# PRODUCTION OF FUNCTIONAL FOOD USING BACTERIAL FERMENTATION

**ZAKI, HALA MOHAMED, F.A. SALEH AND A.I. AHMED**

*Special Food and Nutrition Department, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt.*

(Manuscript received 27 July 2004)

***Abstract***

A special combination of waste materials such as green collared leaves by-product, salted cheese whey and molasses (80/10/10, W/W/W) (GWM), to investigate the production of some beneficial microorganisms biomass. At the begning, GWM was heat-treated at 85 °C for 15 min, cooled to 40 °C and incubated with some lactic acid bacteria and bifidobacteria. Ten lactic acid bacteria and bifidobacteria were used in this study. *L. helveticus, L. casie,* yoghurt culture YC-X11, *B. longum, B. lactis* and *B. bifidum* showed relatively high growth ability and activity. Among all investigated bacteria *L.helveticus*, recorded a higher decreasing effect on pH values (pH 4.5) followed by *B. lactis* (pH 4.6) after 48 h of incubation period. The viable count of bacteria were higher than $10^4$ cfu/g. However this fermented GWM (nutra green) is considered to be value-added bioingredient functional food. It's also containing new type of probiotic bacteria to be used in food patterns. The nutra green for instance was used in preparation of what so-called green yoghurt with an acceptable morphological test and expected higher nutritional value.

## INTRODUCTION

Lactic acid bacteria is a group of microorganisms that of great role in milk processing and milk product industries. The microorganism is an important part of biotechnology. The greatest role of these microorganisms in our modern life is its efficiency in recycling wastes to be valuable matter rather than to be a great source of pollutants. Moreover, Lactic acid bacteria produced bacteriocin which includes nisin, pediocin and heleveticin. Bacteriocins used to inhibit undesirable microorganisms in food, but only nisin is produced industrially and is licensed for use as a food preservative in a partially purified form (Parente and Ricciardi, 1999). Mixture of organic acids produced by lactic acid bacteria such as acetic, caproic, formic, propionic, butyric and n- valeric acids, acting in a synergistic way, were responsible for the antimould activity. Caproic acid plays a key role in inhibiting mold growth (Corsetti *et al.*, 1998).Several species of Bifidobacterium are

considered be the most important among the probiotic organisms; Bifidobacteria is a non-pathogenic bacteria, which inhibit the intestinal tracts pathogens in humans (Kurmann and Rasic, 1991). Among the reported beneficial effects of consuming certain strains of bifidobacteria or metabolites enhanced immune response, balancing of colonic microbial, vaccine adjuvant effect, reduction of facal enzymes implicated in cancer initiation, treatment of diarrhea associated with travel, antibiotic therapy, control of rotavirus, synthesis of vitamins, reduction of serum cholesterol, antagonism against food-borne pathogens, tooth decay organisms and amelioration of lactose malabsorption symptoms (Marin *et al.,* 1997). Whey is the main by-product of the dairy industries. The salted whey (5-10% salt) is a result of Domiati cheese manufacture as an example of Egyptian mass production. Internationally, whey is produced in the region of 130 million ton per year in 1992 with 3% expected annual increase thereafter (Zadow, 1992). Zaied (1997) and El-Gindy (1997) showed that about 500,000- 1.000.000 tons per year of whey were produced in Egypt during that year. Whey generally contains about 6 to 6.5 total solids, which represents almost half the total solids of milk from which it derives; these are in general lactose, protein, minerals, salts, vitamins and traces of fat. Whey is utilized to produce lactose, organic acid, amino acid, single cell protein; gibberellic acid, B-galactosidase, flavour compounds and protein concentrate (Reed and Nagodawithana, 1991). On the other side, green wastes if not use well be an in important source of pollution. Alternatively, they may become a greatest source of proteins. This protein and its conjugated nutrients can be used as carriers for these starter cultures of health promotion. That combination is expected to be food product of good balance with health aspect. Here we are attempting to introduce a new starter culture of specific nutritional properties based on the implication of proteins derived from certain recycling process. This work was aimed to produce beneficial microorganisms biomass using industrial food wastes.

## MATERIALS AND METHODS

-Salted cheese whey and pastaralization bafflo's milk were obtained from the milk processing unit, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt.

