USING MORINGA LEAVES POWDER IN PRODUCTION OF PROBIOTIC YOGHURT

Ola F. El-Sayed¹, H.S.M. El-Taweel¹, A.A. El-Shibiny² and M.M.K. Metwally*²


ABSTRACT

Moringa leaves powder was added during the manufacture of probiotic yoghurt at level (0.5%) before pasteurization. Three probiotic yoghurt treatments were prepared as follows: yoghurt without moringa served as control (T₁), yoghurt + moringa (T₂) and yoghurt + moringa + 10% mango pulp (T₃). Yoghurt were inoculated with 2% lactic acid bacteria (LAB) and 2% L. acidophilus and incubated at 42°C until complete coagulation (pH 4.8), then stored at 5°C up to 14 days. Chemical, microbiological and sensory properties of the produced yoghurt were carried out. The level of 0.5% moringa was found to be the best ratio. The results showed that pH values and moisture content (%) decreased during storage period for all treatments, while the values of titratable acidity, total solids (%), protein content (%), fat content (%), antioxidant activity and total phenolic content were increased and the treatments (T₂ and T₃) had values greater than control yoghurt (T₁). Microbiological analysis indicated that the addition of 0.5% moringa leaves powder and 0.5% moringa leaves powder +10% mango pulp stimulate the growth of LAB and probiotic culture (L. acidophilus). On the other hand Yeasts and moulds, Coliform and Sporeforming bacteria were not detected in all treatments up to the end of storage period. Moreover the addition of 0.5% moringa leaves powder + 10% mango pulp increased the acceptability of product more than the addition of moringa alone up to the 14th day of storage at 5°C.

Kew words: Moringa, production, yoghurt.

INTRODUCTION

Yoghurt is a coagulated dairy product obtained by the lactic acid fermentation of milk by bacteria i.e. Streptococcus thermopiles (ST), Lactobacillus delbrueckii ssp. bulgaricus (LB) (Fadela et al., 2009). Addition of these two cultures resulted in acidification of milk and produce of aromatic compounds (Sahan et al., 2008). Although these microflora have been found to be valuable for human as they help in maintaining health and nutrition. Also efforts have been placed on developing yoghurt containing probiotic cultures like Lactobacillus acidophilus (LA) and B. bifidus (BB) (Vinderola and Reinheimer, 2000). Probiotic cultures are live microbial food ingredients that are beneficial for human health (Salminen et al., 1999), which includes improvement of intestinal microbial balance which results in the inhibition of bacterial pathogens, reducing the risk of colon cancer, in the inhibition of bacterial pathogens, reducing the risk of colon cancer, improving the immune system, lowering serum cholesterol levels (Saarela et al., 2002), alleviation of lactose intolerance and nutritional enhancement (Alizadeh and Ehsani, 2008).
Mango (Mangifera indica L.) is a seasonal fruit grows in tropical regions and is regarded as one of the most important fruits of Asia.

The nutritional importance of mango is mainly due to its high amounts of b-carotene, a carotenoid which provides various health benefits, including provitamin A and antioxidant activity (Harnkansujarit and Charoenrein, 2011). Mango contains a variety of phytochemicals and nutrients. The fruit pulp is high in prebiotic dietary fiber, vitamin C, diverse polyphenols and provitamin a carotenoids (Ajila and Prasada Rao, 2008).

Moringa oleifera is referred to as a "Miracle tree" or "Wonder tree" (Kasolo et al., 2010) of significant socio economic importance because of its several nutritional, pharmacological (Caceres et al., 1991) and industrial application (Makkar and Becker, 1996).

The leaves of this plant contain high amount of vitamin B complex, calcium, potassium, iron and protein. Also, they contain all of the essential amino acids in good proportion (Mishra et al., 2012).

Moringa oleifera leaves are active against the growth of bacteria such as: E. coli, S. aros, P. aeruginosa and B. cereus as these organisms range from pathogenic and oxygenic organism liable to cause food borne illnesses and food spoilage. It can be used as evaluable drug in the treatment of infections caused by E. coli and P. aeruginosa (Abalaka et al., 2012).

MATERIALS AND METHODS

Materials

Fresh cow's milk was obtained from the herd of Badwy farm of El-Arish, Egypt. Average chemical composition of milk (3% fat, 3.35% protein, 12.6% T.S) were determined according to AOAC (2011).

