LIST OF CONTENTS

5. SUMMARY AND CONCLUSTION

Consumption of probiotic bacteria via food products is an ideal way to re-establish the intestinal microflora balance. Since the renewed interst in probiotics, different types of products were proposed as carrier food for probiotic microorganisms by which consumers can take large amounts of probiotic cells for the therapeutic effect.

The current investigation aimed to study:-

Acceleration of fermentation carried out by a combination of mixed culture:-

(A): *St. salivarius subsp. thermophilus +L. delbrueckii subsp. bulgaricus*

(B): *Bifido bacterium spp.*

(C): *L. acidophilus*

(D): *Bifidobacterium spp. A*

(E): *L. acidophilus + A*

(F): *Bifidobacterium spp + C+A*

The first part included: six samples of fresh buffaloes milk were heated at 95˚C for 15 min. followed by cooling to 40°C, than inoculated with mixed culturer, then inoculated until coagulation. The resultant fermented milk was stored under refrigeration, then tested by Rheological, Chemical, Microbiological and Organoleptic properties.

The obtained results could be summarized as follows:

1- Preparation of probiotic fermented buffaloes milk with mixed cultures of yoghurt and probiotic bacteria:-

- Coagulum strength and syneresis:

The coagulum strength (g/100g) and syneresis (ml/100g)**.** The crud tension and synersis of treatment for (F) were: 28.15 and 0.7, 0.9, 1.6, 1.90 and 2.30 after 10, 30, 60, 90 and 120 min respectively. While they were for treatment (A) 29.18 and0.6, 0.8and 1.6, 1.80 and 2.20 after 10, 30, 60, 90 and 120 min.

- Acidity development:

Acidity developed in (A), (D), (E), (F) complete after 4 hr of the beginning of fermentaion. Treatment (B) was complete after 8 hr, while in (C) it was complete after 12 hr.

- Chemical analysis:-

Probiotic fermented milk made with D and A gave the high acidity. Low pH-values and low lactose content during storage at 5±2˚C. while (C) and (E) recorded the highest total nitrogen (T. N) and (C) recoreded highest siluble nitrogen (S.N) while (F) recorded highest Non-protein nitrogen (N. P.N.) content during cold storage.

- Microbiological analysis:-

-Total viable bacterial count:-

Generally br seen that the number of bacteria in the examined samples characterized with gradual increase by lengthning the storage period until 6 days. While after 6 days the number of bacteria in the examined samples were lower number.

-Count of aerobic spore forming bacteria:

aerobic spore forming bacteria could be detected in all of the examined treatments whether fresh or during storage.

- Moulds and yeasts count:

Moulds and yeasts could not be detected in the control and all the examined varients after manufacture and after 3 days of cold storage. However, moulds and yeasts could be detected and counted after 6 days in (A), (B), (C) and (F) expect to (D) and (E) were absent. However, moulds and yeasts could be detected after 9 days in all treatments.

- Lipolytic and probiotic bacteria:

Both of the lipolytic and proteolytic bacterial counts were higher in all the treatments from fresh days to 6 days and low from 9 days to 15 days. Its apparent that the proteolytic bacteria are considerably higher in numbers than the lipolytic ones.

- Detection of coliform and *Staphylococcus aureus*:

Both of coliform and *Staphylococcus aureus* were not detected whether in fresh or stored control and probiotic fermented buffaloes milk.

- Survival of probiotic and lactic acid bacteria in probiotic fermented milk during storage period for 15 days:-

St. thermophilus count slightly decreased during the first week and gradually decreased until the end of storage period *L. bulgaricus, L. acidophilus* and *Bifidobacterium ssp*. were decreased with all storage period.

- Organoleptic properties:-

The sensory evaluation scores decreased during cold storage. However, the flavour increased up to 6 day then decreased finally (F) was the best samples than (D) and (A).

- The second part inculated:-

2. Preparation of probiotic fermintation milk with some juices:-

The supplementation with the examined juices (Mango, Strawberry, Guava, banana and Raswberry) with treatment (F) 6% incoculated.