- Molasses were supplied from Ibex international company.

- Green collared leaves were obtained from local fields of Monophyea Governorate.

- Starter cultures: *Bifidobacterium bifidum* Bb-11, *Bifidobacterium lactis* (Bb-12), *Bifidobacterium longum* Bb-46, YC-X-11 (*Lactobacillius dulbrueekii* sub sp. *bulgaricus, Streptococcus salivarius* sub sp. *thermophilus), Lactobacillius heliveticus* 02 and *Lactobacillius casie* were supplied by Chr. Hansen Laboratories, Copenhagen, Denmark.
- Green collared leaves waste were prepared according to Ahmed and Ibrahim (1990), by freezed to destroy the cells and consequently the cell contents released. These contents minced, diluted with water (1:1 w/v) and homogenitied. The resulted green pulp (GP) utilized in the experiments. The mixer of GP, cheese whey and molasses (80, 10, 10%, w/w/w) (GMW) has been used as the bulk medium to produce the beneficial bacteria.
- Plain and probiotic yoghurt were prepared according to the method of Tamime and Robinson, (1985).
- *L. heliviticus* and *L. casie* were counted using MRS agar, while, Bifidobacteria were determined by MRS agar + 0.05% L-cystein-HCl according to the method of Dinaker & Mistry (1994), whereas, the count of yoghurt culture (YC-X-11) were determined according to the method of Lee *et al.* (1973).
- Moisture and protein content were determined by the method described by A.O.A.C. (1990).
- Sugars and organic acids were analyzed by high performance liquid chromatography (HPLC) according the method of Black and Bagley (1978) and Adhikari *et al.* (2000), respectively.
- Sensory evaluation was carried out by a regular score panel according to Tamime and Robinson (1985).
- Statistical analysis Data are presented as means and standard deviation. The significant differences among yoghurt treatments were evaluated using a one-way ANOVA to analyze the points of sensory evaluation by Least Significant Different (LSD) at $P < 0.05$

## RESULTS AND DISCUSSION

Initial pH of growth medium ranged between 6.25 to 6.70 are listed in Table (1). Within 24 h, pH was decreased to about 5.35 to 4.59. Little less acidic pH was observed after 48 h. It was observed that, YC-X-11 bacteria achieved little less pH movement. While, *L. heliveticus* gave the lowest value of pH, Table (1). These results

are in agreement with Jihan Kassem (2000) and Ghaly *et al.*(2003) who used cheese whey for producing *Lactobacillus heliveticus* at pH 4.5 and 5.5. Sneath (1986) mentioned that the optimum pH for initial growth of bifidobacterium was 6.5-7.0, mainwhile no growth was recorded at pH 4.5-5.0 or 8.0-8.5.

Production of organic acids by different starter culture during fermentation of GMW are shown in Table (2). The results indicated that production of organic acids can be influenced by the kind of starter cultures during fermentation period. Both *L. heliveticus* and YC-X-11 intensified lactic acid as fermentation period set up from 24 h to 48 h. while, it reduced acetic acid after the same period (Table 2). However, *L. casie, Bifidobacterium* Bb-12 and *Bifidobacterium* Bb-46 diminished lactic acid and built up acetic acid after the same period, (Table 2). On the other hand, *Bifidobacterium* Bb-11 stepped up Lactic acid and acetic acid after the same period (Table 2).

It is worthy to mentioned, that the results in Table 2 indicate number of starter cultures that exceeded the level of production of lactic acid and acetic acid which considered to be antimicrobial factors. Corsetti *et al.* (1998) mentioned that after incubation for 48h at 30 °C, organic acids were optimally produced when the initial pH of the WFH broth medium was 6.0 the production of the inhibitory compounds began to existed after 12h of incubation, but rapidly increased, especially for caproic, formic and acetic acids.

Data in Table (3) express count of different starter culture during fermentation of GMW. In general, bacterial count (cfu/g) of different starter culture sharply intensified as fermentation period (h) built up from 24h to 48 h except YC-X-11 and *Bifidobacterium* Bb-11, Table (3). Even last bacteria slightly increased after the same period.

