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Research Article
Identify the Type of Milk Used in Soft Cheese in Egypt

[1]Hassan Mohamed Sobhy, [2]Hassan Mohamed Hassan Hassaan and [2]Sameh Mohamed Shedeed

[1]Department of Animal Resources, African Research and Studies Institute, Cairo University, Giza, Egypt
[2]Department of Dairy Technology, Animal Production Research Institute, Ministry of Agriculture, Dokki, Giza, Egypt

Abstract

This study deals with the investigation the impact of the addition of Skim Milk Powder (SMP) to cheese manufactured, kariesh cheese was manufactured from fresh cow's skim milk as control and reconstituted milk from Skim Milk Powder (SMP) with different way for coagulation ability produce kariesh cheeses. The SMP were added at different ratios to fresh cow's skim milk for making of kariesh cheeses, both of them were reconstituted at a ratio of (a) Skim milk powder 1:2 cow skim milk, (b) Skim milk powder 1:1 cow skim milk and (c) Skim milk powder 2:1 cow skim milk. Resultant cheeses were evaluated for their chemical. The Total Solids (TS) content in reformulated cheese was increased by using the acidic-enzymatic coagulation in all kariesh cheeses manufactured from fresh cow's skim milk or reconstituted milk from Skim Milk Powder (SMP). The titratable acidity content was increased in all kariesh cheeses manufactured by acid coagulation, the ash/moisture content decrease in acid starter cheese. The cheese that contained SMP or UF with any formula, obtained with the incorporating between YC, GDL and rennet were distinguished with the presence of the glycomacropeptide (GMP) band. From this way it can be said that any of the dairy product that contains the amino acid "Glycomacropeptide (GMP)" content in the whey proteins can say that this product either factory of milk, whether full or skimmed powder or using technique ultrafiltration.

Key words: Kariesh cheese, skim milk powder, heat treatment, glycomacropeptide, ultrafiltration technique

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Corresponding Author: Hassan Mohamed Sobhy, Department of Animal Resources, African Research and Studies Institute, Cairo University, Giza, Egypt

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Kariesh is the most popular soft cheese in Egypt. It comprises about 50% of white soft cheese produced in Egypt (Hegazy *et al*., 2012). It is acid cheese coagulation made from skimmed cows and buffalo's milk or buttermilk form sour cream, often made on small farmers. It is considered to be one of the most important traditional Egyptian dairy products, commonly made in the Egyptian countryside, especially in small villages as low-income people such as farmers use kariesh cheese in their diet owing to its high protein content, low fat and price. Initially, it is made from laban khad (i.e., fermented buttermilk) or from sour defatted milk, laban rayeb. Later, it is prepared from fresh whole milk placed in earthenware jars and left undisturbed, the fat rises to the surface and skimmed milk in the lower. After 24-36 h, the creamy layer is skimmed off and the clotted skimmed milk (Laban rayeb) is poured onto reed mats or into small cheese molds. After a few hours, the ends of the mat are tied and some whey drain out. The pressed curd is permitted to drain further and the squeezing process until the desired texture is obtained, the curd is then cut into pieces and salted. Recently, where centrifugal separators were applied, this cheese is conventionally produced by acid coagulation of mechanically skimmed milk by culturing with lactic acid bacteria. Due to the increasing health conscious of consumers, kariesh cheese very popular because of its remarkable health quality as only known relatively fat free cheese consumed by Egyptians. It is often recommended for persons suffering from obesity, cholesterol and heart diseases. The increasing demand for it by Egyptian consumers is mainly attributed to its lower price (Abou-Donia *et al*., 1975). The quality and composition of kariesh cheese may vary considerably due to such factors as: The quality and composition of the clotted skim milk, the method of manufacture, the time required to complete the drainage of whey, the quality of salt added and the method of handling the finished cheese (Fahmi, 1950; El-Gendy, 1983; Abou-Donia, 1984, 1991, 1995, 1999a, b, 2008). Dried milk "Whole or skim" has been widely introduced to most of the market dairy products, e.g., liquid and fermented milks and soft and hard cheeses. Several trials were carried out to produce kariesh cheese either partially or completely from reconstituted skimmed milk powder. The use of dried skimmed milk for traditional kariesh cheese manufacture was studied by El-Safty *et al*. (1976a, b). They found that, using dried milk in cheese making increased the yield, moisture and acidity of resultant cheese.