- Coagulum strength and synersis of probiotic fermented milk affected with the addition of some juices:

The results indicated that the crud tension and synersis were not much affected by using different with the addition of different juices. The curd tension and synersis were with Guava 36.5% (g/100g), 0.9, 1.4, 1.9, 2.40 and 2.60 (ml/100g) after 10, 30, 60, 90 and 120 min respectively.

While the crud tenstion and synersis were with strawberry 27.90 (g/100g) 0.8, 1.0, 1.5, 2.10 and 2.32 (ml/100g) after 10, 30, 60, 90 and 120 min, respectively.

Acidity development:

Probiotic fermnted milk with strawberry and Raswberry were of shorter incubation time than other additives which was 4 hrs, but other treatments stayed for 4.5 hrs.

Chemical analysis:-

The probiotic fermented milk with Strawberry, Raswberry and control gave the highest acidity, lowest pH-values and lactose levels and highest total nitrogen.

While samples with Strawberry and Guava gave the highest non-protein nitrogen (N.P.N).

Microbiological analysis:

Total viable bacterial count:

The bacterial count increased from 6.7 $x10^3$ to 7.2 $x10^3$ cfu/ml in the contral sample after 3 days, from 6.89 $x10^3$ to 7.8 $x10^3$ from 7.2$x10^3$ to 8.1 $x10^3$ from 6.85$x10^3$ to 7.5 $x10^3$ from 6.90$x10^3$ to 7.3 $x10^3$ and from 7.0 $x10^3$ to 7.90 $x10^3$ cfu/ml for treatment Mango, Strawberry, Guava, banana, and Raswberry, in the same order.

- Moulds and yeasts count:-

Moulds and yeasts were absent in all the treatmrnts of after manufactures and after 3 days of cold storage. However, moulds and yeasts could be detected after 6 days in all treatment.

- Lipolytic and proteolytic bacteria:

The lipolytic and proteolytic bacterial counts were higher in fermented milk with the addition of some fruit juices than in control.

Detection of coliform and *Staphylococcus aureus*:

All samples of fermented milk with the addition of some fruit juices. Whether fresh or stored were not contained coliform and *Staph. Aureus*.

Survival of probiotic and lactic acid bacteria in probiotic fermented milk with the addition of some fruit juices:

The highest values were 86x 10^7 cfu/ml and 85x 10^7 cfu/ml from *St. thermophilus* in the Strawberry and Raswverry, respectively from fresh samples, will *L. bulgaricus* were 79$x10^7$ cfu/ml and 78$x10^7$ cfu/ml at the same time from this

samples in addition *L. acidophilus* and *Bifido ssp*. were $96.0x10^7$, $91x10^7$, $56.0x 10^7$, $56.0x 10^7$ and $55.0x 10^7$ cfu/ml respectively from fresh samples (S) and (R). After that the values were decreased in all samples during storage period 3, 6, 9 and 15 days respectively.

Organoleptic properties:

Strawberry and Rasberry treatment had the highest scoring points wheather when it was fresh or after cold storage.

The third point included:

- Preparation of probio tic fermented buffalos milk with (glucose, Honey, Diet-Sweet, sucrose, and wheat) of yoghurt.

Whey protein (2%) was added buffaloes milk after heated at 95˚C for min. followed cooling at 40˚C. the treated milk inoculated with 6% starter (*St. thermophilus* + *L. del brueckii susp. bulgaricus*) + (*L. acidophilus*) + (*Bifidobacterium ssp*) 1: 2.5: 2.5 respectively. The mixture was divided into six equal volumes, one of its used as control (no additives), the different carbohydrates were added separately to the other and incubated at 40˚C until fermintation. The resultant fermented milk was stored at 5±2˚C and were Rheological, chemical, microbiologically and organoleptically analyzed.

