RESULTS AND DISCUSSION

Effect of adding peptone to permeate agar on total bacterial counts

Results in **Table (8)** and **Figure (4)** recorded the effect of adding different concentrations of peptone to permeate [to increase the N content] on total bacterial count of milk compared with Nutrient agar medium.

It is obvious that Nutrient agar medium (as control) gave $289x10^4$ cfu/ml which represents 100% of bacterial number present in the tested milk samples. On the other hand, permeate agar counted only 17.6 and $23x10^4$ cfu/ml in Autoclave and Arnold sterilization, respectively for the same examined sample as a control. It is well clear that Nutrient agar has C/N ratio 8/1 while permeate agar has 48/1. Since many investigators reported the importance of C/N ratio as provide materials, it is of importance to modify the permeate C/N ratio by adding different concentrations of peptone or peptone + beef extract. By enrichment of permeate agar with 5, 7.5 and 10 g peptone/L and 10 g peptone + 3 g beef extract as a source of nitrogen, the counted bacteria increased to be 109, 164, 200 and 243 x 10^4 cfu/ml in the medium sterilized by Autoclave and 132, 184, 221 and 263 x 10^4 cfu/ml in that sterilized by Arnold consequently. The percentages of counted bacteria on permeate agar and enriched permeate agar were 37.7, 56.7, 69.2 and 84.1% (Autoclave sterilization) and 45.7, 63.5 and 91.0% (Arnold sterilization) in the same order. The permeate indicated 93.9% and 88.9% (Autoclave and

Arnold sterilization, respectively) decrease in the bacterial count compared with the standard medium. The percent of decrease was reduced by fortification of permeate agar to reach only 30.81 and 23.5% (Autoclave and Arnold sterilization, respectively) by adding 10 g peptone/L and 15.9 and 9.0% (Autoclave and Arnold sterilization, respectively), when fortified with 10 g peptone + 3 g beef extract. The results indicate that addition of peptone as a soluble nitrogen source improved the C/N ratio to be nearly as the control, thus, it enhanced the bacterial growth.

Analysis of variance indicated that there were highly significant differences for total bacterial count between the treatments ($P<0.01$).

The results are in agreement with the findings of **Wasserman (1960)** who reported that the non-coagulable N in heat and acid treated whey was not utilized for growth by various strains of lactic acid bacteria.

Murad (1998) also reported that the medium containing peptone produced the highest activity of *L. delbreukii* subp. *bulgaricus* when he comprised five MRS broth medium.

The results also coincided with **Bronstein and Monte (1998)** as they had got a good cell concentration of *Lactococcus lactis* subsp. *lactis* on whey permeate medium supplemented with 0.5% peptone in addition to 0.5% yeast extract.

Thus, it was clear that when permeate used as a culture medium it did not provide most types of milk bacteria with their nutritional requirements. Therefore, it was necessary to find cheap sources of nitrogen in order to supplement the deficiency of permeate nitrogen

needed for the bacterial growth. This is the main aim of the present study.

Table (8): Effect of adding different concentrations of peptone to permeate on total bacterial counts (counts x 10^4).

Treatment	C/N Ratio	Autoclave sterilization			Arnold sterilization		
		T.B.C c.f.u. /ml	Counted Bacteria %	Decrease in counts %	T.B.C c.f.u. /ml	Counted Bacteria %	Decrease in counts %
Nutrient agar	8/1	289	100	0			
Permeate agar	48/1	17.6	6.1	93.9	32	11.1	88.9
Permeate agar +							
a) 5 g peptone	20.5/1	109	37.7	62.3	132	45.7	54.3
b) 7.5 g peptone	18.4/1	164	56.7	43.3	184	63.7	36.3
c) 10 g peptone	10/1	200	69.2	30.8	221	70.5	23.5
d) 10 g peptone + 3g beef extract	8.9/1	243	84.1	15.9	263	91.0	9.0
L.S.D. at 0.05		12.83			3.58		
L.S.D. at 0.01		18.24			5.09		

C/N ratio = Percent of carbon to nitrogen
cfu = colony forming units
TBC = Total bacterial counts

