produced proteolytic enzymes, those were responsible for the texture and flavor development would be more active. Similar observations were repoted by **[46]**.

Table (4): Ripening indices of Edam cheese dipped using different coating formulas during ripening

Ripening index	Ripening Period (day)	Edam cheese dipped with coating formula							
		C	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**
Titratable acidity %	**Green**	0.73^{Ab}	0.73^{Ad}	0.73^{Ac}	0.72^{Ac}	0.72^{Ac}	0.73^{Ac}	0.74^{Ad}	0.72^{Ad}
	30	0.93^{Ab}	0.94^{Ac}	0.92^{Ab}	0.94^{Ab}	0.92^{Ab}	0.92^{Ac}	0.93^{Ac}	0.94^{Ac}
	60	1.88^{Ba}	1.92^{Ab}	1.91^{Aa}	1.91^{Aa}	1.92^{Aa}	1.93^{Ab}	1.93^{Ab}	1.92^{Ab}
	90	1.89^{Ca}	2.11^{Aa}	1.93^{Ba}	1.92^{Ba}	1.93^{Ba}	2.08^{Aa}	2.09^{Aa}	2.08^{Aa}
pH value	**Green**	5.63	5.63	5.62	5.62	5.63	5.63	5.62	5.63
	30	5.48	5.47	5.46	5.46	5.45	5.46	5.45	5.46
	60	5.26	5.22	5.22	5.24	5.24	5.23	5.22	5.24
	90	5.15	5.11	5.15	5.14	5.14	5.12	5.13	5.12
Soluble nitrogen / Total nitrogen (SN/TN) %	**Green**	7.58^{Aa}	7.56^{Ad}	7.58^{Ad}	7.57^{Ad}	7.56	5.57^{Ad}	5.58^{Ad}	5.58^{Ad}
	30	9.10^{Ac}	9.08^{Ac}	9.11^{Ac}	9.10^{Ac}	9.11^{Ac}	9.06^{Ac}	9.08^{Ac}	9.07^{Ac}
	60	11.18^{Ab}	11.24^{Ab}	11.31^{Ab}	11.32^{Ab}	11.31^{Ab}	11.26^{Ab}	11.27^{Ab}	11.26^{Ab}
	90	12.28^{Aa}	13.39^{Ba}	13.26^{ABa}	13.27^{ABa}	13.26^{ABa}	13.31^{Ba}	13.33^{Ba}	13.32^{Ba}

***See Table (1) for details.**

On the contrary, **[47]** and **[41],** found that, uncoated semi hard Kashar cheese had higher levels of water-soluble nitrogen and ripening index compared to other casein coated cheese samples at in the end of storage**.** Nevertheless, **[52]** reported that, although ripening index values of all samples were similar until the 60th day, they were on the 90th day higher in the uncoated *versus* coated ones. Likewise, **[49]** observed that, during the ripening process of cheese, the level of soluble nitrogen components determined the harmful effect of the edible film coating on the rate and extent of proteolysis quantitatively in cheese. However, all of this may be due to the interference of external factors, such as yeasts and fungi in the cheese maturation, especially that the cheese was not coated *i.e.,* the cheese surface was not protected against the moisture loss and rather the post contamination.

2. Microbiological situation

2.1. Lactic acid bacterial (LAB) count

Ripening agents perform lactose, protein, and fat breakdown. LAB is one among the cheese-ripening ingredients. They support the texture, flavor, and preservation of cheese quality. LAB counts were therefore crucial to determining if a detrimental impact of TMP based on edible film made with CMC or palmitic acid and containing different essential oils coating on growth of these microorganisms. Table (5) refers that, there were non-significant differences in the LAB count among all fresh Edam cheese treatments and control. The LAB counts were significantly lower in uncoated control cheese samples compared with other cheese treatments along ripening period reflecting that Edam cheese dipping whether with different TMP-films or VIWAX was markedly associated with increase their viable LAB count along ripening. The reduced aeration inside the cheese ecosystem may be the cause of the microaerophilic lactic acid bacteria's ability to survive when present in TMP-treated cheese. Additionally, the TMP coating lowers the cheese's oxygen partial pressure. Similar observations were reported by **[42]**. These results are in accordance with those found by **[50], [51], [52]** and **[38],** who noticed that, counts of *Lactococcus* spp. and *Lactobacillus* spp. in coated cheese were significantly higher than those of its uncoated counterparts. They suggested also that, the presence of coating offers better growth conditions or survival of those strains, presumably by reducing permeability to oxygen and increasing the water activity (a_w) factors that otherwise would limit their growth in uncoated cheese. Generally, the use of some preservatives such as essential oils (thyme, cinnamon, and marjoram) in TMP solution which used for dipping Edam cheese did not appear any significant effect on LAB growth in cheese samples. These results showed coating with TMP based on edible film made with CMC or palmitic acid as polymer and containing different essential oils (thyme, cinnamon, and marjoram) as preservatives to be had no harm influence on the growth of microorganisms, those are necessary for the maturation of cheese. Similar findings were noticed by **[41],** who showed that, coating the semi hard Kashar cheese samples with casein, casein/natamycin and natamycin solutions did not have any negative influences on the growth of cheese maturation microorganisms. These results obtained by **[52]** implied that, the edible antimicrobial coatings in cheese did not inhibit the starter microflora, which remained at high levels throughout ripening.

