Improvement of non–traditional white soft cheese made from fresh milk fortified with adding skim milk powder and vegetable oils using different ratios of starter culture

By

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Abstract

Non – traditional white soft cheese was made by using 20% cocoa butter substitute (CBS) and various ratios (1, 2, and 3%) of starter culture (T1 , T2, and T 3) containing (*Lactobacillus casei* NCAIM B01137, *Lactococcus lactis* ssp *lactis* and *Lb. delbrueckii* ssp *bulgaricus*). The produced cheeses were stored refrigerated at (5 – 7^{o}C) and were analyzed for chemical, microbiological and organolyptic properties when fresh and during interval storage periods up to 6 weeks. The obtained data of chemical analysis reveled that the total solid content of the resultant cheeses was high in the cheeses made with starter culture, a wide significant differences in fat content was noticeable between control and other treatments. However, protein, lactose, salt, ash content and acidity were high as in the treatments than the control. During the storage there was an decrease in TS, fat, protein and lactose content of all cheese samples as the storage period progressed, but the titratable acidity has been increased. On the other hand, addition of starter culture resulted an increase in SN, TVFA, soluble tyrosine and soluble tryptophan for all the treatments either when fresh or allover the storage, and it was more pronounced in the cheeses that made with high levels of starter culture, which recorded the highest values (T1 and T3). The bacteriological analysis of cheese recorded a proportional increase of total bacterial count according to the level of starter added up to the 4th week and decreased till the end of storage. Yeasts & moulds and coliform groups were not detected in all fresh cheese samples. Moulds and yeasts appeared after the 4th week and increased up to the end of storage period.

The score of the panelists was high as high for the control cheese when fresh but T2 possessed the highest score point at the end of the storage. So, it could be recommended that good improved non–traditional white soft cheese made from whole mixed milk fortified with skim milk powder CBS using 2% of starter culture.

Keywords: soft white cheese, non-traditional cheese, starter culture, CBS.

Introduction

White soft cheese is one of the most appreciated cheeses in Egypt. This type of cheese produced either by enzymatic or acidic coagulation of fresh milk or reconstituted skim milk powder with vegetable oils. Three different processes can be used to produce this cheese on a commercial scale. The traditional method, using of ultrafiltration (UF) and non–traditional process.

The quality of cheese depends on the species of milk producing animal also depends on the breading policies, *i.e.* pure or mixed breeding. Also, the production of non–traditional cheeses depends on the type of ingredients involved in cheese formula. Manufacture of cheese from recombined milk is sometimes necessary in countries or regions with low or fluctuating supplies of fresh milk (Newstead, 1993).

Substitution of milk fat with vegetable oils in the manufacture of some dairy products represents a new trend in the dairy industry, because of vegetable oils are cheaper than milk fat which reduces the cost of cheese production to some extent (Abo-El-Naga et al., 1994).

In addition, vegetable oils could be used in many dairy products to meet medical and consumers' demands. Many health organizations have recommended that total dietary fat intake should be decreased to reduce mortality from cardiovascular disease, which is a major cause of death (American Heart Association, 1984; Enser, 1995). Cheeses are popular dairy products but questions raised about the effects of saturated fatty acids (SFAs) and cholesterol (high in milk fat) on coronary disease that caused many consumers to limit their consumption of cheeses (Yu & Hammond, 2000).

Current recommendations are to increase dietary vegetable oils on a therapeutic interaction measure with a significant role in preventive medicine, to increase the ratio of polyunsaturated fatty acids (PUFAs) to saturated fatty acids (SFAs), lower serum cholesterol (and hence LDL cholesterol) and thus indirectly prevent atherosclerosis (Diniz ***et al.,*** 2004).

Due to using different vegetable oils, the quality of non–traditional white soft cheese will varied according the type of vegetable oil used. Thus, as the constituents of the curd, e.g. fat, salt and water are held within the casein network it is important in monitoring cheese quality. In non–traditional white soft cheeses there are some defects seems to be evidence especially in the flavour. To improve the quality of these types of cheeses, this work was conducted to select the proper ratios of lactic starter culture which will be recommended in cheese making to overcome taste and flavour defects of these cheeses.

Experimental

As maintained elsewhere, the best type of cheese was that made from cocoa butter substitute "CBS" but needs some improvement to be similar to the control one. So, improved non–traditional white soft cheese was manufactured using 20% cocoa butter substitute and various ratios (1, 2 and 3%) of starter culture (T1, T2 and T3) of the following mixed starter culture*:* (*Lactobacillus casei* NCAIM B01137, *Lactococcus lactis* ssp. *lactis* and *Lb. delbrueckii* ssp. *bulgaricus*) according to the flow chart (Fig 1). The resultant cheeses were stored in brine solution (5%) at refrigerator temperature up to 6 weeks and were examined for chemical, microbiological and organoleptic properties when fresh and during interval storage periods 2, 4 and 6 weeks. All the experimental repeated three times and the average results were tabulated.

MATERIALS AND METHODS

1. Materials:

Table (1) Row materials used in the manufacture of non-traditional white soft cheeses

Materials	Supplier
Raw milk	Fresh mixed milk (cow and buffalo′s, 1:1) used in this study was obtained from the herds of Faculty of Agriculture, Moshtohor, Benha University, Egypt
Skim milk powder (SMP)	Low heat skim milk powder was purchased from local market, which imported from California Dairies, Inc, Fresno, California, USA.
Shortening (Palm oil)	Pure palm oil was obtained from MIGOP Company, Suez, Egypt
Coconut oil	Pure coconut oil premium quality ”Meizan Brand” was purchased from local market, which imported from B.G.I.O. Edible oils (SDNBHD) paser gooding, Maliza
Cocoa butter substitute	Super "ERCOAT CBS" cocoa butter substitute was obtained from International Egyptian Food Company (IEFCO Egypt), Attaqa, Suez, Egypt.
Stabilizer	Lacta–815 was obtained from Misr Food Additives (MIFAD) Company, Giza, Egypt.
Salt	Commercial fine grade salt was obtained from the Egyptian Salt & Minerals Company (EMISAL), Egypt.
Rennet	Microbial rennet powder (Formase TL2200) was obtained from Chr. Hansen’s Laboratories, Copenhagen, Denmark.
Calcium chloride	Calcium chloride was obtained from El-Nasr Pharmaceutical Chemicals Co., Cairo, Egypt.
Starter culture	Pure strain of *Lactobacillus casei* NCAIM B01137 was obtained from National Collection Agricultural Institute of Microbiology. Starter culture contains *Lactococcus lactis* ssp. *lactis* and *Lb. delbrueckii* ssp. *bulgaricus* was obtained from Chr. Hansen’s Laboratories, Horsholm, Denmark.