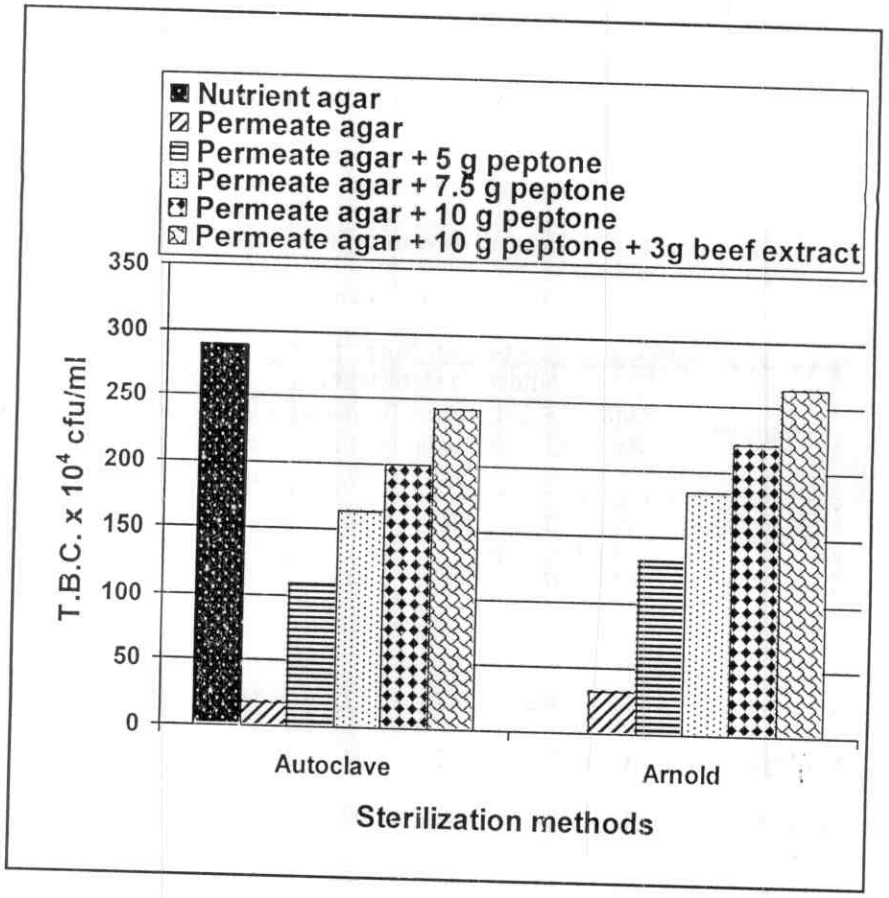

Fig. (4): Effect of adding different concentrations of peptone to permeate on total bacterial counts (counts x 10^4).

Effect of adding skim milk to permeate agar on total bacterial counts

The effect of supplementation of permeate agar with different concentrations of skim milk on total bacterial count compared with the Nutrient agar is presented in **Table (9)** and **Figure (5).**

The obtained bacterial counts of the examined milk samples were 144, 8.3, 19.9, 28 and 32 x 10^4 cfu/ml on Nutrient agar, permeate agar fortified with 0.0, 5, 10 and 15% skim milk, respectively.

The results also show that when permeate agar used as a medium for milk bacterial count, the counted bacteria represented 5.76% compared with Nutrient agar medium, which considered as a standard medium. The percentage of decrease in bacterial growth was 94.24% on permeate agar, while by enrichment of the permeate agar with skim milk as a source of nitrogen, the nutritional characteristics of the permeate was improved resulting in relatively higher bacterial count of the examined milk sample and the decrease in the counted bacteria reached to 77.08% with the addition of 15% skim milk.

From the statistical analysis, it is clear that there are highly significant differences for total count of bacteria between treatments ($P<0.01$).

Generally, whey permeate alone is poor medium with a relatively low buffering capacity. Adding skim milk to permeate agar with different concentrations induces some increments of the counted bacteria especially when added separately to sterilized medium to avoid the effect of Maillard reaction in addition to improving C/N ratio. The best results were noticed by Arnold than Autoclaved sterilization. These results confirmed **Beshkov *et al.* (1969)** who

observed that whey supplemented with 10% skim milk produced the highest yield of vitamin B12 by *Propionibacterium shermenii* in deproteinized whey medium.

Also, **Sultan (1977)** stated that the addition 5 - 20% of raw milk with enhanced the growth of bacteria and increased lactic acid production by 20%.

Parente and Zottola (1991) found that the addition of 0.1%, dried skim milk and 0.5% yeast extract to whey permeate improved the growth and activity of *L. bulgaricus. L. holveticus*, and *Str. thermophilus* and made whey permeate based media comparable with 11% dried skim milk as a medium for lactic acid starter.

Table (9): Effect of adding different concentrations of skim milk to permeate medium compared with nutrient agar medium on total bacterial counts using autoclave or Arnold sterilization (counts x 10^4).

Treatments	C/N ratio	Autoclave sterilization						Arnold sterilization					
		Together *			Separately**			Together *			Separately**		
		T.B.C c.f.u./ml	Counted bacteria%	Decrease in count %	T.B.C c.f.u./ml	Counted bacteria%	Decrease in count %	T.B.C c.f.u./ml	Counted bacteria%	Decrease in count %	T.B.C c.f.u./ml	Counted bacteria%	Decrease in count %
Nutrient agar	8/1	144	100	0.0				-	-	-	-	-	-
Permeate agar	48/1	8.3	5.76	94.24				17.0	11.8	88.2			
Permeate agar +													
a) 5% skim milk	28/1	19.9	13.82	86.18	26.3	18.26	81.74	21.6	15.0	85.0	29.2	20.28	79.72
b) 10% skim milk	23/1	28.0	19.44	80.56	37.0	25.69	74.31	30.0	20.83	79.17	41.0	28.47	71.53
c) 15% skim milk	17.5/1	33.0	22.92	77.08	42.0	29.17	70.83	35.0	24.31	72.92	46.0	31.94	68.06
L.S.D. at 0.05			3.15			3.25			3.40			3.24	
L.S.D. at 0.01			4.58			4.73			4.95			4.71	

* sterilized skim milk and permeate agar together.
** sterilized skim milk and permeate agar separately.
C/N ratio = Percent of carbon to nitrogen.
cfu = colony forming units.
TBC = Total bacterial counts.

