International Journal of Food Science and Nutrition Engineering 2015, 5(1): 15-23
DOI: 10.5923/j.food.20150501.03

Effect of Clove Oil on Physicochemical and Microbiological Characteristics of Egyptian Ras Cheese (Romy) during Storage

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Abstract Substances to fungal decontamination of Ras cheese during storage by using some natural substances. Egyptian Ras (Kefalotyri or Romy) cheese was manufactured from standardized buffaloes' and cows' milk (3.5% fat) with modified traditional method and dry salting around forty-five day. After complete salting process, the cheese wheels were painted on external surface of wheels (approximately 2–3 Kg) by clove oil and ripened at 59.9°F (15.5ºC) for three months. Weight and volume of Ras cheese wheel decreased gradually until the end of the storage period (3rd month) after salting. The untreated wheels decreased in the volume compared with the treated ones with clove oil. The moisture (M%) was gradually decreased for untreated and treated Ras cheese, in the end of the storage period, while the fat on dry matter (F/DM%), salt/cheese serum%, total nitrogen TN%, soluble nitrogen on total nitrogen SN/TN% and non-protein nitrogen on total nitrogen NPN/ TN% tended to increase with advanced storage. The total bacteria count (TBC), yeast and fungi (Y&F) were very high in the beginning of Ras cheese manufacture, while, after salting these values of (Y&F) were decreased in the wheels collecting from the surface and the middle of Ras cheese wheels, these values were decreased because of salting processing. The treatment with clove oil reduced the (TBC) and (Y&F) in the surface of both treatments after 1st, 2nd and 3rd month of ripening, while, the treatment without oil increased gradually of the (TBC) and (Y&F) in the surface of both treatments after the end of the storage. It was observed that, there were no observed fungi on the surface of both treatments which treated with clove oil during storage period. The texture and appearance scores were not significantly ($p \leq 0.05$) different in treated and untreated Ras cheese samples during ripening periods. Both treatment of both treatments have high significant ($p \leq 0.05$) of flavor after 3rd month of ripening in both treatments. This observation, encourage to use this oil for the further studies and it will be used in the commercial scale, as well as, this oil considered an antifungal edible coatings oil and improve the shelf life of cheese. Point of view study can recommend use of volatile oils of medicinal plants as natural substances to prevent and overcome the fungal contamination on Ras cheese during ripening period this is may be very useful from economic point of view.

Keywords Egyptian ras cheese, Physico-Chemical properties, Microbiological examination and organoleptic properties

1. Introduction

Ras cheese is an Egyptian hard cheese made from cows and buffalos milk, the composition of the cheese has been described by [23], it is required a long period of time to develop the full flavor and texture of ripened cheese [14]. The dry cheeses are placed in ripening rooms in the production area, where ripening takes place at a nearly constant relative humidity (90–95%) and temperature (9–12°C).

Penicillium verrucosum, P. commune, *Aspergillus flavus* and *A. niger* isolated from Ras cheese by [13]; [20]; [12] and [3]. Some *Aspergillus* sp. is xerophilic fungi and is responsible for many cases of food and feed contamination.

Clove oil is extracted from *Eugenia caryophyllata* (also known as *E. aromatica*, *E. carophyllus* and *Syzygium aromaticum*) of the Myrtaceae family. The main chemical components of clove oil are eugenol, eugenol acetate, ISO-eugenol and caryophyllene [6] and [17]. The inhibitory effect of clove on growth and aflatoxin production by *A. flavus* has been reported. In maize grain, clove oil was effective against aflatoxin formation by *A. flavus* after 10 days under favourable conditions for mycotoxin production. The ability of oregano oil to inhibit growth of *A. flavus*, *A. ochraceus* and *A. niger* has been evaluated previously [33].