2. Methods:

2.1. Cheese manufacture:

Non–traditional white soft cheese was manufactured by using the method as described in Fig (1); Non–traditional white soft cheese was produced using a mixture of 10% skim milk powder (SMP), 20% [cocoa butter substitute (T1), palm oil (T2), coconut oil (T3) and palm oil &coconut oil (T4)] and whole milk (1:1). The cheeses were produced according to flow diagram shown in Fig (2) in section material and methods. However, the control (traditional cheese made from whole mixed milk, 1:1) was manufactured according to (Fahmi and Sharara, 1950). The resultant cheeses were analyzed for chemical and organoleptic

properties when fresh comparing to the control one (traditional white soft cheese). All the experimental repeated three times and the analysis done in duplicated.

Table (2): Gross chemical composition of raw milk, skim milk powder and vegetable oils used for non-traditional white soft cheese making.

Component	Raw milk	Skim milk powder (SMP)	Palm oil	Coconut oil	Cocoa butter substitute (CBS)
Moisture (%)	86.10	3.94	0.41	0.74	0.22
Protein (%)	3.42	35.34	0.42	0.83	0.94
Fat (%)	4.77	0.4	97.5	96.5	97.1
Lactose (%)	4.83	53.67	–	–	–
Ash (%)	0.75	6.65	1.67	1.94	1.73
Titratable Acidity (%)	0.17	–	–	–	–
pH	6.66	6.72	6.79	6.75	6.81
Specific gravity	1.03	–	–	–	–

2.2. Methods of analysis:

Chemical analysis:

(Total solids, fat contents and Salt contents), (Titratable acidity, ash contents and pH values), Lactose content, Total nitrogen (TN) and Soluble nitrogen (SN) contents of milk and cheeses were determined according to the method described by BSI (1989). AOAC (1995). Lawrence (1968) and AOAC (1995).

Soluble tyrosine & tryptophan were determined spectrophotometrically according to Vakaleris and Price (1959) and Total volatile fatty acids (TVFA) according to Kosikowski (1978)

Microbiological analysis:

Total bacterial counts, Yeast & mould counts and Coliform groups were determined according to the method described by APHA (2004), International Dairy Federation (IDF) (1990) and IDF (1991).

Organoleptic properties evaluation:

Samples of fresh cheeses were organoleptically evaluated according to the scheme described by, IDF (1995). The evaluations were carried out by regular scoring panel (15 panelists) of staff members at Food Science Department, Faculty of Agriculture, Benha University and Dairy Research Dept., Food Technology Research Institute, Agriculture Research Center, Cairo, Egypt.

Statistical analysis:

Statistical analysis was performed according to the user's guide given by **SAS Institute (1998).**

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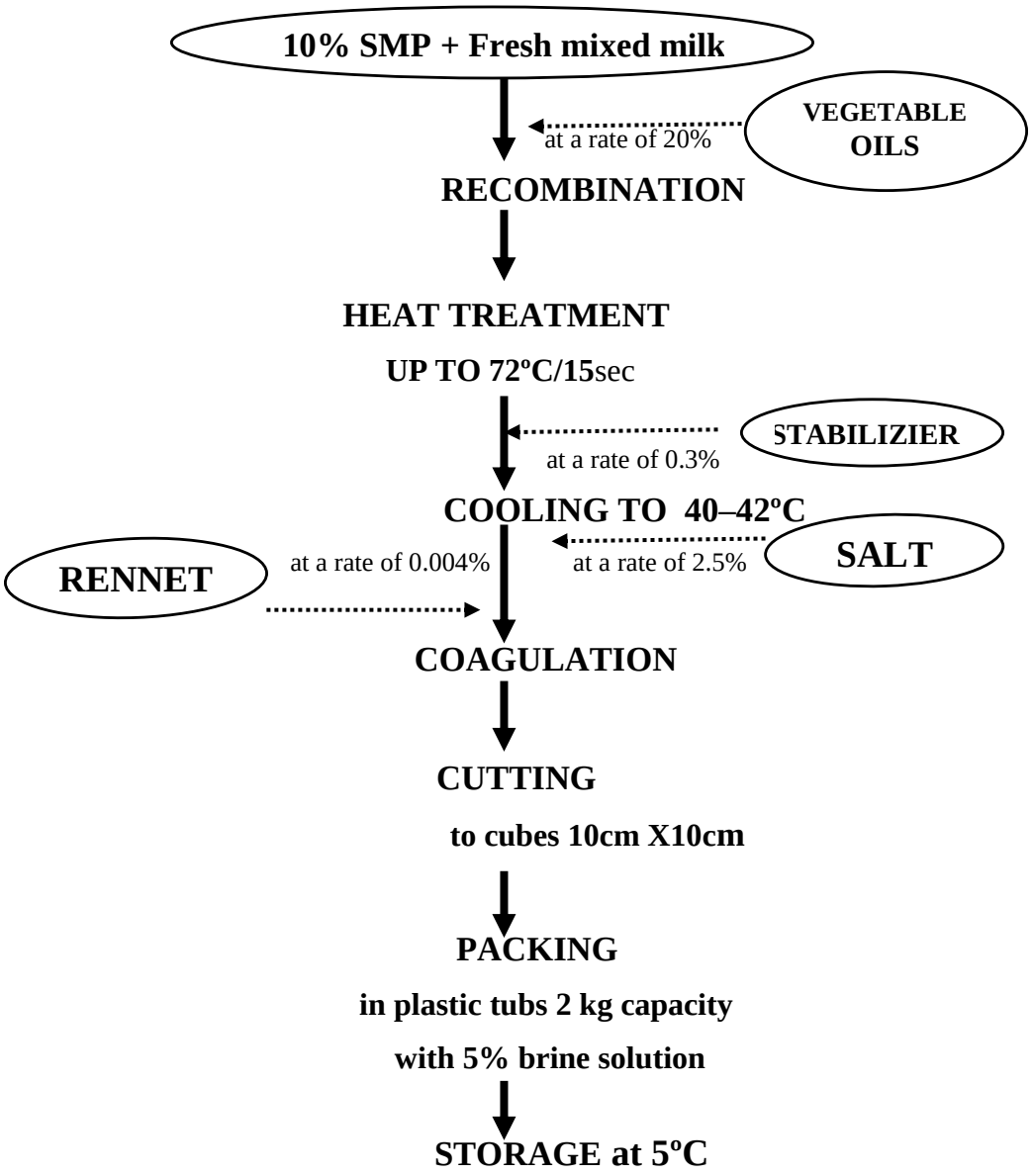