Different methods were developed to detect addition of milk or skim milk powder to fresh skim milk of rennet whey, a by-product of the dairy industry. Assays developed for the analysis of casein/whey protein ratio included indirect methods, based on determination of certain protein fractions and direct methods, which separate protein mixtures into components. Indirect determination of whey protein to casein protein ratio included polarographic (Lechner and Klostermeyer, 1981) and 2nd and 4th derivative spectroscopy methods (Meisel, 1990; Miralles *et al*., 2000) Direct determination of protein fractions is based on effective but laborious electrophoretic, chromatographic and immunoturbidimetric (Greiner *et al*., 1985; Meisel and Carstens, 1989; Recio and Olieman, 1996; Bordin *et al*., 2001).

Recently, detection of addition of manipulation of milk with whey has been focused on the analysis of glycomacropeptide (GMP) also known as caseinomacropeptide (CMP) (De Souza *et al*., 2000; Ferreira and Oliveira, 2003). It is a bioactive 64 amino acids residues glycopeptide released enzimatically in whey from κ-casein by the action of chymosin during cheese making (Eigel *et al*., 1984).

Ferreira and Oliveia (2003) and UHT milks (Recio *et al*., 2000) as well as to monitor the renneting process (Reid *et al*., 1997; Calvo, 2002). Despite the fact that generally, whey addition of milk does not represent a health hazard, it was shown that supplementation of infant formula with GMP enhance the absorption of trace minerals (Kelleher *et al*., 2003). Consequently, reduction of trace minerals in formulas is needed in order to avoid possible adverse effects of excess dietary intake because of supplemental GMP. For that in view, the present study was planned to investigate the use of skimmed milk powder as a substitute of fresh skim milk in kariesh cheeses making with the economic advantages and low fat UF choice of high quality as well as good diet and functional properties, due to a reduced fat content.

This study was conducted to evaluate the properties of such cheese coagulated using different procedures and to select a simple, rapidly and with minimal sample preparation technique in order to detect GMP in cheese.

MATERIALS AND METHODS

Materials: Fresh cow's milk was obtained from EL-Geimiza station belongs to Animal Production Research Institute (8.9% total solids, 0.5% fat, 3.5% protein, 4.9% lactose, 0.7% ash and pH 6.7). Skimmed milk powder was obtained from local market (produced of Australian dairy products, Pty Ltd., Australia) (96.2% total solid). Used in the manufacture of kariesh cheeses. Pure yoghurt starter culture of (YC-mix 495

LYO 100 DCU) was produced by Danisco, France. Glucono-δ-lactone (GDL) was produced by Roquette Freres Company (Lille-France). Dried microbial rennet powder (R) was produced by Hannilase (CHY-Max powder extra) and it was purchased from Chr. Hansen's Lab., Denmark. Salt was obtained from local market.

Manufacture of kariesh cheese: Fresh whole cow's milk was pasteurized at 75±1°C/15 sec and cooling to 40°C, then, separated to obtain cow's skim milk, which heating at 75±1°C/15 sec (control) and then cooled to 32±1°C. Divided into 6 equal portions. The 1st portion (control) was inoculated with 2% of freshly activated yoghurt starter culture (YC) and incubated at 40°C up to curdling. The 2nd portion was coagulated by 2.5% Gouconca Delta Lactone (GDL). The 3rd portion was inoculated with Rennet (R) and incubated at 38°C up to curdling. The 4th portion was inoculated with 2% activated YC starter culture+2.5% GDL. The 5th portion was inoculated with 2% activated YC starter culture+rennet (R) at the foraging level and incubated at 38°C up to curdle. The 6th portion was coagulated by 2.5% GDL+rennet (R) at the forgoing level at 40°C.