The changes in GMW fermented yield, is of cell weight and protein%, are shown in Table (4). It worthy to mention that *Bifidobacterium* Bb-12 achieved the highest yield, followed by *Bifidobacterium* Bb-11. However, the lowest yield was obtained by either *Bifidobacterium* Bb - 46 or *L. heliveticus*.

Similarly, the most increasing of cell weight resulted in *Bifidobacterium* Bb-12, but the least one was *Bifidobacterium* Bb-46 (Table 4).

Data in Table (4) exposed protein percentage as affected by starter cultures. It was observed that the maximum value of protein has been realized by *L. heliveticus* as compared to the protein value of GMW (2.41%). While *Bifidobacterium* Bb-46

gave the minimum value of protein (4.24%)as compared with protein% of GMW, (Table 4).

The yield of starter cultures increase may be attributed to the supplementation of the cheese whey complex to natural nutrients such as green collared leaves addition to the molasses that enhances the growth and yield production of lactic acid bacteria and bifidobacterium. This result is in accordance with the result obtained by Ghaly *et al.* (2003).

Data in Table (5) show sugars profile of fermented GMW using different starter cultures during fermentation period. It was found that both glucose and fructose declined after 24 h of fermentation. This means that the different starter cultures consumed these sugars to produce organic acids such as lactic and acetic acids which are clear in Table (5). In contrast, after 48 h of fermentation *L. casie*, *Bifidobacterium* Bb-12 and *Bifidobacterium* Bb-46 just consumed glucose. In contrary, glucose intensified by another starter cultures such as YC-X-11 and *Bifidobacterium* Bb-11. This increase is most probably refers to the hydrolysis of sucrose to glucose and fructose. From the obvious results, it can be concluded that the starter cultures have different capabilities in consuming or producing the sugars at different duration of time according to the microbial environment.

These cultures have been employed to produce varied yoghurt new form comparing to the regular one.

Sensory evaluation of yoghurt dealed with different starter cultures is shown in Table (6). It was carried out by a regular score panel according to the method described by (Tamime and Rebbonson, 1992). The degree of flavour acceptability, appearance and texture or consistency in stirred yoghurt; respectively and smell (odor) are shown in Table (6). In organolyptic tasting all treatment showed significance difference between samples and plain control except *Bifidobacterium* Bb-12 as regards to flavor. Concerning appearance, *L. heliveticus*, *L. casie* and *Bifidobacterium* Bb-12 showed significant differences, while the other treatments appeared to be similar to the plain control. On other words, no significance difference was obtained between the all starter cultures and plain control in to texture, Table (6). In contrary, *L. casie* showed significance difference in compared to plain control regarding to smell odor, but the rest treatments appeared no significance difference between the samples and plain control, (Table 6).

In contrast, addition of strawberry to the different starter cultures yoghurt has disappeared the significance differences between the all starter cultures when compared to undertaken strawberry control regarding the flavor as seen in Table (6). However, either *L. casie* or YC-X-11 still showed significance difference in flavor. It was worthy to mention that all starter cultures seen to be at no significance difference with strawberry control both in appearance and smell or order as clear in Table(6). In contrary, only *Bifidobacterium* Bb-12 appeared no significance difference between this treatment and strawberry control concerning texture. In all cases the starter cultures showed significant texture difference between the samples and strawberry control as reported in Table (6).

Table 1. Effect of different starter culture on pH values of GMW during fermentation period.

| Starter cultures | Initial pH* | Fermentation period (h) | |
|---|---|---|---|
| | | 24 | 48 |
| | | pH values | |
| *L. heliveticus* | 6.38 | 4.59 | 4.54 |
| *L. casie* | 6.70 | 4.81 | 4.77 |
| YC-X-11 | 6.25 | 5.35 | 5.28 |
| *Bifidobacterium* Bb-11 | 6.56 | 4.84 | 4.82 |
| *Bifidobacterium* Bb-12 | 6.62 | 4.65 | 4.58 |
| *Bifidobacterium* Bb-46 | 6.60 | 4.78 | 4.67 |

* after addition of starter culture

Table 2. Production of organic acids by different starter culture during fermentation of GMW.

