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The Effect of Adding Microbial Transglutaminase Enzyme to Cheese Milk on the Quality of Halloumi-Like Cheese Made From Recombined Skimmed Milk Powder

Hamad, M.N.F.*, Abdel-Kader, Y.I., Hoda F. El-Ashrey****

**Department of Dairying, Faculty of Agriculture, Damietta University*

***Dairy Technology Department, Animal Production Research Institute, Agriculture Research Center, Dokki, Giza, Egypt*

Abstract

The effect of adding microbial transglutaminase enzyme (MT-Gase) to the recombined skimmed milk (SMP) in the production of proper Halloumi - like cheese, three treatments was applied. (P) control cheese without enzyme, P1= 0.5 gm enzyme per liter cheese milk while P2= 1.0 gm enzyme per liter of cheese milk. The three treatments were traditionally processed into Halloumi-like cheese which preserved in 13% brine for 60 days. The intervals of taking the samples were at zero time, 15, 45 and 60 days periods. Samples were chemically, rheologically and organoleptically analyzed. The results indicated that adding MT-Gase to the curd of the milk increased the yield, improved rheological aspects, and markedly improved the organoleptic characters of the cheese. The best treatment was P1 treatment, including 0.5 gm MT-Gase per 1 liter cheese milk.

Keywords: Halloumi cheese, Microbial transglutaminase enzyme, Recombined skimmed milk powder.

1. Introduction

Halloumi cheese is one of the most favorable cheese in the Arab world. The native land of making the cheese of sheep and goat milk in Cyprus. It is a semi-hard rindless cheese kept in 13% brine. The industry of cheese has been qualified by Anifantakis and Kaminarides (1983). Nowadays it is processed from cow, buffalo, and admixture of milk. Ashour Naema (2018) succeeded in making Halloumi cheese from cow, buffalo, and admixture of them, also she inspected adding lecithin as emulsifier for improving its quality. MT-Gase enzyme is recently produced under commercial scales, it has the capability to bind the terminals of amino acids of different proteins forming the bridge covalent bonds which leads to enhance the functional properties of the protein. Better quality of yoghurt and soft cheese, with better rheological properties were obtained when the MT-Gase was applied (Ozer, et al., 2007).

Saadat Sohaila (2017) found that the use of enzyme with 1.5% SMP increased the yield and improved the quality of the cheese as compared with SMP without enzyme. MT-Gase enzyme had the ability to make the bridge covalent bonds between the terminal residues of certain amino acids, highly improving the physical and functional properties of many protein foods.

The advantages of MT-Gase treatments was proofed trough enhancing the characterized of yield, water holding capacity (WHC), texture, rheology, and sensory properties of cheese, without changing in its chemical composition (Gharibzahedi et al., 2018, Romeih and Walker, 2017). Reactions in the process of making cheese was catalyzed by MT-Gase, the functional properties of proteins, such as solubility, water holding capacity, emulsifying capacity, foaming, viscosity, elasticity, and gelation were markedly modified the catalyzing act of the MT-Gase

*Corresponding author e-mail: dr_mnour@du.edu.eg; (Hamad, Mohamed Nour-Eldin Farid Farid).
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(Martins et al., 2014, Wang et al., 2018). The formation of intra- and intermolecular covalent crosslinks that lead to enhancement in the yield, texture, rheology, and sensory properties, was influenced by MT-Gase inclusion in cheese (Gharibzahedi et al., 2018). The main objectives of this study were to determine the proper amount of MT-Gase and its rate of addition either 0.00, 0.50 or 1.00 / gm per one liter of cheese milk, on the yield, chemical, rheological and organoleptic properties of the resultant Halloumi cheese.

Materials and Methods

1 - Materials

1.1. Skimmed milk powder: it produced by Dairy America/California USA, and it was reconstituted as 10% total solids for cheese milk.

1.2. Milk protein concentrate (MPC) 70% total protein was MPC was imported from USA.

1.3. Dehydrogenated kernel Palm oil (melting point 42±1°C) was imported from Malaysia.

1.4. Tri-sodium citrate (TSC) was imported from Germany.

1.5. Yoghurt starter (*Streptococcus salvarius sub sp. thermophilus, Lactobacillus delbreuckii sub sp. Bulgaricus*) and Yo-Mix 495 LYO 250 DCY culture were obtained from Hansen's Lab, Denmark.

1.6. Rennet: Animal calf rennet (Al-Malekah) trademark was bought from Damietta local market (30 ml/100 kg milk).

1.7. Kitchen salt (Sodium Chloride) was bought from EL-Nasr Company Alexandria, Egypt.

1.8. Microbial Transglutaminase: Ca+2-independently MT-Gase from *Streptoverticulum mobaraense* (Activa®YG) obtained from Ajinomoto foods Europe SAS, France. it contained glutathione (GSH) at the ratio of 8% and its specific activity was 100 U/1gm of powder.

2 - Methods

2.1. Experimental procedures

2.1.1. Preparation of control sample:

The powder milk was added to hot water (50-55°C) by the ratio of 1:10 liter. Then, 3.5 % palm oil was added and 1.0% MPC was sprinkled. The admixture was stirred well in a blender with a velocity of 1300 rpm for 40 minutes to get a homogenous recombined milk. A representative sample was taken for chemical analyses. 0.02% Calcium Chloride and 3% starter were added up to the homogenous milk at 35°C. The cheese milk was left for 40 minutes in order to develop its acidity. Rennet was add up to the cheese milk and left for 30-40 minutes in order to obtain complete coagulation. The coagulum was cut into 1×1×1 cm pieces by American knives.