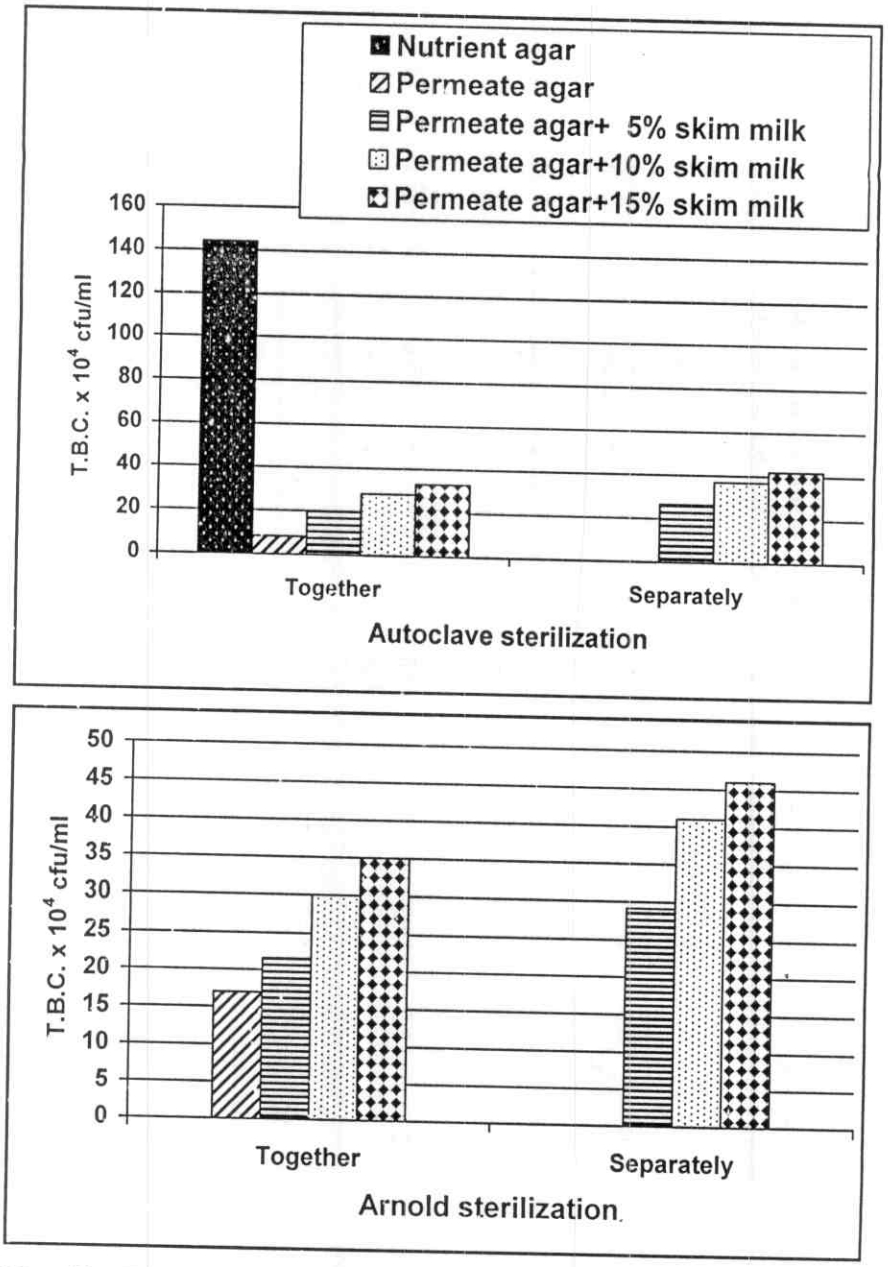

Fig. (5): Effect of adding different concentrations of skim milk to permeate medium compared with nutrient agar medium on total bacterial counts using autoclave or Arnold sterilization (counts x 10^4).

Effect of adding baker's yeast to permeate agar on total bacterial count

Table (10) and **Figure (6)** recorded, the effect of adding different amounts of baker's yeast under different conditions to permeate on the milk total bacterial counts compared with Nutrient agar as a standard medium.

The bacterial count of milk on Nutrient agar medium was 130 x 10^4 cfu/ml of the examined milk samples, while the permeate agar Autoclave and Arnold sterilized revealed only 9.7 and 13.5 x 10^4 cfu/ml, respectively, which represents, 7.46 and 10.38% of the counted bacteria with a reduction of 92.54 and 89.62% in the same order comparing with the standard medium and the differences were highly significant ($P<0.01$). The addition of baker's yeast with levels of 5, 10, 15 and 20 g/L directly boiled in permeate induced slight increase in counts to be 15.4, 23.8, 25.8 and 26.2 x 10^4 cfu/ml, respectively for the Autoclave sterilized and 17.8, 26.0, 28.7 and 29.6 x 10^4 cfu/ml in the same order for Arnold sterilized. Comparing with permeate agar medium, adding the same quantities previously mentioned to permeate followed by an incubation period of 15 min at 37°C, another increase in counts were obtained to be 20.50, 46.54 and 52 x 10^4 cfu/ml for the Autoclave sterilized samples while the counts were 24.1, 57.64 and 66 x 10^4 cfu/ml for the Arnold sterilized by adding 5, 10, 15 and 20 g/L baker's yeast, respectively. A noticeable increase in counts of the bacteria of tested milk sample were found by adding 15 g baker's yeast/L of permeate after the activation period of yeast for 15 min at 37°C for Autoclave and Arnold sterilized to give

41.54 and 49.23% of bacteria enumerated on the standard Nutrient agar consequently. This indicates that baker's yeast addition under certain conditions may achieve best results in increasing the permeate ability for promoting growth of milk bacteria. This improvement of permeate agar medium by adding baker's yeast can be attributed to the formation of nutritional substances such as soluble nitrogen, vitamins, minerals or other soluble matters during yeast growth in the incubated period. Also, addition of baker's yeast improved the C/N ratio to be 20.6, 16.7, 13.2 and 10.8/L for the different concentrations of baker's yeast in order.