Skim milk powder (96% TS, product of Dairy America TM) USA, was obtained from the local market.

Direct Vat Starter (DVS) yoghurt culture was obtained from CHR-Hansen's laboratorie, Denmark, under commercial name type (FD-DVS-YC-X11) containing Streptococcus thermophiles and Lactobacillus delbrueckii spp. Bulgaricus.

Probiotic bacteria strain Lactobacillus acidophilus (DSM20384) was obtained from Egyptian Microbial Culture Collection (EMCC) at Cairo Microbiological Resources Center (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University.

Mango (Mangifera indica) fruit and sugar were obtained from local market of El-Arish, Egypt.

Moringa oleifera leaves was obtained from Cautia farm of North Sinai, Egypt.

Methods

Preparation of Additions

Preparation of mango pulp

Mango pulp (0.27% fat, 0.51% protein, 81.3% T.S) was obtained manually after thorough washing and peeling of the skin and blended to get smooth and then pasteurized at 90°C for 10 min according to the procedure mentioned by Vijayalakshmi et al. (2009).

Preparation of moringa leaves powder

The collected leaves were spread on a clean curtain cloth and kept at room temp.

The selected room for shade drying was well ventilated by natural current of air. The leaves took about six to seven days to dry completely and became crispy and brittle to touch then blended in a blender to get powder according to the procedure mentioned by Delong (2003).

Preparation of probiotic culture

Strain Lactobacillus acidophilus (DSM 20384) was activated in MRS broth according to De Man et al. (1960).
Manufacture of Yoghurt

Yoghurt was made from standardized cow's milk according to Tamime and Robinson (1999) as shown in diagram (A).

Diagram (A) – Manufacture of yoghurt

Fresh cow milk (3% fat, 3.35% protein, 12.6% TS).

Methods of Analysis

Yoghurt samples were analyzed chemically, microbiologically and organoleptically when fresh and after 7 and 14 days of storage at 5°C.

Chemical Analysis

pH values were measured using Jenway pH meter with Jenway spear electrode No: 29010 (Jenway limited Gransmore Green, Felsted, Dunmow, England).

Titratable acidity, total solids, total protein and fat were determined according to the method described by AOAC (2011). Moisture content was calculated using the regular equation as follows:

Moisture (%) = 100 – Total solids

Measurement of antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) inhibition assay was carried out according to methods described by Li et al. (2009). Total phenolic content (TPC) of the previously prepared yoghurt samples were determined using the Folin–Ciocalteau by method described by Li et al. (2009).

Microbiological analysis

Preparation of all samples for microbiological examination was carried out as described by Frazier and Foster (1961).

Lactobacillus acidophilus and Lactobacillus delbrueckii ssp. Bulgaricus were determined using MRS agar medium as described by De Man et al. (1960).

Streptococcus thermophiles was determined by using M17 selective medium as described by Krusch et al. (1987). Plates were incubated at 37°C for 48hr.

Moulds and Yeasts Count

Were determined on oxytetracycline glucose yeast extract agar medium as suggested by Harrigan and Mcconce, (1966). Plates were incubated at 25°C for 3 days.

Coliform group

Were determined according to the American Public Health Association (1978). Appropriate dilutions of samples were plated on Mac Conk's agar medium and incubated at 37°C for 48hr.

Organoleptic properties

Organoleptic properties of yoghurt samples were evaluated according to Tamime and Robinson (1999).

RESULTS AND DISCUSSION

Chemical Analysis of Yoghurt

Based on the results presented in Table 1. Generally, pH of all yoghurt samples decreased during storage up to 14 days. This phenomena was due to the growth of lactic acid bacteria and the production of lactic acid, which was due to the especial synergistic effect between Lac. spp and Strep. spp. (Yousef et al., 2013).

Also, there were slightly differences in pH values between control yoghurt and treated yoghurts during the storage period. These results were in agreement with those obtained by Vijayalakshmi et al. (2009).

It was clear from Table 1 that acidity values of all treatments increased during the progress of storage period.