The mode of action of essential oils as antimicrobial agent are depending on multi target mechanisms such as; damage bacterial cell due to disruption of cytoplasmic membrane, electron flow, active transport and coagulation of cell

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Published online at http://journal.sapub.org/food

contents. Several previous reports were focused on the antimicrobial properties of essential oils. In fact, many essential oils are known to be active against a wide variety of microorganisms, including Gram-positive and Gram-negative bacteria and fungi. Toxic effects on membrane structure and function have been generally used to explain the antimicrobial action of essential oils, as a result of their lipophilic character, which preferentially partition from an aqueous phase into membrane structures. This results in membrane expansion, increased membrane fluidity and permeability, disturbance of membrane-embedded proteins, inhibition of respiration, and alteration of ion transport processes [32]. Clove essential oils were used for inhibitory activity against important spoilage microorganisms of intermediate moisture foods. It has been listed as a ‘‘Generally Regarded As Safe’’ substance by the United States Food and Drug Administration when administered at levels not exceeding 1500 ppm in all food categories [18]. Additionally, the World Health Organisation (WHO) [18] has established the acceptable daily human intake of clove oil at 2.5 mg/kg body weight for humans. It is safe, effective, and relatively inexpensive. In addition, humans have used clove oil for centuries, as an anaesthetic for toothaches, headaches and joint pain. The consumption of clove oil can reduce many health risks. Also, it is used throughout the world for applications ranging from food flavoring. It was found to be an effective antioxidant *in vitro* assays including reducing power and superoxide anion radical scavenging, hydrogen peroxide scavenging and metal chelating activities. It can be used for minimizing or preventing lipid oxidation in food and prolonging the shelf life of food and pharmaceuticals [18]. Clove oil is considered as the most common herbs with strong antimicrobial activity. The addition of essential oils containing chemical compounds such as carvacrol, cinnamaldhyde, eugenol and camphor which are identified as the major chemical components responsible for exerting antimicrobial activity. Some scientists confirmed the antimicrobial activity of essential oils from oregano, thyme, sage, rosemary, clove, coriander, garlic and onion against both bacteria and molds [27].

The aim of this study was examined the inhibitory effect of clove oil against the microbial growth naturally on the Ras cheese wheels during the storage period (three months). Physical properties, organoleptic properties, microbiological examination and chemical composition of Egyptian Ras cheese was also studied.

2. Materials and Methods

Fresh whole cows’ and the buffaloes’ milk that used in this study was obtained from El-Ghazy laboratory, Elsawalem, Damietta, Egypt. Local rennet 0.7N, “Elamel Elmasry“ was obtained by Ayman Haekel Company. Iodized salt, produced by El-Nasr Saline’s Co, Alex. was used. Other pure chemicals for measuring acidity, fat, protein, etc.…, were obtained from El-Gomheria Chemical Company, Mansoura, Egypt. Commercial clove oil was purchased from a local market, namely Cloves tested for their effect on fungal growth.

2.1. Ras Cheese Making

In an attempt to imitate the widely used method among the cheese manufacture in El-Ghazy laboratory, Elsawalem, Damietta for making Ras cheese. The procedure suggested by [1] and [2] for making Ras cheese was adopted. Standardized milk (3.5% fat) was heated to 32ºC and sufficient rennet was added in the proportion of 2.5g per 100kg milk to complete coagulation in 30 – 40 minutes. The coagulum was cut into small pieces about the size of chickpea grains and then vigorously stirred. The temperature of the vat was then raised to 45ºC over a period of around 40 – 50 minutes, and gently stirring was maintained throughout. After the curd had settled, the whey drained out (acidity≈0.14%), salt was sprinkled over the curd at a ratio of 1% (w/w), and the curd was manually pushed to the sides of the fat. Molds, lined with cheesecloth, were filled with sufficient curd to produce one finished cheese, and manual pressure was applied to expel some of the remaining whey. Light mechanical pressure follows over the next four hours at which point the cheese was reversed in the press and left under pressure for overnight (approximately 18 hours) (Fig. 1). The cheese wheels were then removed from the moulds and cloths and placed in the salting chamber (Fig. 2). After draining for a further day at ambient temperature, the surfaces of each cheese wheels were coated with a small amount of dry salt (Fig. 3).