2.1.2. Manufacture of Halloumi cheese by the addition of two levels of Transglutaminase enzyme:

The milk was heated up to 63°C/15 minutes, and cooled down to 35°C. Then milk was inoculated with starter culture at the level of 3% (w/v) and held until the pH 5.5 was attained. At this stage rennet was added to the cheese milk. According to Özer et al. (2013) Transglutaminase enzyme was added after 5 minutes from the addition of rennet to give the chance for renneting. Coagulation was completed, and the curd was cut into 1×1×1 cm pieces, then the curd was leave for 2 minutes until the cheese whey was separated by squeezing. The manufacturing steps are shown in the following chart.

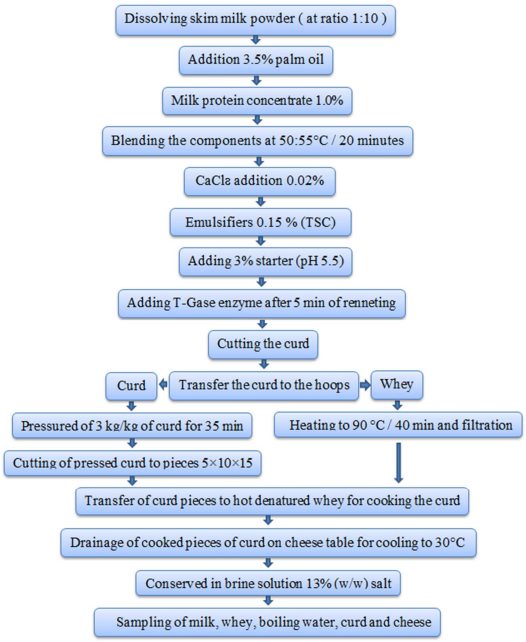

Figure 1: Flow diagram showing steps undertaken in the Halloumi cheese making from skimmed milk powder with 3.5% palm oil by the addition of Transglutaminase enzyme

2.2. Cheese Yield:

Actual cheese yield was calculated as the ratio of mass of the cheese obtained on the mass of the milk used in its preparation the cheese expressed in kg. L-1(100kg) of milk whereas adjusted cheese yield (at 52% moisture content) was calculated by the

following formula as (Shakeel et al., 2003).

Adjusted yield (kg. L^{-1}(100kg) = 100 – Moisture actual level divided by 100 – Moisture desired level X 100 (Actual cheese yield)

2.3. Chemical analysis:

2.3.1. Moisture content, Titratable acidity (TA), pH value and fat content for milk and cheese were described by AOAC (2000).

2.3.2. Salt content was determined by Kosikowski (1970).

2.3.3. Ash content: It was determined using the electric muffle furnace at 550ºC as described by AOAC (2000).

2.3.4. Total nitrogen (TN) and Soluble nitrogen (SN) content of milk, cheese, whey and boiling water were analytically described by Kjeldahl according to Ling (1963).

2.3.5. Total volatile fatty acids (TVFAs) content were determined as described by Kosikowski (1978). (TVFA) value was expressed as ml of NaOH N/10 per 100gm of cheese.

2.4. Textural Characteristics of the cheese:

Experimental cheeses were estimated by texture analyzer Lab pro (FTC TMS-pro, USA). The cheese samples presented to the instrument were each of 30 mm diameter and 20mm elevation. A TA15-45° Perspex cone was used as a plumbline with a permeation of 10 mm at 1mm/s. Samples were permitted to be in equilibrium at room temperature for around 30-45 min. before testing. Textural characteristics were described by Kosikowski (1982) and Kpodo et al. (2014), in which the following terms are shown as follow:

- Hardness is the force required to achieving changing in shape or distorting.
- Cohesiveness is the force of interior bonds making up the cheese shape.
- Springiness is the ratio of distorted material returns to its main shape at removal of the distorting force.
- Adhesiveness is the strength in demand to pull plumbline from sample.
- Chewiness is the power required to chew the food product to a state where it is ready for swallowing: it is expressed in the terms of hardness × cohesiveness × springiness.
- Gumminess is the power required to disintegrate a semisolid food for swallowing.

2.5. Organoleptic Evaluation: Samples from fresh and stored Halloumi cheese were organoleptically scored by ten panelists experienced in the evaluation of dairy products. The panelist scored for the flavor (50 points), body and texture (35 points), and appearance (15 points) as described by Nelson and Trout (1981).

Results and Discussion

Effect of adding MT-Gase on the Property of Halloumi – Like – cheese:

Table (1) showed the composition of compound cheese milk. In Table (1); results revealed that TS of (P1) are higher than (P), this may be due to the addition of MT-Gase. It is clear that protein, P/DM, and casein are higher for (P1) treatment as compared with (P) treatment. Fat percent was adjusted at 3.5% for the standardized treatments. Casein and casein/fat ratio values were higher for (P1) treatments as compared with (P) treatments. These values are closer to those recorded by Han and Spradin (2000), they found that the addition of MT-Gase had improved the efficiency of protein retaining. Similar trend observed with Naeim et al. (2012) and Othman et al. (2015) and Ossman (2018).