Moreover, there were slightly differences in acidity values between control yoghurt and treated yoghurts during the storage period (Lamoureux et al., 2002) the increase in acidity attributed to the decrease in lactose content and post acidification
$T_1$
- Addition of skim milk Powder (3%)
- Pasteurization (95°C/5min)
- Cooling (42°C)
- Inoculation with 2% yoghurt Culture + 2% *L. acidophilus*
- Filled into plastic containers
- Incubation at 42°C until complete coagulation
- Storage in refrigerator at 5°C for 14 days

$T_2$
- Addition of skim milk powder (3%)
- Pasteurization (95°C/5min)
- Addition of moringa leaves powder (0.5%)
- Cooling (42°C)
- Inoculation with 2% yoghurt culture + 2% *L. acidophilus*
- Filled into plastic containers
- Incubation at 42°C until complete coagulation
- Inoculation (2% yoghurt culture + 2% *L. acidophilus*)
- Storage in refrigerator

$T_3$
- Addition of skim milk powder (3%)
- Pasteurization (95°C/5min)
- Addition of sugar (6%)
- Pasteurization 95°C/5min
- Cooling (42°C)
- Addition of mango pulp (10%)
- Cooling (42°C)
- Filled into plastic containers
- Incubation at 42°C until Complete coagulation
- Storage in refrigerator At 5°C for 14 days
Table (1): pH and Acidity values of yoghurt fortified with moringa during storage up to 14 days at 5°C

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Storage (day)</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T1</td>
</tr>
<tr>
<td>pH</td>
<td>Fresh</td>
<td>4.60</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>4.18</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>4.14</td>
</tr>
<tr>
<td>Acidity (%)</td>
<td>Fresh</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>0.9</td>
</tr>
</tbody>
</table>

T1: control (milk)  T2: milk + moringa  T3: milk + moringa + mango + sugar.

especially by *L. delbreuckii* ssp. *bulgaricus* during the storage period. It was clear from Table 2 that the T.S of probiotic yoghurt slightly increased in all treatments during storage and the control treatment was lower than the others allover the storage period.

These results are similar to the values obtained by Hashim (2007). Moreover, the increase in total solids content during storage period attributed to the loss of moisture (Tamime, 1978). Also the protein content of all treatments gradually increased during storage and the control treatment was lower than the others allover the storage period. These results were in agreement with those obtained by Salem et al. (2013).

It was clear from Table 2 that the fat content of all treatments were slightly increased gradually during the progress of storage and the control treatment was lower than the others as storage period proceeded.

Increasing fat content in all treatments during storage may be due to the loss of moisture. These results are in agreement with those obtained by Ismail et al. (2006).

It is clear from this Table 2 that the moisture content of all treatments decreased gradually during the progress of storage. The values of control treatment was higher than the other treatments during storage period.

The decrease in moisture contents of all treatments allover the storage period was probably due to the increase in total solid values.

**Evaluation of Antioxidant activity (%) and Total phenolic content (TPC)**

It is clear from Table 3 that the antioxidant activity in fresh yoghurt made with moringa (T2) exhibited higher significant scavenging activity followed by yoghurt made with moringa + mango (T3) while the plain yoghurt was found to have a lower scavenging effect. High potential of antioxidant activity of treatments may be due to that they are rich in photochemical contents, which possessed high antioxidant.

On the other hand, DPPH radical scavenging activity of all treatments dropped at the 7th and the 14th days of storage period. Data presented in Table 3 show that the total phenolic content (TPC) of yoghurt fortified with moringa and moringa + mango were significant higher than plain yoghurt. Moreover, at the 7th day of storage period the TPC of all samples decreased significantly and also at the 14th day of storage this may be due to the decreased in pH values throughout storage period.
### Table (2): Chemical analysis of yoghurt fortified with moringa during storage up to 14 days at 5°C

<table>
<thead>
<tr>
<th>Chemical analysis (%)</th>
<th>Storage (day)</th>
<th>Treatment</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>13.2</td>
<td>14.6</td>
<td>18.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total solids</td>
<td>7</td>
<td>14.3</td>
<td>15.7</td>
<td>19.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>15.9</td>
<td>16.8</td>
<td>20.5</td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>3.5</td>
<td>3.9</td>
<td>4.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>7</td>
<td>3.6</td>
<td>4.2</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>3.8</td>
<td>4.5</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>3.2</td>
<td>3.3</td>
<td>3.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>7</td>
<td>3.3</td>
<td>3.5</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>3.4</td>
<td>3.6</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>86.8</td>
<td>85.4</td>
<td>81.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moisture</td>
<td>7</td>
<td>85.7</td>
<td>84.3</td>
<td>80.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>84.1</td>
<td>83.2</td>
<td>79.5</td>
<td></td>
</tr>
</tbody>
</table>

T<sub>1</sub>: control (milk)  
T<sub>2</sub>: milk + moringa  
T<sub>3</sub>: milk + moringa + mango + sugar.