| Starter cultures | Fermentation period (h) | | | |
|---|---|---|---|---|
| | 24 | | 48 | |
| | Lactic acid % | Acetic acid % | Lactic acid % | Acetic acid % |
| *L. heliveticus* | 2.58 | 2.74 | 3.87 | 0.63 |
| *L. casie* | 2.96 | 1.11 | 2.79 | 1.36 |
| YC-X-11 | 1.75 | 1.33 | 2.89 | 0.48 |
| *Bifidobacterium* Bb-11 | 2.41 | 1.23 | 2.94 | 1.68 |
| *Bifidobacterium* Bb-12 | 3.27 | 1.26 | 2.96 | 1.36 |
| *Bifidobacterium* Bb-46 | 2.57 | 0.85 | 2.45 | 1.22 |

Table 3. Count of different starter culture during fermentation of GMW.

| Starter cultures | Fermentation period (h) | |
|---|---|---|
| | 24 | 48 |
| | Bacterial count* CFU/g | |
| *L. heliveticus* | 74.8 | 82.6 |
| *L. casie* | 11.65 | 25.6 |
| YC-X-11 | 1.3 | 1.6 |
| *Bifidobacterium* Bb-11 | 25.6 | 25.9 |
| *Bifidobacterium* Bb-12 | 19.6 | 21.3 |
| *Bifidobacterium* Bb-46 | 45.9 | 65.7 |

* log CFU/g fermented WGM

Table 4. Changes in GMW fermented yield and protein % during 48 h of fermentation by different starter culture.

| Starter cultures | Yield (gram) | Yield increasing | Protein % | Protein increasing |
|---|---|---|---|---|
| WGM* | 10.43 | - | 2.41 | - |
| *L. heliveticus* | 10.82 | 0.39 | 7.79 | 3.94 |
| *L. casie* | 10.92 | 0.49 | 5.18 | 1.33 |
| YC-X-11 | 10.94 | 0.51 | 5.78 | 1.93 |
| *Bifidobacterium* Bb-11 | 10.97 | 0.54 | 5.78 | 1.93 |
| *Bifidobacterium* Bb-12 | 11.35 | 0.92 | 6.46 | 2.61 |
| *Bifidobacterium* Bb-46 | 10.81 | 0.38 | 4.24 | 0.39 |

* weight of WGM before fermentation.

Table 5. Sugars profile of fermented GMW using different starter cultures before and during fermentation period.

| Starter cultures | Fermentation period | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 24 h | | | | | 48 h | | | | |
| | Sugars % | | | | | | | | | |
| | Sucrose | Lactose | Glucose | Galactose | Fructose | Sucrose | Lactose | Glucose | Galactose | Fructose |
| Zero time* | 2.29 | 0.15 | 1.47 | 0.66 | 0.26 | -- | -- | - | -- | -- |
| *L. heliveticus* | 2.82 | 0.19 | 0.15 | 0.91 | 0.24 | 2.08 | 0.14 | 0.61 | 0.72 | 0.23 |
| *L. casie* | 2.62 | 0.17 | 0.75 | 0.61 | 0.25 | 2.08 | 0.14 | ND | 0.86 | 0.22 |
| YC-X-11 | 2.04 | 0.14 | ND | 0.57 | 0.20 | 2.24 | 0.15 | 1.28 | 0.71 | 0.23 |
| *Bifidobacterium* Bb-11 | 1.82 | 0.12 | ND | 0.40 | 0.19 | 0.56 | 0.04 | 0.47 | 0.49 | 0.12 |
| *Bifidobacterium* Bb-12 | 1.87 | 0.12 | 0.43 | 0.51 | 0.21 | 3.24 | 0.22 | ND | 0.89 | 0.24 |
| *Bifidobacterium* Bb-46 | 0.85 | 0.06 | 0.68 | 0.14 | 0.12 | 2.72 | 1.81 | ND | 1.00 | 0.24 |

* GMW before fermentation

ND = not detected.