Table 1
Chemical composition of recombined cheese milk

Type of milk	pH	TS%	Fat%	Fat/DM%	Protein%	Protein/ DM%	Casein%	Casein/ Fat ratio	Ash%
P	6	11.9	3.5	29.4	3.1	26.05	2.449	0.70	0.86
P_1	6.4	12.8	3.5	27.3	3.8	29.69	3.002	0.86	0.88

P: Skim milk Powder 1:10 with 3.5% Palm oil + 1.0% MPC
P1: Skim milk Powder with 3.5% Palm oil +1.0 % MPC = (Recombined milk) + MT-Gase

Table (2) deals with the gross chemical composition of cheese milk using 0.15% Tri sodium citrate as emulsifier. The three types of cheese milk P, P1 and P2 were processed into Halloumi cheese, following the standard method of processing. Results indicated that, the treatment with MT-Gase to recombined milk decreased TS, Fat and protein loss into the whey and the boiling water. This is due to the action of the enzyme in binding water, lipid, and proteins into cheese. As the concentration of enzyme increased the loss in the content of whey and boiling water decreased. F/DM of the whey were (12.08, 6.71 and 5.32%) for P, P1 and P2 treatments respectively. Respective values for protein loss per dry matter (P/DM) were (23.31, 20.94 and 21.27%) for P, P1 and P2 treatments, respectively. Similarly, F/DM of the boiling water were (37.99, 22.31 and 11.36% for P, P1 and P2 treatments) respectively.

El-Kholy, et al. (2020) estimated that TS%, Fat%, TP% of whey drained from cheese of different treatments as affected by MT-Gase treated milk, as the amount of MT-Gase increased, the loss of TS, fat, and protein in the whey decreased. While Mostafa heba (2020) concluded that the treatment with MT-Gase and rennet reduced the curd strength and cheese hardness, also increased the loss of proteins, and fat in the whey. Also, she concluded that the best way to add the enzyme was later than coagulation and curd cutting. MT-Gase led to various changes in cheese like increasing the amount of protein in curd and decreasing protein and TS in the whey. Also, Sayadi et al. (2013) showed that transglutaminase-catalyzed crosslinking of whey proteins in cheese yield, it has been anticipated to support the protein network of cheese and herewith holds more serum within the cheese yield.

Table 2
Effect of adding MT-Gase enzyme to cheese milk on the gross chemical composition of whey and boiling water

Components / Treatment		Total solid %	Fat %	Fat / DM %	Total Nitrogen%	Total Protein%	Total Protein /DM %	Ash %
P	**Whey**	6.620	0.8	12.08	0.302	1.927	29.11	0.388
	Boiling water	1.053	0.4	37.99	0.054	0.345	32.72	0.064
P_1	**Whey**	5.960	0.4	6.71	0.189	1.206	20.23	0.298
	Boiling water	0.8965	0.2	22.31	0.042	0.268	29.89	0.048
P_2	**Whey**	5.642	0.3	5.32	0.165	1.053	18.66	0.224
	Boiling water	0.880	0.1	11.63	0.038	0.242	27.55	0.046

P: Control (without MT-Gase enzyme)
P1: 0.5 gm. MT-Gase enzyme per one liter of cheese milk
P2: 1.0 gm. MT-Gase enzyme per one liter of cheese milk

Effect of Microbial Transglutaminase on the total solids, moisture of Halloumi cheese:

Table (3) showed that adding MT-Gase to cheese increased TS contents of cheese. As the amount of enzyme

increased TS of the cheese increased. As the storage period advanced TS of cheese gradually increased. Total solids of fresh / 60 days old cheese values were (44.77/50.05%), (47.13/52.88%) and (49,79/55.08%) for P, P1 and P2 respectively. Moisture decreased clearly as the storage period progressed, respective values of fresh/60 days old were (55.23/49.95%), (52.87/47.12%) and (50,21/44.94%) for P, P1 and P2. The increase of the ratio of total solids owing to increasing protein, fat, and ash content in the cheese yield. This was caused by adding the MT-Gase enzyme. Similar trend have been reported by Gómez et al. (2020) who recorded that moisture value was significantly increased with MT-Gase addition. Similar results obtained by El-Metwally (2017) who concluded that recombined milk (SMP + 3.5% palm oil) of fresh cheese had 46.14 % T.S, when 1.0% MPC was added the total solids increased to reach 48.10%.

Yagoub et al. (2016) they reported that (TS) of Mozzarella cheese is affected by palm oil and the period of storage. TS of cheese raised with the advance in the period of storage. Sample with 3% palm oil reported the highest TS (51.36%) comparison with values 2% (50.05%), 1% (48.45%), and 0% palm oil (46.50%). Also, Karzan et al. (2016) showed that the treatment with MT-Gase led to increase dry matter in cheese yield. MT-Gase caused a significant ($P<0.05$) increasing in protein and TS in cheese (Desá and Luiz, 2010). In this field, EL Sharaihy (2008) concluded that adding SMP or WPC to cheese milk led to increasing in TS of soft cheese either when fresh or after ripening. Increasing SMP or WPC ratio led to highly increasing in TS of the cheese.

Effect of MT-Gase amount on Fat and Fat/DM of the cheese:

Any type of cheese affected by fat content, it can also affect and be responsible for the smoothness and hardness of the body and texture. The addition of MT-Gase enzyme to recombined milk slightly increased the values of fat content during storage period. Respective values for Fat/DM of fresh/60 days old were (41.54/40.96%), (40.31/39.90%) and (39,96/39.47%) for P, P1 and P2 respectively.

El-Kholy, et al., (2020) concluded that with increasing the level of MT-Gase led to increase in (Fat/DM) content as compared with control Kashkaval cheese, also It was noticed that F/DM content of all cheese treatments increased significantly ($P\leq 0.05$) as storage period advanced. Also, Ashour naema (2018) illustrates changes of different values of fat contents of Halloumi cheeses. Fat content increased for all treatments as ripening period advanced. Osman (2018) found that the addition of n to soft white cheese affected the values of fat content compared to the control cheese. Also, Gemici and Onek (2017) illustrated that adding MT-Gase to half-fat Kashar cheese at level 2 U g1 was without effect excepting fat and TS.