Figure 1. Ras cheese in moulds during pressing process

By the following day, most of this salt will had be absorbed into the cheese, so that the wheels were turned and the dry salting process repeated once again. This dry salting produce was continued for a period of around forty-five day, either every other day or once every three days. After completely salting process, the surface of cheese wheels was painted by clove oil and ripened at 60°F (15.5ºC) for three months in a digital incubator (Switc, MPM Instraents s.r.t.,

Bernareggiol, Made in Italy), thereafter samples were taken after one, two and three months.

Figure 2. Fresh Ras cheese wheels in the salting chamber

Figure 3. Salting Ras cheese wheels

2.2. The Sampling Procedure

The sampling procedure during the time of the experiment was as following: Eight cheese wheels were made in this experiment. The first one, was used as a control and will be referred to it in the following experiment as salting zero time. Then, the seven cheese wheels removed to the salting chamber. At the end of the dry salting process (45 day), the second cheese wheel was used as a control also, but will be referred to it in the following experiment as salting 1.5 months. The six cheese wheels were divided into two groups, the first group, was treated with clove oil by painting the surfaces of each cheese wheel and the second group were bot treated with clove oil. All six cheese wheels were stored at 15.5ºC for three months in a digital incubator. Thereafter, samples were taken after one, two and three months. Two samples were taken from every cheese wheel for the microbiological examination. The first sample was taken by scraping the cheese wheel's surface under aseptic conditions and the second sample was taken from the middle of the cheese wheel by cutting it using a sterilized knife.

2.3. Chemical Analysis

Ras cheese was analyzed for total solids (TS), fat, total nitrogen (TN), soluble nitrogen (SN) and non-protein nitrogen contents (NPN) according to [26]. Salt contents of Ras cheese were estimated using the Volhard method according to [30].

2.4. Microbiological Analysis

Poured plate method was used, one ml of suitable folds serial dilutions of all cheese samples were inoculated onto a plate containing potato dextrose agar (PDA) medium (three replicates). Approximately fifteen ml of PDA medium at about 50°C was poured in each plate, then thoroughly mixed and left for solidification. The plates were incubated at 25°C for 5 days. After the incubation period, developed colonies were counted per each plate. The mean count of plates was recorded to represent fungal count [4]. Total bacterial count of Ras cheese was determined according to [4] using nutrient agar medium [10]. It consists of (g/L): beef extract 3 g; peptone, 5 g; agar agar, 15 g; distilled water 1 L and sterilized by autoclaving at 121ºC for 15 min. The pH was adjusted to 6.8, plates were incubated at 30°C for 3 days before counting.

2.5. Sensory Evaluation

This was carried out by at least 30 panelists and staff (both sexes, ages ranging between 18 and 65 years old) in Dairy science and Microbiology Departments, Faculty of agriculture, Damietta University, according to the scheme recommended by [23], taking into consideration the maximum attainable scores were 60 points out of 100 for flavour, 30 points for body and texture and 10 points for the general appearance.

2.6. Statistical Analysis

PROC GLM procedure of the Statistical Analysis Systems [31] was used to analyze the Least-Squares Means (LSM) and Standard Errors (SE) for different treatments and the differences between means were detected by Duncan`s Multiple Range Test [11].

3. Results and Discussion

Table 1. The means and standard deviation of some physical properties, chemical composition and organoleptic evaluation in Ras cheese wheels in total storage period

Weight (Kg)	Height (cm)	Diameter (cm)	Volume (cm^3)	M(%)
2.55±0.05	12.48±0.28	16.40±0.08	2624.73±31.19	31.85±0.77
F/DM(%)	**Salt/cheese serum(%)**	**TN(%)**	**SN/TN(%)**	**NPN/T(%)**
44.61±0.66	7.87±0.52	3.59±0.09	7.25±0.65	3.64±0.24
Flavor	**Body and texture**		**Appearance**	
53.98±1.48	26.76±0.60		8.60±0.16	

Average wheel weight (Kg), wheel height (cm), wheel diameter (cm), wheel volume (cm^3) was 2.55±0.05, 12.48±0.28, 16.40±0.08 and 2624.73±31.19, respectively. Average percentages of M, F/DM, salt/cheese serum, TN, SN/TN and NPN/TN were 31.85.60±0.77, 44.61±0.66 and

7.87±0.52, 3.59±0.09, 7.25±0.65 and 3.64±0.24, respectively (Table 1), while, the average of Ras cheese flavor and body and texture and appearance scores were 53.98±1.48, 26.76±0.60 and 8.60±0.16, respectively (Table 1).