Fig (1): Flow diagram of making white soft cheese using non–traditional method with addition of skim milk powder and vegetable oils

Results and Discussion

Chemical composition:

Total solids content:

The chemical composition of improved non–traditional white soft cheeses made with different ratios of starter culture during storage at 5±2ºC up to 6 weeks are shown in Table (3). Total solids content of fresh cheese samples was 36.06, 44.70, 44.98, 43.68 and 43.75% for C1, C2, T1, T2 and T3 cheeses, respectively. The total solids content was slightly lower in C1 than all treatments; this is mainly due to the adjustment of non–traditional white soft cheeses composition during making of such cheeses. Addition of starter cultures to non–traditional white soft cheese milk increased the total solids content of the resultant product. During cold storage at 5±2ºC, total

solids content of all cheese samples was gradually decreased in all treatments to be 34.06, 42.15, 41.29, 40.90 and 40.79% for C1, C2, T1, T2 and T3, respectively at the end of the storage period. This particular behavior of total solids changes may be attributed to the ability of cheese to take up water from surrounding brine. This ability might be due to changes in the casein structure during storage. Similar results were obtained by Mashaly *et al.,* (1983); Hallal & Al-Omar (1987) and Karakus & Alperden (1995).

The analysis of variance for the cheese total solids content showed significant differences among the storage period with LSD 1.236 at level of 5%. However, there were significant differences between the control and other treatments at level of 5% analysis of variance and LSD was 1.382.

Fat and F/DM contents:

Results recorded in Table (3) represent the changes of fat and fat/dry matter (F/DM) contents of improved non–traditional white soft cheeses as affected by adding different ratios of starter culture during storage at 5±2ºC up to 6 weeks. The fat content of the fresh cheese of different treatments was 17.17, 27.50, 27.33, 27.25 and 27.00% for C1, C2, T1, T2 and T3, in the same order. The data indicated that C2 (non–traditional cheese without starter culture) had the highest fat content, while the traditional cheese (C1) had the lowest fat content but comply the Egyptian Standard (2005) for soft cheese.

The fat content of different treatments was slightly decreased during storage and they were 16.38, 26.83, 26.50, 25.75 and 25.25% for C1, C2, T1, T2 and T3, by the end of storage period, in the same order. This could be due to fat hydrolysis and increases in losses fatty acids in the pickling solution (Degheidi *et al.,* 1998). Moreover, there was an opposite relationship between the fat content and the moisture content. The same trend was observed by Mashaly *et al.,* (1983); Hallal & Al-Omar (1987) and Mehanna *et al.*, (2002).

During the storage period, F/DM content was gradually increased with extending the storage period of all treatments. Similar trend was noticed by Salama (2004). From statistical analysis of variance there were significant differences due to both treatments and the storage periods between the control cheese (C1) and other treatments with LSD 0.452 at 5% variance.

On the other hand, there was non significant difference between cheese treatments either when fresh or during the storage period at level of 5%.

Protein and P/DM contents:

The results presented in Table (3) clear the changes in protein content and the ratio of protein/dry matter for improved non–traditional white soft cheese samples as affected by adding different ratios of starter culture compared with the control during storage at 5±2ºC up to 6 weeks. The fresh cheese samples contained protein content of 10.91, 7.72, 7.76, 7.45 and 7.34% for C1, C2, T1, T2 and T3 cheeses, respectively. It was noticeable that the fresh control (C1) had the highest total protein content of all treatments; this is due to the higher protein content (3.42%) in fresh milk, used in making control cheese.

The total protein was slightly decreased during the interval storage periods to be 10.43, 7.68, 7.57, 7.22 and 6.76% for C1, C2, T1, T2 and T3, after 2 weeks of storage period.

By the end of storage period also, there was a decrease in protein content to be 10.21, 7.30, 7.17, 6.48 and 6.12%, in order. This decrease may be due to the proteolysis to soluble matters during storage. The obtained results reflect that there were significant differences due to both treatments and storage period. Among all treatments, cheese made without starter culture (C1) then, C2 had the highest protein content either fresh or during the cold storage. These results are in agreement with Abou-Donia (1981); Kehagias *et al.,* (1995) and El-Abd *et al., (*2003).

From analysis of variance for protein data it was clear that there were significant differences between the control cheese and treatments at a level of 5% variance, also, there were significant differences between all the cheeses either control (C1 & C2) or cheese treatments among the storage period with LSD of 0.038.

Lactose content:

Results recorded in Table (3) represent the changes in lactose content of improved non–traditional white soft cheese samples as affected by adding different ratios of starter culture when fresh or during storage at 5±2ºC up to 6 weeks. Lactose content of fresh cheeses was 3.59, 5.41, 5.38, 5.19 and 5.05% for C1, C2, T1, T2 and T3, in order. Lactose content dramatically decreased after 2 weeks to be 3.51, 4.46, 4.04, 3.98 and 3.75% for the same previous sequence. The obtained data indicated that there were highly significant differences between the control cheese and the treatments with starter culture. This could be related to the original ratio of lactose in the milk. The differences were when fresh and allover the storage period, also, between all the cheese treatments.

Adding starter culture to cheese milk resulted low lactose content due to the action of starter culture, which was clear in the cheeses made with 2% and 3% starter culture. Along the storage period, lactose content was significantly decreased to be 2.36, 3.79, 3.52, 3.29 and 3.17% for C1, C2, T1, T2 and T3, respectively by the end of storage period (6 weeks). This decrease attributed to the growth and activity of starter culture microflora and acid development which increased greatly during storage. The obtained results have the same trend of the findings of Abd El-Halim (2007).