Skimmed Milk Powder (SMP) was mixed with fresh skimmed cow's milk at a ratio of (a) Skim milk powder 1:2 fresh cow skim milk, (b) Skim milk powder 1:1 fresh cow skim milk and (c) Skim milk powder 2:1 fresh cow skim milk. After reconstituted the total solids were required in cheese (not less than 25% TS). All formula were heat treated at 72°C/15 sec, cooled to the suitable temperature and then divided into 6 equal portions. The 1st portion (control) was inoculated with 2% of freshly activated YC starter culture and incubated at 40°C up to curdling. The 2nd portion was coagulated by 2.5% GDL. The 3rd portion was coagulated with rennet (R) and incubated at 38°C up to curdling. The 4th portion was inoculated with 2% activated YC starter culture+2.5% GDL, the 5th portion was inoculated with 2% activated YC starter culture+rennet (R) at the foraging level and incubated at 38°C up to curdle. The 6th portion was coagulated by 2.5% GDL+rennet (R) at the forgoing level at 40°C.

Skimmed cow's milk was firstly heat treated at 72°C/15 sec, cooled immediately 50°C at which the UF-process was run. The resultant retentate (concentrated to give the milk solids content desired in the final kariesh cheese) was subjected to another heat treatment (72°C/15 sec), cooled to the suitable temperature. Then divided into 6 equal portions as mentioned before and filled into plastic containers. The 6th portion was technically behaved as done with the foregoing formula of the dried dairy ingredients.

Chemical analysis: Moisture, total nitrogen (using micro-Kjeldahl method), ash (using Thermolyne, type 1500 Muffle Furnace) contents and titratableacidity were determined in milk and resultant kariesh cheeses according to the methods described in AOAC (2007). As well as fat using Gerber method (Ling, 1963), lactose were determined by reduction methods. Lactose = Total solids-(fat+protein+ash), pH values (using pH meter, Hanna Instruments, Italy Srl).

Sodium dodecil sulphate polyacrylamide gel electrophoresis (SDS PAGE): The method of Weber and Osborn (1969) was applied for protein extraction. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (1970) as modified by Studier (1973). The SDS-PAGE patterns of milk proteins were identified according to Basch *et al.* (1985), Farrell *et al.* (2004) and Gab-Allah (2011). A discontinuous buffer system was carried out according to Bollag and Edelstein (1994).

RESULTS AND DISCUSSION

Chemical properties: Data given in Table 1 illustrate the coagulation ability produce kariesh cheeses from fresh skimmed cow's milk was heated treatment, at 75°C/15 sec. Data indicated that, kariesh cheeses made with yoghurt starter showed an increase pH value, TN/TS and fat/TS; but, decreased in lactose/moisture and TS contents, than cheese with another type of coagulation. Also, kariesh cheeses made with starter and rennet, GDL or mixed were characterised by low contents of fat/ST and TN/TS; but high contents of pH and lactose/moisture, which were not significantly differed from one to another as compared with cheeses with starter only this appeared in Fig. 1.

That could be attributed to the application of GDL that was in the dried form, while the lyophilized bacterial starter cultures were reconstituted in UHT skimmed for activation before adding in to coagulate the reconstituted formula and hence the concentration of cheese components were slightly diluted, increasing in TS contents in treats with starter and rennet due to the action of rennet and the expulsion of whey. Similar trends were found by Abou-Dawood (2002), Korish and Abd Elhamid (2012) and Hussein and Shalaby (2014). Concerning the fat content, no difference between treatments because the similarity in the fat/TS content to all treatments in this study.

