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6-SUMMARY

The present study was designed in three parts in order to evaluate the nutritional value and biological effects of some bacteria used in the production of fermented milk (part I & part 2). However part III was a trail to improve the viability of *B. bifidum* in yoghurt using different concentrations of honey or ascorbic acid, and to evaluate the sensory properties of the resultant fermented milk products.

In the first two parts, Albino rats were fed for six weeks on a commercial chow diet and drank water (control) or fed on chow and drank solution of one of 8 fermented milk products made using yoghurt culture (*S. thermophilus* & *L. bulgaricus*) or *L. acidophilus* alone or a mixture of them with *B. infantis*, *B. longum* or *B. bifidum* strains. The investigated parameters were rats' growth properties, concentrations of plasma and liver lipids, activities of the antioxidative enzymes catalase and superoxide dismutase (SOD), and concentration of thiobarbituric acid reactive substances (TBARS) in liver mitochondria and red blood cells (RBCs). The bacterial numbers of these strains and the pathogenic staphylococci and coliform bacteria in rats' small and large intestine and feces were also determined.

In the third part of the study, milk was fortified either with 1, 2 or 3% of honey or with 50, 100 or 150 mg/kg of ascorbic acid and fermented milks were mad using a mixed culture of yoghurt and *B. bifidum*. The effect of these materials on the

viability of the culture strains and sensory properties of the resultant fermented milk products were investigated during 8 days of cold storage in a refrigerator at $7^{\circ}\text{C}\pm 2$. A brief synopsis for the obtained results is shown henceforth.

6-1-PART I: EFFECT OF *IN VIVO* ADMINISTRATION OF YOGHURT OR YOGHURT AND *BIFIDOBACTERIA*.

1-Body weight gain significantly increased in all rats fed the culture milk products compared to the control group however; relative liver and spleen weights were comparable among all rats groups.

2-All fermented milk products showed a hypolipidemic action demonstrated by a significant reduction in the concentration of plasma cholesterol, triglycerides and phospholipids by (22-29%), (19-30%) and (25-52%), respectively.

3-No significant differences were found among all groups in plasma LDL-cholesterol concentration, atherogenic indices and the ratio between HDL- cholesterol and total cholesterol although the fermented milk products tended to lower plasma LDL-cholesterol concentration.

4-The addition of *bifidobacteria* to yoghurt tended to enhance the hypolipidemic effect of the resultant cultured products.

5-Feeding the cultured milk products significantly decreased the concentration of cholesterol, triglycerides and phospholipids in rats' liver by (25 - 28%), (33-41%) and (13-24%), respectively compared to the control group.

6-No significant differences were found between rats fed the control diet and that fed yoghurt in the activities of catalase, SOD and concentration of TBARS in red blood cells (RBC). The rats fed the diets supplemented with a mixed culture of yoghurt and *bifidobacteria* resulted in higher catalase and SOD activities and lower TBARS concentration in their RBC although it was not always significant.

7-All cultured milk products resulted in higher catalase activity in rats' liver than did the control group. However, rats fed the milk fermented with yoghurt culture plus *B. infantis* exhibited the highest catalase activity than the other fermented milk groups. On the other hand, hepatic SOD activity and concentration of mitochondrial TBARS were not significantly different among all rat groups.

8-There were considerable variations among rat groups in their small and large intestinal and fecal content of lactic acid bacteria, *bifidobacteria*, *staphylococci* and coliforms. The rats fed yoghurt or the milk fermented with yoghurt culture and *bifidobacteria* resulted in higher lactic acid and *bifidobacteria* counts compared to the control group. Milk fermented with *B. longum* or *B. bifidum* were more effective to reduce the count of *staphylococci* and coliform bacteria compared to the other groups.

6-2-PART II: EFFECT OF *IN VIVO* ADMINISTRATION OF MILK FERMENTED BY *L. ACIDOPHILUS* OR *L. ACIDOPHILUS* AND *BIFIDOBACTERIA*.

1-Rats fed the cultured milk products exhibited significantly higher final body weight and body weight gain than the control group. However, rats' relative liver and spleen weights were similar among all groups.

2-All the fermented milk products showed a hypolipidemic effect in rats' plasma. The cultured milk products resulted in a significant reduction of plasma cholesterol and triglycerides by (18-35%) and (6-33%), respectively compared to control. The addition of *bifidobacteria* to *L. acidophilus* enhanced the hypolipidemic effect of the resultant milk products. The rats fed the milk fermented by a mixed culture of *L. acidophilus* and *B. bifidum* showed the highest reduction in cholesterol and triglycerides concentration compared to the other groups.

3-In comparison to the control group, concentrations of plasma HDL- and LDL-cholesterol fractions were significantly lowered in the experimental groups fed the fermented milk products. The atherogenic indices and the ratio between HDL-cholesterol and total cholesterol were not significantly different among all groups.

4-The cultured milk products significantly decreased the concentration of cholesterol and triglycerides in rats' liver by (59-64%), and (30-59%), respectively compared with the control group. The milk fermented with *L. acidophilus* or a mixture of *L.*

acidophilus and *B. longum* or *B. bifidum* showed the highest hypolipidemic effect compared to the other fermented milk products.