Salt and salt-in-moisture contents:

Table (3) show the salt content as well as salt-in-moisture phase of improved non–traditional white soft cheese during storage at 5±2ºC up to 6 weeks. The salt in fresh cheeses recorded 2.72, 2.63, 2.53, 2.62 and 2.54% for C1, C2, T1, T2 and T3, respectively. Salt considered as one of the soluble materials in water phase and therefore, higher salt will be with higher moisture retention in cheese curd.

Salt-in-moisture of fresh cheeses was 4.25, 4.75, 4.60, 4.65 and 4.51% for C1, C2, T1, T2 and T3, in order. The moisture retention in the cheese was affected by the acidity development which also affected by the ratio of starter culture used.

By the end of storage period, salt and salt-in-moisture contents were 3.80, 3.34, 3.12, 3.28, 3.43% and 5.76, 5.77, 5.31, 5.54, 5.80% for C1, C2, T1, T2 and T3, respectively. The increase of salt content of all cheeses may be due to the increasing of moisture content in all treatments, whereas, it recorded 65.94, 57.85, 58.71, 59.10 and 59.21% in the same previous order. These findings are in agreement with Abd El-Halim *et al.,* (2007) who stated that higher salt will be with

Table (3): Chemical composition of improved non–traditional white soft cheese using different ratios of starter culture during storage at 5±2ºC.

Storage period (week)	Treatments				
	C1	C2	T1	T2	T3
			T.S (%)		
0	36.06^{B}	44.70^{A}	44.98^{A}	43.68^{A}	43.75^{A}
2	35.17^{B}	43.19^{A}	42.93^{A}	42.84^{A}	42.35^{A}
4	34.25^{B}	42.90^{A}	42.48^{A}	41.19^{A}	41.46^{A}
6	34.06^{B}	42.15^{A}	41.29^{A}	40.90^{A}	40.79^{A}
Mean	**34.89**	**43.24**	**42.92**	**42.15**	**42.09**
L.S.D at 5%:	**Treatment = 1.382**	**Storage = 1.236**	**Treatment × Storage = N.S (Non Signifiant)**		
			Fat (%)		
0	17.17^{D}	27.50^{A}	27.33^{A}	27.25^{A}	27.00^{A}
2	17.00^{D}	27.25^{A}	27.17^{A}	27.17^{A}	26.75^{A}
4	16.50^{D}	27.00^{A}	26.67^{A}	26.50^{A}	26.17^{A}
6	16.38^{D}	26.83^{A}	26.50^{A}	25.75^{A}	25.25^{A}
Mean	**16.76**	**27.15**	**26.92**	**26.67**	**26.29**
L.S.D at 5%:	**Treatment = 0.452**	**Storage = 0.537**		**Treatment × Storage = N.S (Non Signifiant)**	
			F/DM (%)		
0	47.61	61.52	60.77	62.39	61.72
2	48.34	63.09	63.28	63.41	63.17
4	48.18	62.94	62.77	64.34	63.11
6	48.08	63.66	64.18	62.96	61.91
Mean	**48.05**	**62.80**	**62.75**	**63.28**	**62.48**
			Protein (%)		
0	10.90^{A}	7.72^{B}	7.76^{B}	7.45^{B}	7.34^{B}
2	10.43^{A}	7.68^{B}	7.57^{B}	7.22^{B}	6.76^{D}
4	10.38^{A}	7.38^{B}	7.21^{B}	6.64^{C}	6.21^{D}
6	10.21^{A}	7.30^{B}	7.17^{B}	6.48^{C}	6.12^{D}
Mean	**10.48**	**7.52**	**7.43**	**6.95**	**6.61**
L.S.D at 5%:	**Treatment = 0.043**	**Storage = 0.038**		**Treatment × Storage = N.S (Non Significant)**	
			P/DM (%)		
0	30.23	17.27	17.25	17.06	16.78
2	29.66	17.78	17.63	16.85	15.96
4	30.31	17.20	16.97	16.12	14.98
6	29.98	17.32	17.36	15.84	15.00
Mean	**30.04**	**17.39**	**17.31**	**16.47**	**15.68**
			Lactose (%)		
0	3.59^{F-I}	5.41^{A}	5.38^{A}	5.19^{AB}	5.05^{B}
2	3.51^{F-I}	4.46^{C}	4.04^{DE}	3.98^{DE}	3.75^{E-H}
4	2.55^{K}	4.24^{CD}	3.84^{DEF}	3.63^{FGH}	3.42^{HIJ}
6	2.36^{K}	3.79^{EFG}	3.52^{G-J}	3.29^{IJ}	3.17^{J}
Mean	**3.00**	**4.48**	**4.20**	**4.02**	**3.85**
L.S.D at 5%:	**Treatment = 0.151**	**Storage = 0.135**	**Treatment × Storage = 0.302**		
			Salt (%)		
0	2.72^{G}	2.63^{G}	2.53^{G}	2.62^{G}	2.54^{G}
2	3.07^{DEF}	2.91^{F}	3.02^{EF}	3.13^{C-F}	3.15^{CDE}
4	3.78^{A}	3.15^{C-F}	3.07^{DEF}	3.27^{BCD}	3.28^{B-E}
6	3.80^{A}	3.34^{BC}	3.12^{C-F}	3.28^{BCD}	3.43^{B}
Mean	**3.34**	**3.01**	**2.94**	**3.08**	**3.10**
L.S.D at 5%:	**Treatment = 0.098**	**Storage = 0.088**	**Treatment × Storage = 0.797**		

Table (3): Continued.