Effect of MT-Gase amount on total protein and P/DM of Halloumi cheese

The previous studies showed that when MT-Gase added to cheese milk, protein content of cheese increased, and there were a positive relationship between concentration of MT-Gase and the protein content. It is well known that the main activity of the MT-Gase is the formation of bridge cross linking between the residues of amino acids. It seems that the MT-Gase tied a part of whey proteins with the casein. These results are in agreement with Ossman (2018), Gazar (2007), Mahmood and Sebo (2009), Naeim et al. (2012), Othman et al. (2015) and Saadat (2017).

P/DM values were of fresh / 60 days old were (44.60/43.60), (45.62/45.25) and (46,39/46.93) for P, P1 and P2 respectively. The crosslinking reaction catalyzed by MT-Gase enhances the texture, also the nutritional properties, essential amino acid content, and protein efficiency ratio of the cheese (EL-kiyat et al., 2021). in addition, Mostafa heba (2020) reported that proteins have the best importance on the forming of network structures specifically for fermented dairy products. The present results were in line with Ashour Naema (2018) who stated that the protein content gradually increased the apparent increase is owing to the increase in total solids. Yang, et al. (2016) mentioned that MT-Gase crosslinking is a helpful way to improve the physical functionality of protein. In addition, Sandra et al. (2011) found that Halloumi type cheese prepared from Skimmed Goat Milk and UFGM was observed with significantly ($P<0.05$) higher protein content than other variants which indicates that ultrafiltration increases the protein retention in cheese However, the protein retention decreased with increasing fat content and UFGM-4% and 5% possessed significantly similar ($P>0.05$) protein to goat milk cheese.

Table 3
Effect of MT-Gase enzyme amount on TS, Fat, F/DM, P and P/DM% of Halloumi cheese during 60 days of storage

Properties (%)	Storage Period (days)	Treatments		
		P	P_1	P_2
TS	Fresh	44.77	47.13	49.79
	15	46.01	48.99	51.25
	30	47.74	50.60	52.71
	45	49.03	52.04	54.07
	60	50.05	52.88	55.06
Moisture	Fresh	55.23	52.87	50.21
	15	53.99	51.01	48.75
	30	52.26	49.40	47.29
	45	50.98	47.96	45.93
	60	49.95	47.12	44.94
Fat	Fresh	18.60	19.00	19.9
	15	19.10	19.64	19.75
	30	19.65	20.20	20.28
	45	20.12	20.78	20.86
	60	20.50	21.10	21.18
Fat/DM	Fresh	41.54	40.31	39.96
	15	41.51	40.09	38.54
	30	41.16	39.92	38.47
	45	41.04	39.93	38.58
	60	40.96	39.90	38.47
Protein	Fresh	19.97	21.50	23.10
	15	20.22	22.33	24.24
	30	20.93	23.03	24.88
	45	21.44	23.54	25.46
	60	21.82	23.93	25.84
Protein/DM	Fresh	44.60	45.62	46.39
	15	43.94	45.58	47.30
	30	43.84	45.51	47.20
	45	43.73	45.23	47.09
	60	43.60	45.25	46.93

P: Control (without MT-Gase enzyme)
P1: 0.5 gm. MT-Gase enzyme per one liter of cheese milk
P2: 1.0 gm. MT-Gase enzyme per one liter of cheese milk

Cheese yield:

Estimating the yield of cheese depends on the fat and casein of milk as well as the moisture content of the cheese. Consumers always search for high quality cheese while processor are very keen about the yield which translated into money. Table (4) deals with the yield of the three cheese treatments (P, P1 and P2) when fresh, 15, 30, 45, and 60 days of storage. MT-Gase adding slightly increased the yield of fresh and 60 days old cheese. Also, losses in cheese weight during storage was the lowest for enzyme treatments (P1 and P2), being 10.30, 11.40 and 12.31 for fresh cheese of P, P1and P2. Respective values for 60 days old cheese were 8.60, 9.61 and 10.37%, respectively.

The importance of MPC in quality and cheese yield:

Also, in this field El-Metwally (2017) stated that recombined milk with MPC gave 10.5 % yield higher than its fresh cow counterpart, Addition of 1.0% MPC highly increased the yield to reach 11.60 % for Mozzarella cheese, this is because MPC is a casein donor and partly acted as emulsifier. The addition of emulsifier partly increased the cheese yield. The highest was for 0.15 % TSC. The addition of MPC to recombined milk raised the yield from 10.5 to 11.60 %. Cheese yield is one of the most important economic aspects of cheese manufacturing. In this field, the content of fat and casein in the milk used to make any type of cheese is the main factor in the production efficiency and quality of cheese (Mona, et al., 2011).

Role of MT-Gase in yield of the cheese:

El-Kholy, et al. (2020) recorded that cheese milk treated with 0.7 g/L MT-Gase (T5) had higher ($P \leq 0.05$) yield than control low fat Kashkaval cheese and other treatments treated with MT-Gase. Also, Ossman (2018) recorded significant increase in the actual and adjusted yields when the addition of MT-Gase 10 minutes after renneting. The yield was increased as increasing the level of MT-Gase up to 80 units. The addition of MT-Gase 15 minutes after renneting led to markedly increase in the actual and adjusted yields, the yield was increased as increasing of the level of MT-Gase up to 80 units. In this field,

Desá and Luiz (2010) showed that the impact evaluation of MT-Gase on the properties of cheese curd, noticed that the sample making by added MT-Gase seven minutes after the rennet addition recorded the greatest result in terms of forming cheese curd. Statistical analysis showed difference of MT-Gase concentration on firmness of soft-white cheese curd. However, Özer et al., (2007) reported that MT-Gase increase the cheese yield and decrease synereseis. For all treatments, actual yield markedly increased during the first month of storage in yogurt treatments.