3.1. Physical Properties of Treated and Untreated Ras Cheese Wheels with Clove Oil during Storage Period

Table 2 shows the changes in the physical examination of Ras cheese wheels during storage. Weight and volume of Ras cheese wheels were varied according to the treatment of salting, where the weight of the Ras cheese wheel decreased from 2.95±0.14 kg in the fresh Ras cheese wheel to 2.57±0.14 kg in the wheel after salting, and the loss of weight was 12.88%. The weight of untreated Ras cheese wheels with clove oil was 2.52±0.14, 2.45±0.14 and 2.43±0.14 after 1st, 2nd and 3rd months, respectively, but the weight of treated Ras cheese wheels with clove oil was 2.55±0.14, 2.48±0.14 and 2.45±0.14 over the same period, respectively. Similar finding were reported [15].

The volume was decreased from 3017.54±11.37 cm^3 in the fresh Ras cheese wheel to 2565.53±11.37 cm^3 in the cheese wheel after salting, and the shrinkage of volume was 14.98%. The volume of untreated Ras cheese wheels was 2578.18±11.37, 2565.35±11.37 and 2553.90±11.37 after 1st, 2nd and 3rd months, respectively, but the volume of treated Ras cheese wheels with clove oil was 2580.16±11.37, 2574.90±11.37 and 2566.53±11.37 over the same period, respectively. It was observed that, the untreated wheels decreased in the volume compared with the treated wheels with clove oil.

The treatment of clove oil prevented the water evaporation from wheels. Also, the loss of weight was taken the same pattern of shrinking of volume. Similar results were obtained [9] who studied the effect of cheese coating (Canestrello Pugliese cheese) with a thin layer of extra-virgin olive oil to prevent moisture loss undergone ripening; also, mechanical and dynamic-mechanical properties were studied. It showed changes in the texture and in the fundamental physical properties during ripening time, both in the external and the internal portions of the cheese, and decrease of the moisture content, and pH and an increase of the elastic modulus and the storage modulus, during ripening, and both for the internal and external portions of the Canestrello cheese. Also, results demonstrated that, for the first 60 days of ripening the elastic modulus of the outer region was significantly higher than that of the inner region.

3.2. Chemical Examination of Ras Cheese

The changes in M%, F%, Salt/cheese serum%, TN%, SN/TN% and NPN/TN% of Ras cheese wheels from different treatment followed the pattern of changes during ripening (Table 3). The average moisture of fresh Ras cheese was 38.86±1.21 compared to 30.22±1.21 and 30.40±1.21 for untreated and treated Ras cheese wheels after 3 months of ripening after salting. The reason for this loss may be attributed to the second stage of salting, done during this period, thus the moisture content tended to decrease. The examination of market Ras cheese, had a mean moisture of 34.82% [34].

The average fat contents on dry matters were 39.33±0.22 for fresh Ras cheese and 46.42±0.22 and 47.84±0.22 for untreated and treated Ras cheese after 3 months of ripening after salting, respectively. The market Ras cheese had a lower average of 48.56% [34].

Ras cheese wheels are salted at two stages, before and after pressing. In the first stage, the heated curd is lightly salted in brine. The second stage, continuing for almost 45 days after manufacture, is the most effective. The average salt on cheese serum contents of ripened cheese were 1.67±0.09 versus 9.37±0.09 and 9.75±0.09 for untreated and treated Ras cheese after 3 months after salting. There is a gradual increase in salt content to the second month, and then it remains almost constant until the end of the ripening period. Dry salting of Ras cheese is a slow process. Besides their unsuitability for large-scale production, resultant cheeses would definitely lack uniformity in salt content. Salting by brine solution or any other method should be considered. Similar trends were obtained [16]; [21]; [28]; [2]; [5] and [29].