			Salt-in-moisture (%)		
0	4.25	4.75	4.60	4.65	4.51
2	4.73	5.12	5.29	5.47	5.46
4	5.75	5.52	5.33	5.55	5.61
6	5.76	5.77	5.31	5.54	5.80
Mean	**5.12**	**5.29**	**5.13**	**5.30**	**5.35**
			Ash (%)		
0	4.22^{A}	3.64^{B}	3.49^{B}	3.56^{B}	3.73^{B}
2	4.42^{A}	3.72^{B}	3.55^{B}	3.57^{B}	3.77^{B}
4	4.54^{A}	3.76^{B}	3.80^{B}	3.69^{B}	3.82^{B}
6	4.58^{A}	3.86^{B}	3.82^{B}	4.02^{B}	3.83^{B}
Mean	**4.44**	**3.75**	**3.67**	**3.71**	**3.79**
L.S.D at 5%:	**Treatment = 0.122**	**Storage = 0.109**	**Treatment × Storage = N.S (Non Significant)**		
			Titratable acidity (%)		
0	0.24^{D}	0.28^{D}	0.33^{D}	0.36^{D}	0.40^{D}
2	0.30^{D}	0.31^{D}	0.38^{D}	0.43^{D}	0.45^{D}
4	0.49^{C}	0.43^{D}	0.55^{B}	0.61^{A}	0.69^{A}
6	0.58^{B}	0.56^{B}	0.63^{A}	0.72^{A}	0.82^{A}
Mean	**0.40**	**0.40**	**0.47**	**0.53**	**0.59**
L.S.D at 5%:	**Treatment = 0.046**	**Storage = 0.041**	**Treatment × Storage = N.S (Non Significant)**		
			pH values		
0	6.71^{A}	6.63^{A}	6.57^{A}	6.52^{A}	6.45^{A}
2	6.68^{A}	6.61^{A}	6.54^{A}	6.46^{A}	6.41^{A}
4	6.14^{C}	6.13^{C}	6.04^{C}	5.96^{C}	5.82^{C}
6	6.09^{C}	6.12^{C}	6.02^{C}	5.84^{C}	5.78^{C}
Mean	**6.41**	**6.37**	**6.29**	**6.20**	**6.12**
L.S.D at 5%:	**Treatment = 0.083**	**Storage = 0.074**	**Treatment × Storage = N.S (Non Significant)**		

C1 (control 1): mixed cow & buffaloʻs milk (1:1) without starter culture
C2 (control 2): 20% CBS + 10% SMP without starter culture
T1: 20% CBS + 10% SMP + 1% starter culture
T2: 20% CBS + 10% SMP + 2% starter culture
T3: 20% CBS + 10% SMP + 3% starter culture
Values with the same letters are non significant different.

higher moisture retention in cheese curd. The increase of salt was due to the immigration of salt from surrounding brine solution into the cheese (Karakus and Alperden, 1995).

The analysis of variance for salt content of the produced cheeses cleared that there were highly significant differences either between the treatments or during the storage period with LSD of 0.098 and 0.088 for the treatments and storage period, respectively at level of 5%.

Ash content and Ash/DM percent:

Ash content and Ash/DM of the produced cheeses when fresh and during storage at 5±2ºC up to 6 weeks are presented in Table (3). Ash content of fresh samples was 4.22, 3.64, 3.49, 3.56 and 3.73% for control and improved non–traditional white soft cheeses C1, C2, T1, T2 and T3, in order. Results indicated that ash content was high (4.22%) in C1 (mixed cow and buffaloʻs milk, 1:1 without starter culture).

On the other hand, there was a difference between C1 and the other treatments (C2) and T1 to T3 with starter culture The differences in ash content of cheeses are related to the differences of moisture content in resultant cheeses. Ash content ranged from 3.82 to 4.58% by the end of the storage for T1 and C1, respectively.

The increase in ash content of improved non–traditional white soft cheese treatments during storage period could be attributed to the changes in moisture and acidity of these cheeses. Similar results were recorded by Abd El-Halim (2007).

Titratable acidity (TA):

The titratable acidity of cheese naturally depends on milk constituents, in addition, acidity developed during storage because of lactose fermentation in cheese. Acidity values of fresh cheeses recorded 0.24, 0.28, 0.33, 0.36 and 0.40% as lactic acid for C1, C2, T1, T2 and T3, respectively. These results clearly indicated that C1 cheese (mixed cow and buffalo′s milk 1:1 without starter culture) and C2 cheese (20% CBS + 10% SMP without starter culture) had low values of acidity, while the cheeses made with adding starter culture showed high values, due to the growth of starter culture microorganisms. In addition, it was noticeable that the development of acidity was proportional to the amount of added starter to cheese milk.

On the other hand, adding of 2% starter culture (T2) and 3% starter culture (T3) caused a remarkable increase in acidity to be 0.36 and 0.40% respectively, at zero time. Titratable acidity of all improved non–traditional white soft cheeses was increased by increasing the storage period to reach maximum values by the end of storage period. The changes occurred in TA during storage period are mainly due to the consistent ability of microorganism in fermenting lactose to different acids. The correspondence values of acidity after 6 weeks of storage were 0.58, 0.56, 0.63, 0.72 and 0.82% for C1, C2, T1, T2 and T3 cheeses, on order. The results are in agreement with those obtained by Abdeen (2000); Mehanna *et al.,* (2002); El-Abd *et al.,* (2003); El-Alfy *et al.,* (2004b) and Abd El-Halim *et al.,* (2007)

From the statistical point of view the results of titratable acidity indicated that there were significant differences between all the treatments including the control (C1) either when fresh or during the storage period.

pH values:

The pH values of improved non–traditional white soft cheese with various ratios of starter culture during storage at 5±2ºC up to 6 weeks are also presented in Table (3). Fresh cheese showed pH values of 6.71, 6.63, 6.57, 6.52 and 6.45 for C1, C2, T1, T2 and T3, respectively. In contrary to acidity, the pH values were lower in cheese made with starter culture than that of control cheese, on the other hand, a noticeable decrease in pH values was found with increasing the percentage of added starter as the acidity of cheese treated with 1, 2 and 3% starter culture.

From statistical analysis point of view it could be concluded that the variations of pH values between the treatments were significantly different among the storage period. However, there were non significant differences between pH values of cheese samples either when fresh or after 2 weeks of storage. By the end of storage period (6 week) there were non significant differences between the control and the treatments.

The variation of pH values of different cheeses are related to the variations of the initial pH values of both milk and starter culture used in cheese making. The addition of lactic acid bacteria promoted the acidity development of soft cheese. During the interval storage periods of improved non–traditional white soft cheese, the pH values showed gradual decrease up to 6 weeks then become almost steady (El-Kenawi, 1983 and Salama & Shahein, 2002).

Ripening parameters:

Soluble nitrogen content (SN) and SN/TN:

Soluble nitrogen content and its proportion to the total nitrogen of improved non–traditional white soft cheese were tabulated in Table (4). SN content of fresh cheeses was 0.225, 0.215, 0.219, 0.217 and 0.223% for C1, C2, T1, T2 and T3, in order.

The control cheese (C1), T2 and T3 had exhibited remarkable higher values of SN than T1 and C2 through the interval storage periods. From the fore mentioned results, it could be seen that addition of starter cultures to the cheese caused significant increase in SN content.