The statistical analysis showed that, the differences of total bacterial count between Nutrient agar and all treatments were highly significant ($P<0.01$).

However, **Dawood *et al.* (1987)** found slight decrease of counted bacteria after long period (12 hrs) of incubation for baker's yeast with whey medium. They attributed this to the production of some yeast products as alcohols or other accumulative materials that may induce an inhibitory effect on growing of milk bacteria and they recommended the activation period to be 15 min. These results are in line with those of **Bronstein and Monte (1998)** and **Abdeltif (2000).** So, the addition of baker's yeast to permeate medium followed by a short-time of incubation at 37°C/15 min was found to be the best treatment as an activation period to reduce the formation of any inhibitory substances and to give the required function of bacterial growth.

Table (10): Bacterial counts by using nutrient agar and permeate agar (after adding different amounts of beaker's yeast, and thus sterilized directly or after incubated period of 15 min at 37°C (counts x 10^4).

Treatments	C/N ratio	Boiling after direct addition						After incubated for 15 min					
		autoclave			Arnold			Autoclave			Arnold		
		T.B.C c.f.u./ml	Counted bacteria%	Decrease in count %	T.B.C c.f.u./ml	Counted bacteria%	Decrease in count %	T.B.C c.f.u./ml	Counted bacteria%	Decrease in count %	T.B.C c.f.u./ml	Counted bacteria%	Decrease in count %
Nutrient agar	8/1	130	100	0.0	-	-	-	-	-	-	-	-	-
Permeate agar	48/1	9.7	7.46	92.54	13.5	10.38	89.62	-	-	-	-	-	-
Permeate agar +													
a) 5g beaker yeast /L	20.6/1	15.4	11.85	88.15	17.8	13.69	86.31	20.5	15.77	84.23	24.1	18.54	81.46
b)10g beaker yeast /L	16.7/1	23.8	18.31	81.69	26.0	20.0	80.0	46	35.38	64.62	57	43.85	56.15
c)15g beaker yeast /L	13.2/1	25.8	19.85	80.15	28.7	22.10	77.6	54	41.54	58.46	64	49.23	50.77
d)20g beaker yeast/L	10.8/1	26.2	20.12	79.85	29.6	22.77	77.23	52	40.0	60.0	66	50.77	49.23
L.S.D at 0.05			4.31			2.16			3.27			3.05	
L.S.D at 0.01			6.13			3.08			4.65			4.34	

C/N ratio = Percent of carbon to nitrogen
cfu = colony forming units
TBC = Total bacterial counts