Data given in Table 1 illustrate the coagulation ability produce kariesh cheeses from Skimmed Milk Powder (SMP) mixed with fresh skimmed cow's milk (SM). Both of them were reconstituted at a ratio of (a) Skim milk powder 1:2 cow skim

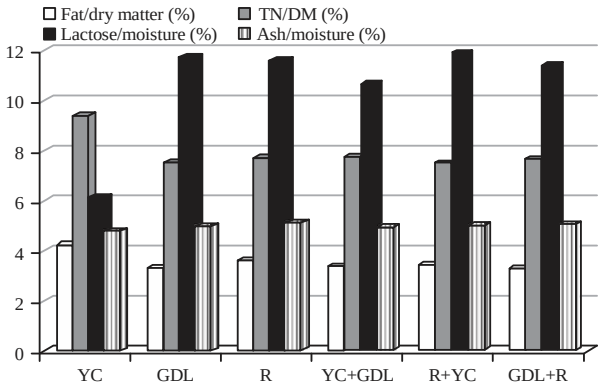

Fig. 1: Chemical properties of kariesh cheeses manufacturing from skimmed cow's milk were heated at 75 °C

Table 1: Chemical properties of kariesh cheeses manufacturing from skimmed cow's milk were heated at 75 °C, ultrafiltrtion skimmed cow's milk and powder (SMP) mixed with fresh skimmed cow's milk (SM)

Heat treatments	Types of coagulation	TS (%)	Fat/dry matter (%)	TN/DM (%)	Lactose/moisture (%)	Ash/moisture (%)	pH	Titratable acidity (%)
75°C	YC	23.4	4.27	9.38	6.18	4.79	4.5	0.83
	GDL	25.6	3.32	7.53	11.73	5.00	5.0	0.80
	R	26.2	3.63	7.72	11.61	5.12	5.0	0.50
	YC+GDL	25.0	3.40	7.77	10.68	4.99	4.7	0.89
	R+YC	25.9	3.48	7.50	11.94	5.06	4.8	0.70
	GDL+R	25.7	3.31	7.69	11.41	5.07	5.2	0.65
UF milk	YC	25.8	6.20	10.51	3.76	5.54	5.15	0.87
	GDL	26.2	5.73	9.96	5.29	5.64	5.29	0.95
	R	26.3	5.70	10.19	4.74	5.71	5.16	0.67
	YC+GDL	25.9	6.18	10.09	4.71	5.57	5.2	0.85
	R+YC	26.0	6.15	10.40	3.99	5.68	5.32	0.67
	GDL+R	26.2	5.73	9.97	5.18	5.72	5.34	0.68
SMP:SM 1:2 (A)	YC	24.2	4.13	8.61	8.36	4.70	4.6	0.90
	GDL	26.13	2.87	7.26	13.00	4.98	5.1	0.82
	R	26.41	2.84	7.48	12.73	5.01	5.6	0.52
	YC+GDL	25.22	2.58	7.71	11.41	4.87	4.7	0.89
	R+YC	26.11	2.87	7.44	12.51	5.04	4.7	0.68
	GDL+R	26.21	2.86	7.36	12.81	5.03	5.3	0.67
SMP:SM 1:1 (B)	YC	26.18	3.44	8.08	11.08	4.88	4.7	0.85
	GDL	26.9	2.42	7.17	14.02	5.06	5.11	0.80
	R	27.19	2.76	7.44	13.38	5.22	5.15	0.53
	YC+GDL	26.23	2.48	7.47	12.72	5.02	5.0	0.88
	R+YC	26.8	2.80	7.43	13.10	5.14	5.1	0.76
	GDL+R	26.88	2.79	7.35	13.33	5.17	5.2	0.66
SMP:SM 2:1 (C)	YC	24.7	4.05	8.57	8.69	4.86	4.5	0.90
	GDL	25.7	2.92	7.38	12.29	5.01	5.0	0.84
	R	26.9	2.42	7.51	13.05	5.23	5.5	0.50
	YC+GDL	26.0	2.50	7.42	12.64	5.00	4.7	0.88
	R+YC	26.2	2.67	7.66	12.06	5.15	4.8	0.71
	GDL+R	26.7	2.62	7.28	13.37	5.18	5.3	0.67