2. 2. Yeasts and molds count

As shown in Table (5), yeasts and molds count in green Edam cheese samples were no existence log cfu /g for control and all treatments. This might be due to high sanitation and hygienic conditions adapted during the manufacturing process.. During ripening, yeasts and moulds counts gradually increased in all Edam cheese samples up to the end maturing period (90 days). The increase was more obvious in uncoated cheese (C). It is indicating that, Edam cheese dipping, whether with TMP based on edible film made with palmitic acid as polymer containing different essential oils (thyme, cinnamon, and marjoram) as preservatives during ripening or even coating by VIWAX helped reduce growth and reproduction opportunities leading comparatively to lower yeasts and molds counts. Moreover, the dipping of Edam cheese with TMP edible film with different essential oils during ripening storage markedly reduced, *versus* VIWAX, the count of yeasts and moulds in the resultant cheese. This is may be due to the condition that could be related to a lack of oxygen within TMP coated Edam cheese. Another reason could be due to the effect of different essential oils as antifungal agents in growth of different yeasts and moulds. **[53]** stated that, Na-caseinate film-coated Kashar cheese samples could not be counted after 60 days, whilst uncoated samples could not be counted after 90 days. **[54]** confirmed that, by covering the surface of the ricotta with a chitosan-whey protein edible film, microbial contamination growth was inhibited, and the shelf life of the product was increased. **[55]** On the 26th day of storage at 4C, no fungi were found in the cheese samples wrapped with the active film, whereas fungal growth was found in the control samples on the 16th day of storage. Also, **[45]** showed that, there was significant increase in the yeasts and molds population of uncoated Cheddar cheese as compared to milk protein film wrapped cheese samples at any point of storage. **[38]** stated that, although the initial yeasts and molds were not detected , after 120 days, when 2% chitosan-treated Ras cheese was compared to uncoated cheese (control), fungal growth was reduced by 1.5 logarithmic orders of magnitude. **[37]** reported that, The counts of yeasts and molds in Saloio cheese were 4.53 and 6.06 (log cfu g1), respectively, while natamycin+chitosan-coated cheese had a lower mould/yeast rate (4.950.27) on the 27th day of storage. **[52]** examined the antibacterial agent efficiency of edible films made from whey protein isolate, glycerol, and natamycin. The viable cell count experiment revealed the incorporation of natamycin in the film had a cidal impact on *Yarrowia lipolytica*. **[54]** notied that, natamycin had an early fungicidal impact on *Saccharomyces cerevisiae* when it was tested for its ability to kill yeast in Port Salut cheese.

Table (5): Microbiological quality (log CFU/g) of Edam cheese dipped using different coating formulas during ripening

Microbial count (log CFU/g)	Ripening Period (day)	Edam cheese dipped with coating formula							
		C	T1	T2	T3	T4	T5	T6	T7
Lactic acid bacterial	Fresh	6.45^{Ac}	6.46^{Ad}	6.44^{Ad}	6.45^{Ad}	6.46^{Ad}	6.45^{Ac}	6.44^{Ac}	6.45^{Ac}
	30	6.63^{Aa}	6.67^{Ac}	6.63^{Ab}	6.64^{Ab}	6.64^{Ab}	6.65^{Ab}	6.64^{Ab}	6.65^{Ab}
	60	6.57^{Cb}	6.81^{Aa}	6.72^{Ba}	6.72^{Ba}	6.71^{Ba}	6.82^{Aa}	6.84^{Aa}	6.81^{Aa}
	90	6.34^{Cd}	6.72^{Ab}	6.53^{Bc}	6.53^{Bc}	6.54^{Bc}	6.69^{Ab}	6.68^{Ab}	6.69^{Ab}
Yeasts and molds	Fresh	<10	<10	<10	<10	<10	<10	<10	<10
	30	<10	<10	<10	<10	<10	<10	<10	<10
	60	1.92	<10	<10	<10	<10	<10	<10	<10
	90	3.34	1.36	1.43	1.44	1.43	1.39	1.38	1.39