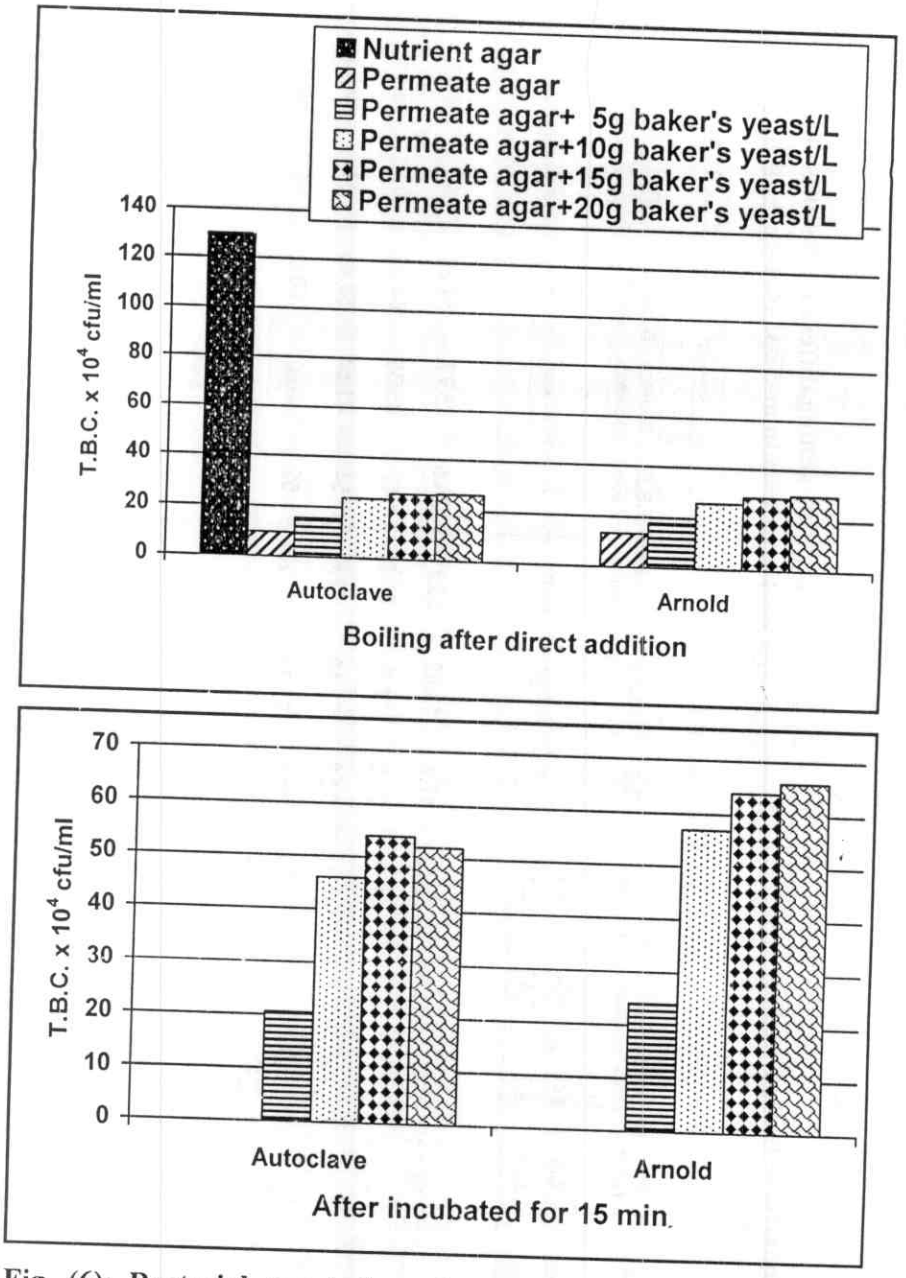

Fig. (6): Bacterial counts by using nutrient agar and permeate agar (after adding different amounts of Baker's yeast, and thus sterilized directly or after incubated period of 15 min at 37°C (counts x 10^4).

Effect of adding whey protein and liquid rennet to permeate agar on total bacterial counts

Table (11) and **Figure (7)** point out the effect of adding different amounts of whey protein and commercial liquid rennet (to increase the soluble nitrogen of the formed media) on the total bacterial count. It was obvious that Nutrient agar medium shows $113x10^4$ cfu/ml for the examined milk sample. While, permeate agar Autoclave and Arnold sterilization gave only 12.7 and 14.7 x 10^4 cfu/ml.

A noticeable increase in counts were induced with supplementing permeate with different amounts of whey proteins treated with rennet. By adding 1 and 3 ml rennet to permeate with 5 g whey protein, it gave count of 54 and 25.5 x 10^4 cfu/ml, respectively (Autoclave sterilized), while it was counted 78 and 73 x 10^4 cfu/ml with Arnold sterilization in the same order. By adding 1 and 3 ml rennet to permeate with 10 g whey protein, the total bacterial count decreased than that of 5 g as they were 44 and 16.7 x 10^4 cfu/ml, respectively in Autoclave sterilization while it was 66 and 47 x 10^4 cfu/ml in Arnold sterilization method. Increasing the amount of whey protein to be 15 g gave counts of 21.6 and 13.8 x 10^4 cfu/ml with addition of 1 and 3 ml rennet, respectively with Autoclave sterilized, while it counted 40 and 30 x 10^4 cfu/ml in Arnold sterilized method in order.

Variance of total bacterial count between Nutrient agar and all treatments was highly significant ($P<0.01$).

It was obvious that supplementation of permeate agar medium with whey protein concentrate and rennet promoted bacterial growth, thus, its counts increased. Addition of whey protein concentrate improved medium quality as it increased the soluble nitrogen level, leading to an increase in the assimilation index.

These results are in accordance with those given by **Leh and Charles (1989); Urbiene and Leskauskaite (1990) and Bury *et al.* (1998b). Bury *et al.* (1999)** claimed that the growth promoting effect of bacteria by addition of whey concentrate to the medium was not due to the increase in lactose, but it was affected by the protein supplementation.

Addition of rennet with whey protein concentrate which contains proteolytic enzymes (rennin and pepsin), which increase the whey soluble nitrogen as the commercial rennet usually contains pepsin enzyme (**Amer *et al.*, 1980**).

Results revealed that the increase in counted bacteria was more pronounced in the case of using 1 ml rennet/L than 3 ml/L. **Dawood *et al.* (1987)** attributed this to the effect of preservative compounds in the liquid commercial rennet, which inhibit the growth of some milk bacteria. They confirmed this by replacing a dried rennet tissues instead of liquid rennet as they observed that the bacterial counts increased by increasing the amounts of added dry rennet.

Increasing the amount of hydrolysed whey protein to 10 or 15 g/L caused reduction in the enumerated bacterial counts and it was pronounced in the case of 15 g than 10 g. This may be due to the release of some inhibitory substances with high concentrations. This

coincided the findings of **Carminati *et al.* (1994)** who reported that amino acids have both stimulating and inhibiting effects on the metabolic activity of lactic acid bacteria.

Thus, it can be concluded that the total bacterial counts of the standard medium (Nutrient agar) can be attained through a simple and cheap method by using permeate as a main substrate for preparing the new medium. This simple method involved fortification of permeate with 5 g whey protein/L, adjusted to pH 6.8 and treated by 1 ml commercial rennet/L for 24 hrs at 37°C before the usual preparation as previously described in materials and methods and finally sterilized by Arnold method.

Table (11): Effect of adding different amounts of whey protein and liquid rennet to permeate agar and incubation for 24h at 37°C on the total bacterial count (counts x 10^4).