Table 6. Organolyptic evaluation of plain and strawberry yoghurt containing ferment GMW using different starter cultures.

| Starter cultures | Flavor point 50 | Appearance Point 20 | Texture Point 20 | Smell order Point 10 | Total Point 100 |
|---|---|---|---|---|---|
| Plain | | | | | |
| Control | 47.6±1.5 | 17.9±1.5 | 17.8±1.5 | 8.4±1.1 | 91.7 |
| *L. heliveticus* | 38.7±4.7* | 14.6±3.7* | 15.7±3.0 | 8.1±1.5 | 77.1 |
| *L. casie* | 35.1±6.1* | 14.3±4.4* | 16.4±2.3 | 6.9±1.4* | 72.7 |
| YC-X-11 | 36.6±8.7* | 15.8±2.9 | 15.8±1.7 | 8.0±1.4 | 76.2 |
| *Bifidobacterium* Bb-11 | 37.0±7.2* | 15.3±2.7 | 15.8±2.7 | 7.3±1.6 | 75.4 |
| *Bifidobacterium* Bb-12 | 35.7±7.4* | 14.4±3.4 | 16.7±1.7 | 7.3±1.3 | 74.1 |
| *Bifidobacterium* Bb-46 | 33.4±6.9* | 15.8±2.9 | 16.8±2.2 | 7.8±1.5 | 73.8 |
| Strawberry | | | | | |
| Control | 46.9±1.8 | 17.5±1.7 | 18.3±1.5 | 9.0±0.7 | 91.7 |
| *L. heliveticus* | 41.9±6.6 | 16.0±2.6 | 15.4±3.5* | 8.4±1.6 | 81.7 |
| *L. casie* | 40.7±5.9* | 16.2±1.8 | 15.6±3.4* | 8.1±1.7 | 80.6 |
| YC-X-11 | 40.2±6.1* | 15.1±1.8 | 15.6±2.6* | 8.1±1.8 | 79.0 |
| *Bifidobacterium* Bb-11 | 41.4±7.2 | 15.2±2.8 | 15.6±3.5* | 8.3±1.8 | 80.5 |
| *Bifidobacterium* Bb-12 | 41.6±5.3 | 15.2±2.5 | 15.1±2.7* | 8.3±1.5 | 80.2 |
| *Bifidobacterium* Bb-46 | 43.1±5.7 | 15.7±3.1 | 16.9±2.6 | 8.4±1.5 | 84.1 |

*the mean is significant at ($p<0.05$)

## REFERENCES

1. A. O. C. C. 1990. Association of Official Analytical Chemists, Official method of Analysis. Fifteenth Edition Published by the Association of Official Analytical chemists INC.2200 Wislon Boulevard, Suite400. Arlington, Virginia, 22201.

2. Adhikari, K., Mustapha, A. and Grun, I. U. 2000. Viability of microencapsulated bifidobacteria in set yoghurt during refrigerated storage. J. Dairy Sci 83:1946-1951.

3. Ahmed, A.I.S. and Ibrahem, E.M. 1990. "Leaf protein by-product effects on yeast growth" Agric Res. Rev.68(2)389-395.

4. Black, L. T. and Bagley, E. B. 1978. Determination of oligosaccharides in soybeans by high pressure liquid chromatography using an internal standard. J. of the American Oil Chemists Society Vol.55(2):228.

5. Corsetti, A.; Gobbetti, M., Rossi, J. and Damiani, P. 1998. Antimould activity of sourdough lactic acid bacteria: identification of a mixture of organic acids produced by *Lactobacillus sanfrancisco* cB1. Appl Microbiol Biotechnol. 50:253-256.

6. Dinaker, P. and Mistry, V.V. 1994. Growth and viability of *Bifidobacterium bifidum* in chedder cheese. J. Dairy Sci, 77(10): 2854-2864.

7. El–Gindy, S. M. 1997. A review on handling and proper management of byproducts from the food industry. Dairy Technology Section. Acadimy of Seintific Research and Technology (in Arbic).

8. Ghaly, A. E., Tango, M. S. A. and Adams, M. A. 2003. Enhanced lactic acid production from cheese whey with nutrient supplement addition. Agricultural Engineering International: the CIGR Journal of Scintific Research and Development Manuscript FP02009.

9. Jihan Kassem, M. 2000. Reducing pollution from whey by the production of single cell protein. Thesis MSc. Of Dairy Science and Technology, Faculty of Agriculture, Cairo University.