Mahmood and Sebo (2009) and Cozzolino et al. (2003) investigated that Adding MT-Gase before rennet improves the yield and properties of the cheese, but when adding MT-Gase and rennet together the protein, fat content, hardness, and curd strength decreased in the whey. In addition, Lauber et al. (2000) treated raw skim milk with MT-Gase and reported that the yield of yogurt increased.

Table 4
Effect of MT-Gase enzyme amount on the yield of resultant cheese during storage period (60 day)

Treatments	P	P_1	P_2
Yield of fresh cheese %	10.30%	11.40%	12.31%
Yield of ripened cheese (60 day) %	8.60%	9.61%	10.37%
Losses of yield %	15.68%	13.21%	12.01%

P: Control (without MT-Gase enzyme)
P1: 0.5 gm. MT-Gase enzyme per one liter of cheese milk
P2: 1.0 gm. MT-Gase enzyme per one liter of cheese milk

Effect of MT-Gase enzyme amount on titratable acidity, pH, salt, and ash of Halloumi cheese:

Titratable acidity (TA) and pH:

From table (5) MT-Gase addition had no noticeable effect on TA and pH values. Meanwhile the TA values increased, and pH decreased during the storage period. The relation between them is reversible.

In this field, Pham et al., (2021) reported that enzyme-treated fresh cheese had a slight decrease in TA values during the first week of storage. Increase in TA values during storage. Actually, this reflected the formation of isopeptide bonds between γcarboxamide groups (-(C=O) NH_2) of glutamine residue side chains and the ε-amino groups (-NH_2) of lysine residue side chains with subsequent release of ammonia (NH_3). After one week, the TA of these samples was increased. TA of all products increased after 28 days of storage.

It was noticed that there was a continuous increase in acidity and a decrease in pH values during the storage period, this mainly because the evolution of lactic acids by lactose fermentation by starter culture bacteria El-Kholy, et al. (2020). Similar results have been reported by Ossman (2018) illustrated that MT-Gase adding to cheese milk, did not significantly influenced by the acidity and PH values. PH values of different cheese took the opposite trend of acidity; the MT-Gase had no effect on salt content. Also, Saadat Sohaila (2017) observed that as the storage period advanced, acidity values slightly increased, although the cheese was kept at frozen temperature. The addition of enzyme to cheese milk had no marked effect on acidity values of cheese.

Salt and Ash content:

Salt and ash % contents very related to each other. The MT-Gase enzyme increases the values of salt and ash slightly.

Ossman, (2018) concluded that the changes in contents of salt% and ash% of different cheeses as affected by addition of MT-Gase. This apparent increase of salt is due to the increasing in DM. The same trend of results was also noticed by Gazar (2007), Ozer et al. (2013) and Othman et al. (2015). The salt level increased to range between 2.358 to 3.132% by advancing the storage period.

Concerning Ash content of cheese not affected by MT-Gase. Similar to salt content, as the storage period advanced, the ash content was also increased. The analysis of variance showed that the percentage of MT-Gase had not affected on salt and ash of soft white cheese, while the difference was found for salt and ash during storage period for each treatment Gazar (2007), Othman et al. (2015).

Saadat Sohaila (2017). The addition of MT-Gase to cheese milk had not marked effectively on salt content, which is affected by the moisture content, salt content ranged between 1.60 and 1.75%. This result is agreed with Gazar (2007).

Table 5
Effect of MT-Gase enzyme amount on the TA, pH, ash and salt contents of Halloumi cheese through 60 days of storage

Properties	Storage Period (days)	Treatments		
		P	P_1	P_2
TA %	Fresh	0.720	0.730	0.740
	15	0.750	0.760	0.780
	30	0.770	0.780	0.800
	45	0.810	0.810	0.830
	60	0.820	0.830	0.850
pH	Fresh	5.400	5.380	5.300
	15	5.355	5.335	5.275
	30	5.320	5.300	5.240
	45	5.290	5.260	5.210
	60	5.250	5.225	5.190
Salt %	Fresh	2.358	2.531	2.632
	15	2.731	2.742	2.842
	30	2.900	2.955	2.973
	45	3.080	3.104	3.124
	60	3.132	3.152	3.266
Ash %	Fresh	3.124	3.380	3.442
	15	3.243	3.562	3.700
	30	3.543	3.696	3.860
	45	3.665	3.898	3.908
	60	3.876	3.980	4.056

P: Control (without MT-Gase enzyme)
P1: 0.5 gm. MT-Gase enzyme per one liter of cheese milk
P2: 1.0 gm. MT-Gase enzyme per one liter of cheese milk

Effect of MT-Gase enzyme amount on the Total nitrogen, Soluble nitrogen of the cheese:

Table (6) shows that the effect of adding MT-Gase to the cheese milk samples increased the TN owing to increase in protein ratio, the TN values was (3.13, 3.37, 3.62%) and (3.42, 3.75, 4.05%) for fresh and during 60 days storage period for treatment P, P1 and P2 respectively.

The WSN content of cheese which is regarded as a measure of proteolysis during storage, as the unites of MT-Gase increased the amount of WSN continuously decreased for all treatment respectively. Values were (0.27, 0.25, 0.24%) and (0.41, 0.37, 0.33%) for fresh and during 60 days storage period for treatment P, P1 and P2 respectively. Comparable results have been reported by El-Kholy, et al. (2020) who recorded that adding of MT-Gase at all levels were increased significantly TN, in comparison with control full-fat and control low-fat Kashkaval cheese. The TN content increased during storage among all treatments. This increase might be due to the loss of moisture content during the ripening.