***See Table (1) for details.**

2.3. Spoilage and pathogenic bacteria

2.3.1. Salmonella spp., Staphylococcus aureus and coliform incidence

Salmonella spp. and *Staphylococcus aureus* could not be detected in all Edam cheese samples (control and treated cheese) either fresh or along the ripening period. Also, all Edam cheese treatments and control samples whether fresh or during ripening period were free from coliform bacteria. This may be due to the effective heat treatment of the different Edam cheese milk samples and different raw materials (72ºC for 15 sec) used in cheese manufacture and superior hygiene standards throughout production and storage. In addition, it could be due to the impact of acidity on various cheese samples, which is crucial in lowering the rate of coliform and harmful bacterial development as mentioned by **[53]**.

Otherwise, it could be concluded that the Edam cheese samples dipped on TMP with different essential oils enhanced the shelf-life of Edam cheese while being stored and were effective at limiting the growth of spoiling germs. It is assumed that a portion of essential oils' antibacterial action, The following list includes potential processes via which EOs may prevent bacterial growth: A breakdown of the bacterial outer membrane or phospholipid bilayer, changes to the fatty acid composition, an increase in membrane fluidity that allows potassium ions and protons to leak through, interference with glucose uptake, inhibition of enzyme activity, or cell lysis are just a few of the changes that can occur.

3. Texture profile parameters

The parameters of texture profile present in Table (6) demonstrated that, the hardness values of different green Edam cheese samples ranged between 15.91 and 17.33 (N), while, the hardness values ranged from 19.20 to 24.42 (N) in different Edam cheese samples after 90 days of ripening. Control Edam cheese was the hardest cheese (24.43 N) among all experimental Edam cheese, while (T1) had the lowest hardness value (19.20). Hardness was reversely affected by the moisture content in experimental Edam cheeses. The same trend was recorded in the values of gumminess and chewiness criteria. On the other hand, springiness and cohesiveness values were comparable in all treatments. These findings were comparable to those obtained by **[58], [59]** and **[60]**.

Table (6): Texture profile parameters of Edam cheese dipped using different coating formulas during ripening

Parameter	Ripening Period (day)	Edam cheese dipped with coating formula							
		C	T1	T2	T3	T4	T5	T6	T7
Hardness (N)	Green	15.95	15.92	15.91	15.94	15.95	15.92	15.94	15.93
	30	17.33	17.26	17.21	17.23	17.24	17.22	17.26	17.22
	60	20.85	18.23	18.34	18.38	18.35	18.41	18.39	18.41
	90	24.43	19.20	19.42	19.39	19.38	19.42	19.43	19.42
Cohesiveness (B/A area)	Green	0.61	0.63	0.62	0.63	0.62	0.62	0.63	0.64
	30	0.62	0.66	0.63	0.63	0.64	0.63	0.64	0.65
	60	0.67	0.71	0.65	0.64	0.65	0.68	0.69	0.68
	90	0.71	0.76	0.73	0.72	0.73	0.74	0.74	0.74
Gumminess (N)	Green	9.94	9.92	9.94	9.93	9.92	9.94	9.93	9.94
	30	10.22	10.15	10.14	10.16	10.17	10.18	10.17	10.18
	60	12.32	11.32	11.36	11.36	11.37	11.35	11.33	11.34
	90	13.15	12.25	12.27	12.28	12.27	12.26	12.24	12.26
Chewiness (N.mm)	Green	7.94	7.93	7.94	7.93	7.95	7.95	7.93	7.94
	30	8.66	8.67	8.68	8.66	8.67	8.66	8.68	8.67
	60	8.95	8.87	8.82	8.83	8.83	8.85	8.84	8.85
	90	9.65	8.92	8.84	8.82	8.84	8.89	8.88	8.88
Springiness (N.mm)	Green	0.62	0.63	0.62	0.63	0.62	0.62	0.63	0.64
	30	0.66	0.68	0.63	0.63	0.64	0.63	0.64	0.65
	60	0.71	0.76	0.72	0.73	0.72	0.73	0.75	0.74
	90	0.79	0.82	0.76	0.75	0.76	0.78	0.79	0.78