By the end of storage period, SN content recorded 0.278, 0.252, 0.272, 0.290 and 0.307% for C1, C2, T1, T2 and T3, respectively. The analysis of variance for SN clarified that there were significant increases of SN by advancing the storage period. This increase could be due to the activity of protenases and peptidases released from starter culture microorganisms, which resulted in higher proteolysis of cheese. This will improve the sensory properties of the produced cheeses. These findings are in harmony with the results obtained by (El-Sissi, 2002; El-Zeiny & Metwally, 2002 and Salama, 2004).

Also, it was noticeable that the cheeses made using 2% and 3% starter culture exhibited the highest SN after 6 weeks of storage period. This is due to the higher amount of starter which hydrolyses the cheese protein; therefore, it may enhance the cheese flavour in such treatments.

The ratios of SN/TN shown in Table (4). The SN/TN recorded 13.16, 17.77, 18.01, 18.59 and 19.39% for C1, C2, T1, T2 and T3, in order when fresh. During storage the SN/TN increased and being more noticeable by the end of storage period (6 weeks), whereas, it reached 17.38, 22.03, 24.20, 28.57 and 31.98% for the same previous order. The pronounced values of SN/TN were for the cheeses made using starter culture can be attributed to their effect on the protein degradation, as it was proportional to the amount of added starter. The obtained results are in agreement with those obtained by El-Alfy *et al.,* (2004).

Analysis of variance for SN data show that there were significant differences between the control cheese (C1) and the other cheese treatments either when fresh and during the interval storage periods with LSD of 0.019 and 0.018 for treatments and storage period, respectively at level of 5% of variance.

Total volatile fatty acids (TVFA):

The total volatile fatty acids content of improved non–traditional white soft cheese when fresh and along storage period is presented in Table (4). Values of TVFA in fresh treatments were 7.93, 6.40, 6.53, 7.67 and 7.87 ml 0.1N NaOH/100g cheese for C1, C2, T1, T2 and T3, in order. There was an increase during the interval storage periods and by the end of storage period (6 weeks) there was marked increase of the TVFA for all the treatments and it was more noticeable for cheeses using the high levels of starter culture. The values of TVFA reached to be 17.07, 11.93, 15.93, 18.13 and 20.87 ml 0.1N NaOH/100g cheese at the end of storage period for C1, C2, T1, T2 and T3, respectively. The gradual increase in TVFA of all treatments during storage is mainly due to the activity of starter culture and its enzymes, as well as, the rennet enzymes.

The trend of these results are in accordance with Abdeen (2000), El-Zeiny & Metwally, (2002) and El-Abd *et al.* (2003).

Table (4): Ripening parameters of improved non–traditional white soft cheese using different ratios of starter culture during storage at 5±2ºC.

Storage period (week)	Treatments				
	C1	C2	T1	T2	T3
			SN (%)		
0	0.225^{C}	0.215^{C}	0.219^{C}	0.217^{C}	0.223^{C}
2	0.248^{AB}	0.231^{C}	0.235^{C}	0.246^{B}	0.262^{AB}
4	0.264^{A}	0.246^{B}	0.250^{B}	0.270^{A}	0.282^{A}
6	0.278^{A}	0.252^{B}	0.272^{A}	0.290^{A}	0.307^{A}
Mean	**0.254**	**0.236**	**0.244**	**0.256**	**0.269**
L.S.D at 5%:	**Treatment = 0.019**	**Storage = 0.018**	**Treatment × Storage = N.S (Non Significant)**		
			SN/TN (%)		
0	13.16	17.77	18.01	18.59	19.39
2	15.17	19.19	19.80	21.73	24.72
4	16.23	21.28	22.12	25.96	28.95
6	17.38	22.03	24.20	28.57	31.98
Mean	**15.49**	**20.07**	**21.03**	**23.71**	**26.26**
			TVFA (ml 0.1N NaOH/100g cheese)		
0	7.93^{D}	6.40^{C}	6.53^{C}	7.67^{D}	7.87^{D}
2	13.07^{B}	7.80^{D}	9.73^{C}	10.87^{C}	12.13^{B}
4	15.60^{B}	10.80^{C}	13.87^{B}	14.67^{B}	18.00^{A}
6	17.07^{A}	11.93^{C}	15.93^{B}	18.13^{A}	20.87^{A}
Mean	**13.42**	**9.23**	**11.52**	**12.84**	**14.72**
L.S.D at 5%:	**Treatment = 2.145**	**Storage = 1.919**	**Treatment × Storage = N.S (Non Signifiant)**		
			Soluble tyrosine (mg/100g)		
0	15.74^{H}	16.93^{H}	17.66^{GH}	15.84^{H}	16.18^{H}
2	17.72^{FGH}	18.29^{FGH}	20.66^{C-F}	18.42^{E-H}	18.11^{FGH}
4	19.96^{D-G}	18.59^{E-H}	21.49^{CD}	20.19^{C-G}	23.02^{BC}
6	21.24^{CDE}	21.67^{CD}	25.07^{AB}	24.61^{AB}	26.59^{A}
Mean	**18.67**	**18.87**	**21.22**	**19.77**	**20.98**
L.S.D at 5%:	**Treatment = 1.278**	**Storage = 1.143**	**Treatment × Storage = 2.556**		
			Soluble tryptophan (mg/100g)		
0	6.52^{GH}	6.28^{H}	6.69^{GH}	6.72^{GH}	6.82^{FGH}
2	7.57^{E-H}	7.42^{E-H}	7.66^{E-H}	8.09^{D-H}	6.92^{FGH}
4	8.33^{D-G}	7.57^{E-H}	8.56^{DEF}	10.99^{BC}	11.97^{B}
6	8.79^{DE}	7.87^{E-H}	9.71^{CD}	11.84^{B}	14.74^{A}
Mean	**7.80**	**7.29**	**8.16**	**9.41**	**10.11**
L.S.D at 5%:	**Treatment = 0.760**	**Storage = 0.680**	**Treatment × Storage = 1.521**		

C1 (control 1): mixed cow & buffalo's milk (1:1) without starter culture
C2 (control 2): 20% CBS + 10% SMP without starter culture
T1: 20% CBS + 10% SMP + 1% starter culture
T2: 20% CBS + 10% SMP + 2% starter culture
T3: 20% CBS + 10% SMP + 3% starter culture
Values with the same letters are non significant different.