10. Kurmann, J. A., and rasic, J. L. 1991. The health potential of products containing bifidobacteria. Page 117 in Therapeutic Properties of fermented milks. Robinson, R.K ed. Elsevier Appl. Sci., London, England.

11. Lee, S. Y., Vedemuthu, E. R.; Washam, C.J. and Reinbold, B. W. 1973. An agar medium for the differential enumeration of yoghurt starter bacteria. J. Milk Food Tech., 9(37):272-275.

12. Marin, M. L., Lee, J. H.; Murtha, J., Ustunol, Z. and Pestka, J. J. 1997. Deferential cytokine production in conal macrophage and t-cell lines cultured with Bifidobacteria. J of Dairy Sci. vol.80, No.11,2713-2720.

13. Parente, E. and Ricciardi, A. 1999. Production, recovery and purification of bacteriocins from lactic acid bacteria, Appl. Microbiol Biotechnol 52: 628-638.

14. Reed, G. and Nagodawithana, T. W.1991. Yeast Technology. P.31,70 and 273.AVI Publishing Co., New York.

15. Sneath, P.H.A., Mair, N. S. Elisabeth Sharpe, M. and Holt, J. G. 1986. Bergeys manual of systematic bacteriology. Vol.2. Williams and Wilkins, London.

16. Tamime, A. Y. and Robinson, R.K. 1985. Yoghurt, Science and Technology. Paramo Press, Oxfored, England.

17. Zadow, J. G. 1992. Lactose utilization. Food Res. Quarterly. 51:1-2.

18. Zaid, K. A. 1997. A genetically improved lactose whey fermentation by *Saccharomyces cerevisiae* hybrids. Annals Agric. Sci. Ain Shams Univ., Cairo,42:429-434.

# إنتاج أغذية وظيفية باستخدام التخمرات البكتيرية

**هالة محمد زكى على محمد - فرج على صالح - على إبراهيم أحمد**

*قسم الأغذية الخاصة والتغذية - معهد بحوث تكنولوجيا الأغذية - مركز البحوث الزراعية .*

الخلطة المستخدمة فى هذه التجربة تتمثل فى استغلال منتجات ثانوية يتوالى إنتاجها فى البيئة دون الاستفادة منها، مما يؤدى إلى تلوث البيئة، وتشمل هذه المنتجات الثانوية ورق الكرنب الأخضر (كمنتج ثانوى) والمولاس والشرش المالح و بنسبة (٨٠/١٠/١٠، بالوزن) ويرمز لها GMW وقد استخدمت هذه الخلطة كبيئة لإنتاج بعض البكتيريا النافعة بعد أن عوملت هذة الخلطة (GMW) بالحرارة على درجة ٨٥° م لمدة ١٥ دقيقة ثم بردت الى ٤٠° ثم حضنت مع بكتيريا حامض اللاكتيك وبكتيريا البيفيدو( Bifidobacteria) . وفى هذه الدراسة تم استخدام عشرة أنواع من هذه البكتيريا من بينهم ٦ أنواع أظهرت القدرة على النمو والنشاط فى هذه الخلطة GMW وتشمل هذه الأنواع:-

ثلاثة أنواع من بكتيريا حامض اللاكتيك وهى (*L. helveticus, L .casie* and *L. acidophilus*)

أما الثلاثة أنواع الأخرى تمثل بكتيريا البيفيدو وتشمل (*B .Longum, B .lactis* and *B. bifidum*)

ومن بين كل البكتيريا المستخدمة سجلت *L. helveticus,* أقل القيم فى الـ pH وهى (pH4.5 ) يليها *B. lactis* أعطت (pH4.6 ) بعد ٤٨ ساعة من فترة التحضين. أظهر العد الكلى للبكتيريا فى كل الأنواع المختبرة أعلى من ١٠ $^{٤}$ خلية/جرام. وتعتبر هذه الخلطة الغذائية المتخمرة ذات قيمة حيوية غذائية. وهذه الخلطة أيضا تحتوى على نوع من البكتيريا تنتج مواد مفيدة صحيا. وهذة الخلطة استخدمت فى تجهيز ما يسمى بالزبادى الاخضر وزادت من قيمة الغذائية وكان مقبولا من النواحى الظاهرية بالإضافة إلى احتواءة على ما يجعلة غذاء وظيفيا.