***See Table (1) for details.**

4. Sensory attributes

The judging scores given in Table (7) indicated that, until first 60 days of all Edam cheese ripening, appearance, body and texture and flavor scores among treatments were statistically similar for each other. After 60 days of ripening, there were significant differences in all scores of the foregoing sensory attributes among all treatments. Whereas, the dipping of Edam cheese with any TMP films, whether based on edible film made with CMC or palmitic acid with different essential oils (thyme, cinnamon, and marjoram), improved scores of previous criteria, namely appearance, body and texture and flavor. At the beginning of storage ripening period, the total sensory scores of different Edam cheese samples were 78, 76, 77, 78, 76, 76, 78 and 77 points for control (C), T1, T2, T3, T4, T5, T6 and T7 Edam cheeses respectively. In the beginning of the ripening, total organoleptic scores were statistically similar between all Edam cheese samples. After 90 days of cheese ripening, total scores became 65, 92, 92, 92, 92, 93, 94 and 93 points for C, T1, T2, T3, T4, T5, T6 and T7 Edam cheeses in order. From the results, it could be seen that, as ripening period progress the total scores of all Edam cheese dipped with any one of different TMP edible films besides, Edam cheese sample coated by VIWAX gradually significantly increased. While, uncoated Edam cheese showed gradually increased in total score till 60 days of ripening period then gradually significantly decreased till the end of repining period. Therefore, it could be concluded that sensory characteristics of Edam cheese samples kept their quality by dipped with TMP films with containing different essential oils.

Table (7): Sensory scores of Edam cheese dipped using different coating formulas during ripening

Sensory criteria	Ripening Period (day)	Edam cheese dipped with coating formula							
		C	T1	T2	T3	T4	T5	T6	T7
Appearance (10)	Green	7	7	7	7	7	7	7	7
	30	7	8	8	8	8	8	8	8
	60	6	8	7	8	8	8	8	8
	90	5	8	8	8	8	8	8	8
Body and texture (40)	Green	33	33	33	33	33	33	33	33
	30	34	35	37	36	35	36	37	36
	60	35	36	37	37	36	37	36	36
	90	30	37	36	36	37	37	38	37
Flavor (50)	Green	38	36	37	38	36	36	38	37
	30	40	41	41	42	41	41	41	41
	60	45	46	47	47	47	47	47	47
	90	30	47	48	48	47	48	48	48
Total scores (100)	Green	78^{Ac}	76^{Ac}	77^{Ac}	78^{Ac}	76^{Ac}	76^{Ac}	78^{Ad}	77^{Ac}
	30	81^{Bd}	84^{Ab}	86^{Ab}	86^{Ab}	84^{Ab}	85^{Ab}	86^{Ac}	85^{Ab}
	60	86^{Ba}	90^{Aa}	91^{Aa}	92^{Aa}	91^{Aa}	92	91^{Ab}	91^{Aa}
	90	65^{Bd}	92^{Aa}	92^{Aa}	92^{Aa}	92^{Aa}	93^{Aa}	94^{Aa}	93^{Aa}

***See Table (1) for details.**

Sensory tests were not performed because surface deterioration with yeasts and molds, as well as other contaminating microbes, made sensory inspection of uncoated cheese after 90 days of storage unsafe. The results agree with **[38]** who reported that, there were substantial differences in Ras cheese's overall organoleptic quality as a result of chitosan coating and ripening period. Chitosan-coated Ras cheese received significantly higher total organoleptic ratings than control uncoated Ras cheese. **[49]** noticed that, the shelf-life of the products was increased by coating Göbek Kashar cheese with edible film made of chitosan and chitosan/whey protein. This coating reduced microbial development. Also, **[54]** concluded that coating the surface of the ricotta cheese with an edible film made of chitosan and whey protein inhibited the growth of microorganisms and increased the shelf life of the product packaged in modified atmosphere. Edam cheese coating with edible film improved texture maintenance and didn't appear to change sensory properties. It's likely that other dairy products will also provide the advantages provided by the biopolymers.

CONCLUSION

Finally, it could be recommended upon the previous results that, TMP based on edible film made with palmitic acid as polymer containing different essential oils (thyme, cinnamon, or marjoram) as preservatives could be used as dipping film in Edam cheese coating. Those edible films may be useful for keeping the moisture during ripening period besides improve its marketing quality and safety and produce high quality of Edam cheese.

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