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Effect of Glucono-δ-Lactone and Rennet on Improvement of Kareish Cheese Characteristics

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Abstract:

Kareish cheese was made by using 5% starter culture as control to study the effect of partial substitution of starter culture by Glucono-δ-Lactone (GDL) (0.5, 1.0 and 1.5 % in milk) on the fresh Kareish cheese properties and its whey. The study indicated that the total solids, acidity and total protein increased by increasing the GDL substitution ratio, and also increased the yield of fresh Kareish cheese till 1.0% GDL and then decreased with 1.5% replacement. The coagulation time was decreased by increasing the GDL from 239 minuets for the control to 216 minuets by using 1.5% GDL. The treatment which gave the best results was served as a control to study the effect of adding different rates of rennet (0.25, 0.50 and 0.75 ml rennet / kg milk). Results indicated that by increasing the rennet being added, an increase in both total solids and total protein were occurred , also slightly decreased acidity and resulted in the highest decease in coagulation time from 120 to 50, 44 and 31 minutes for 0.0, 0.25, 0.50 and 0.75 ml rennet, respectively. Also by increasing the added rennet, a decrease of Kareish cheese yield at 0.25 ml of 24.21% and an increase of 25.74% by adding 0.50 ml rennet, then decreased by adding 0.75 ml rennet to (21.01%). Sensory evaluation showed that Kareish cheese manufactured by 4% starter culture + 1% GDL + 0.5 ml rennet gained the highest total scores.

Keywords: Kareish cheese, Glucono-δ-Lactone , rennet and sensory evaluation

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Introduction:

Kareish cheese is one of the most popular soft fresh skimmed milk, lactic cheese. It contains most of the skim milk constituents including protein, small amount of sugar, some of water, soluble vitamins and most of the calcium and phosphorus (Abou-Donia, 2008). Kareish cheese is an acid coagulated white soft cheese made from skim milk with soft composition, white curd and slightly salty. It is one of the most popular cheeses consumed in Egypt and Arabian countries, especially the countryside owing to its high protein, low fat and price. This cheese is an excellent source of protein, amino acids, calcium, phosphorus, vitamins and many micronutrients. Environmental conditions prevailing during storage, combined with the composition of the cheese often create possibilities for extensive development of mould on cheese surface, which reduces considerably its quality. Therefore, Kareish cheese will be the most promising food to avoid the health problems associated with fat. The farm-house manufacture of this cheese depends on the removal of whey from natural acid coagulated skim milk using a cheese mat and dry salting. The production of Kareish cheese by this method needs at least 4-5 days, which markedly decreases its microbiological quality. Many modifications have been tried to improve cheese quality (Reps et al., 2002) and (Francois et al., 2004). Fahmi (1960) used combined acid and rennet coagulation and reported that it gave Kareish cheese a smooth texture and was more appealing than cheese made by the traditional method.

Glucono-delta-lactone (GDL) is an internal ester that spontaneously hydrolyzed to form gluconic acid with first-order reaction hydrolysis kinetics (de Kruif, 1997). GDL is the cyclic 1,5-intramolecular ester of D-gluconic acid. Milk acidification has been simulated by the use of GDL (Pablo Sebastia´n Rimada and Analı´a Graciela Abraham, 2006). Lucey *et al.*, (1997) used GDL as an acidogenic at 0.517 or 0.376% (w/v), giving pH values after 24 hours of approximately 4.2 and 4.6, respectively. Acidification was performed at three gelation temperatures, i.e. 20, 30 or 40°C. Gels were made from solutions with or without added NaCl.

The use of GDL avoids some of the difficulties associated with starter bacteria, such as variable activity and variation with the type of culture used. In addition, during gelation with GDL, the final pH of the system is a function of the amount of lactone added, where as starter bacteria produce acid until they are inhibited by the low pH (Lucey *et al.*, 1998).

Therefore, the present work carried out to elucidate the effect of adding glucono-delta-lactone and rennet to milk on the improvement of Kareish cheese qualities.