milk, (b) Skim milk powder 1:1 cow skim milk and (c) Skim milk powder 2:1 cow skim milk that the premixed kariesh cheese whether using coagulated by GDL, rennet or mixed, possessed TS content higher than those coagulated by the bacterial starter cultures. That could be attributed to the application of GDL was in the dried form, while the lyophilized bacterial starter cultures were analyzed lactose to lactic acid to coagulate the reconstituted formula and hence the concentration of cheese components, Increasing TS contents in treatments with starter and rennet due to the action of rennet and the expulsion of whey. Similar trends were found by Abou-Dawood (2002), Korish and Abd Elhamid (2012) and Hussein and Shalaby (2014).

Kariesh cheese made with yoghurt starter showed increase in fat/TS, TN/TS contents and titratable acidity percentage, but decrease in pH values and ash/moisture than

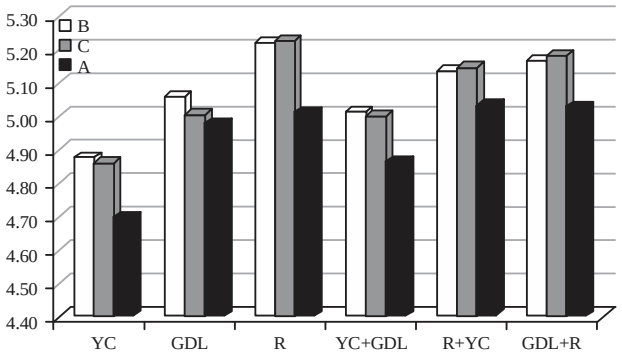

Fig. 2: Ash/moisture content of kariesh cheeses manufacturing from skimmed cow's milk mixed with skimmed milk powder

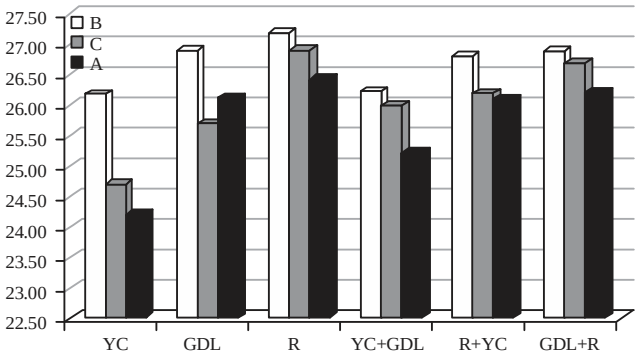

Fig. 3: TS content of kariesh cheeses manufacturing from skimmed cow's milk mixed with skimmed milk powder

cheese with other treatment. The acidity development in different base mixtures of kariesh cheese compared to fresh skim milk. Regarding the TN/TS content, data stated that the YC-cheese treatment possessed the highest TN/TS content compared to other kariesh treatments. The TN/TS content of kariesh cheese made using the other formula (B) and (C) (Table 1) was higher than that of the first one. Total TN/TS of treated kariesh cheeses was significantly affected by the addition of SMP, due to the higher TN/TS content in the SMP.

The GDL-kariesh cheese whether made using the 1st or 2nd formula possessed TN/TS content lower than those coagulated by the bacterial starter cultures. This could be attributed to the presence of 2.5% GDL within the TS content of GDL-cheese. The acidic-enzymatic coagulation led to obtain the higher TN/TS content than other coagulation type except cheese coagulated by the bacterial starter cultures. That could be ascribed to the retention of whey TN/TS as well as the released glycomacropeptide in the cheese matrix due to the absence of whey drainage step.