Table 2. Physical examination of Ras cheese during storage*

Storage periods			Parameters of Ras cheese wheels					
			Weight Kg	Height Cm	Diameter Cm	Volume Cm^3	Shrinkage of volume%	Lose of weight%
Salting		0	2.95±0.14	16.00±0.15^a	15.50±0.15^b	3017.54±11.37^a	----	----
		1.5st	2.57±0.14	12.00±0.15^b	16.50±0.15^a	2561.31±11.37^b	14.98	12.88
Ripening	1st	Untreated	2.52±0.14	11.99±0.15^b	16.55±0.15^a	2578.18±11.37^b	14.56	14.58
		Treated	2.55±0.14	12.00±0.15^b	16.55±0.15^a	2580.16±11.37^b	14.49	13.56
	2nd	Untreated	2.45±0.14	11.96±0.15^b	16.53±0.15^a	2565.35±11.37^b	14.99	16.95
		Treated	2.48±0.14	11.99±0.15^b	16.54±0.15^a	2574.90±11.37^b	14.67	15.93
	3rd	Untreated	2.43±0.14	11.95±0.15^b	16.50±0.15^a	2553.90±11.37^b	15.36	17.62
		Treated	2.45±0.14	11.98±0.15^b	16.52±0.15^a	2566.53±11.37^b	14.95	16.95
p-value			0.24	≤ 0.001	≤ 0.01	≤ 0.001		

*Means within the same row (a, b and c) with different superscripts differed significantly (P<0.05).

Table 3. Chemical examination of Ras cheese during storage*

Storage periods			Chemical examination of Ras cheese wheels					
			M %	F/DM%	Salt/cheese serum%	TN%	SN/TN%	NPN/TN%
Salting		0	38.86±1.21^{a}	39.33±0.22^{e}	1.67±0.09^{g}	3.15±0.07^{e}	3.17±0.06^{h}	1.58±0.04^{g}
		1.5st	34.76±1.21^{b}	39.69±0.22^{e}	6.80±0.09^{f}	3.22±0.07^{de}	3.73±0.06^{g}	2.48±0.04^{f}
Ripening	1st	Untreated	31.29±1.21^{cb}	44.43±0.22^{d}	8.15±0.09^{e}	3.33±0.07^{cde}	5.10±0.06^{f}	3.00±0.04^{e}
		Treated	31.35±1.21^{cb}	45.81±0.22^{cb}	8.43±0.09^{d}	3.43±0.07^{cd}	5.54±0.06^{e}	3.49±0.04^{d}
	2nd	Untreated	30.59±1.21^{c}	45.31±0.22^{c}	9.18±0.09^{c}	3.50±0.07^{cb}	8.57±0.06^{d}	4.28±0.04^{c}
		Treated	27.35±1.21^{c}	48.05±0.22^{a}	9.61±0.09^{ab}	3.66±0.07^{b}	9.01±0.06^{c}	4.64±0.04^{b}
	3rd	Untreated	30.22±1.21^{c}	46.42±0.22^{b}	9.37±0.09^{bc}	4.18±0.07^{a}	10.52±0.06^{b}	4.54±0.04^{b}
		Treated	30.40±1.21^{c}	47.84±0.22^{a}	9.75±0.09^{a}	4.28±0.07^{a}	12.38±0.06^{a}	5.14±0.04^{a}
p-value			≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001

M%: Moisture percentage; F/DM%: Fat on Dry Matter percentage; TN%: Total Nitrogen percentage; SN/TN%: Soluble Nitrogn on Total Nitrogen percentage and NPN/TN%: Non-Protein Nitrogen on Total Nitrogen percentage.
*Means within the same row (a, b, c, d, e, f, g and f) with different superscripts differed significantly (P<0.05).

The higher total protein in cheese may be due to lower fat as well as partial denaturation of soluble proteins by heat and their retention in the resulting para-casein curd [25] and [24].

Results indicate that Ras cheese wheels had higher initial soluble nitrogen on total nitrogen coefficient of 3.17±0.06 versus 3.73±0.06% for cheese after salting (1.5st month). Rate of increase in soluble nitrogen was less for cheese, due to the effect of heat processing on the natural flora and enzymes in milk known to affect protein degradation.