Materials and Methods:

Fresh buffalos' skim milk was obtained from Dairy Department, Faculty of Agriculture, Mansoura University. Skim milk composition was 0.26 ,10.62, 6.41, 9.50,5.06 and 3.51 % of fat, total solids, pH, solid not fat , lactose and total protein, respectively. Lyophilized starter culture of (*Str. thermophilus* and *L. delbrueckii subsp. bulgaricus*) was obtained from Hansen Laboratories – Denmark, and kept under suitable conditions until use, liquid calf rennet was obtained from local market (0.5 N), glucono - delta- lactone (GDL) is

a commercial product by Roquette Freres Company, Lille-France. All the chemicals used for different analyses in this study were of the highest purity. Kareish cheese manufactured according to Fahmi (1960). Chemical analyses of milk and whey (Total solids, fat content, total protein, S. N. F content and lactose percent) were determined by Lacto stare (made in Germany 2001).Cheese samples were taken for organoleptic properties, chemical and microbiological analyses at zero time. Total nitrogen using Kjeldahl method was estimated as described by Ling (1963). pH value of cheese was measured by using a digital pH-meter Janway 3010 – England. 20 gm of cheese samples were softened by mixing with the same amount of distilled water and the whole homogenous was left 5 minutes before measurement. Total solids were determined according to the British Standard Institution (B.S.I.) method (1955).Organoleptic properties of fresh Kareish cheese by ten trained panelists from the staff members of the Dairy Department of Faculty of Agriculture, Mansoura University by evaluating the each cheese sample and used a quality rating scores card for evaluation of flavor (50 points) ,body and texture (35 points) and color & appearance (15 points) as described by Nelson and Trout (1965). *Staphylococcus sp.* was counted by using Staphylococcus medium 110. The plates were incubated at 37°C for 24 -36 hours and examined for the appearance of orange colonies. Coliform bacteria were counted by using MacKonky agar. The plates were incubated at 37°C for 24 hours. Microbial groups as recommended in Standard Methods for the Examination of Dairy Products (Marshall, 1992). Statistical analyses of data were subjected to analysis of variance and the least significant differences (LSD) at 5% probability using Statistical Package for the Social Science (SPSS) software for windows. All determinations carried out in tri-replicates.

Results and Discussions:

Total solids, pH values and total protein of resultant fresh Kareish cheese were presented in Table (1) as affected by partial substitution of starter culture by glucono delta lactone. An increase of GDL % from 0.0% in the control with (5% starter culture) to 1.5% with (3.5% starter culture) resulted in a significant increase at ($p<0.05$) of total solids, pH and total protein to reach 25.75, 6.01 and 15.51, respectively. These results are in harmony with those obtained by Abd El-Gawad *et al,.* (2011) which demonstrated that adding of glucono-δ-lactone, (GDL) alone to Mozzarella cheese milk decreased moisture more than using GDL with yoghurt starter as acidulate. This finding was in contrast of those obtained by (Ismail *et al.,* 2007) who reported that pre-acidification of milk with GDL decreased TS, and TN values of the resultant Mozzarella cheese. This might be due to rapidly hydrolyzed to gluconic acid and resulted in a rapid reduction of the pH during the first 1000 min, after which the pH decreased steadily (Lucey *et al.*, 1998) and (Braga *et al.*, 2006).

Table1. Chemical characteristics of fresh Kareish cheese as affected by adding starter culture, GDL and rennet to cheese milk.

Compositions / Treatments	T.S%	PH	T.P%
	Starter		
S(control)	24.72[d]	5.31[d]	14.63[d]
S1	25.23[c]	5.35[c]	15.32[c]
S2	25.51[b]	5.38[a]	15.43[b]
S3	25.75[a]	6.01[b]	15.51[a]
Treatments	Rennet		
R (control)	25.51[d]	5.38[b]	15.43[d]
R1	25.63[c]	5.82[a]	16.30[c]
R2	25.71[b]	5.84[a]	16.48[b]
R3	25.74[a]	5. 87[a]	16.83[a]

Means with the same column with different superscript (a,b,c and d) are significantely different ($p< 0.05$).