Figure 2 shows that the ash/moisture content were not differences due to the type of coagulation but was significantly affected by addition of SMP in the formula. However, there was decrease in TN/TS content of treatments with increasing the added ratio of SMP in the formula used in this study, where the kariesh cheese, those made using SMP possessed higher ash/moisture content than those made using the mixed with SM. That may be because of the higher ash/moisture content of SMP.

Kariesh cheese coagulated by yoghurt starter possessed the lowest ash/moisture content when compared with those coagulated by any one of the treatment due to the nature of GDL (1% impurity) and rennet, especially when used in the dried from. The data indicated also that during storage the ash/moisture content increased in all treatments including YC-cheese. This increase could be due to the decrease in moisture content occurred in all treatments.

Data given in Table 1 reveal that, all factors studied had led to significant differences in all criteria determined. The TS content of kariesh cheeses made using enzymatic coagulation was the highest followed by that of the GDL one, while that made by YC starter alone obtained the lowest TS content this is show in Fig. 3.

These differences could be ascribed essentially to the changes occurred as a result of whey drainage applied in the

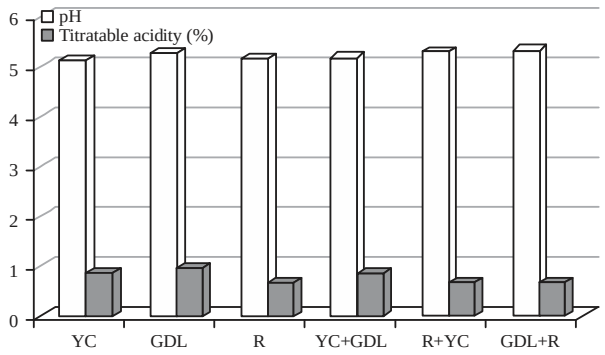

Fig. 4: pH and titratable acidity value of kariesh cheeses manufacturing from ultrafiltrtion skimmed cows

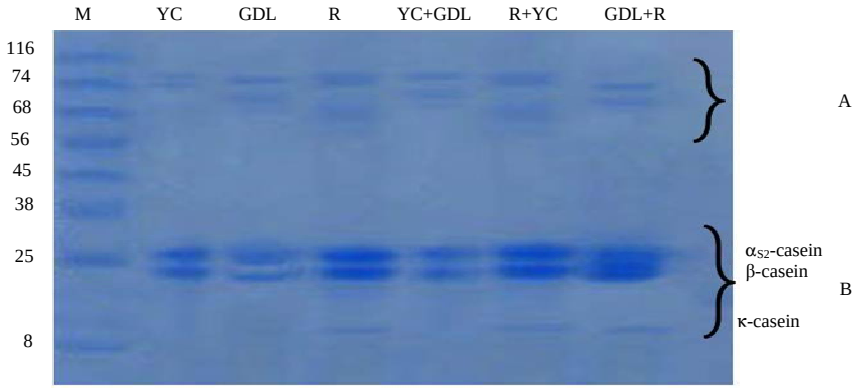

Fig. 5: SDS-PAGE electrophoretogram of kariesh cheeses from manufacturing skimmed milk treated at 75°C

conventional procedure. Moreover, the enzymatic coagulation incorporated with the acidic one made the curd to hold more moisture. The YC-kariesh cheeses was characterized with the highest TN/TS content followed by those of enzymatic-ones. The GDL-cheese came in the latest order containing the lowest TN/TS content. Moreover, the use of acidic coagulation alone or incorporating with the enzymatic in kariesh cheese making led to obtain TN/TS content higher than given when the coagulation was applied the enzymatic one.

Regarding the fat/TS content of kariesh cheeses was differences occurred due to the manufacture procedure were significant, where the fat content of UF-kariesh cheese was higher than conventional one which lost some fat during whey drainage. Neither the source of acidic coagulation nor the resultant kariesh cheese. Concerning the ash/moisture content percentage, UF technique led to increase the ash/moisture content in cheese, which was in the highest level compared with that of conventional one.