Treatments	C/N Ratio	Autoclave sterilization			Arnold sterilization		
		T.B.C c.f.u. /ml	Counted Bacteria%	Decrease in count	T.B.C c.f.u. /ml	Counted Bacteria %	Decrease in count%
Nutrient agar	8:1	113	100	00.0	-	-	-
Permeate agar	48:1	12.7	11.2	88.8	14.7	13.0	87.0
Permeate agar + 5 g whey protein/L +	37:1						
a) 1 ml rennet /L		54	47.8	52.3	78	58.4	41.6
b) 3 ml rennet /L		25.2	22.3	77.7	73	64.6	35.4
Permeate agar + 10 g whey protein/L +	26:1						
a) 1 ml rennet /L		44	38.9	61.1	66	58.4	41.6
b) 3 ml rennet /L		16.7	14.8	85.2	47	41.6	58.4
Permeate agar + 15 g whey protein /L +	15:1						
a) 1 ml rennet /L		21.6	19.1	80.9	40	35.4	64.6
b) 3 ml rennet /L		13.8	12.2	87.8	30	26.5	73.5
L.S.D. at 0.05		4.99			3.54		
L.S.D. at 0.01		6.96			4.93		

C/N ratio = Percent of carbon to nitrogen
cfu, colony forming units
TBC = Total bacterial counts

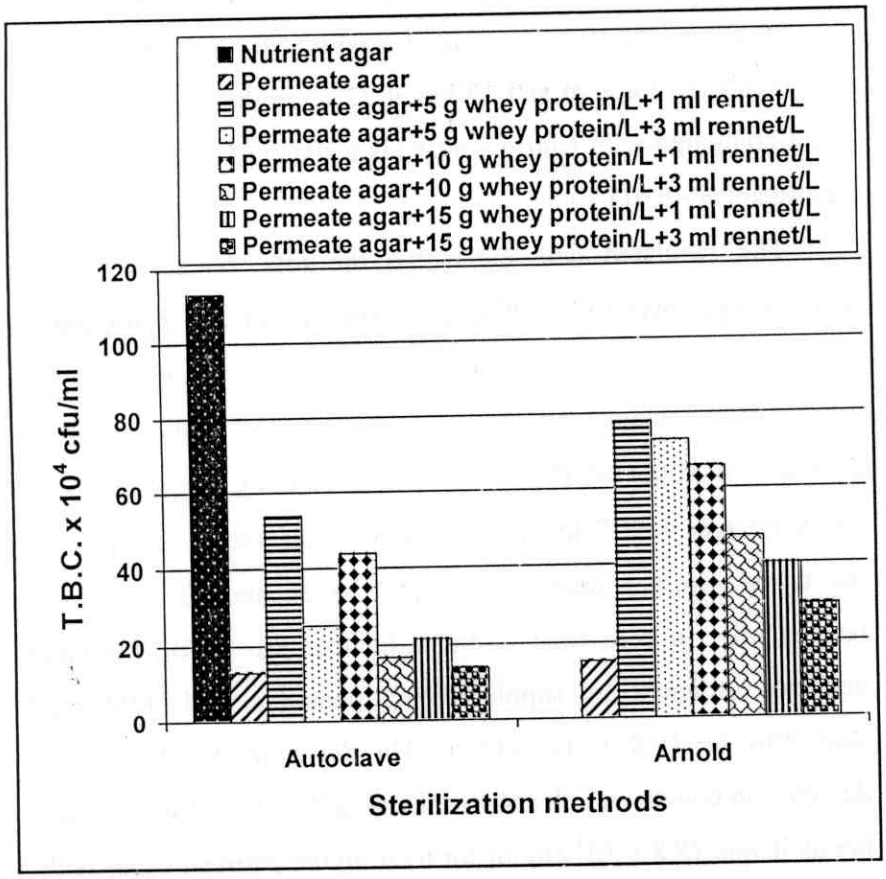

Fig. (7): Effect of adding different amounts of whey protein and liquid rennet to permeate agar and incubation for 24h at 37°C on the total bacterial count (counts x 10^4).

Effect of adding yoghurt starter to permeate agar on total bacterial counts

a) Effect of different amounts of the starter:

Table (12) and **Figure (8)** indicate the effect of adding different concentrations (5, 10 and 15%) of yoghurt starter to permeate agar then incubated for zero and 12 hrs at 37°C on the milk total bacterial count compared with Nutrient agar (as a standard medium) were high significant ($P<0.01$).

The tabulated data clear that the total bacterial counts on Nutrient agar were 132 x 10^4 cfu/ml. This counts was decreased when counted on permeate agar to be 26.1 x 10^4 cfu/ml. showing only about 19.8% of the counted bacteria on the standard which resulting a decrease of about 80.2%. It is obvious that by supplementing the permeate agar with 5 and 10% yoghurt starter, the bacterial counts of the tested samples were increased to be 38 and 57 x 10^4 cfu/ml, respectively, in zero time (without incubation). While a noticeable decrease in count by supplementing permeate with 15% yoghurt starter to be 19.8 x 10^4 cfu/ml. On the other hand, pronounced decrease in counts was clear by prolonging the incubation period to 12 hrs as it was 18.8 x 10^4 cfu/ml for treating the permeate agar with 5% yoghurt starter and 20.5% x 10^4 cfu/ml for treating the permeate with 10% yoghurt starter. By increasing the inoculum of the permeate medium to 15% yoghurt starter, the bacterial counts were decreased to be 18.9 x 10^4 cfu/ml after incubation for 12 hrs. The percentage of decrease in count was 85.8, 84.5 and 85.7% when the incubation

period was 12 hrs for treating permeate with 5, 10 and 15% yoghurt starter, respectively.

Table (12): Effect of adding different concentrations of yoghurt starter to permeate agar during incubation for 12 hr on the total bacterial count (counts x 10^4).