- Coagulum strength and synersis of probiotic fermented milk affected with different carbohydrates:-

The changes in coagulum strength in samples from control, glucose, honey, diet-sweet, wheat and sucrose were 31.09, 29.91, 28.78, 29.39, 28.73 and 31.14 respectively. The amount of released whey increased with progress of synersis time from 10 to 120 min.

- pH-value and titratable acidity during preparation process:-

The treatments were resulted in adecreased in the titratable acidity and increase in pH values the titratable acidity of the control was 0.73 and pH-value was 4.81 after 4.5 (h) while the treatments glucose, sucrose and honey were titratable acidity 0.73, 0.70 and 0.71 and pH-value were 4.80, 4.85 and 4.79 respectively after 4.0 (h).

Chemical composition of yoghurt with the addition of different carbohydrates after manufacture and during the storage period:-

pH-values and titratable acidity:-

its clear that control and treatments had the highest content of titratable acidity in the fresh and after cold storage. On the other hand pH-values in treatments decreased after fresh 3, 6, 9, and 15 days.

Lactose content:-

The decline of lactose after 6 days the lactose content gradually decreased in all samples in the order and during the time storage.

Total nitrogen (T.N) content:

The probiotic fermented milk with different carbohydrates were resulted increase in the (T.N) content in control, glucose, sucrose, Honey, wheat and Diet-sweet.

Soluble nitrogen (S.N) content:

The treatments, which the higher level of wheat, control and source.

Non-protein nitrogen (N.P.N) content:-

Data presented that the fermented that the fermented milk with addition of different carbohydrates had highest value of (N.P.N) content.

Microbiological analysis:

Total viable bacterial count:

The count of total bacterial cfu/ml slightly increased during the first 3 days of cold storage in the probiotic fermented milk with different carbohydrates.

Furthermore, viable counts of total bacterial cfu/ml more decreased after 3 days and up to the end of cold storage.

Count of aerobic sporeforming bacteria:

All the treatments decreased after storage period.

Moulds and yeasts counts:

Moulds and yeasts were absent in all of the examined treatments, whether fresh of 3 days of cold st orage except treatments glucose, sucrose and wheat. However, moulds and yeasts could be detected after 6 days of all the examined treatments except cotrol sample. The treatment sucrose highest count after 6, 9 and 15 days of cold storage.

Lipolytic and proteolytic bacteria:-

The treatments were hughest lipolytic and proteolytic bacterial count in fresh and after 3 days, then decreasing the level after 6, 9 and 15 day from stronge period.

Detection of coliform and *Staphylococcus aureus*:

Both of Staph. Aureus and coliform were not detected whether in fresh or stored variants of yoghurt prepared in this work.

Survival of probiotic and lactic acid bacteria:-

The population of viable yoghurt organisms increased initially after manufacture reached a maximum and then decreased in the product during refrigerated storage.

Organoleptic properties:

The control, glucose and Sucrose gaves the highest total score points. While the treatment wheat was lowest total score after fresh, 3, 6, 9 and 15 days in cold storage.

RECOMMENDATIONS

According to the obtained results, we recommended with the following for preparation of probiotic fermented milk:-

1. Using the probiotic bacteria together with lactic acid bacteria (yoghurt bacteria), where the starters composed of (*Bifidobacterium spp*.) + (*L. acidophilus*) + (*St. thermophilus* + *L. bulgaricus*) 2.5: 2.5:1, respectively reduced the incubation time to 4 hours (similar to yoghurt starter). Using starter was characterized with high viability for probiotic bacteria and recorded the highest degrees for organoleptic evaluation.
2. Supplementation of probiotic fermented milk with some juices (Strawberry, Raswberry, with probiotic bacteria together with lactic acid bacteria 6% for preparation of probiotic fermented milk resulted in reduction of incubation period and elevated the viability of used bacteria to probiotic level. The supplemented (Strawberry, Raswberry) recorded the highest degrees in organoleptic evaluation.
3. Supplementation of probiotic fermented milk with (Sucrose, glucose) and added whey protein 2% resulted in reduction of incubation period and recorded the highest degrees in organoleptic evaluation.