In Table 1 and Fig. 4, the UF-cheese had TA% lower and hence pH value higher than those conventional one. That could be attributed to the relatively lower water activity suffered the bacterial growth in UF-retentate. While, the relatively higher pH value may be due to the considerable high buffering capacity occurring in cheeses made by the UF process (Glover, 1985; Mistry and Kosikowski, 1984; Omar, 1987; Patel *et al*., 1986; Srilaorkul *et al*., 1989). The GDL-cheese gained the highest TA% followed by YC-cheeses. Moreover, the acid produced in kariesh cheese by the acidic coagulation alone was higher than when it was made by the acidic-enzymatic coagulation system.

SDS-PAGE electrophoretogram of kariesh cheese: According to results presented in Fig. 5, heat treatment has different effects on the protein content. This indicates that the milk treated at 75°C have significant influence on the milk protein content. The SDS-profiles of the heat-treated samples could be divided into two zones. Zone A contains high molecular weight components (Molecular weight 68-95 kDa), whereas zone B corresponds to (Molecular weight 10-30 kDa) fractions (Fig. 6). Zone B contains the most important milk protein, including α_{s2}-casein, β and κ-casein.

Figure 6 shows that SDS-profiles of the ultrafilitarating samples could be divided into two zones. Zone A contains

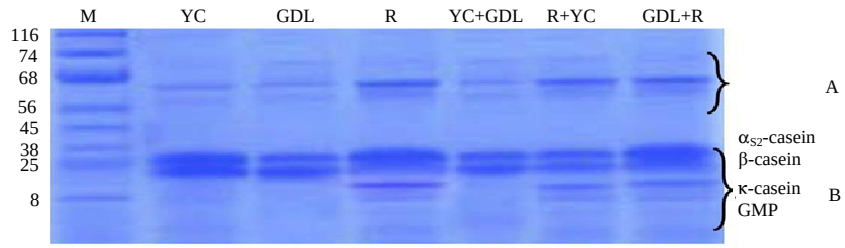

Fig. 6: SDS-PAGE electrophoretogram of kariesh cheeses manufacturing from ultrafiltrtion skimmed cow's milk

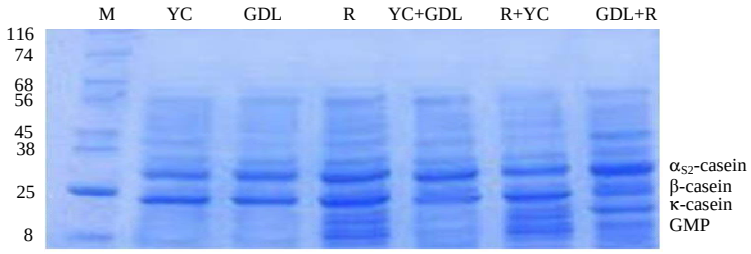

Fig. 7: SDS-PAGE electrophoretogram of kariesh cheeses manufacturing from skimmed milk powder mixed with skimmed cow's milk 1:2

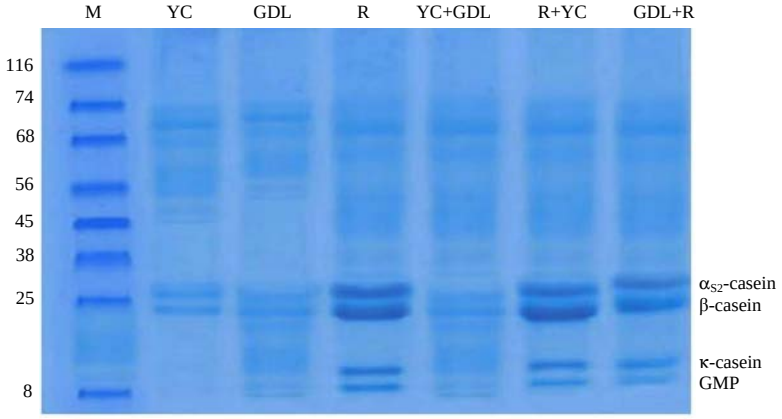

Fig. 8: SDS-PAGE electrophoretogram of kariesh cheeses manufacturing from skimmed milk powder mixed with skimmed cow's milk 2:1

high molecular weight components (Molecular weight 72-91 kDa), whereas zone B corresponds to (Molecular weight 10-30 kDa) fractions (Fig. 7). Zone B contains the most important milk protein, including α_{s2}-, β-, κ-casein and glycomacropeptide (GMP).