Besides these two ripening agents, the amount of decomposed protein and the degree of decomposition would also depend on age, moisture, acidity, and type of cheese [24]. After 3 months of ripening after salting, the soluble nitrogen coefficients were 10.52±0.06 and 12.38±0.06 for untreated and treated Ras cheese wheels, respectively. The corresponding values at the end of 6 months were 19.50 and 15.54%, [34]. The percentages of non-protein nitrogen were 1.58±0.04 in the fresh Ras cheese wheel to 2.48±0.04 in the cheese wheel after salting, while, these values gradually increased from of Ras cheese wheels untreated were 3±0.04, 4.28±0.04 and 4.54±0.04 after 1st, 2nd and 3rd months, respectively, but in Ras cheese wheels treated were 3.49±0.04, 4.64±0.04 and 5.14±0.04 after the same period, respectively. Generally, the Salt/cheese serum%, TN%, SN/TN% and NPN/TN% tended to increase with advanced storage. Similar trends were obtained by [16]; [21]; [28]; [2]; [5] and [29].

As obtained in Table 4, correlations coefficients between chemical composition of Ras cheese after salting without oil (above the diagonal) and clove oil (lower the diagonal) were positive and highly significant (P<0.005) between some components, and vice versa for others. Negative coefficients and high significant (P<0.005) were shown among the percentage of M-salt/cheese serum, F/DM, TN, SN/TN and NPN/TN (r=-0.942***, -0.930***, -0.775**, -0.974*** and -0.954***, respectively) for Ras cheese without oil samples. A positive significant coefficient (P<0.005) was between the percentage of F/DM and salt/cheese serum (r=0.786***), TN and F/DM (r=0.857**), SN/TN-salt/cheese serum, F/DM and TN (r=0.953***, 0.927*** and 0.833**, respectively), NPN/TN-salt/cheese serum, F/DM, SN/TN (r=0.977***, 0.857** and 0.978***, respectively). The lowest positive coefficient were shown among the percentage of TN and salt/cheese serum (r=0.683*) and NPN/TN and TN (r=0.714*) for Ras cheese without oil samples. On the other side, lower the diagonal, a positive significant coefficient (P<0.005) were found between the percentage of SN/TN and NPN/TN (r=0.972***), TN-SN/TN and NPN/TN (r=0.949*** and 0.866**), salt/cheese serum-F/DM, SN/TN and NPN/TN (r=0.974***, 0.887*** and 0.953***, respectively), F/DM-SN/TN and NPN/TN (r=0.819**, and 0.916***, respectively). The lowest positive coefficient were showen among the percentage of salt/cheese serum and TN (r=0.705*) and F/DM and TN (r=0.621*) for Ras cheese painted with clove oil samples. Negative coefficients and non-significant (P<0.005) were showed among the percentage of salt/cheese serum and M (r=-0.149^{ns}), F/DM and M (r=-0.321^{ns}) M-TN, SN/TN and NPN/TN (r=-0.086^{ns}, -0.125^{ns} and -0.183^{ns}, respectively) for Ras cheese painted with clove oil samples.

3.3. Microbiological Examination of Ras Cheese during Storage

Results of microbiological examination of Ras cheese during storage are presented in Table 5. Results showed that, the total bacterial count (TBC) and the total yeast and fungi count of Ras cheese wheels were varied according to the treatment of salting and clove oil. The count of total bacteria, yeast and fungi were very high in the beginning of Ras cheese manufacture being 6.16±0.21 and 3.15±0.37 (log_{10} cfu/g), respectively. After salting (1.5 month) the values of total yeast and fungi count were decreased to 3.11±0.19 and 2.65±0.21 (log_{10} cfu/g) in the wheels collected from the surface and the middle, respectively, while, the total

bacterial count collected from the surface and the middle of Ras cheese wheels were 6.13±0.18 and 6.12±0.21 (log_{10} cfu/g), respectively