El- Aidie et al. (2019) stated that the higher SN in cheese made with fat replacer may be attributed to excessive protein breakdown occurring through the growth of cheese microflora and proteolytic enzyme activity that eventually associate with extra moisture retained by fat replacers. In this field, Saadat Sohaila (2017) reported that the incorporation of enzyme to cheese milk slightly decreased the SN of cheese. This is due to the formation of bridge bonds and the confirmation of firm matrix decrease the tendency to protein degradation.

Fenelon et al. (2000) Explained that there was an inverse relationship between the extent of primary protein degradation and the fat content of cheese milk. Also, Motoki and Seguro (1998) cleared that MT-Gase enzyme stimulate an acyl-transfer reaction between the carboxyamide group of peptide bound glutamine residues (acyl donors) and an assortment of primary amines (acyl acceptors), overall amino group of lysine remains in proteins. In addition, Katsiari & Voutsinas (1994) illustrated that WSN and SN level in Feta cheese made from ewe's milk was decreased when comparable with control cheeses.

Effect of MT-Gase enzyme amount on the Ripening Index:

It has known that the increasing in Ripening Index (SN/TN) Indicates an increase in the settlement rate of the resulting cheese, SN/TN was taken as a measure of protein coefficient hydrolysis. SN/TN for fresh Halloumi cheese and during 60 days ripening were (8.63/ 7.41/ 6.62) and (11.99/ 9.87/ 8.15) for P, P1 and P2, respectively (as shown in table 5). In addition, Kavas et al., (2004) who reported that, the formal ripening index of palm oil cheese increased during the storage.

Effect of MT-Gase enzyme amount on the TVFA

TVFA is one of component of ripening indices, TVFA of different cheeses behaved to some extent similarly to SN Table (6) the progress of ripening

time led to increase the TVFA. On the other hand, the addition of MT-Gase increased the TVFA, as the percentage elevated the TVFA increased. The values increase from (6.90, 7.20, 7.90) to (12.88, 12.04, 12.76) ml NaOH (N/10) /100gm cheese for the treatments P, P1and P2 respectively during the 60 days period storage.

El-Kholy, et al., (2020) found that total volatile fatty acids (TVFA) affected with fat content of the cheese, thus control full-fat cheese had the highest value among all other treatments while fresh or during storage progress. These results due to its higher fat ratio than the low-fat cheese treatments. Low-fat Kashkaval cheese containing MT-Gase exhibited higher ratio of TVFA in comparison with control low-fat Kashkaval cheese. Also, TVFA content increased while increasing the MT-Gase ratios added. Similar to the results of Ashour naema (2018) found that as the ripening time advanced on Halloumi cheese, TVFA values are gradually increased. TVFA values of 60 days old cheeses were 9.3, 8.3, 8.7, 9.3 and 9.4 ml NaOH (N/9) /100gm Halloumi cheese, it seem that type of starter had no marked effect on TVFA.

Table 6
Effect of MT-Gase enzyme amount on TN, SN, SN/TN and TVFA of Halloumi cheese during 60 days of storage

Properties	Storage Period (days)	Treatments		
		P	P_1	P_2
TN%	Fresh	3.13	3.37	3.62
	15	3.17	3.5	3.8
	30	3.28	3.61	3.9
	45	3.36	3.69	3.99
	60	3.42	3.75	4.05
WSN%	Fresh	0.27	0.25	0.24
	15	0.33	0.28	0.27
	30	0.37	0.31	0.28
	45	0.40	0.35	0.32
	60	0.41	0.37	0.33
SN/TN%	Fresh	8.63	7.41	6.62,
	15	10.41	8.00	7.11
	30	11.28	8.59	7.18
	45	11.90	9.49	8.02
	60	11.99	9.87	8.15
TVFA	Fresh	6.90	7.20	7.90
	15	8.68	8.56	9.42
	30	10.24	10.44	10.64
	45	11.84	12.02	12.14
	60	12.88	12.04	12.76

P: Control (without MT-Gase enzyme)
P1: 0.5 gm. MT-Gase enzyme per one liter of cheese milk
P2: 1.0 gm. MT-Gase enzyme per one liter of cheese milk
TVFA = ml NaOH (N/10) for 100gm cheese

Effect of MT-Gase enzyme amount on the textural properties of Halloumi cheese during 60 days of storage:

Table (7) represents the addition of MT-Gase enzyme enhances the textural properties such as:

Hardness (g):

Defined as a measure of the amount of force required to compress the sample of the cheese and related to the strength of the cheese matrix, also it is defined as a force required to attain a given deformation. After 60 days of storage at refrigerator hardiness values decreased, Fresh and 60 days old cheese had (3245.98/3650.27), (3340.60/3896.86), and (3432.44 and 3968.86 N.) for P, P1, and P2 respectively. Ziarno and Zaręba (2020) showed that adding MT-Gase 12 hours before fermentation led to increase hardness of the yoghurt treated with MT-Gase compared with the control yoghurt. The main effect of MT-Gase in cheese is to forming extra isopeptide bonds, produce a gel network with smaller aggregates and pore sizes (Imm et al., 2000). Ashour naema (2018) observed that for all Halloumi cheese treatment as the brining time advanced hardness values gradually increased, the rate of hardness increase was the highest for control cheese as compared with lecithin treatments since rate of increase was less, as the ratio of lecithin increased the rate of increase in hardness was less. Also, Mazuknaite et al. (2013) found that adding T-Gase in making cottage cheese without using rennet gives a better hardness than control cheese. Simillar results have been reported by, Darnay et al. (2017) reported that adding MT-Gase to semi-hard cheese produced increasing in hardness values. The present findings are agreed Yüksel and Erdem (2010) who noticed

that the treated Yogurt with MT-Gase led to increasing hardness through the storage period, while untreated samples remained without changing during ripening.