The increase of TVFA content enhanced the development of cheese taste especially that made with adding high levels of starter culture.

There were significant differences either between the treatments or storage period, whereas, the interaction between the treatments and storage period had no significant effect on the TVFA content at level 5% of variance.

Soluble tyrosine content:

Data recorded in Table (4) represent the soluble tyrosine content of improved non–traditional white soft cheese samples during storage at 5±2ºC up to 6 weeks. The soluble tyrosine content of fresh cheese from different treatments was 15.74, 16.93, 17.66, 15.84 and 16.18mg/100g for C1, C2, T1, T2 and T3, respectively. The increase of soluble tyrosine continued to be 21.24, 21.67, 25.07, 24.61 and 26.59 mg/100g for C1, C2, T1, T2 and T3, respectively by the end of storage period (6 weeks). The progressive increase of tyrosine content was proportional to the amount of starter culture added to the cheese milk. This can be attributed to the protein degradation resulting in releasing more tyrosine which is used as ripening index for cheese. These results are in agreement with those given by El-Alfy, (1988).

There were significantly and consistently increase either with advancing the storage period or between control cheese and different cheese treatments.

Soluble tryptophan content:

The soluble tryptophan content of improved non–traditional white soft cheese when fresh and along the storage period is presented in Table (4). The tryptophan content of fresh cheeses was 6.52, 6.28, 6.69, 6.72 and 6.82 mg/100g for C1, C2, T1, T2 and T3, respectively. During the interval storage periods, soluble tryptophan increased gradually and by the end of storage period (6 weeks) reached to be 8.79, 7.87, 9.71, 11.84 and 14.74 mg/100g for C1, C2, T1, T2 and T3, in the same order. Also, the increase of soluble tryptophan was proportional to the percentage of the added starter culture. These results are in agreement with those obtained by El-Alfy *et al.,* (2004). According to the analysis between either the controls cheese or treatments, there were clear and highly significant differences also during the storage period with LSD 0.760 and 0.680 for the treatments and storage period, respectively.

In a conclusion from the results of ripening indices, there was a variation between the control cheeses and that made with added starter culture. Also, between the treatments there was a variation due to the percent of starter culture added.

Microbiological quality:

Total bacterial counts:

Table (5) show the effect of different ratios of starter culture on total bacterial counts (TBC) of improved non–traditional white soft cheese during storage period. In fresh cheese, total bacterial counts were 1.83, 0.97, 1.86, 2.03 and 2.45 X 107 (cfu g^{-1}) for C1, C2, T1, T2 and T3, respectively. It is clear that all treatments with starter culture possessed higher bacterial counts than that of control cheeses (C1 & C2).

From the presented data, as it is expected that treatment with starter culture have high bacterial counts due to the culture microorganisms. Among treatments, T3 with added 3% starter culture had the highest counts of bacteria.

It is noticeable that through progress of storage, the TBC of all cheeses increased up to the 4th week and it was nearly proportional to the level of starter added. The total bacterial counts after 4 weeks were 6.88, 7.29, 10.50, 10.67 and 11.63 X 107 (cfu g^{-1}) for C1, C2, T1, T2 and T3, respectively and then decreased till the end of storage period to be 6.17, 6.10, 8.97, 9.45 and 10.33 X 107(cfu g^{-1}) in the same order. Similar trends were observed by El-Alfy *et al.,* (2004) for Feta like–cheese. This decrease would be evidently attributed to the increase in titratable acidity, which inhibits the rate of bacterial growth. These findings agree with those obtained by Gafour (2005).

Yeast & mould counts were not detected in all fresh cheeses either controls or improved non–traditional white soft cheese treatment. However, Yeast and mould counts could be detected in C1 and C2 after 2 weeks of storage period. After 4 weeks, yeast and moulds appeared in all improved non–traditional white soft cheeses. The yeast and mould counts increased rapidly in all cheeses during storage up to the end of storage period, as they reached to 3.5, 1.83, 2.33, 5.67 and 3.16 X 10^2 (cfu g^{-1}) for C1, C2, T1, T2 and T3, respectively.

Yeast & mould counts:

Appearance of yeasts & moulds may be due to some post contamination during handling and storage of cheeses. These results are in accordance with those obtained by Jordano *et al.,* (1991) and Abo Iaina (2003) who stated that the counts of yeast & mould were corresponded with the low pH values appropriated for enhancing their growth.

Coliform groups:

Coliform groups were examined in all improved non–traditional white soft cheese and could not be detected in all samples when fresh and along the storage period for (6 weeks).This is due to the efficient heat treatment of cheese milk and sanitary conditions during the cheese making and storage. These results are in accordance with those given by El-Alfy *et al.,* (2004).

Organoleptic properties:

Table (6) show the changes in organoleptic properties of improved non–traditional white soft cheese. The obtained results reveal that the average flavour score for all fresh cheeses recorded 41.6, 38.5, 39.2, 40.0 and 39.6 for C1, C2, T1, T2 and T3, respectively. It is clear that cheeses made with starter culture were less or had the same score. However, the lowest score was for that cheese made from skim milk powder, different vegetable oil without starter culture (C2). During the interval storage periods there was an improvement for flavour in all the cheese treatments and recorded 40.5, 39.0, 40.7, 40.6 and 40.4, in order after 2 weeks.

By the end of storage (6 weeks) there was a clear decrease in flavour of all stored cheeses to be 40.9, 38.0, 39.0, 40.3 and 39.9 for C1, C2, T1, T2 and T3, in the same sequence. Also, these results cleared that cheese made with 2% starter was closed to the control cheese made from normal milk (C1) while control cheese made from skim milk powder, different vegetable oil without starter (C2) had the least flavour score.

From such results it could be concluded that adding of starter improved the cheese flavour either when fresh or during the intervals storage periods. The organoleptic score of flavour correlated with the increases happened in TVFA and SN of cheeses with starter culture. From statistical analysis of variance for flavour score data, there were significant differences either between treatments or the intervals storage period *($p < 0.05$).*

In respect to the average score points of body & texture of all produced cheeses they were closed each to other whereas they recorded 31.9, 31.5, 32.1, 32.4 and 31.9 for C1, C2, T1, T2 and T3, respectively. The average score became slightly high after 2 weeks of storage as it was 32.2, 31.8, 32.2, 32.7 and 32.1 in order.