These values were decreased because of salting processing. The treatment with clove oil reduced the count of yeast and fungi in the surface area of Ras cheese wheels after the 1st month of ripening from 3.14±0.19 (log_{10} cfu/g) in the untreated wheel to 3.06±0.19 (log_{10} cfu/g) in the treated wheel, but these values in the middle portion were 2.66±0.21 (log_{10} cfu/g) and 2.72±0.21 (log_{10} cfu/g), respectively. The total bacterial count took the same pattern of yeast and fungal count. It was observed that, clove oil reduced the counts of yeast, fungi and bacteria in all experiments. Moreover, there were no observed fungal growth on the surface of Ras cheese wheels which treated with clove oil during storage period (Figs. 4 - 6).

Ten essential oils were tested [19] against thirteen fungal species isolated from Ras cheese and he found that, clove oil had a very strong effect on the isolated fungi. The highest effect was in case of clove oil followed by cinnamon oil while basil oil was the last one. The highest values of inhibition zone were in case of cloves oil in all fungal species. *Penicillium* sp., *A. glaucus* and *A. niger* gave the highest values being 45.00, 34.52 and 33.01 mm, respectively.

These results can explaine by the observation of [6] who reported that, the chemical composition of clove contained eugenol ($C_{10}H_{12}O_2$), which considered a very strong factor on fungal species. These component are known as antimicrobial substances against several microorganism.

The total bacterial count in the surface area of untreated Ras cheese wheels after 1.5 month of salting and after 1st, 2nd and 3rd month of ripening were significantly higher ($P<0.05$) than those in the treated Ras cheese wheels, while, the total yeast and fungal count in the surface area of untreated Ras cheese wheels after the 1st and 2nd month of ripening were significantly higher ($P<0.05$) than those in the treated Ras cheese wheels. On the other hand, total count of bacteria and total yeast and fungal count on the surface and middle area of untreated and treated Ras cheese wheels were significantly higher ($P\leq0.001$) than those in the ripening period (three months after salting).

Figure 4. Untreated and treated Ras cheese wheels with clove oil after one month and stored at 15.5ºC

Table 4. Correlation coefficients between chemical composition of Ras cheese after salting without oil (above the diagonal) and clove oil (lower the diagonal)

	Salt/cheese Serum%	F/DM%	M%	TN%	SN/TN%	NPN/TN%
Salt/cheese Serum%	---	0.786**	-0.942***	0.683*	0.953***	0.977***
F/DM%	0.974***	------	-0.930***	0.875**	0.927***	0.857**
M%	-0.149^{ns}	-0.321^{ns}	------	-0.775**	-0.974***	-0.954***
TN%	0.705*	0.621	-0.086^{ns}	------	0.833**	0.714*
SN/TN%	0.887***	0.819**	-0.125^{ns}	0.949***	------	0.978***
NPN/TN%	0.953***	0.916***	-0.183^{ns}	0.866**	0.972***	------

Table 5. Microbiological examination of treated and untreated Ras cheese with clove oil during storage*

Storage periods			Microbiological examination of Ras cheese wheels			
			Y&F (log_{10} cfu/g)		TBC (log_{10} cfu/g)	
			Surface	Middle	Surface	Middle
Salting		**Zero time**	--------	3.15±0.37^{a}	-------	6.16±0.21^{a}
		1.5 month	3.11±0.19^{b}	2.65±0.21c	6.13±0.18^{a}	6.12±0.21^{b}
Ripening	**1st**	**Untreated**	3.14±0.19^{ab}	2.72±0.21^{cb}	6.14±0.18^{a}	6.12±0.21^{b}
		Treated	3.06±0.19^{c}	2.66±0.21^{c}	5.99±0.18^{b}	5.96±0.21^{d}
	2nd	**Untreated**	3.14±0.19^{a}	2.75±0.21^{b}	6.14±0.18^{a}	6.13±0.21^{b}
		Treated	2.99±0.19^{d}	2.66±0.21^{c}	5.75±0.18^{c}	5.99±0.21^{cd}
	3rd	**Untreated**	3.14±0.19^{a}	2.72±0.21^{cb}	6.14±0.18^{a}	6.13±0.21^{b}
		Treated	2.99±0.19^{d}	2.66±0.21^{c}	5.72±0.18^{c}	5.99±0.21^{c}
p-value			≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001

TBC: Total bacterial count and Y&F: Yeast and fungi.
*Means within the same row (a, b, c and d) with different superscripts differed significantly ($P<0.05$).