S: Kareish cheese made with 5% starter .

S1: Kareish cheese made with 4.5% starter+0.5% G.D.L

S2: Kareish cheese made with 4% starter+1.0% G.D.L

S3: Kareish cheese made from 3.5% starter+1.5% G.D.L

R: Kareish cheese made from 4% starter+1.0% G.D.L

R1: Control +0.25ml rennet

R2: Control +0.5ml rennet

R3: Control +0.75ml rennet

Table (1) shows that by increasing the added rennet rate to the control which has 4.0% starter culture and 1.0% of GDL recorded the highest yield of cheese and the shortest coagulation time, and gradually increased the total solids , pH values and total proteins with 25.74, 5.87 and 16.83 % respectively, when 0.75 ml rennet was used. These increase is significant at ($p<0.05$).

Changes in both coagulation time and cheese yield of fresh Kareish cheese as affected by adding GDL in different percent was presented in Table (2). It could be observed that the coagulation time were 239, 216, 120 and 99 minutes for control (5% starter culture), S1, S2 and S3 respectively. The lowest coagulation time recorded for S3 treatment (3.5% starter + 1.5% GDL) with 99 mints. The decrease in the coagulation time are significant at ($p<0.05$). On the other hand, It has been observed that adding GDL caused an significant increase at ($p<0.05$) in cheese yield gradually from 21.48% for the control to 25.23% for S2 (4% starter +1.0%GDL), afterward, Kareish cheese yield % decreased to 23.73% for S3 (3.5% starter +1.5%GDL) .These results are in agreement with those obtained by (Abd El-Rafee *et al.,* 1998), who stated that the yield of Mozzarella cheese made using Mucor miehei rennet, was the lowest compared with using the other type of coagulants. Theses changes are significant at ($p<0.05$).

Table 2. Yield of kareish cheese and coagulation time as affected by adding of golocono-delta-lactone,rennet, and starter culture .

Compositions / Treatments	G.D.L %	Coagulation time(minutes)	Cheese yield %
	Starter		
S(control)	0.0 GDL	239[a]	21.48[d]
S1	0.5 GDL	216[b]	23.50[c]
S2	1.0 GDL	120[c]	25.23[a]
S3	1.5 GDL	99[d]	23.73[b]
Treatments	Rennet		
R (control)	0.0 ml rennet	120[a]	25.23[b]
R1	0.25ml rennet	50[b]	24.21[c]
R2	0. 5ml rennet	44[c]	25.74[a]
R3	0.75ml rennet	31[d]	21.01[d]

Means with the same column with different superscript (a,b,c and d) are significantly different ($p< 0.05$).

S: Kareish cheese made with 5% starter .
S1: Kareish cheese made with 4.5% starter+0.5% G.D.L
S2: Kareish cheese made with 4% starter+1.0% G.D.L
S3: Kareish cheese made from 3.5% starter+1.5% G.D.L
R: Kareish cheese made from 4% starter+1.0% G.D.L
R1: Control +0.25ml rennet / Kg milk
R2: Control +0.5ml rennet / Kg milk
R3: Control +0.75ml rennet / Kg milk

Data in Table (2) showed that adding the rennet in different rats caused a significant decrease at ($p<0.05$) of the coagulation time of Kareish cheese from 120 mints for control (4% starter +1.0%GDL) to 50, 44 and 31 mints for R1 (25 ml), R2 (0.5 ml) and R3 (0.75 ml) respectively. On the other hand, it was observed that adding of rennet (25 ml) decreased the cheese yield from 25.23 % for control to 24.21 % by adding 25 ml rennet, then it increased to 25.74 % by adding 0.5 ml rennet, and when adding excess of rennet, the Kareish cheese yield decreased to reach 21.01%. These changes significantly differ at ($p<0.05$).