Exhibits that, the band density of all fractions were obviously influenced either by the manufacture procedure ultrafilitared cheese or the coagulation material whether the type of bacterial starter culture or organic acid whether alone or incorporated with the rennet. When the pattern of UF-cheese obtained with the incorporating between YC, GDL and rennet were distinguished with the presence of the glycomacropeptide (GMP) band. That could be due to the retention of this peptide into cheese matrix as a result of the absence of the whey drainage step. The healthy multi-functional spectra contributed to this incorporated peptide were reviewed by Fayed (2012) and Neelima *et al.* (2013).

Figure 7-9 shows that manufacturing cheese with skim milk powder incorporation with skim milk in different formula, It can investigate that the major proteins α_{s2}-, β-, κ-casein can be present in clear bands with increasing the concentrations of skimmed milk. When the pattern of the cheese that contained SMP with any formula, obtained with

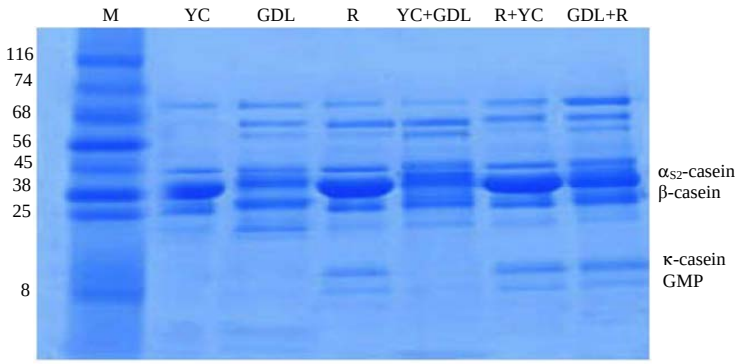

Fig. 9: SDS-PAGE electrophoretogram of kariesh cheeses manufacturing from skimmed milk powder mixed with skimmed cow's milk 1:1

the incorporating between YC, GDL and rennet were distinguished with the presence of the glycomacropeptide (GMP) band. That could be due to that the presence of glycomacropeptide (GMP) constitutes a marker of containment of SMP with whey powder (Galindo-Amaya *et al*., 2006). The results obtained demonstrate that investigation of GMP by SDS-PAGE in milk, constitute a sensitive and specific parameter to detect milk adulteration with whey, to levels as low as 1%, something that cannot be revealed only with the evaluation of the physical-chemical parameters of milk. The polyacrylamide gel electrophoresis sodium dodecylsulfate (PAGE-SDS) has been extensively used to separate native and denaturalized proteins. In various countries this has been useful to detect milk adulteration with whey, which is a fraud according to the current legal standards. The presence of glycomacropeptide (GMP) constitutes a marker of adulteration. It is released into the serum due to the hydrolysis of κ-casein peptide catalyzed by rennin in cheese elaboration. Galindo-Amaya *et al*. (2006). For detecting the addition of whey in milk, several analytical approaches based on determination of whey protein to casein protein ratio and/or GMP analysis have been described. In the present investigation the PAGE-SDS technique was used to identify GMP as an adulterant in commercial skim milk powder. The GMP was detected in the selected skim milk powder.

CONCLUSION

The results showed that milk adulteration continues to be a concern and efforts should be made by authorities and producers to protect product authenticity. The described procedure is a useful tool for routine detection of fraudulent manipulation of milk and dairy products with whey.

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