5- There was a significant increase in the activity of catalase of RBC from rats fed the milk fermented with *L. acidophilus* and *B. infantis* or *B. bifidum* compared to the control group. However the other fermented milk products did not exert such effect. In contrast the fermented milk products increased the activities of SOD compared to the control group. All fermented milk products tended to decrease the TBARS in RBC

6- All the fermented milk products resulted in higher catalase and SOD activities in rats' liver than did the control group. The milk fermented with *L. acidophilus* and *B. bifidum* exhibited the highest catalase and SOD activities than the other fermented milk products. On the other hand, concentration of liver mitochondrial TBARS did not differ significantly among all groups although it tended to decrease in rats fed the cultured milk products compared to the control group.

7- In comparison with the control group rats fed the cultured milk products resulted in a considerable increase of their small and large intestinal and fecal content of *L. acidophilus* and bifidobacteria. However, the count of *staphylococci* and coliforms significantly decreased. The coliform bacterial count significantly decreased in rats fed the milk fermented with *L. acidophilus* and *B. longum* or *B. bifidum* than the other fermented milk fed groups.

6-3-PART III: IMPROVING THE VIABILITY OF *B. BIFIDUM* IN FERMENTED MILK.

6-3-1-(A) The effect of different levels of honey on yoghurt-bifidum fermented milk during refrigerator storage.

1-The fresh fermented milk added with honey resulted in slightly higher acidity values than the control. The acidity gradually increased during the 8 days of storage in all samples with slightly higher values for the cultured product with 1 and 2 % of honey. The pH values are in consistent with the acidity development.

2-Pronounced gradual increase in the total volatile fatty acids (TVFA) was found in all fermented milk during eight days of cold storage. The addition of honey by 2 and 3% to the yoghurt-bifidum fermented milk resulted in elevation of TVFA in the fresh product.

3-Addition of honey to yoghurt-bifidum fermented milk significantly increased the acetaldehyde content in the fermented milk during the first 2 days of storage period. After 2 days of cold storage all fermented milk samples showed a gradual reduction of its acetaldehyde content till the end of the storage period.

4-Lactic acid bacterial count gradually increased during refrigerated storage up to the fourth day in all samples. Addition of honey increased the viability *L. bulgaricus* and *S. thermophilus*. The maximum bacterial counts were found with 2

% honey in the cultured milk products. The bacterial count decreased by increasing the storage period.

5- Addition of honey significantly increased the viability of *B. bifidum* bacteria. However, the addition of 2 and 3% of honey to the milk were more effective than the addition of 1% in this respect throughout the storage period. All fermented milk samples showed a gradual increase of the viability of *bifidobacteria* up to 4 days of cold storage and then declined till the end of the storage period.

6- No significant sensory differences were found between the control samples and the cultured milk added with 1 or 2% of honey. However, the addition of 3% of honey resulted in lowered scores for flavour and appearance with no effect on body and texture of the resultant product.

6-3-2-(B) The effect of different levels of ascorbic acid on yoghurt-bifidum fermented milk during refrigerator storage.

1- All fresh fermented milk products added with ascorbic acid resulted in higher acidity than the control sample. The acidity gradually increased during the 8 days of refrigerated storage in all samples without significant differences among groups. The increasing of ascorbic acid content in the cultured milk products resulted in a higher reduction of the pH throughout the storage period. The addition of ascorbic acid by 150 mg/kg milk was more effective in reducing the pH value than the other treatment.

2-A pronounced gradual increase in TVFA was found in all fermented milk products during the eight days of cold storage. The data show a slight effect of ascorbic acid on the production of TVFA during storage period.

3-Addition of ascorbic acid to yoghurt-bifidum fermented milk significantly increased the acetaldehyde content in the fresh fermented milk products. After 2 days of cold storage all fermented milk samples showed a remarkable increase of its acetaldehyde content thereafter, the acetaldehyde concentration gradually reduced till the end of the storage period. No remarkable significant differences were found among all cultured products during cold storage.

4-Addition of ascorbic acid increased the viability of yoghurt bacteria (*L. bulgaricus* and *S. thermophilus*). The fermented milk with vitamin C resulted in higher yoghurt bacterial counts in the fresh samples compared to the control. All samples resulted in a gradual bacterial count increase up to 4 days of storage followed by a gradual reduction till the end of storage period. The maximum yoghurt bacterial counts were found with 50 mg ascorbic after 4 days of storage. Increasing the storage period at a refrigerator decreases the yoghurt bacterial count.

5-The addition of ascorbic acid to the cultured milk products increased the viable bifidobacterial count throughout the 8 days of cold storage compared to the control. The control and 50 mg /kg ascorbic acid added fermented milk showed a gradual bifidobacterial count increase up to 4 days of storage followed by a reduction to the

end of storage period. In the fermented milk added with 100 and 150 mg ascorbic acid the bacterial count reduction started after 2 days of storage.

6-The milk fermented with yoghurt culture mixed with *B. bifidum* had a significant high sensory score compared to the milk made with yoghurt culture only. On the other hand addition of ascorbic acid by 50 mg /kg to the cultured products obtained sensory scores comparable to that for the control. The addition of 100 and 150 mg vitamin C /kg milk resulted in extremely lower sensory scores compared to the other cultured milk products.

7-CONCLUSION

1-This study demonstrated and agrees with other studies demonstrated the beneficial nutritional and biological of consumption of fermented milk.

2-The inclusion of *bifidobacteria* mainly *B. longum* or/ and *B. bifidum* in the production of yoghurt or acidophilus milk is highly recommended for enhancement the physiological effect of the resultant product.

3-Fortification of yoghurt, acidophilus milk or bifidobacteria-cultured milk or their combinations with 2% honey or/and 50 mg/kg of ascorbic acid (vitamin C) will increase its nutritional value and improve the viability of their bacterial strains during refrigerated storage.