Chemicals composition of whey output from Kareish cheese manufacture as affected by both partial substitution of GDL and adding rennet at different rates are shown in Table (3). Data in Table (3) showed that the total solids, PH values, solids not fat and total protein were decreased by increasing the GDL amount, while the whey lactose increased with decreasing GDL due to the fact that glucoic acid formed whenever it grew, the less activity rate of starter bacteria to produced lactic acid which increases lactose in whey

Theses results are not in agreement with those obtained by (Ismail et al;2007), who stated that whey of GDL Mozzarella cheese had higher TS and TN values than that from, cheese made without adding GDL, and are in agreement with those finding in pH values. It could also be observed from the same Table that the total solids%, solids not fat and total protein of whey decreased by increasing the rate of adding rennet. On the other hand, both of pH values and lactose percentage increased from 4.48 to 4.80 and from 1.03 to 0.89, respectively. The different among all treatments are significant at ($p<0.05$).

Table3. Chemical compositions of Kareish cheese whey as affected by adding starter culture, GDL and rennet

Compositions / Treatments	T.S%	pH	S.N.F%	Lactose	T.P%
	Starter				
WS(control)	3.91[a]	4.71[a]	2.90 [a]	1.03[d]	0.88[a]
WS1	3.52[b]	4.50[b]	2.86 [b]	1.14[c]	0.85[b]
WS2	3.42[c]	4.48[c]	2.56 [c]	1.21[b]	0.83[c]
WS3	3.21[d]	4.38[d]	2.38 [d]	1.24[a]	0.80[d]
Treatments	Rennet				
WR (control)	3.42[a]	4.48[d]	2.56[a]	1.21[a]	0.83[a]
WR1	2.81[b]	4.61[c]	1.78[b]	0.80[d]	0.81[a]
WR2	2.72[c]	4.69[b]	1.41[c]	0.85[c]	0.74[b]
WR3	2.42[d]	4.80[a]	1.23[d]	0.89[b]	0.64[c]

Means with the same column with different superscript (a,b,c and d) are significantely different ($p< 0.05$).

WS: Kareish cheese whey made with 5% starter .
WS1: Kareish cheese whey made with 4.5% starter+0.5% GDL
WS2: Kareish cheese whey made with 4% starter+1.0% GDL
WS3: Kareish cheese whey made from 3.5% starter+1.5% GDL
WR: Kareish cheese whey made from 4% starter+1.0% GDL
WR1: Control +0.25ml rennet / Kg milk
WR2: Control +0.5ml rennet / Kg milk
WR3: Control +0.75ml rennet / Kg milk

Data in Table (4) show the sensory evaluation of fresh Kareish cheese manufactured by different ratios of GDL, starter culture and rennet. From these data it could be observed that control (s) with 5% starter culture gained the highest score of color and appearance, followed by R2 (4% starter + 1% GDL +0.5 ml rennet), also both S2 with 4% starter culture + 1% GDL and R2 with 4%starter culture +1% GDL + 0.5 ml rennet gained the same and the highest score of body and texture with an average of 34 points. However the flavor out of 50 point recorded the highest score for R2 treatment with (4% starter culture + 1% GDL + 0.5 ml of rennet). Data also indicated that R2 had the highest total scores with an average of 94 points followed by S2 treatment with an average of 92 points, while the lowest total sensory scores were given to both S1 and S3 with an average of 65 points. These results are in agreement with those of Ismail et al. (2007) who reported that sensory evaluation of Mozzarella cheese made using a mixture of 0.25% yoghurt starter and 0.37% GDL gained the highest scores points for all types of milk treatments. Statistically there are no significant differences found between S1 and S3, also between S2 and R2.

Table4. Organoleptic properties of kareish cheese as affected by different percentage of starter, GDL and rennet.

Properties / Treatments	Color& Appearance (15)	Body &Texture (35)	Flavour (50)	Total (100)
Treatments	Starter			
S(control)	14^{a}	29^{b}	43^{b}	86^{b}
S1	8^{c}	25^{c}	32^{c}	65^{c}
S2	12^{b}	34^{a}	46^{a}	92^{a}
S3	8^{c}	25^{c}	32^{c}	65^{c}
Treatments	Rennet			
R (control)	12^{a}	34^{a}	46^{a}	92^{a}
R1	10^{b}	22^{c}	41^{b}	73^{c}
R2	13^{a}	34^{a}	47^{a}	94^{a}
R3	9^{b}	30^{b}	38^{c}	77^{b}

Means with the same column with different superscript (a,b,c and d) are significantely different ($p< 0.05$).