Table (5): Microbiological quality of improved non–traditional white soft cheese using different ratios of starter culture during storage at 5±2ºC.

Storage period (week)	Treatments				
	C1	**C2**	**T1**	**T2**	**T3**
	Total bacterial counts X 10^7 (cfu g^{-1})				
0	1.83	0.97	1.86	2.03	2.45
2	5.74	4.48	7.69	8.37	9.00
4	6.88	7.29	10.50	10.67	11.63
6	6.17	6.10	8.97	9.45	10.33
	Yeast & mould counts X 10^2 (cfu g^{-1})				
0	N.D	N.D	N.D	N.D	N.D
2	0.50	0.33	N.D	N.D	N.D
4	2.17	1.17	0.83	2.00	2.17
6	3.50	1.83	2.33	2.67	3.16

C1 (control 1): mixed cow & buffaloٍs milk (1:1) without starter culture
C2 (control 2): 20% CBS + 10% SMP without starter culture
T1: 20% CBS + 10% SMP + 1% starter culture
T2: 20% CBS + 10% SMP + 2% starter culture
T3: 20% CBS + 10% SMP + 3% starter culture
Values with the same letters are non significant different.

After 4 weeks of storage, the score of body & texture decreased due to increase of cheese moisture which causes weakness in the body. By the end of storage period (6 weeks) all the cheeses had more or less the same score. Analysis of variance for body & texture data clears that there were significant increase for body & texture score during interval storage periods only after 2 weeks then starts to decrease till the end of storage.

Appearance of improved non–traditional white soft cheese score clear that all the fresh cheeses almost had more or less the same score whereas, it recorded 18.2, 18.0, 17.9, 18.0 and 17.9 for C1, C2, T1, T2 and T3, respectively. However, this score starts to decrease allover the interval storage periods as it became 16.7, 17.4, 17.5, 17.5 and 17.6, in order.

From total score and overall acceptability, there were variations either between the treatments or during the intervals storage period. The total score points indicated that cheeses made with 2 and 3% starter culture were comparable to the traditional control cheese (C1). Several investigators (Awad *et al.,* 2001; Salama & Shahein, 2002 and Salama, 2004) reported that the various starters play an important role in flavour development and improved the organoleptic properties in cheese. Statistical analysis for the obtained data indicated that there were significant

differences between the control (C1 & C2) and the other treatments for their flavours and body & texture at level of variance 5% either when fresh or among the storage period.

Table (6): Organoleptic properties of improved non–traditional white soft cheese using different ratios of starter culture during storage at 5±2ºC.

Characteristics	Storage period (week)	Treatments				
		C1	C2	T1	T2	T3
Flavour (45)	0	41.6^{AB}	38.5^{G}	39.2^{D-G}	40.0^{A-G}	39.6^{C-G}
	2	40.5^{A-F}	39.0^{EFG}	40.7^{A-F}	40.6^{A-F}	40.4^{A-F}
	4	42.0^{A}	38.7^{FG}	40.9^{A-E}	41.4^{ABC}	41.3^{A-D}
	6	40.9^{A-F}	38.0^{A-E}	39.0^{B-G}	40.3^{A-F}	39.9^{C-G}
	Mean	**41.3**	**38.6**	**40.0**	**40.6**	**40.3**
	L.S.D at 5%:	**Treatment = 0.822**		**Storage = 0.735**		**Treatment × Storage = 0.164**
Body& texture (35)	0	31.9^{AB}	31.5^{AB}	32.1^{AB}	32.4^{A}	31.9^{A}
	2	32.2^{A}	31.8^{AB}	32.2^{A}	32.7^{A}	32.1^{AB}
	4	31.2^{B}	31.7^{AB}	31.8^{B}	32.1^{AB}	31.8^{B}
	6	31.2^{A}	31.7^{AB}	31.7^{A}	31.8^{B}	30.7^{B}
	Mean	**31.6**	**31.7**	**32.0**	**32.3**	**31.6**
	L.S.D at 5%:	**Treatment = 0.645**		**Storage = 0.577**		**Treatment × Storage = 1.289**
Appearance (20)	0	18.2^{A}	18.0^{A}	17.9^{A}	18.0^{A}	17.6^{A}
	2	17.4^{A}	18.1^{A}	18.0^{A}	17.9^{A}	18.4^{A}
	4	17.1^{A}	17.5^{A}	17.8^{A}	18.0^{A}	17.7^{A}
	6	16.7^{A}	17.7^{A}	17.5^{A}	17.5^{A}	17.8^{A}
	Mean	**17.4**	**17.8**	**17.8**	**17.9**	**17.9**
	L.S.D at 5%:	**Treatment = N.S (Non Significant)**			**Storage = N.S (Non Significant)**	**Treatment × Storage = N.S (Non Significant)**
Total (100)	0	91.7^{A}	88.0^{A}	89.2^{A}	90.4^{A}	89.1^{A}
	2	90.1^{A}	88.9^{A}	90.9^{A}	91.2^{A}	90.9^{A}
	4	90.3^{A}	87.9^{A}	90.5^{A}	91.5^{A}	90.8^{A}
	6	88.8^{A}	87.4^{A}	88.1^{A}	89.5^{A}	88.4^{A}
	Mean	**90.2**	**88.1**	**89.7**	**90.7**	**89.8**
	L.S.D at 5%:	**Treatment = N.S (Non Significant)**			**Storage = N.S (Non Significant)**	**Treatment × Storage = N.S (Non Significant)**

C1 (control 1): mixed cow & buffalo's milk (1:1) without starter culture
C2 (control 2): 20% CBS + 10% SMP without starter culture
T1: 20% CBS + 10% SMP + 1% starter culture
T2: 20% CBS + 10% SMP + 2% starter culture
T3: 20% CBS + 10% SMP + 3% starter culture
Values with the same letters are non significant different.

Conclusion:

From such study it could be conclude that the addition of starter cultures to non–traditional white soft cheese milk caused an increase in SN, TVFA, the soluble tyrosine and soluble tryptophan content which improved the cheese quality. The panelists gave the highest score for control C1 when fresh, while T2 possessed the highest at the end of the storage period (6weeks). Therefore, it

could be recommended to use starter culture to produce improved non-traditional white soft cheese and especially that made with 2% tarter culture.

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