Treatments	C/N ratio	Incubation period					
		0 hr.			12 hr.		
		T.B.C c.f.u./ ml	Counted bacteria %	Decrease in count %	T.B.C c.f.u./ ml	Counted bacteria %	Decrease in count %
Nutrient agar	8:1	132	100	00	-	-	-
Permeate agar	48:1	26.1	19.8	80.2	-	-	-
Permeate agar +							
a) 5% yoghurt starter	39:1	38	28.8	71.2	18.8	14.2	85.8
b) 10% yoghurt starter	29:1	57	43.2	56.8	20.5	15.5	84.5
c) 15% yoghurt starter	18:1	19.8	15	85	18.9	14.3	85.7
L.S.D. at 0.05		4.47			2.76		
L.S.D. at 0.01		6.51			4.01		

C/N ratio = Percent of carbon to nitrogen
cfu, colony forming units
TBC = Total bacterial counts

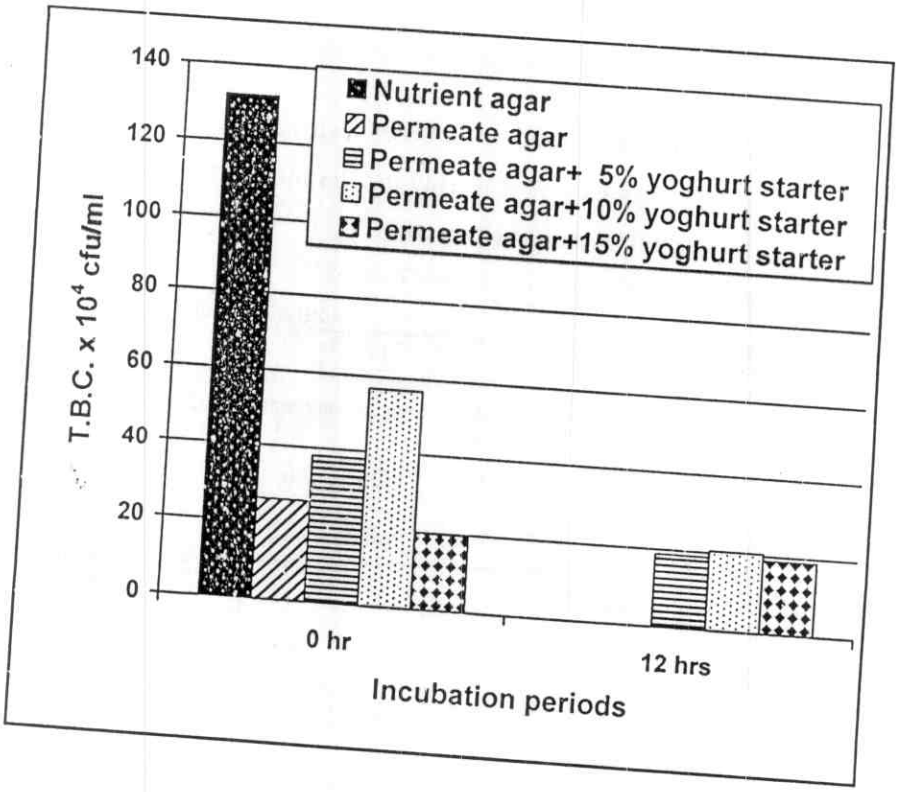

Fig. (8): Effect of adding different concentrations of yoghurt starter to permeate agar during incubation for 12 hr on the total bacterial count (counts x 10^4).

The obtained results are in accordance with **Beshkov (1969), Sultan (1977) and Dawood *et al.* (1987)** who reported that the addition of yoghurt starter may increase the soluble nitrogen which encourages the growth of other organisms resulting an increase in the counted bacteria. Also, **Stojsavljevic *et al.* (1971)** and **Rasic *et al.* (1971)** stated that during the fermentative process by the yoghurt starter, there is a significant increase in the free amino acids concentration. Free amino acids are readily available by many organisms in the medium and stimulates its growth. On the other hand, the reduction in counts by prolonging the incubation time may be due to some metabolic substances formed during the hydrolysis. In addition, the yoghurt starter organisms hydrolyse lactose to glucose and galactose, the glucose is firstly and easily consumed by bacteria while the latter is accumulated in the medium to cause an inhibitory effect on some other milk organisms (**O'leary and Woychik, 1976**) and also this can be confirmed through the previous results. This explain why the enriched permeate agar medium with different concentrations of yoghurt starter did not reach the counts as on Nutrient agar medium.

b) Effect of different incubation periods:

Table (13) and **Figure (9)** indicate the effect of incubation period (zero, 30, 60, 180 and 360 min) of permeate agar supplemented with 10% yoghurt starter at 37°C on the bacterial count compared with Nutrient agar.

The tabulated data show that, on Nutrient agar medium the examined milk sample enumerated total bacterial count of 157 x 10^4 cfu/ml, while on permeate agar, with Autoclave and Arnold sterilization, it was only 13.9 and 20.0 x 10^4 cfu/ml, respectively. An observed increase in counts was obtained by the addition of 10% yoghurt starter, but the highest increase was achieved after 60 min incubation period. The counts of bacteria were 65, 73, 82, 75 and 70 x 10^4 cfu/ml with Autoclave sterilized method and 75, 81, 93, 85 and 77 x 10^4 cfu/ml with Arnold sterilized through the incubation intervals of zero, 30, 60, 180, and 360 min, consequently. Accordingly, the counted bacteria after the present treatments were 41.40, 46.60, 52.23, 47.77 and 44.58% Autoclave sterilization and the corresponding counts using Arnold sterilization were 47.77, 51.59, 59.29, 54.14 and 49.04% compared with that obtained on Nutrient agar. The percentage of decrease on the same base took the opposite trend to give 58.60, 53.50, 42.77, 52.23 and 55.41% (Autoclave sterilized) and 52.23, 48.41, 40.76, 45.86 and 50.96% (Arnold sterilized) with the same sequence.