S: Kareish cheese made with 5% starter .
S1: Kareish cheese made with 4.5% starter+0.5% G.D.L
S2: Kareish cheese made with 4% starter+1.0% G.D.L
S3: Kareish cheese made from 3.5% starter+1.5% G.D.L
R: Kareish cheese made from 4% starter+1.0% G.D.L
R1: Control +0.25ml rennet / Kg milk.
R2: Control +0.5ml rennet / Kg milk.
R3: Control +0.75ml rennet / Kg milk.

Microbiological Analyses:

coliform bacteria and *staphylococcus sp.* not detected in resultant fresh Kareish cheese and whey in all treatments.

Conclusions:

Results of the present investigation are of practical value. The use of G.D.L for Kareish cheese maneufacture was advantageous due to improvement Kareish cheese properties and had high rate of dressing by added GDL and rennet to Kareish cheese milk. The results highlighted the possibility of S2 and R2 .The developed treatment was evaluated and proved to be of good quality, long shelf life and could be kept at 4°C without significant microbial growth or loss of the product color & texture during manufacture and storage of Kareish cheese.

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استخدام الجلوكونو- دلتا- لاكتون والمنفحة لتحسين خواص الجبن القريش

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تم تصنيع جبن قريش بإضافة ٥% بادئ كعينة مقارنة لدراسة تأثير إضافة جلكونو-دلتا-لاكتون علي صفات الجبن والشرش الناتج . تم استبدال جزئي للبادئ المستخدم بمادة جلكونو-دلتا-لاكتون بنسبة ٠,٥و١و١,٥% ولقد أوضحت الدراسة أن زيادة النسبة المستبدلة من جلكونو-دلتا-لاكتون أدت إلي زيادة الجوامد الصلبة الكلية والحموضة و البروتين الكلي.كما ادي ذلك الي زيادة نسبة تصافي الجبن الناتج حتى نسبة استبدال ١% وبعدها حدث نقصان عند نسبة استبدال ١,٥% كما أدي ذلك إلي انخفاض ملحوظ في زمن التجبن من ٢٣٩ دقيقة لعينة المقارنة إلي ٩٩ دقيقة لنسبة الاستبدال ١,٥% جلكونو-دلتا-لاكتون . أيضا تمت دراسة اثر إضافة معدلات مختلفة من المنفحة ٠,٢٥و٠,٥٠و٠,٧٥ مل / كجم لبن إلي اللبن المستخدم في تصنيع الجبن القريش بإضافة ٤% بادئ +١% جلكونو-دلتا-لاكتون والتي أعطت أفضل نتائج حيث أوضحت نتائج الدراسة أنة بزيادة المنفحة المضافة حدثت زيادة في كل من الجوامد الصلبة الكلية وبالتالي البروتين الكلي أيضا أدي ذلك إلي انخفاض طفيف في الحموضة ، أيضا أدي ذلك إلي انخفاض شديد في زمن التجبن من ١٢٠ دقيقة (٠,٠ مل منفحة) إلي ٥٠ دقيقة (٠,٢٥ مل منفحة) إلي٤٤ دقيقة (٠,٥ مل منفحة) إلي ٣١ دقيقة (٠,٧٥ مل منفحة) ، في نفس الوقت حدث انخفاض في تصافي الجبن عند إضافة ٠,٢٥ مل منفحة ثم ما لبس أن زاد إلي ٢٥,٧٤% ثم انخفض مرة أخري إلي ٢١,٠١% ل ٠,٥ و ٠,٧٥ مل/كيلو لبن الصناعة علي التوالي. وأوضحت نتائج التقييم الحسي أن المعاملة **R2** (٤% بادئ +١ % جلكونودلتالاكتون + ٠,٥ مل منفحة) حصلت علي اعلي تقييم حسي.