Springiness (mm):

Defined as the ratio of which a distorted material returns to its main form when removing the distorting strength Bourne, (1978) and Szczesniak et al., (1963). From table (6) it is clear that springiness values are higher in cheese made from milk treated with MT-Gase, Increasing the ratio of MT-Gase led to a noticeable increase in springiness values. On the other hand, the springiness of the control treatment reduced during the first 60 days of ripening. In this field Hebishy et al., (2021) showed that adding MT-Gase had a significant positive effect on the springiness of mozzarella cheese. Topcu et al., (2020) mentioned that adding MT-Gase led to change springiness ratio slightly during storage period, but the control cheese springiness ratio was clearly reduced more than the other samples. Also, García-Gómez et al. (2019) found that adding MT-Gase to cheese treatments made with chymosin led to significantly increased in springiness values.

Adhesiveness (g):

Defined as the negative area for the first bite, representing the work necessary to pull the compressing plunger away from the sample. From Table (7) Adhesiveness increased during storage period for all treatments, The addition of MT-Gase enzyme increased adhesiveness, Values of adhesiveness are (-50.77/-44.98), (-54.50/-46.85) and (-60.22 and -52.40 gm) for fresh and 60 days old cheese of P, P1 and P2 treatments respectively. The obtained data are in accordance with Hebishy et al. (2021) showed that adding MT-Gase had a significant positive effect on the adhesiveness of mozzarella cheese. Abou-Soliman et al. (2017) found that Using milk treated with MT-Gase, the adhesiveness values increased due to the increase in the cross-linking between the proteins, also a noticeable increase in the adhesiveness after h days of storage of the yogurt samples. In this field El-metwally (2017) who reported that the ratio of adhesiveness increased after 28 days of storage for all Mozzarella cheese samples. This increase may be due to the increasing of total solids of the cheese.

Cohesiveness (%)

Defined as the force of internal bonds forming the body of the product Bourne (1978) and Szczesniak et al. (1963).

From Table (7) It's clear that as the storage period decreased the Cohesiveness ratio decreased too, when MT-Gase was added. Our results are inconsistent with Ossman (2018) who found that as storage period advanced the cohesiveness ration decreased, the chemical and biochemical changes caused by the MT-Gase that determine the morphological and structural appearance of the cheese also had a clear effect on the moisture content and consistency of the cheese after 60 days of storage. The present results were in line with Ashour Naema (2018) who reported that the Cohesiveness ratio parallel increased until storage period ended in Halloumi cheese. Similar trend has been reported by El-Metwally (2017) who stated that Ratio of cohesiveness decreased when 3.5% palm oil was added to mozzarela cheese from recombined milk. The addition of 0.15% emulsifiers increased the ratio of a cohesiveness. Also, storage at 20°C for 60 days decreased the ratio of cohesiveness. The ratio of cohesiveness of buffalo recombined milk are less than SMP recombined milk. A noticeable increased Cohesiveness values were found with MT-Gase treatment compared to the control cheese such results are in agreement with Naeim et al. (2012), Othman et al. (2015) and Ozer (2013)

Gumminess (Kg):

Defined as the power required to disintegrate a semi-solid food for swallowing Szczesniak et al. (1963) and Bourne (1978). Addition of MT-Gase slightly increase the cheese values, the gumminess decreased from (686.92/644.60), (830.98/788.90) and (852.95/794.06) for P, P1 and P2 for fresh and 60 days storage respectively. Our data are in accordance with Ossman (2018) who noticed that adding MT-Gase to cheese milk, highly increased the gumminess of cheese to be 449.53, 704.74, 882.68 and 992.71 g/sec for LFC20, LFC40, LFC60 and LFC80, respectively. Also, Ashour Naema (2018) reported that the gumminess on Halloumi cheese defined as the product of hardness X cohesiveness.

Chang et al. (2011) concluded that increasing MT-Gase concentration, the hardness and gumminess ratio of soybean cheese was significantly increased. In addition, Zaky (2014) found that Gumminess value of fresh cheeses ranged between 207 and 531N the lowest was for control, while the highest was for 3% skim milk powder addition. In general, the addition of SMP increased gumminess. The storage period decreased the value of gumminess for all treatments.

Chewiness (kg X mm):

Defined as the power wanted to chewing a food product to a state where it is ready for swallowing: (Hardness × cohesiveness × springiness) Bourne (1978) and Szczesniak et al. (1963).

Chewiness values increased gradually by the addition of MT-Gase in fresh Halloumi cheese. On the other hand, values decreased by storage time from

(460.38/478.86/488.11) to (384.90/365.46/350.80) for P, P1 and P2 respectively. Our data is in accordance with Salinas-Valdés et al. (2015) found that the treatment with MT-Gase was significantly increased the level of chewing, also led to a slight structural improvement compared to the control treatment. García-Gómez et al. (2019) observed that adding MT-Gase to rennet, a significant increase was found in the values of hardness, chewing and springiness in the cheese samples.

Chewiness is known as the required energy amount to chew a solid food material that is associated to other textural properties including hardness, cohesiveness and springiness (Prakasan et al., 2015 and Salinase-valdes et al., 2015). In addition, Zaky (2014) found that adding SMP and vegetable oil increased chewiness. There is a relationship between chewing and hardness in cheese, as by increasing the hardness, the value of chewing increases (Beal and Mittal, 2000).