The differences of total bacterial count between Nutrient agar and all treatments were statistically high significant ($P<0.01$).

The increase in total milk bacterial counts on permeate medium fortified with 10% yoghurt starter by prolonging the incubation time until 60 min may be due to the increase of soluble nitrogen and free amino acids (**Rasic *et al.*, 1971**). Also, the starter organisms fermented lactose to lactic acid, causing a change in the pH of the media.

The decrease in total milk bacterial count on permeate medium fortified with 10% yoghurt starter by prolonging the incubation time more than 60 min may be due to the presence of some bacterial metabolic substances formed by the added starter or from accumulation of other inhibitory materials (such as galactose, lactic acid ... *etc*) which prevent some milk bacteria from growth resulting a reduction in the counted bacteria.

The results are in accordance with **O'Leary and Woychik (1976)** they stated that, addition of yoghurt starter organisms hydrolysis lactose to glucose and galactose, the glucose is firstly and easily consumed by bacteria while the latter is accumulated in the medium to cause an inhibitory effect on some other milk organisms.

On the other hand, the reduction in lactose content was increased with incubation period. Lactose content was 5.6% in zero time, while it was found to be 5.1, 4.6, 4.1 and 3.8% after incubation periods of 30, 60, 180 and 360 min, respectively. This decrease may be due to the effect of fermentation by yoghurt starter.

Table (13): Effect of adding 10% yoghurt starter to permeate agar for different periods on total bacteria count (count x 10^4).

Treatments	Lactose %	Autcclave sterilization			Arnold sterilization		
		T.B.C. c.f.u /ml	Counted bacterial %	Decrease in count %	T.B.C. c.f.u /ml	Counted bacterial %	Decrease in count %
Nutrient agar	-	157	100	00.0			
Permeate agar	5.6	13.9	8.85	91.1	20.0	12.74	87.26
Permeate + 10% yoghurt starter							
a) Zero time	5.6	65	41.40	58.60	75	47.77	52.23
b) 30 min.	5.1	73	46.50	53.50	81	51.59	48.41
c) 60 min.	4.6	82	52.23	47.77	93	59.24	40.76
d) 180 min.	4.1	75	47.71	52.23	85	54.14	45.86
e) 360 min.	3.8	70	44.58	55.41	77	49.04	50.96
L.S.D. at 0.05		4.02			2.94		
L.S.D. at 0.01		5.82			4.27		

TBC = Total bacterial counts
cfu = colony forming units

In all previous experiments, the media were sterilized using Autoclave and Arnold methods. It was found that the growth of bacteria always more better when Arnold method used than the Autoclave methods.

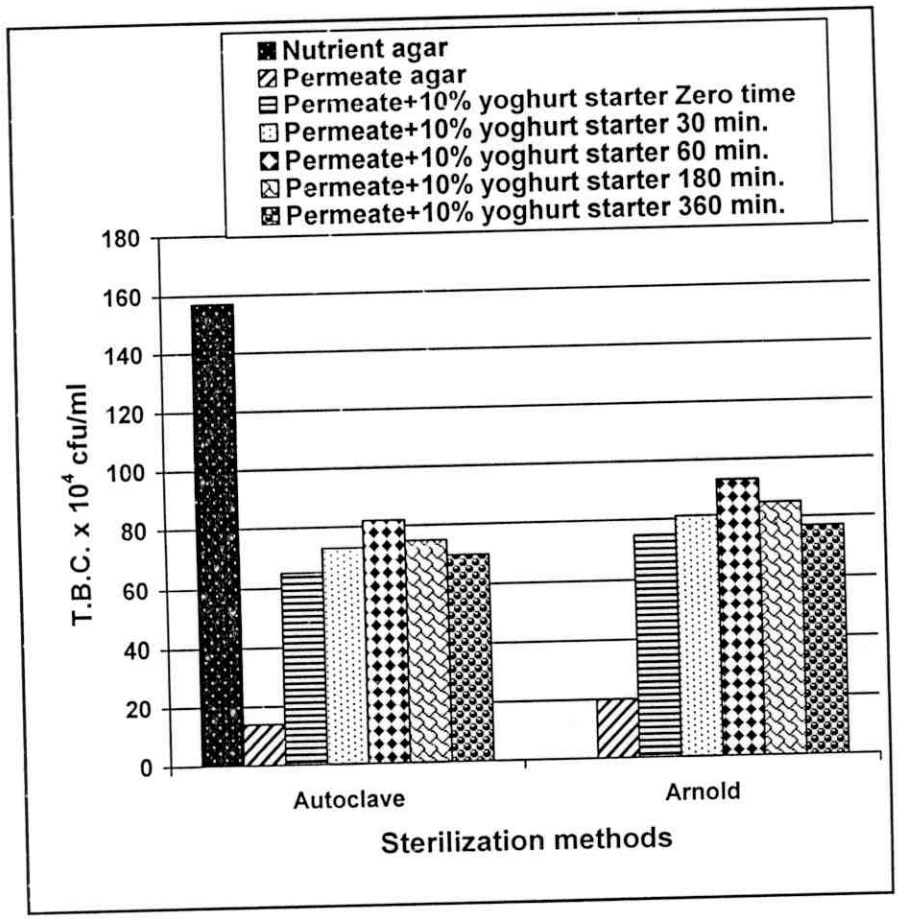

Fig. (9): Effect of adding 10% yoghurt starter to permeate agar for different periods on total bacteria count (count x 10^4).