### Table (3): Antioxidant activity (%) and Total phenolic content (TPC) of yoghurt fortified with moringa during storage up to 14 days at 5°C

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Storage (day)</th>
<th>Treatment</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>67.34</td>
<td>93.46</td>
<td>87.83</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antioxidant activity (%)</td>
<td>7</td>
<td>43.85</td>
<td>84.63</td>
<td>75.39</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>37.64</td>
<td>77.56</td>
<td>63.84</td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>9.86</td>
<td>15.89</td>
<td>14.71</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPC (mg Gallic acid/100 gm.sample)</td>
<td>7</td>
<td>8.73</td>
<td>15.04</td>
<td>14.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>7.93</td>
<td>13.85</td>
<td>12.96</td>
<td></td>
</tr>
</tbody>
</table>

T<sub>1</sub>: control (milk)  
T<sub>2</sub>: milk + moringa  
T<sub>3</sub>: milk + moringa + mango + sugar.
Microbiological Analysis

Results cleared that there were an obvious differences between treatments of bio-yoghurt in the viable numbers of *S. thermopiles* and *L. bulgaricus* when fresh and during storage period. The counts were increased up to the 7th day then decreased during the progress of storage. The highest count obtained was of yoghurt made with moringa (T2) followed by yoghurt made with moringa + mango (T3) while the lowest count was in control. Moringa alone stimulate the growth of both (*Lactobacillus acidophilus* and *Lactobacillus bulgaricus*) more than moringa + mango. The decline in bacterial counts may be due to the decreasing in the pH value of yoghurt (Yannawa *et al.*, 2014).

These results are in agreement with those obtained by Vijayalakshmi *et al.* (2009), Sharareh *et al.* (2015), Salem *et al.* (2013) moreover Van Tienen *et al.* (2011) suggested that the growth of the probiotics in *M. oleifera*-supplemented yoghurt was found to have a growth-enhancing effect.

It was clear from this Table 4 that the count of *S. thermophilus* increased gradually up to the 7th day of storage then decreased during the progress of storage. The highest count obtained was in yoghurt made with moringa (T2) while the lowest count obtained was in control (T1). These results are in agreement with those obtained by Vijayalakshmi *et al.* (2009), VanTienen *et al.* (2011), Salem *et al.* (2013) and Sharareh *et al.* (2015).

It was clear from the same Table that yeast and mould and coliform group were not detected in all treatments allover the storage period.

Organoleptic properties

Data in Table 5 shows that the total scores of sensory evaluation of all treatments were gradually decreased during storage. This may be due to the increase in the acidity which affect the rheological properties. In general, the values of total sensory evaluation were in the following desending order T3 > T1 > T2. These results are in agreement with those obtained by Madhu *et al.* (2012) and Sharareh *et al.* (2015).

| Table (4): Microbiological analysis of yoghurt fortified with moringa during storage up to 14 days at 5°C |
|-------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Type of culture                                 | Storage (day)                   | Treatments (log (cfu/ml))       |
|                                                 |                                 | T1                              | T2                              | T3                              |
| *L. acidophilus, L. bulgaricus count*           | Fresh                           | 8.32                            | 8.45                            | 8.44                            |
|                                                 | 7                               | 9.42                            | 10.30                           | 10.13                           |
|                                                 | 14                              | 9.30                            | 9.93                            | 9.03                            |
| *S. thermophilus count*                         | Fresh                           | 8.10                            | 8.12                            | 8.11                            |
|                                                 | 7                               | 8.93                            | 10.15                           | 10.04                           |
|                                                 | 14                              | 7.90                            | 9.01                            | 8.93                            |
| *Yeast & Mould and Coliform count*              | Fresh                           | ND                              | ND                              | ND                              |
|                                                 | 7                               | ND                              | ND                              | ND                              |
|                                                 | 14                              | ND                              | ND                              | ND                              |