Table 7

Textural profile of cheese produced from different amount of MT-Gase enzyme on Halloumi cheese during 60 days of storage

Properties	Storage Period (days)	Properties		
		P	P_1	P_2
Hardiness (N)	**Fresh**	3245.98	3340.60	3432.44
	60	3650.27	3896.86	3968.86
Springiness (mm)	**Fresh**	0.5227	0.6246	0.6614
	60	0.4864	0.5685	0.5957
Adhesiveness (g)	**Fresh**	-50.77	-54.50	-60.22
	60	-44.98	-46.85	-52.40
Cohesiveness (Ratio)	**Fresh**	0.3125	0.3082	0.2281
	60	0.2890	0.2780	0.2648
Gumminess (N)	**Fresh**	686.92	830.98	852.95
	60	644.60	788.90	794.06
Chewiness (J)	**Fresh**	460.38	478.86	488.11
	60	384.90	365.46	350.80

P: Control (without MT-Gase enzyme)
P1: 0.5 gm. MT-Gase enzyme per one liter of cheese milk
P2: 1.0 gm. MT-Gase enzyme per one liter of cheese milk

Sensory evaluation as affected by the amount of MT-Gase enzyme on fresh Halloumi cheese and after 60 days of storage

Samples of fresh and 60 days representing all treatments of Halloumi cheese were judged by the 10 panelists, average of their opinions expressed as scoring points were tabulated in Table (8). (P) treatment gained less scoring point because of the poor flavor of SMP product as well the less quality of rheological aspects. the quality is less than (P1&P2) treatments with MT-Gase enzyme. The total scoring points increased as the storage time progress. Color and appearance of fresh cheese ranged between 7, 9 and 8 points out of 15 for P, P1 and P2 after 60 days of storage color and appearance slightly increased to 8, 10 and 10 points out of 15 for P, P1 and P2 (as shown in table 8).

Body & texture of the cheese affected by the addition of MT-Gase enzyme of fresh and 60 days old cheese. The storage slightly improved the body & texture, but still the values are nearer to control cheese, the addition of MPC improved the body & texture, most of judges gave higher scoring points for the fresh and 60 days old cheese because of the smoothness felt during evaluation. By the end of ripening, total scoring points were 73, 80and 78 points out of 100 for control, P1 and P2 respectively.

El-Kholy et al., (2020) concluded that adding MT-Gase to low-fat kashkaval cheese highly enhanced the flavor, body, texture, appearance, and overall acceptability. Results concluded that low fat Kashkaval cheese can be successfully made from milk treated with 0.7 g MT-Gase/L in order to obtain cheese of improved flavor, acceptable texture and good body. Also, Mostafa Heba (2020) concluded that continuously increased of MT-Gase addition led to further decline in yogurt flavor, coarseness, and overall acceptance.

Ossman (2018) showed that the organoleptic characteristics were influenced by the presence of MT-Gase soft white cheese and storage period, as well the appearance sequentially total scoring points decreased. During cheese processing and storage, proteins are changed by the action of rennet and other enzymes originally found in milk or produced by microorganisms. These changes cause the cheese body to become smoother and softer, and its flavor to become richer and fully ripened. It is well known that fat had a very rich flavor which satisfies the consumer as well it highly improves the texture of the cheese, MT-Gase adding to cheese milk coused a remarkable increasing in total score. Such results are in agreement with Saadat Sohaila (2017) who reported that flavor, as the SMP increased the flavor

decreased, because the fat content was decreased, the addition of enzyme improved the flavor because the improving the functional properties of the cheese and holding more moisture. However, Ahmed et al. (2015) recorded that addition MT-Gase enhanced the sensory properties of the resultant cheese specially texture and body was due to the increased ability to hold water and cross-linked protein molecules.

Table 8
Organoleptic properties of fresh and 60 days old cheese made from SMP recombined milk

Treatments	Properties							
	Appearance (15)	Body & Texture (35)	Flavor (50)	Total (100)	Appearance (15)	Body & Texture (35)	Flavor (50)	Total (100)
	Fresh				60 days			
P	7	17	32	56	8	30	35	73
P_1	9	25	35	69	10	32	38	80
P_2	8	23	34	65	10	31	37	78

P: Control (without MT-Gase enzyme)
P1: 0.5 gm. MT-Gase enzyme per one liter of cheese milk
P2: 1.0 gm. MT-Gase enzyme per one liter of cheese milk

Conclusion

Halloumi cheese a rind less semi-hard cheese is well known in Cyprus, Greece, and Middle East. Nowadays many Syrian people live in Egypt, they search for Halloumi cheese with its unique characters, taste, and flavor. No sheep or goat milk are abundant in Egypt. To produce cheaper cheese, SMP, Palm oil, Sodium citrate were tried to produce proper Halloumi cheese. Rheological properties are the most important character in order to rigid compact elastic cheese. Many researches pointed out the advantage of trying emulsifiers, for pasta filata cheese trials. Recently the production of MT-Gase led to use it successfully in the field of food processing. The amount of MT-Gase must be proper to each kind of food production. This research deals with MT-Gase application in cheese produced from recombined skim milk powder. Two concentration (0.5 or 1.0) gm of enzyme per one liter of cheese milk were tried (P1) and (P2) were compared with the control (P). The addition of the enzyme decreased protein, TS and fat loss into the boiling water and whey. In turn the yield of the cheese increased by MT-Gase addition in both the treatments P1 - P2.

Hardness, springiness, and cohesiveness values by the addition of MT-Gase. P1(0.5 gm enzyme / 1 liter of milk) gained the highest points of appreciation. The chewiness, flavor and texture were the best. It is recommended to apply only 0.5 gm enzyme to 1 liter of cheese milk to have high quality cheese with affordable cost.

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