This result is in line of [8] who reported that, eugenol is a major component (approximately 85%) of clove oil. Sub-lethal concentrations of eugenol have been found to inhibit production of amylase and proteases by *B. cereus*. Cell wall deterioration and a high degree of cell lysis were also noted. The hydroxyl group on eugenol is thought to bind to proteins, preventing enzyme action in *E. aerogenes*.

Figure 5. Untreated and treated Ras cheese wheels with clove oil after two month and stored at 15.5ºC

Figure 6. The fungal growth between Untreated and treated Ras cheese wheels with clove oil after two month and stored at 15ºC

3.4. Organoleptic Properties of Treated and Untreated Ras Cheese Wheels with Clove Oil during Storage Period

The organoleptic properties of Ras cheese depend mainly on the method of manufacture, type of milk used for cheese making, salt concentration added and the conditions of storage [2]. Data presented in Table 6 show the organoleptic evaluation of Ras cheese wheels during the storage period. Data revealed that treated and untreated cheese with clove oil significantly improved flavour, body and texture of the 2, 3 months old Ras cheese. The points given for flavour were the highest in cheese after three months. The corresponding scores at the end of ripening period were 58.25 and 58.20 out of 60 points. The present results agree with the results reported before [7], who found that the proteolysis products play an important role in the flavour of cheese by the contribution either directly to cheese taste or indirectly as precursors of flavour components.

Regarding the body and texture (Table 6), the aforementioned trend of results was also noticed. The corresponding scores at the end of the ripening period were differed significantly and reached average values of 28.55 and 28.95 out of 30 points, respectively. In general, an improvement in body and texture was noticed by the increase in ripening time for all cheese samples. This trend of results agrees well with that mentioned [7] for abundance cheese (French type). They were able to establish significant relationships between the texture's properties and the rheological properties linked firstly to the fatty acids composition and proteolysis and secondly to the pH of mature cheese.

Insignificant differences were observed for the "appearance" of the fresh cheese as shown in Table 6, whereas at the end of the ripening period in cheese had a significant higher value being 9.15 and 9.10 points out of 10 for Ras cheese wheels with untreated and treated samples, respectively.

The results showed that the total scores of Ras cheese wheels were 61.87 and 88.29% for fresh Ras cheese wheels and after salting, respectively. The total score increased in Ras cheese wheels stored at temperature (15.5ºC) during three months. At the end of three months storage, the total score was 95.95 and 96.25 for Ras cheese wheels without oil and in Ras cheese wheels painted with clove oil after salting, respectively. The organoleptic properties scores in the untreated and treated Ras cheese wheels after the 3rd month of ripening were higher than those in the treated and untreated Ras cheese wheels in the previous months. Similar trends were obtained [22].

Table 6. Organoleptic properties of Ras cheese during storage

Storage periods			Organoleptic properties			
			Flavor (60)	Body and Texture (30)	Appearance (10)	Total (100)
Salting		0	35.55	19.64	6.68	61.87
		1.5st	54.32	25.70	8.27	88.29
Ripening	1st	Untreated	55.65	26.85	8.95	91.45
		Treated	55.54	27.21	8.75	91.50
	2nd	Untreated	56.75	28.35	8.90	94.50
		Treated	57.55	28.85	8.99	95.39
	3rd	Untreated	58.25	28.55	9.15	95.95
		Treated	58.20	28.95	9.10	96.25

4. Conclusions

Generally, it was observed that, there was no difference between Ras cheese wheels which treated with clove oil and untreated in the organoleptic properties. At the same time, the treatment of clove oil reduced the fungal growth. This observation, encourage to use this oil for the further studies

and it will be used in the commercial scale, as well as, this oil considered an antifungal edible coatings oil and improve the shelf life of cheese.

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