Table 5: Organoleptic properties of yoghurt fortified with moringa during storage up to 14 days at 5°C

<table>
<thead>
<tr>
<th>Sensory parameter</th>
<th>Storage (day)</th>
<th>Treatment</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh</td>
<td></td>
<td>4.95</td>
<td>4.87</td>
<td>4.80</td>
</tr>
<tr>
<td>Appearance (5 marks)</td>
<td>7</td>
<td></td>
<td>4.95</td>
<td>4.87</td>
<td>4.80</td>
</tr>
<tr>
<td></td>
<td>14</td>
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<td>4.90</td>
<td>4.85</td>
<td>4.75</td>
</tr>
<tr>
<td></td>
<td>Fresh</td>
<td></td>
<td>4.93</td>
<td>4.90</td>
<td>4.85</td>
</tr>
<tr>
<td>Body &amp; Texture (5 marks)</td>
<td>7</td>
<td></td>
<td>4.93</td>
<td>4.90</td>
<td>4.85</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>4.90</td>
<td>4.85</td>
<td>4.80</td>
</tr>
<tr>
<td></td>
<td>Fresh</td>
<td></td>
<td>8.85</td>
<td>8.0</td>
<td>9.5</td>
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<tr>
<td>Flavor (10 marks)</td>
<td>7</td>
<td></td>
<td>8.85</td>
<td>8.0</td>
<td>9.5</td>
</tr>
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<td></td>
<td>14</td>
<td></td>
<td>8.80</td>
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<td></td>
<td>Fresh</td>
<td></td>
<td>18.7</td>
<td>17.77</td>
<td>19.15</td>
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<tr>
<td>Total acceptance (20 marks)</td>
<td>7</td>
<td></td>
<td>18.7</td>
<td>17.77</td>
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<tr>
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<td>14</td>
<td></td>
<td>18.6</td>
<td>17.20</td>
<td>18.85</td>
</tr>
</tbody>
</table>

Conclusion

Finally it was concluded, from the previous data that, the addition of 0.5% moringa leaves powder and 0.5% moringa leaves powder +10% mango pulp in the manufacture of pro-biotic yoghurt stimulate the growth of LAB and probiotic culture (L. acidophilus) so increased the nutritional value of yoghurt. Also all treatments had a high positive effect on total phenolic contents and its antioxidant properties. Moreover the addition of 0.5% moringa leaves powder +10% mango pulp in the manufacture of yoghurt increased the acceptability of product more than the addition of moringa alone up to the 14th day of storage at 5°C.

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استخدام مسطح أوراق المورنجا في إنتاج زبادي وظيفي

علي فتحي السيد إبراهيم، أحمد سيد محمد الطويل

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تم تصنيع الزيتاء باستخدام مادة من شعيرية في ثلاثة أجزاء متوازية كجزء من تطوير المكملات كالي: المكملة الأولى: Lactobacillus delbrueckii ssp. bulgaricus و Streptococcus thermophilus

لا تتأثر النوى على المكملات بالطين من %70 و %60 من المادة الصلبة في المكملات وينتظر تأثير تكوين البذور لنقل البذور وفق الخصائص المكملات في المكملات الأولى وفق الخصائص المكملات في المكملات الأولى والثاني: %50 من الزيتاء الممرض للبيئات والبيئات بالبيئات والبيئات كما في المكملات الأولى.

يتأتى النتائج انتقائي في اليوم الأول، وكذلك أثناء التزبني على %65. بعد 47 يوم من التزبني حيث تم عمل اختبارات كيميائية وميكروباتية وولوجية للتحقق من النتائج كالي.

للمكملات، وتم استخدام المكملات الكلاسيكية في مصادر معينة من المكملات مثل L. acidophilus و L. delbrueckii ssp. bulgaricus و Streptococcus thermophilus.

في المكملات، وتم استخدام المكملات الكلاسيكية في مصادر معينة من المكملات مثل L. acidophilus و L. delbrueckii ssp. bulgaricus و Streptococcus thermophilus.

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